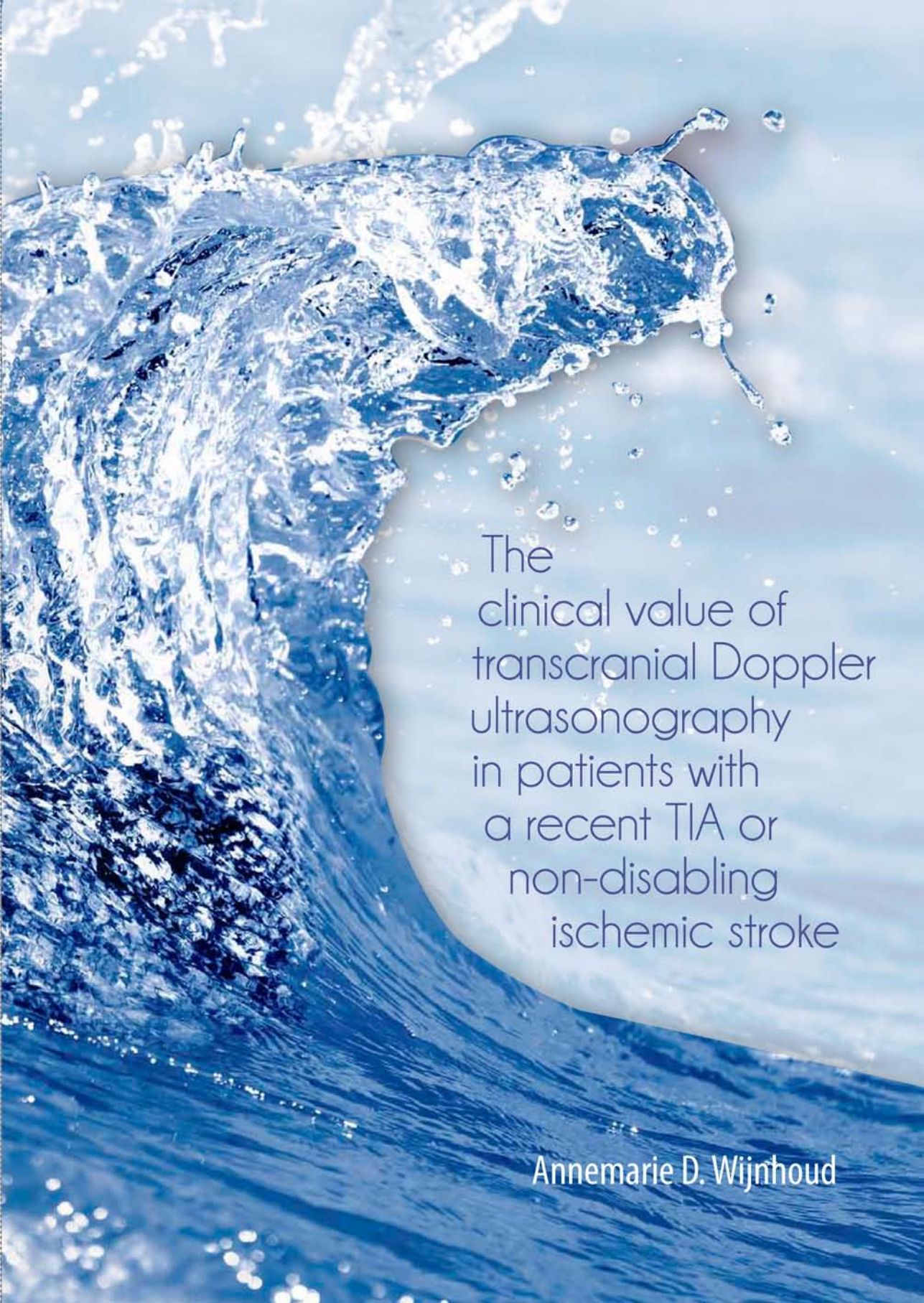
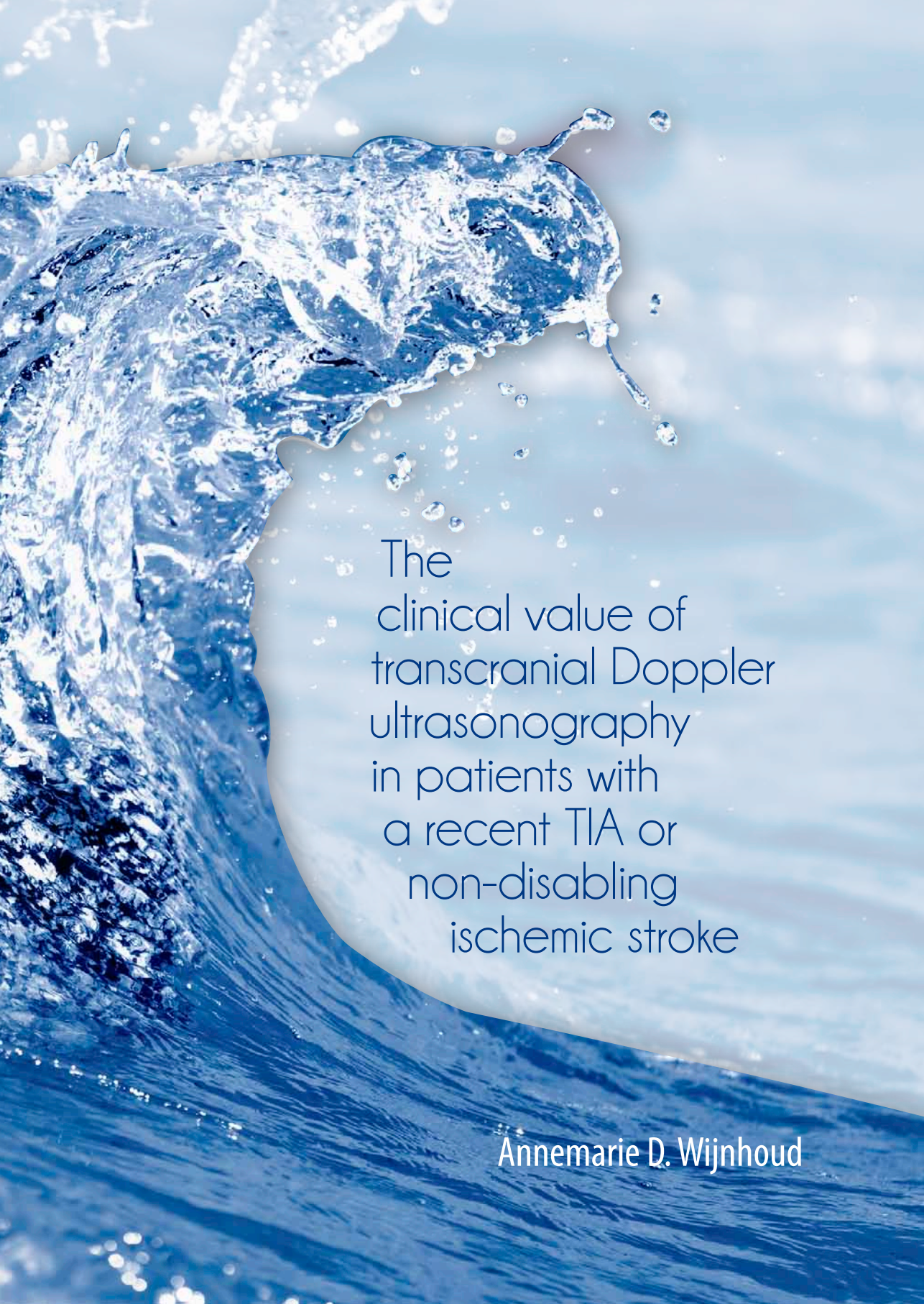


The clinical value of transcranial Doppler ultrasonography in patients with a recent TIA or non-disabling ischemic stroke

Annemarie D. Wijnhoud



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Annemarie D. Wijnhoud

ISBN: 978-94-6169-322-8

Layout and printing: Optima Grafische Communicatie, Rotterdam, The Netherlands

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De klinische waarde van transcranieel Doppler ultrageluid bij patiënten met een recente TIA of licht herseninfarct

Proefschrift

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

prof.dr. H.G. Schmidt

en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
Donderdag 22 november 2012
om 09.30 uur

door

Annemarie Dagmar Wijnhoud
geboren te Zwolle

PROMOTIECOMMISSIE

Promotoren:	Prof.dr. D.W.J. Dippel
	Prof.dr. P.J. Koudstaal
Overige leden:	Prof.dr. A. van der Lugt
	Prof.dr. E.W. Steyerberg
	Prof.dr. J. Stam

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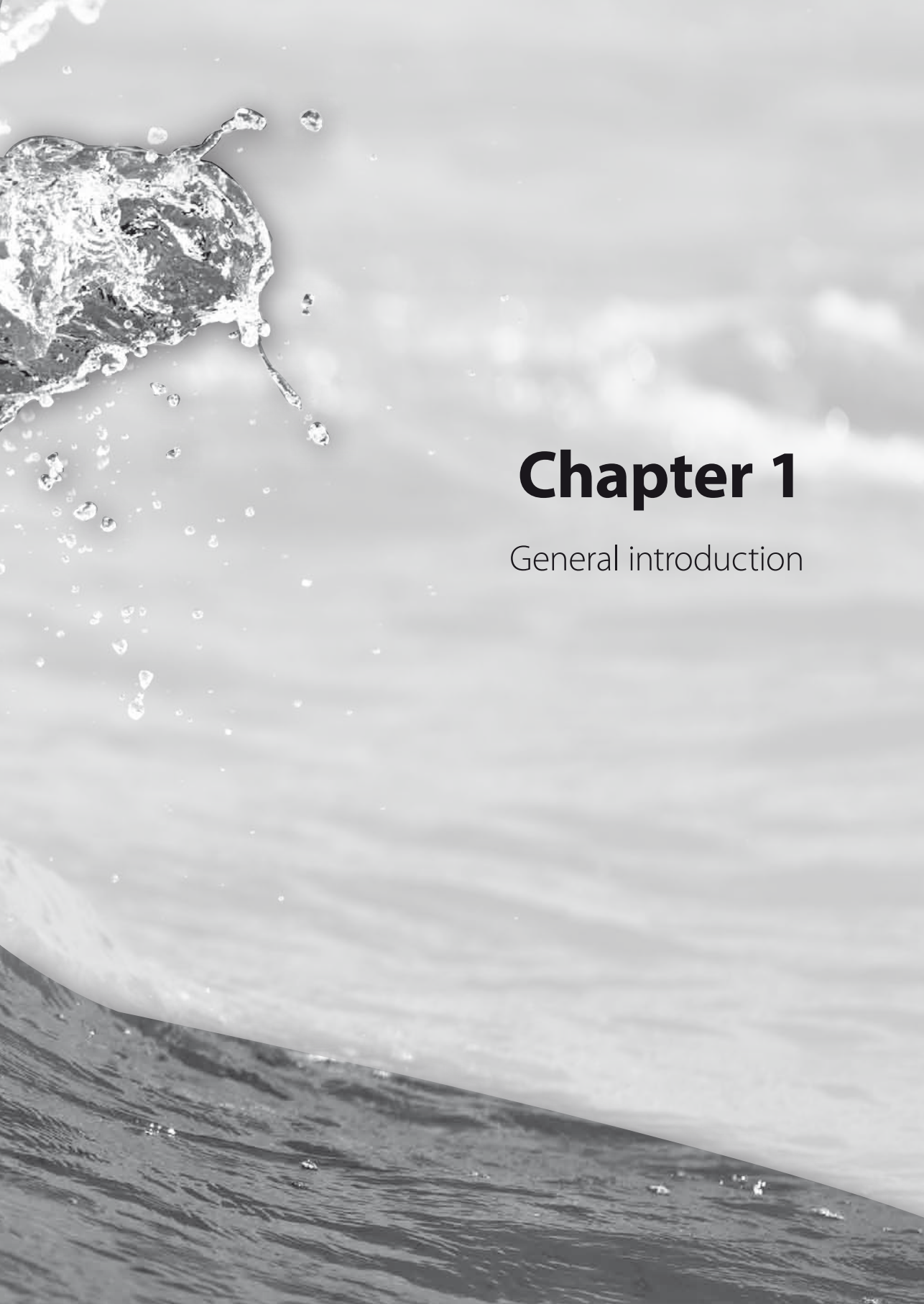

Chapter 1

General introduction

Stroke is the third leading cause of death in developed countries, after heart disease and cancer, and the first cause of disability.[1] Most strokes are ischemic and caused by occlusion of a cerebral artery. This leads to dysfunction and eventually death of brain tissue through lack of oxygen. This results in typical symptoms such as unilateral weakness, language disturbances, unilateral sensory disturbances, hemianopia, ataxia, or impaired speech. In the acute phase of tissue dysfunction, patients can be treated with thrombolytic agents, but treatment should be started within 4,5 hours after onset of symptoms. However, at present, only 25% of patients are eligible for this treatment, and even when patients can be treated, treatment is not always successful.[2] In many patients, cerebral ischemia is only transient and does not result in persistent symptoms and disability. These Transient Ischemic Attacks (TIAs) or minor ischemic strokes offer the opportunity to prevent major, disabling strokes or other vascular events. Secondary prevention is therefore one of the main objectives of stroke management.

Risk of recurrent stroke is approximately 9-16% annually after ischemic stroke. Intensive secondary prevention by use of antiplatelet medication and treatment of high blood pressure and high cholesterol has reduced the annual risk of recurrent events to approximately 4.5%.[3] The most well-known risk factors for stroke or other major vascular events such as myocardial infarction or vascular death are age, male sex, hypertension, diabetes, and smoking. There are many other risk factors such as white matter lesions, carotid or vertebral artery stenosis, and atrial fibrillation. Unfortunately, even with this large number of prognostic factors, it is still hard to discriminate between patients with a high risk of recurrent stroke and patients with a low risk of recurrent stroke. It has therefore been contended that it is important to identify new risk factors for the occurrence of stroke and other major vascular events.[4]

Transcranial Doppler ultrasonography, the main topic of this thesis, offers noninvasive information on flow velocity, cerebral CO_2 reactivity, and the presence of micro-embolic signals. These three parameters may provide new predictors of increased risk of stroke recurrence and other vascular complications. Transcranial Doppler ultrasonography allows the noninvasive investigation of arterial blood flow within the skull.[5] It was first described in 1982 by Aaslid et al.[5] Low frequency (2 MHz) ultrasound can pass through the bone at the level of the temporal window, which is located just above the ear where the temporal bone is the thinnest. Pulsed wave ultrasound is reflected by flowing blood, which reflects the ultrasound wave. This causes a phase shift, wherein the frequency is decreased or increased, depending on which direction the blood is flowing. This frequency change correlates directly with the blood flow velocity. This is called the Doppler effect. With this technique several parameters can be studied in the main cerebral arteries: flow velocities (mean flow velocity, peak flow velocity, and end-diastolic flow velocity), cerebral CO_2 reactivity, and micro-embolic signals (MES), which are described below.

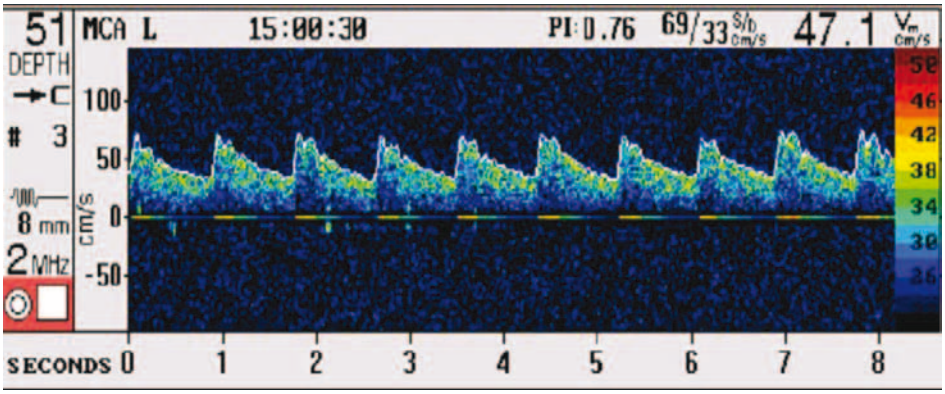

Figure 1: Example of TCD signal: Flow velocity in the left middle cerebral artery. On the X-axis is the timeline (in seconds), on the y-axis the flow velocity in cm/sec.

Flow velocity parameters

As the velocity in the major cerebral arteries is dependent on the heartbeatcycle, peaksystolic flow velocity (PSV) and end-diastolic flow velocity (EDV) can be defined as parameters (Figure 1). With these parameters mean flow velocity (MFV) can be calculated:
MFV= EDV + 1/3(PSV-EDV).

Pulsatility index (PI), a measure of peripheral vascular resistance can be calculated as:
(PSV-EDV)/MFV

High flow velocity parameters are diagnostic for a middle cerebral artery (MCA) stenosis. A mean flow velocity of > 100 cm/s or a PSV of > 140 cm/s is highly suggestive of a MCA stenosis of more than 50%.[6] A MCA stenosis is, however, rare in Caucasian patients.[7,8] patients with a MCA stenosis of more than 50% have an increased risk of recurrent stroke compared to patients without a MCA stenosis. MFV in the MCA is related to age and sex. MFV decreases with age[9] and MFV is higher in women.[10] The relationship with other vascular risk factors in unclear. In healthy elderly patients an increased MFV is related to increased risk of stroke.[11] Before TCD parameters can be studied as diagnostic or prognostic factors, these relationships will have to be investigated.

Cerebral CO_2-reactivity

The compliance of the cerebral circulation, also called the cerebral vasomotor reactivity or cerebral CO_2-reactivity, can be measured.[12] The cerebral CO_2-reactivity measures the flow velocity change in a major cerebral artery during hypercapnia. During hypercapnia distal vasodilatiation occurs and therefore the flow velocity in the observed major artery will have to rise. The cerebral CO_2-reactivity is the percentage increase in flow velocity in the observed

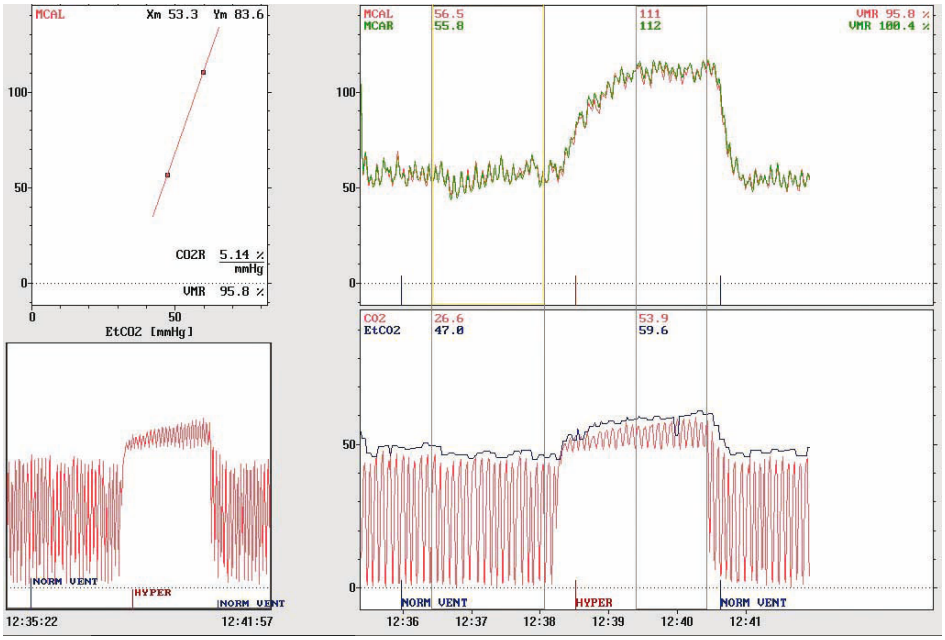

Figure 2: example of Cerebral CO_2-reactivity.The x-axis represents the time line. The upper lines represent the flow velocities in the left (red line) and right (green line) middle cerebral arteries measured in cm/s (y-axis). The lower lines represent the end-tidal CO_2 (blue line, and the actual expired CO_2 concentration (red line). Note that flow velocities increase during hypercapnia and quickly return to normal during normal ventilation. The percentage increase in mean flow velocity during hypercapnia represents the cerebral CO_2 reactivity.

major artery, usually the middle cerebral artery (Figure 2). This is thought to reflect the vascular reserve capacity in the brain.

Cerebral CO_2-reactivity diminishes in patients with a severe carotid artery stenosis or occlusion, and is a predictor of recurrent stroke in these patients.[13,14] It is unknown whether this applies for patients without a carotid artery stenosis. An impaired cerebral CO_2-reactivity is related to various vascular risk factors such as hyperlipidemia and diabetes, and to the presence of white matter lesions on MRI scan of the brain.[15-17] In patients with a lacunar infarction, the cerebral CO_2-reactivity is lower than in healthy controls.[18] This may suggest that an impaired cerebral CO_2-reactivity is a prognostic factor for stroke. The cerebral CO_2-reactivity seems to distinguish between patients with a cortical infarction and patients with a lacunar infarction,[19] but this has not been reproduced. The diagnostic and prognostic value of the cerebral CO_2-reactivity in patients without a carotid artery stenosis is therefore still unknown.

Micro-embolic signals

With continuous TCD registration, circulating micro-embolic signals (MES) can be detected (figure 3). Due to increased scattering and reflection of ultrasound from the embolus, compared

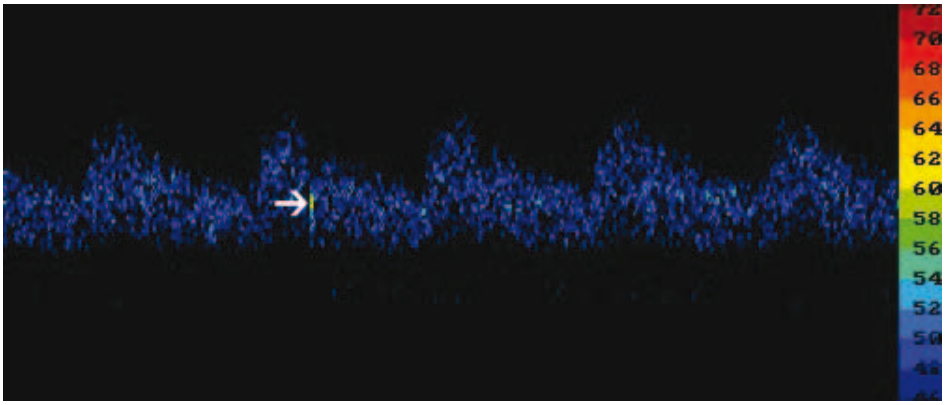

Figure 3: Example of a TCD signal in the middle cerebral artery with a micro-embolic signal (white arrow)

with the surrounding red blood cells, an embolus appears as a short duration high intensity signal within the Doppler flow spectrum.

MES occur more frequently in acute stroke patients with a history of TIA or ischemic stroke[20] and in patients with symptomatic or asymptomatic carotid stenosis.[21-23] In these patients the presence of MES constitutes a higher stroke risk. Higher prevalence rates are reported in patients with atrial fibrillation compared to those without. Patients with a mechanical heart valve have more frequent and louder MESs than those with a biological valve.[24-26] These MES are thougth to be gaseous, not solid.[27] All together MES may be of importance in detecting potential sources of cardiac[28] (or extracranial) embolism.

Furthermore, the presence of MES may play a role in differentiating between stroke subtypes. In patients with lacunar infarction (small vessel disease) MES were observed less often than in patients with non-lacunar infarcts,which are often caused by cardiac embolism or large vessel disease.[29]

Feasibility of TCD investigation

TCD ultrasound is a noninvasive procedure. It takes approximately 30 minutes. The use of headbands and semi-automated MES detection greatly improves its applicability in clinical practise. In approximately 10 to 20% of the patients, no adequate Doppler signal can be obtained.[30] It has been postulated that the thickness of the temporal window plays a crucial role here.

Aim of this thesis

The overall aim of my thesis is to investigate whether it is worthwile to perform a TCD investigation for diagnostic and prognostic purposes on all patients with a recent TIA or minor ischemic stroke.

My research questions are:

1. Can TCD be of diagnostic value in detetecting the presence of a potential cardiac source of embolism and the presence of small vessel disease in patients with a TIA or minor ischemic stroke?
2. Is it possible to identify patients with a high risk of recurrent vascular events after TIA or minor ischemic stroke by means of TCD investigation?

Firstly, we assessed the diagnostic value of TCD in a large cohort of patients with a recent TIA or non-disabling ischemic stroke (Chapter 2.1). We therefore analysed the relationship of the above mentioned TCD parameters with vascular risk factors and evaluated the value of TCD in screening for a potential cardiac cause of embolism (PCSE) and for stroke subtype (Chapter 2.3). In Chapter 2.2 we describe which patients have a high risk of having window failure.

Secondly, we assessed the prognostic value of TCD in patients with a recent TIA or non-disabling ischemic stroke. We therefore analysed the prognostic value of the different TCD parameters for a recurrent major vascular event (Chapter 3.2 and 3.3). We then validated several prediction models on the data from the present study (Chapter 3.1), and evaluated if these TCD parameters improved existing predicition models (Chapter 3.4).

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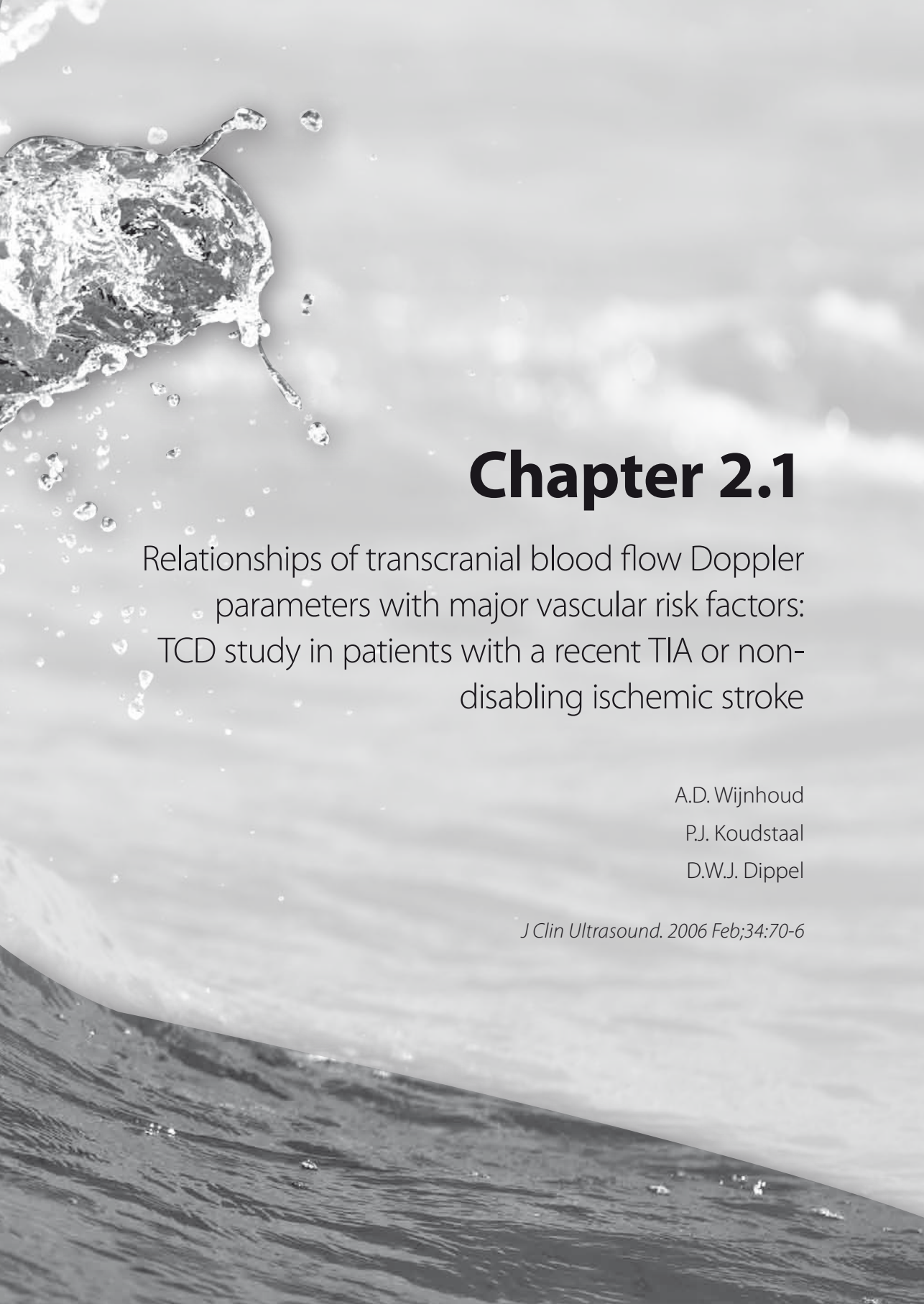

Chapter 2.1

Relationships of transcranial blood flow Doppler parameters with major vascular risk factors: TCD study in patients with a recent TIA or non-disabling ischemic stroke

A.D. Wijnhoud
P.J. Koudstaal
D.W.J. Dippel

J Clin Ultrasound. 2006 Feb;34:70-6

ABSTRACT

Purpose**:** The relationship between intracranial vascular disease and cardiovascular risk factors, such as smoking, hypertension, diabetes mellitus, and total serum cholesterol in patients with recent cerebral ischemia is not well-established. We used transcranial Doppler ultrasonography (TCD) tests as parameters of intracranial vascular disease, and investigated the relationship between these parameters and conventional cardiovascular risk factors.

Methods**:** We prospectively studied 598 patients with a minor ischemic stroke or TIA. In all patients, flow velocities in the left and right MCA, as well as the cerebrovascular CO_2 reactivity were measured by means of TCD. Student's t-test and linear regression analysis were used to determine the relationship between the baseline characteristics, vascular risk factors and TCD parameters.

Results**:** After adjustment for other vascular risk factors, a statistically significant relationship with mean flow velocity in the MCA was found for age (3.5 cm/s/10 yrs of age, 95% C.I.: 2.5 to 4.5 cm/s/10yrs, p<0.0001), sex (-2.9 cm/s for male sex, 95% C.I.: -5.5 to -0.3 cm/s, p=0.03), diabetes (5.6 cm/s for diabetics, 95% C.I.: 2.1 to 9.1 cm/s, p=0.002), and total serum cholesterol (2.4 cm/s per mmol increase in total serum cholesterol, 95% C.I.:1.4 to 3.5 cm/s, p<0.0001). Total serum cholesterol and hypertension were related to cerebrovascular CO_2 reactivity.

Conclusion**:** Cerebral flow velocity is influenced by multiple interacting factors. Results of TCD investigations will have to be adjusted for age, sex, diabetes, and cholesterol when they are used for diagnostic or prognostic purposes.

INTRODUCTION

The relationship between cardiovascular risk factors and intracranial vascular disease in patients with a recent TIA or minor stroke is difficult to ascertain. This is at least partly due to the inaccessibility of particularly small brain vessels, the difficulty to relate, with certainty, brain infarcts on CT or MRI to intracranial disease, and the invasive nature of some radiological imaging procedures. We do know, however, that certain established cardiovascular risk factors, such as hypertension and diabetes mellitus, are related to cerebral white matter lesions[1] and to silent, mostly lacunar, brain infarcts on CT and MRI.[2] Of other cardiovascular risk factors like serum cholesterol level, the relationship with manifestations of intracranial vascular disease still remains to be elucidated.[3]

Ultrasonography may play a role in determining the relationship between these cardiovascular risk factors and intracranial atherosclerosis, since the major arteries within the brain can be relatively easily, non-invasively, and dynamically monitored by means of transcranial Doppler ultrasound (TCD).[4] With TCD, flow velocities in the large arteries in the brain can be easily assessed through the temporal bone window.[5] Moreover, the cerebrovascular reactivity to a vasodilatory stimulus like $CO_{2,}$ can be assessed with TCD.[6] This is thought to reflect the vascular reserve capacity in the brain, and predicts stroke in patients with a severe carotid artery occlusion.[7]

Before TCD parameters could be used as valid diagnostic or prognostic factors, their relationship with conventional cardiovascular risk factors needs to be studied, because these risk factors are likely to influence either arterial flow velocity in the major cerebral arteries, or cerebrovascular reserve capacity, or both. Previous studies have shown that the mean flow velocity in the major arteries in the brain is sex- and age- dependent.[8,9] Some studies have also shown a sex- and age-dependent effect on cerebrovascular reactivity, measured by transcranial Doppler ultrasonography.[10]

We therefore studied the relationship between established cardiovascular risk factors and TCD parameters such as mean flow velocity, peak systolic flow velocity, end diastolic flow velocity, pulsatility index, and cerebrovascular reactivity to CO_2, in a large series of patients with a recent TIA or minor ischemic stroke.

METHODS

Subjects

We studied patients from the Rotterdam TCD study, a prospective study that investigates the diagnostic and prognostic value of TCD in patients with a recent TIA or ischemic non-disabling stroke. Inclusion criteria were a recent (<6 months) TIA or non-disabling ischemic stroke; excluded were patients with a mechanical heart valve and patients with a planned carotid

endarterectomy. Only patients who were over 18 years of age and gave informed consent were included in our study.

The study was approved by the institutional review board and medical ethics committee. Oral and written informed consent was obtained from all participants, and kept on file.

TCD recordings

All patients underwent TCD examination (Multidop X-4, DWL, Sipplingen, Germany). TCD examination was performed within 6 months after TIA or non-disabling ischemic stroke. In all patients peak flow velocity (PSV) and enddiastolic flow velocity (EDV) was measured with a handheld 2 MHz probe in both middle cerebral arteries (MCAs). Mean flow velocity (MFV) was calculated as EDV + 1/3(PSV-EDV). The pulsatility index (PI) was calculated as (PSV-EDV)/MFV. After that, the mean flow velocity in the MCA on both sides was measured continuously with 2x2MHz probes, held in position by a metal headband. Patients breathed through a tightly fitting air mask. Carbon dioxide concentrations in this mask were measured continuously with a carbon-dioxide analyzer (Multinex, Datascope). End-tidal CO_2 pressure was assumed to reflect arterial CO_2.[11] Patients first breathed room air through the mask for at least two minutes until the mean flow velocity in the MCA had stabilized. Patients then breathed 5% carbon dioxide in oxygen for at least two minutes, until the mean flow velocity in the MCA had stabilized for at least 30 seconds. The mean flow velocity in both MCA's was measured during inspiration of normal air and during inspiration of 5% carbon dioxide in oxygen, both times after stabilization of the mean MCA flow velocity. TCD-8 software (DWL) to determine the cerebrovascular CO_2-reactivity (CVR) was used. All data were stored on hard disk for off-line analysis. We calculated the cerebovascular CO_2-reactivity as the percentage rise in mean flow velocity during hypercapnia.

All TCD recordings were done by one investigator (A.W.). Blood pressure was measured automatically with a self-inflating cuff (Dynamap, Datascope, The Netherlands) before and during the TCD recordings.

Laboratory investigations

Total cholesterol, LDL-cholesterol, and HDL-cholesterol levels were measured by means of standard methods, with a Roche/Hitachi 917 (Mannheim, Germany). The cholesterol-HDL ratio was calculated. Blood samples were taken at least five days after the onset of the TIA or ischemic stroke.

Ancillary investigations

All patients underwent routine workup including neurological examination, a CT scan to rule out hemorrhage, and ECG. A duplex-ultrasound scan of the carotid arteries was made in all patients with an anterior circulation stroke.

Risk factors

Hypertension was defined by a regular blood pressure exceeding 140/90 mm Hg during 2 episodes of continuous blood pressure measurement or use of anti-hypertensive drugs. Diabetes was defined by fasting serum glucose over 7.9 mmol/l, or non-fasting serum glucose over 11.0 mmol/l, or use of antidiabetic medication. Regular drinking of alcohol entailed an intake of at least 1 unit per week. Smoking was defined as smoking of at least one cigarette or cigar a day on average.

Statistical analysis

The mean of the right and left flow velocity parameters and the mean of the right and left CVR were used for the analyses if both MCA's could be insonated adequately. In patients with one-sided window-failure, TCD parameters in the contralateral MCA were used. Student's t-test and linear regression analysis, two-tailed, was used to determine the relationship between the baseline characteristics, major risk factors and TCD parameters in order to detect possible confounding factors. A p-value smaller than 0.05 was considered to indicate statistical significance. No adjustments for multiple testing were made. After that, multiple linear regression analysis was used to adjust the relationship between TCD parameters and each risk factor for the effects of the other vascular risk factors.

RESULTS

We included 598 consecutive patients in our study. In 4 patients the diagnosis was revised shortly after inclusion (1 patient had Multiple Sclerosis, 2 had partial epilepsy, and 1 had migraine), which left 594 patients. In 104 of these, we failed to obtain adequate Doppler data due to two-sided window failure or other limitations. Therefore, 490 patients were included in the present analysis. Mean age was 60 years, and two thirds of the patients were men. Approximately half of the patients had had transient symptoms and more than 75% had focal ischemia in the anterior circulation. Almost one third of the patients were current smokers, nearly half of the patients had hypertension, and approximately 17% had diabetes mellitus (Table 1). Nearly 25% of the patients had one-sided window failure. Age in patients with window failure was significantly higher than in patients without window failure (60 ± 13 vs. 71 ± 10, $p<0.001$). Window failure was more often present in female patients than in male patients (31% vs 9%, $p<0.001$). The distribution of hypertension, diabetes, and serum cholesterol level did not differ significantly between patients with window failure and patients without window failure. The proportion of patients who were current smokers was lower in patients with window failure as compared to patients without window failure (16 vs. 28%, $p<0.05$).

Table 1: Patient characteristics.

	Men N=324	Women N=166	All N=490	p
Demographics				
Age, years (mean ± SD)	62 ± 12	56 ± 14	60 ± 13	<0.0001
Stroke characteristics				
TIA	169 (52%)	94 (56%)	263 (54%)	NS
Anterior circulation TIA or stroke	253 (80%)	139 (84%)	392 (82%)	NS
Infarct on CT scan (old or recent)	158 (49%)	65 (39%)	223 (46%)	0.046
Lacunar syndrome	116 (36%)	59 (36%)	175 (36%)	NS
White matter lesions	47 (14%)	14 (8%)	61 (12%)	0.055
Risk factors				
Smoking, present	95 (29%)	43 (26%)	138 (28%)	NS
Alcohol consumption			323 (66%)	
Hypertension	179 (55%)	78 (47%)	257 (52%)	0.088
Diabetes	48 (15%)	29 (17%)	77 (16%)	NS
Previous TIA or stroke	75 (23%)	33 (20%)	108 (22%)	NS
Previous myocardial infarction	43 (13%)	10 (6%)	53 (11%)	0.015
Carotid artery stenosis (≥70%)	30 (9%)	7 (4%)	37 (7%)	0.046
Body-mass-index, kg/m^2 (mean ± SD)	26.2 ± 3.4	26.1 ± 4.7	26.2 ± 3.9	NS
Use of cholesterol lowering medication	83 (26%)	38 (24%)	121 (25%)	NS
Cholesterol				
Total cholesterol,mmol/l (mean ± SD)	5.6 ± 1.1	6.0 ± 1.2	5.7 ± 1.2	<0.0001
HDL-cholesterol, mmol/l (mean ± SD)	1.2 ± 0.4	1.5 ± 0.5	1.3 ± 0.4	<0.0001
LDL-cholesterol, mmol/l (mean ± SD)	3.8 ± 1.1	3.8 ± 1.1	3.8 ± 1.1	NS
Cholesterol-HDL ratio (mean ± SD)	5.0 ± 1.7	4.3 ± 1.6	4.7 ± 1.6	<0.0001

No statistically significant difference was observed between the TCD parameters of the mean of left and right MCA and between the TCD parameters on the symptomatic and the asymptomatic side.

Age

All cerebral blood flow velocity measures (MFV, PSV, EDV), but not cerebrovascular CO_2-reactivity declined significantly with increasing age. The pulsatility index increased significantly with increasing age. After adjustment for other cardiovascular risk factors, these relationships did not change in size or direction. In women the decline in cerebral flow velocity was higher than in men: -5.0 cm/s vs. –2.8 cm/s per 10 years of age.

Sex

Blood flow velocity was higher in female than in male patients. This sex-related difference in cerebral flow velocity was present in younger people. After the age of 60 there was no

Table 2: TCD parameters in a cohort of 490 patients with a TIA or minor stroke.

TCD Parameter	Men N=324	Women N=166	All N=490	P
Mean of both sides				
Mean flow velocity (cm/s)	49 ± 15	56 ± 13	51 ± 14	<0.0001
Peak systolic flow velocity (cm/s)	81 ± 23	90 ± 20	84 ± 22	<0.0001
Enddiastolic flow velocity (cm/s)	33 ± 12	38 ± 12	35 ± 12	<0.0001
Pulsatility index	0.95 ± 0.22	0.89 ± 0.20	0.93 ± 0.22	0.008
Cerebrovascular CO_2-reactivity (% increase in MFV)	33.4 ± 16.2	33.7 ± 15.2	33.5 ± 16.0	NS
Symptomatic side				
Mean flow velocity (cm/s)	49 ± 19	57 ± 15	51 ± 18	<0.0001
Peak systolic flow velocity (cm/s)	80 ± 28	92 ± 23	84 ± 27	<0.0001
Enddiastolic flow velocity (cm/s)	33 ± 16	39 ± 13	35 ± 12	<0.0001
Pulsatility index	0.95 ± 0.25	0.89 ± 0.23	0.98 ± 0.26	0.0426
Cerebrovascular CO_2-reactivity (% increase in MFV)	32.8 ± 16.5	34.6 ± 15.6	33.2 ± 16.2	NS

difference in MFV, PDV, and EDV between men and women. No difference in cerebrovascular CO_2-reactivity was found between male and female patients.

Current smoking

In patients who were still smoking at the time of TCD investigation the MFV and PSV was higher than in patients who had quit smoking or had never smoked at all. After adjustment for age, however, the relationship between smoking and the MFV was not statistically significant anymore.

Hypertension

In hypertensive patients the MFV was lower, and the PI was higher than in normotensive patients unadjusted for other risk factors. These relationships did not remain statistically significant after adjustment for age. The cerebrovascular CO_2-reactivity reactivity was 3.1% ($p<0.03$) lower in patients with hypertension (Table 3), and 4.1% ($p=0.01$) lower after adjustment for the remaining vascular risk factors (Table 4).

Diabetes

MFV and PSV were higher in diabetic patients, whereas PI was lower in diabetic patients. After adjustment for age and other vascular risk factors, these relationships did not change in size or direction and remained statistically significant. Diabetes was not related to cerebrovascular CO_2-reactivity.

Table 3 Relationship of major vascular risk factors with MFV, PI, and cerebral CO_2-reactivity the MCA, measured by transcranial Doppler ultrasound. Unadjusted, linear regression analysis. Effect size per unit of risk factor has been given, with 95% confidence limits.

	Mean flow velocity	Mean PI	Mean cerebrovascular CO_2-reactivity
Age	**-4.0cm/s/10yrs (-5.0 to -3.1)**	**0.10/10yrs (0.08 to 0.11)**	0.7%/10yrs (-0.4 to 1.9)
Male Sex	**-6.0 cm/s (-8.6 to -3.3)**	0.04 (-0.00 to 0.09)	- 0.1% (-3.2 to 3.1)
Current smoking	**3.3 cm/s (0.5 to 6.2)**	**-0.06 (-0.1 to -0.01)**	- 1.9% (-5.1 to 1.4)
Hypertension	**-3.2 cm/s (-5.7 to -0.6)**	**0.06 (0.02 to 0.10)**	**- 3.1% (-6.0 to -0.2)**
Diabetes	**4.0 cm/s (0.5 to 7.5)**	**0.07 (0.01 to 0.12)**	- 0.8% (-4.9 to 3.3)
Total serum cholesterol	**2.5cm/s/mmol (1.4 to 3.6)**	-0.01/mmol (-0.03 to 0.01)	**- 1.3%/mmol (-2.6 to 0.0)**

Table 4 Relationship of major vascular risk factors with MFV, PI, and cerebral CO_2-reactivity the MCA, measured by transcranial Doppler ultrasound. Linear regression analysis adjusted for age, sex, hypertension, diabetes, and total cholesterol. Mean cerebrovascular CO_2-reactivity also adjusted for change in blood pressure during hypercapnia. Effect size per unit of risk factor has been given, with 95% confidence limits.

	Mean flow velocity[a]	Mean PI[b]	Mean cerebrovascular CO_2-reactivity[c]
Age	**-3.5cm/s/10yrs (-4.5 to -2.5)**	**0.08/10yrs (0.07 to 0.10)**	1.1%/10yrs (-0.1 to 2.3)
Male Sex	**-2.9 cm/s (-5.5 to -0.3)**	0.01 (-0.03 to 0.04)	-0.9% (-4.2 to 2.4)
Current smoking	1.7 cm/s (-1.0 to 4.4)	-0.02 (-0.06 to 0.02)	-2.5% (-5.8 to 0.9)
Hypertension	-0.2 cm/s (-2.7 to 2.4)	-0.00 (-0.04 to 0.03)	**-4.1% (-7.3 to -1.0)**
Diabetes	**5.6 cm/s (2.1 to 9.1)**	**0.07 (0.02 to 0.12)**	-0.3% (-4.8 to 4.1)
Total serum cholesterol	**2.4 cm/s/mmol (1.4 to 3.5)**	-0.01/mmol (-0.03 to 0.00)	**-1.3%/mmol (-2.6 to -0.0)**

a. F=16.56, (prob>F)<.00001,
b. F=24.47, (prob>F) <.00001.
c. F= 2.15 (prob>F)=.0377

Total serum cholesterol

Per mmol increase in total serum cholesterol, MFV increased by 2.5 cm/s (95% confidence interval 1.4 to 3.6, p<0.0001), but not after adjustment for age and sex. The cerebrovascular CO_2-reactivity decreased significantly with increasing total serum cholesterol. This was observed after adjustment for remaining vascular risk factors. Twenty-five percent of the patients were using cholesterol lowering drugs (statins) at inclusion in the study. We observed

no statistically significant relationship of cholesterol lowering drugs with mean flow, mean Cerebral CO_2-reactivity or mean pulsatility index.

Other confounding factors

Adjustment for blood pressure during TCD investigation did not change these figures significantly. After exclusion of the 37 patients with carotid stenosis, flow measures and CO_2 reactivity did not change significantly.

DISCUSSION

In this study, we found that cardiovascular risk factors for atherosclerosis were significantly related to flow velocities in the large arteries in the brain in patients with a recent TIA or ischemic stroke. Blood flow velocity in the MCA decreases with age, and is lower in men than in women. Blood flow velocity in the MCA increases with total serum cholesterol, and is increased in diabetics compared to non-diabetics. Cerebrovascular CO_2-reactivity is lower in hypertensive patients and decreases with increasing total serum cholesterol.

Before our results can be accepted, some methodological issues need to be discussed. Firstly, in our study 25% of the patients had an inadequate acoustical window. These patients were predominantly elderly female patients. Although this proportion matches that of other studies,[4,12,13] it implies that our findings are not representative for the entire population of patients with recent TIA or stroke.

Secondly, we were not able to relate the results of Doppler ultrasound to other imaging techniques, such as digital subtraction angiography or MR or CT angiography, which could have corroborated our results. On the other hand, flow velocity and CO_2 reactivity is determined by exactly the part of the intracranial vasculature that cannot be visualized by MR or CT angiography. Thirdly, we calculated the cerebrovascular CO_2 reactivity as the relative increase in MFV in the MCA during hypercapnia. Most studies have used the percentage increase in MFV per kPa CO_2 rise in end-tidal CO_2. Calculating the cerebrovascular CO_2 reactivity per kPa increase in end tidal CO_2 should preferably be done because the MFV is dependent on the end-tidal CO_2.[14] The relationship between the percentages administered CO_2 and increase in MFV is not linear, but is S-shaped, and a maximum increase in MFV is only reached after at least 8% CO_2.[15] Calculating the increase of the MFV per increase in kPa CO_2 would therefore enhance the relationship with other (vascular risk) factors. In our study, however, end-tidal CO_2 measurements failed in substantial number of patients due to technical limitations. Despite the fact that we calculated the cerebral CO_2 reactivity as the relative increase in MFV in the MCA, and not the relative increase in MFV in the MCA per kPa rise in end-tidal CO_2, the relationship of hypertension and cholesterol with the cerebral CO_2 reactivity was statistically significant.

Age- and sex-related differences in cerebral blood flow velocity have been reported previously in population-based studies,[10] in healthy volunteers[14,16,17] and in patients with a previous neurological event.[8] The decline in MFV with increasing age is probably caused by flow reduction in the MCA [8]. Hormonal effects may play an important role in the difference in flow velocities in the MCA between men and women under the age of 60 years.[18] Gender-related differences in MCA diameter could also play a role in this observed difference in flow velocities between men and women.[19] This gender-difference is consistently observed in patients at a younger age, but not in elderly patients.[8,10,14] Since the vessel diameter is not age-related, this probably only plays a minor role in the observed difference in flow velocity. Moreover, Kastrup et al.[20] have reported a difference in cerebrovascular CO_2 reactivity between patients with and patients without hormonal replacement therapy: in patients with hormonal replacement therapy, the cerebrovascular CO_2 reactivity was higher than in patients without hormonal replacement therapy. We could not make this distinction in our study, since very few patients received hormonal replacement therapy. However, in our study no difference in cerebrovascular CO_2 reactivity was found between premenopausal women and postmenopausal women.

Interestingly, the risk factors for atherosclerosis did not have a unidirectional relation with the cerebral flow velocities. Age and sex showed inverse relations with the flow velocity parameters in the MCA, whereas cholesterol level showed a positive relation with the flow velocity parameters in the MCA. This may be explained by the fact that hypercholesterolemia is associated with increased peripheral vascular resistance, which can lead to increased cerebral flow velocity in the MCA.[21]

It is likely that the relationship between hypertension and cholesterol on the one hand and decreased cerebrovascular CO_2 reactivity on the other reflects the presence of small vessel disease. The relationship between small vessel disease and hypertension has been well established.[22] An impaired cerebrovascular CO_2 reactivity has previously been associated with small vessel disease or lacunar infarction.[1,23-25] No interaction with statin treatment was noted.

Our results indicate that the cerebral flow velocity in the MCA is influenced by multiple interacting factors. This implies that in future studies of TCD parameters as a prognostic or diagnostic factor in patients with stroke or TIA, ultrasound findings will have to be adjusted for age, sex, diabetes, total serum cholesterol level, and possibly also for hypertension.

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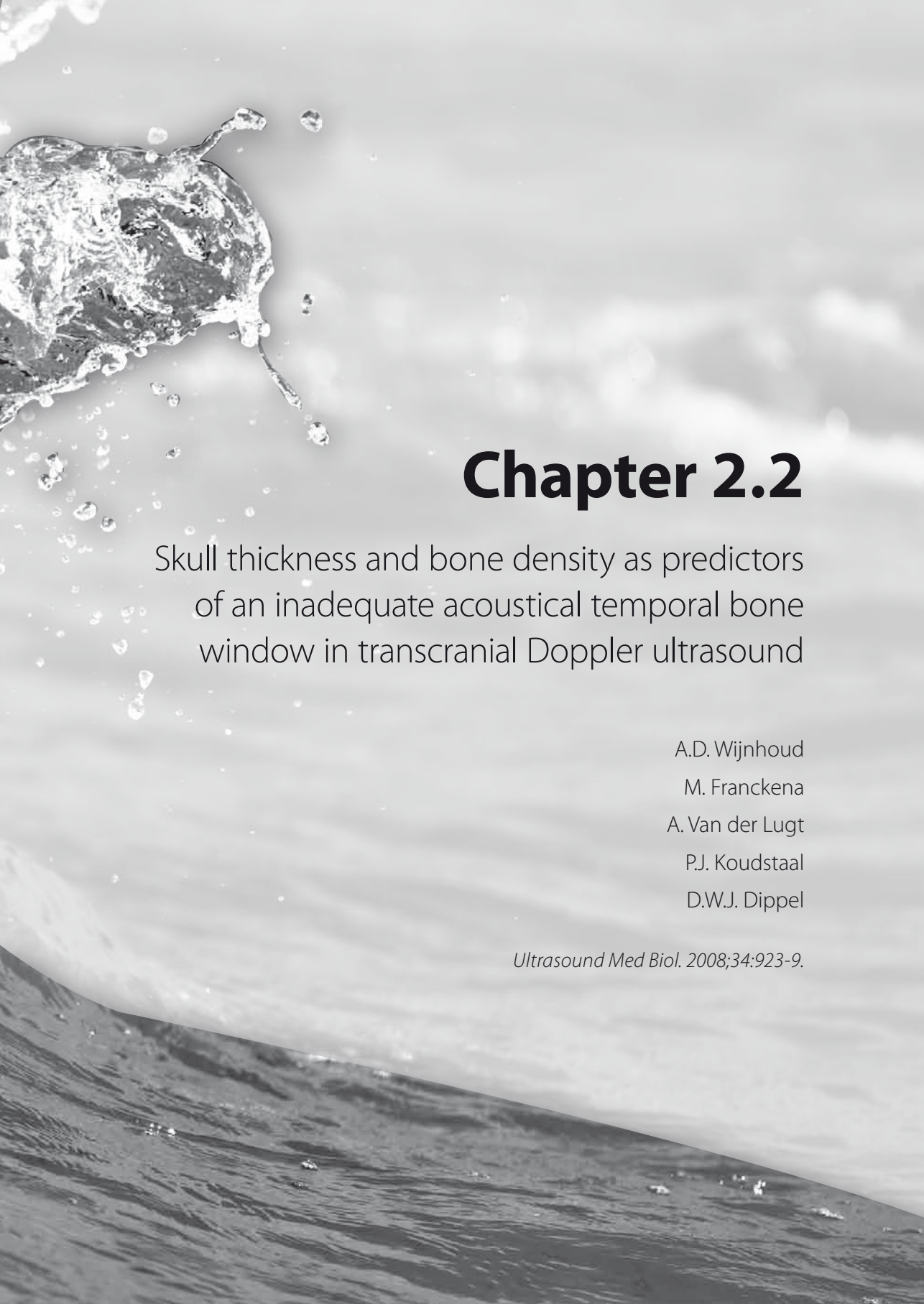

Chapter 2.2

Skull thickness and bone density as predictors of an inadequate acoustical temporal bone window in transcranial Doppler ultrasound

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Ultrasound Med Biol. 2008;34:923-9.

ABSTRACT

Transcranial Doppler (TCD) ultrasonography may provide important diagnostic and prognostic information in patients with ischemic stroke or transient ischemic attack. TCD also enhances the effect of thrombolytic treatment in patients with acute stroke. In some patients, especially elderly women, TCD cannot be performed because of temporal bone window failure (WF). We investigated whether skull thickness or bone density on computed tomography scans predicts WF. In 182 patients with a transient ischemic attack or minor ischemic stroke, skull thickness and bone density measurements were made at the level of the temporal bone window. Multiple logistic regression analysis was used to relate independent variables to WF, and to adjust the estimates for possible confounding factors. TCD signals were absent on the symptomatic side in 22 female and 11 male patients (18%). Both skull thickness and radiodensity at the level of the temporal bone window were strongly related to WF as well as age and female gender. After adjustment according to age and gender, skull thickness at the temporal bone window was an independent prognostic factor of WF (Odds Ratio: 2.3 per mm increase in skull thickness, 95% C.I.: 1.4 to 3.8). Radiodensity of the temporal bone decreased with age in women (-52 HU per 10 years over 50 years of age, 95% C.I.: -73 to -30), but in men (-10 HU per 10 years over 50 years of age, 95% C.I.: -33 to 13), no statistically significant association was observed. We computed probabilities of WF for each patient individually. With a probability cut point of 50%, 33% of the patients with WF and 97% of the patient without WF were correctly identified. The area under the ROC curve of this simple prediction model including age, gender, and skull thickness was 0.88; the area under the ROC curve of a gender-stratified model including age, skull thickness, and radiodensity was 0.90. This difference was not statistically or clinically significant p=0.13). WF is more common in women, because density of the temporal bone in elderly women is low. Absence of WF can be predicted by a combination of three simple parameters: skull thickness, age, and gender. This may help to select patients with ischemic stroke for diagnostic TCD screening and to facilitate targeted delivery of ultrasound-enhanced thrombolysis.

INTRODUCTION

Transcranial Doppler (TCD) ultrasonography can be used to assess intracranial blood flow in a non-invasive way. It may provide important diagnostic and prognostic information in patients with a recent transient ischemic attack (TIA), or minor stroke, and in patients with acute ischemic stroke [1,2]. Furthermore, experimental studies have demonstrated that reperfusion is reached more often and more quickly when intravenous thrombolytic agents are combined with a transcranial ultrasound beam directed at the thrombus [3,4]. More recently, several clinical observations and one clinical phase II trial (CLOTBUST) showed that ultrasound enhances the effect of thrombolytic agents on reperfusion in patients with an acute cerebral infarction in the middle cerebral artery territory. Whether ultrasound enhanced thrombolysis improves outcome in patients with acute ischemic stroke is currently being investigated in a phase III clinical trial (CLOTBUST II) [5,6]. Usually, TCD monitoring can be easily and quickly performed , but in 5–37% of the patients, however, no adequate Doppler signal can be found because of an inadequate acoustic temporal window or window failure (WF) [7-9]. TCD can be a time-consuming investigation in case of an inadequate signal in patients with insufficient temporal bone window.

The temporal bone window is the thinnest area of the lateral skull located closest to the ear. It allows for an ultrasound beam to invade and be reflected. Numerous studies have reported that an inadequate temporal bone window is more common in older women [10-12], and in non-Caucasians[8]. Moreover, an inadequate temporal bone window is also related to thickening of the skull [13-15].

We studied the relationship of skull thickness and density of the temporal bone with WF to determine if WF can be predicted easily and accurately.

METHODS

Study population

We retrospectively studied computed tomography (CT) scans of patients from a prospective cohort study in which we investigated the diagnostic and prognostic value of TCD in patients with a recent TIA or ischemic non-disabling stroke. Inclusion criteria were a recent (<6 months) TIA or non-disabling ischemic stroke. Patients had to be over 18 years of age, and had to give informed consent to be included in this study. The study was approved by the Medical Ethics Committee and Review Board of the Erasmus MC in Rotterdam, The Netherlands. Patients had to be independent in most activities in daily living, corresponding with grade 3 or better on the modified Rankin scale [16,17].

All patients underwent TCD examination and received a routine check up, including a computed tomography (CT) scan, neurological and physical examination, routine laboratory tests, and a duplex of the carotid arteries in case of an anterior circulation stroke or TIA.

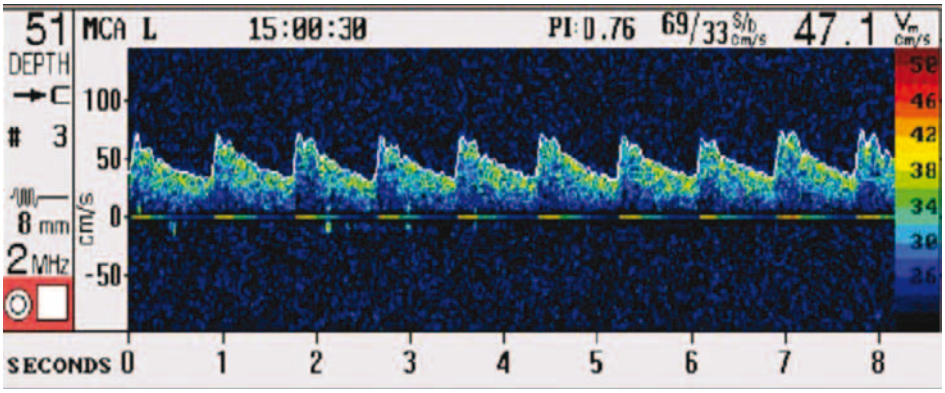

Figure 1: Image of an adequate transcranial Doppler ultrasonography signal of the MCA with a mean velocity of 47.1 m/s, peak-systolic flow velocity of 69 cm/s, and an enddiastolic flow velocity of 33 cm/s.

TCD examination

TCD examination was performed with a Multidop X4 (DWL, Sipplingen, Germany) using a 2 MHz handheld probe. Power was set at 147 mW and increased if necessary to 200 mW. Since the TCD examination was originally performed for different purposes than to study the relationship between CT measurements and WF, we searched for a signal of the middle cerebral artery (MCA) on both symptomatic and asymptomatic side at a depth ranging from 41 – 65 mm for at least 10 minutes. A signal was considered adequate if an envelope could be visualized around the Doppler spectrum for the calculation of the mean, peak and end-diastolic velocities (Figure 1). WF was only considered when neither an MCA signal nor any other arterial signal could be visualized adequately in order to rule out an MCA occlusion. We did not use any contrast agents during the TCD examination. All TCD-examinations were performed by one investigator (A.W.), who was blinded to the results of the measurements performed on the CT-scans.

Quantitative CT scan measurements

All CT scans were made on two Philips Somatom Plus 4 CT scanners (Philips BV, The Netherlands) in Erasmus Medical Center Rotterdam from April 1998 to March 2000. All CT scans were performed as part of routine patient care. The CT scans were made with a slice thickness of 5 mm. The CT scans were recovered from the digital archives in our hospital.

We measured skull thickness and radiodensity at a region of interest (ROI). We defined our ROI in the slice above the sella turcica just above the external meatus. The circular ROI was positioned at the level of the temporal bone window at the thinnest part of the skull. Both skull thickness and radiodensity were measured in the same ROI. Radiodensity measurements were performed by means of a Siemens Magic View® workstation (Siemens AG, Germany). After designating the ROI we calculated the mean radiodensity in Hounsfield Units (HU) for the ROI. To eliminate the contribution of non-bone elements in the ROI and the influence of partial volume effects in measuring the radiodensity, a cut point was determined at a level halfway between

the maximum and the minimum radiodensity in the ROI. This means that all components with radiodensities below the cut point were discarded in the second measurement, only leaving the bony parts of the skull to be taken into account when calculating the radiodensity of the temporal bone window.

Skull thickness was measured by means of a Philips Easy Vision® workstation (Philips BV, The Netherlands). The measurements were carried out three times on either side of the skull, all three close to each other inside the region of interest. On each side three lines were drawn from the outside of the skull to the inside at our ROI and the computer made graphs of the distribution of radiodensities along the lines that were drawn. The width of the bony skull was measured after applying this cut point to distinguish between bone and soft tissue. The mean of these three measurements was calculated. One observer (M.F.) performed the measurements on the CT scans. This observer was blinded to the presence or absence of an adequate TCD signal.

Statistical analysis

We analysed the temporal bone window on the symptomatic side in patients. The analysis was carried out with STATA 8 software (Stata Corporation, College Station, Texas USA). Differences between patients with WF and patients without WF were analysed using parametric tests or chi-square tests. A p-value below 0.05 was considered statistically significant. Multiple logistic regression analysis was used to relate independent variables to the occurrence of WF, and to adjust for possible confounding factors, such as age and gender. We evaluated the relationship between WF and radiodensity and thickness of the temporal bone with a multiple logistic regression model adjusting for age and gender. We then created an interaction factor (skull thickness x radiodensity) to study if thickness and density were dependent on each other, i.c. if thicker bones had less radiodensity. For prediction, two models were created: one with three simple parameters including age, gender, and skull thickness, and a gender-specific model including age, skull thickness and radiodensity. The performance of these models for prediction of WF was assessed by comparing the area under the receiver operating characteristics (ROC) curve. The ROC curve plots 1-specificity versus sensitivity for WF. The area under the ROC curve was calculated to assess the regression model's performance in distinguishing between the presence and the absence of WF. An area of 1.0 represents a perfect test, whereas an area of 0.5 indicates a test with no discriminatory power. The area under the ROC curve is especially meaningful for comparing different tests or prediction models, as we did.[18]. The ROC curve was constructed after computing probabilities for each patient with the multiple logistic regression model.

RESULTS

285 consecutive patients were eligible for inclusion in this study. In 79 patients, the CT-images were not available at the time of study. In 6 patients, scans were made on a different type of CT scanner, and in 8 patients, the CT scan image was technically inadequate. This left 182 patients for analysis. Patients in whom the CT scan was not available for analysis did not differ from included patients with regard to age, gender, occurrence of WF.

WF was present in 33 (18%) patients. Patients with WF were older than patients without WF, 72.4 ± 9.3 vs. 58.8 ± 13.9 years (mean ± SD) . WF was not present in patients under 50 years of age. Patients with WF were more often female than patients without WF (67% vs. 34%, p=0.001) (Table 1).

The mean skull thickness in the study population was 3.1 ± 0.9 mm (mean ± SD). The mean radiodensity at the temporal bone window on the symptomatic side was 1001 ± 163 HU (mean ± SD) (Table 1). Skull thickness was related to age: 0.14 mm increase per 10 years over 50 years of age, 95% C.I.: 0.05 to 0.23, but not to gender: 3.2 ± 0.9 mm in women vs. 3.1 ± 1.0 mm in men. Radiodensity of the temporal bone decreased statistically significant with higher age in women (-52 HU per 10 years over 50 years of age, 95% C.I.: -73 to -30), but not in men (-10 HU per 10 years over 50 years of age, 95% C.I.: -33 to 13) (Fig 2). Ethnicity was neither related to WF nor to skull thickness.

In patients with WF, skull thickness was 3.8 ± 1.1 mm compared to 3.0 ± 0.8 mm (mean ± SD) in patients without WF (p<0.001). Radiodensity of the temporal bone was lower in patients with WF (943 ± 146 HU) than in patients without WF (1014 ± 164 HU (mean ± SD), p=0.022) (Table 1).

Table 1: Baseline characteristics

	Overall	WF	No WF	
No. of patients	182 (100%)	33 (18%)	149 (82%)	
Baseline				
Age (yrs), mean ± SD	61.2 ± 14.2	72.4 ± 9.3	58.8 ± 13.9	p<0.000
Male gender	109 (59.9 %)	11 (33%)	98 (66%)	p=0.001
Caucasian Race	162 (90 %)	30 (91%)	132 (89%)	NS
Risk factors				
Hypertension	76 (41.8%)	17 (52%)	59 (40%)	NS
Diabetes Mellitus	21 (11.7%)	5 (15%)	16 (11%)	NS
Hyperlipidemia	54 (30.9%)	10 (31%)	44 (31%)	NS
Previous MI	22 (12.3%)	5 (16%)	17 (12%)	NS
Intermittent claudication	17 (9.5%)	1 (3%)	16 (11%)	NS
CT measurements				
Skull thickness (mm), mean ± SD	3.1 ± 0.9	3.8 ± 1.1	3.0 ± 0.8	p<0.000
Radiodensity (HU), mean ± SD	1001 ± 163	943 ± 146	1014 ± 164	p=0.022

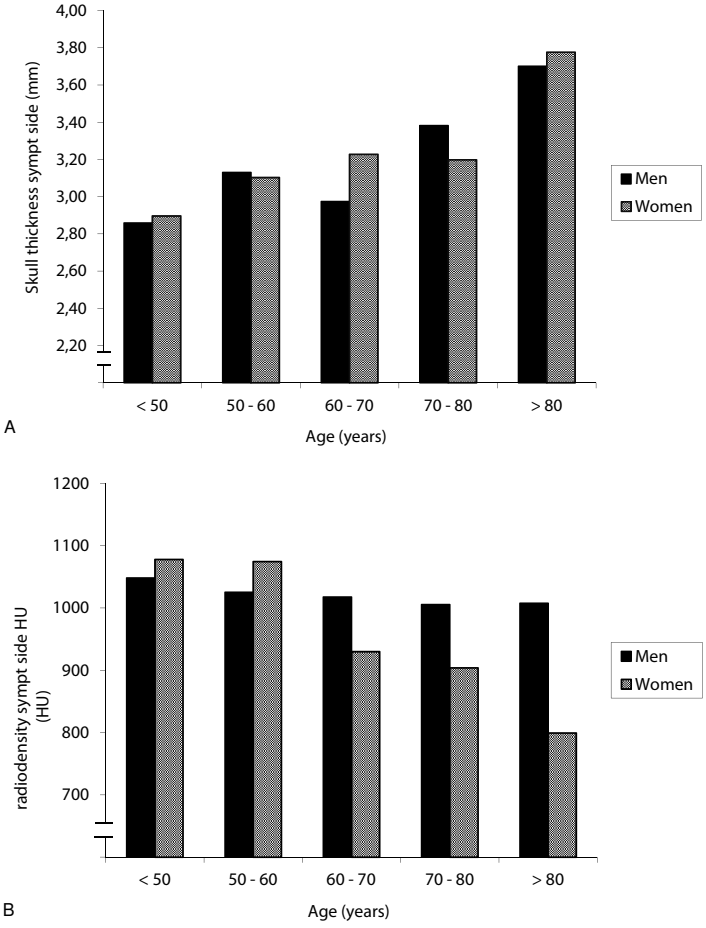

Figure 2: Skull thickness (A) and radiodensity (B) at the level of the temporal bone window per age group for men and women.

After adjustment for age and gender, the relationship between skull thickness and WF did not change (Table 2). Radiodensity of the skull on the symptomatic side, however, was no longer related to WF after adjustment for age and gender (Table 2). We also studied the possibility of interaction between skull thickness and radiodensity of the temporal bone by creating a new variable (skull thickness × radiodensity of the temporal bone). This interaction factor was related to WF, but not after adjustment for age and gender (Table 2).

We then constructed multiple logistic regression models for men and women separately, that included age, skull thickness, and radiodensity of the temporal bone, because radiodensity was very strongly related to age in women. In men, skull thickness, as well as radiodensity was related to WF (Table 3). In women, age and skull thickness were related to WF, whereas radiodensity was not (Table 3).

Table 2: Odds ratios for window failure for skull thickness (A) and radiodensity of the temporal bone (B).

A	Unadjusted	Adjusted for age and gender	Adjusted for age, gender, and radiodensity	Adjusted for age, gender, radiodensity and the interaction factor (skull thickness * density)[†]
Skull thickness[‡] OR (95% C.I.)	2.37 (1.58–3.57)	2.33 (1.44-3.79)	2.49 (1.49-4.17)	3.71 (0.28-49.0)

[†] Odds ratio for WF for the interaction factor (skull thickness * density) was 1.00 (95% C.I.: 0.99-1.01) in this model

[‡] per mm increase in skull thickness

B	Unadjusted	Adjusted for age and gender	Adjusted for age, gender, and skull thickness	Adjusted for age, gender, skull thickness, and the interaction factor (skull thickness * density)
Radiodensity[§] OR (95% C.I.)	0.76 (0.63-0.98)	1.00 (0.76-1.32)	0.86 (0.64-1.16)	0.99 (0.38-2.61) [†]

[†] Odds ratio for WF for the interaction factor (skull thickness * density) was 1.00 (95% C.I.: 0.99-1.01) in this modelc

[§] per 100 HU increase in radiodensity of the temporal bone

Table 3: Odds ratios for window failure, based on a multiple logistic regression models of age, skull hickness, and radiodensity, for men and women separately.

	Window failure symptomatic side OR (95% CI)	
	Men	Women
Age per 10 yrs over 50 yrs	1.71 (0.86 – 3.39)	4.08 (1.84 – 9.01)
Skull thickness per mm	3.58 (1.73 – 7.43)	2.42 (1.01 – 5.80)
Radiodensity per 100 HU	0.65 (0.44 – 0.93)	1.20 (0.79 – 1.83)

Table 4: Odds ratios for window failure based on a logistic regression model of age, gender, and skull thickness.

	Window failure symptomatic side OR (95% CI)
Age per 10 yrs over 50 yrs	2.58 (1.63 – 4.07)
Female gender	5.94 (2.21 – 15.9)
Skull thickness per mm	2.33 (1.44 – 3.79)

We calculated probabilities of WF for each patient individually, by means of a multiple regression model including age, gender, and skull thickness. In this model, age, sex, and skull thickness were independently related to WF (Table 4). Female gender was the strongest predictor of the presence of WF. With a probability cut point of 50%, 33% of the patients with WF were correctly identified as having WF, and 97% of those without WF were correctly identified as having no WF. The positive predictive value with this probability cut point was 69%, the negative predictive value was 87%. With a probability cut point of 90% the positive predictive value increased to 100%, but the negative predictive value was 83%. The area under the ROC curve of the simple prediction model including age, gender, and skull thickness was 0.88; the area under

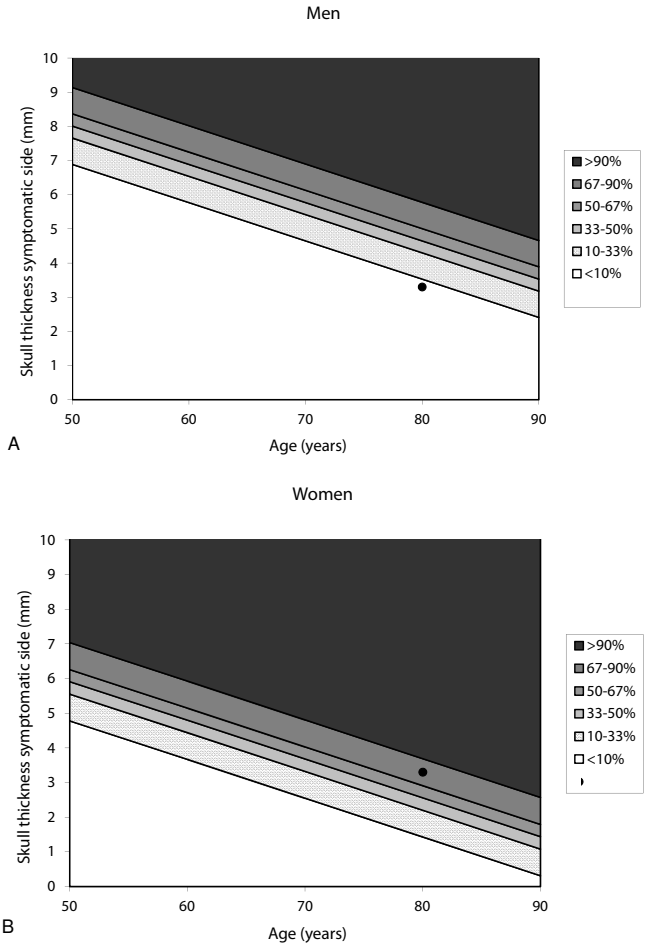

Figure 3: Dependency of cut points of skull thickness (mm) on age and gender to predict window failure with different probabilities for men (A) and for women (B). In women, the cut point is lower than in men. With this figure, estimation of the probability of WF for an individual patient can be made. For example, in an eighty-year old male patient with a temporal bone thickness of 3.3 mm, the chance of WF would be less than 10% (black dot in figure 2A). If this patient would be female, the probability of WF would increase to approximately 80%.

the ROC curve of a gender-specific model including age, skull thickness, and radiodensity was 0.90. This difference was not statistically or clinically significant p=0.13)

We calculated probability cut points of skull thickness for WF, as a function of age based on the simple logistic regression model including age, sex, and skull thickness. These results are shown in Figure 3. With this figure, estimation of the probability of WF for an individual patient can be made. For example, in an eighty-year old male patient with a temporal bone thickness of 3.3 mm, the chance of WF would be less than 10% (black dot in Figure 3A). If this patient would be female, the probability of WF would increase to approximately 80% (black dot in Figure 3B).

DISCUSSION

Our study confirms that skull thickness at the level of the temporal window is related to inadequacy of the ultrasound signal. Furthermore, skull thickness is more closely related to WF than radiodensity of the temporal bone. A decreased radiodensity of the bone at the temporal window was also related to WF, but not significantly so, after adjustment for age and gender.

Some methodological issues need to be discussed. First, selection bias could have occurred, since skull thickness and radiodensity measures could not be carried out in all patients, as the CT scan was not available for analysis at the time of our study. However, patients in whom the CT scan was not available for analysis did not differ from included patients with regard to age, gender, and occurrence of WF. We therefore think that the CT non-availability represents random error and does not bias the results of our study. Secondly, we did not use any contrast agents during TCD investigation. Contrast agents enhance assessment of intracranial cerebral arteries, however, the original protocol for this study did not allow for use of contrast agents.

In our study, age and gender are strongly related to WF. This confirms the results of previous studies [7,8,19]. We used a simple method to determine skull thickness and density of the temporal bone window in vivo. Our observation that the temporal bone was thicker in women than in men is in concordance with other studies [13,20]. Skull thickness in relation to WF has been measured in postmortem studies [21], and in clinical studies. [13,20]. These studies have shown that skull thickness is related to the presence of WF. This can probably be explained by the notion that ultrasound is scattered by the cancellous bone in the diploe [22]. Moreover, the thickness of the cancellous bone is related to the presence of WF [21].

In our study, radiodensity of the temporal bone was related to age, but only in women. Unadjusted, radiodensity was related to WF on the symptomatic side, but this relationship was neutralized by female gender. We therefore conclude that the high incidence of WF in elderly women is probably caused by loss of skull density. However, gender-stratified logistic regression models including radiodensity, did not predict WF more accurately than the simple logistic regression model including age, gender, and skull thickness. Recently, Kwon et al. [20] have described the relationship between inhomogeneity of temporal bone and WF. Most probably, loss of density leads to energy loss due to scattering of the ultrasound beam, and therefore to an inadequate Doppler signal [23] .

We showed that a simple logistic regression model of age, gender, and skull thickness could predict absence of WF in nearly all patients correctly. The positive predictive value, however, was only 69%, where 100% is desirable, since the percentage of false positive predictions needs to be as low as possible. Our study results may be particularly helpful when ultrasound enhanced thrombolysis has been proven to improve clinical outcome. CT measurements, that take approximately 5 minutes of time, may be helpful in avoiding TCD measurements in patients with a high probability of WF. This is important especially when this service is not available on a 24-hour basis. If skull thickness were to be used as a screening test to identify patients with

probable bone window failure, then the cut point should depend on the disutility of missing a patient with window failure and missing a patient without window failure. If both disutilities are considered equal, then a cut point of 50% should suffice. If thrombolysis is proven to be enhanced by TCD, and missing a patient without window failure is ten times worse that missing one with window failure, then the cut point would be 90%. We chose 90% to estimate how many patients should be screened. In our study, none of the men had a probability of more than 90% of having window failure. This means that in practice, only women would have to be screened for WF. No woman under 70 years of age had a probability of more than 90% of having WF. Only 21 women in our study were older than 70 years of age, of whom 3 had a probability of more than 90% of having WF, which means that the number needed to screen is 7 (female) patients.

Our relatively large study provides quantitative estimates of the contribution of skull density and thickness parameters to the occurrence of window failure. We conclude that both thickness and density of the temporal bone are related to the presence of WF, but that skull density does not independently predict WF. Absence of WF can be predicted accurately in nearly all patients with ischemic stroke or TIA by means of three simple parameters: skull thickness, age and gender. This may help to select patients for diagnostic TCD screening and perhaps in the near future, to facilitate targeted delivery of ultrasound-enhanced thrombolysis.

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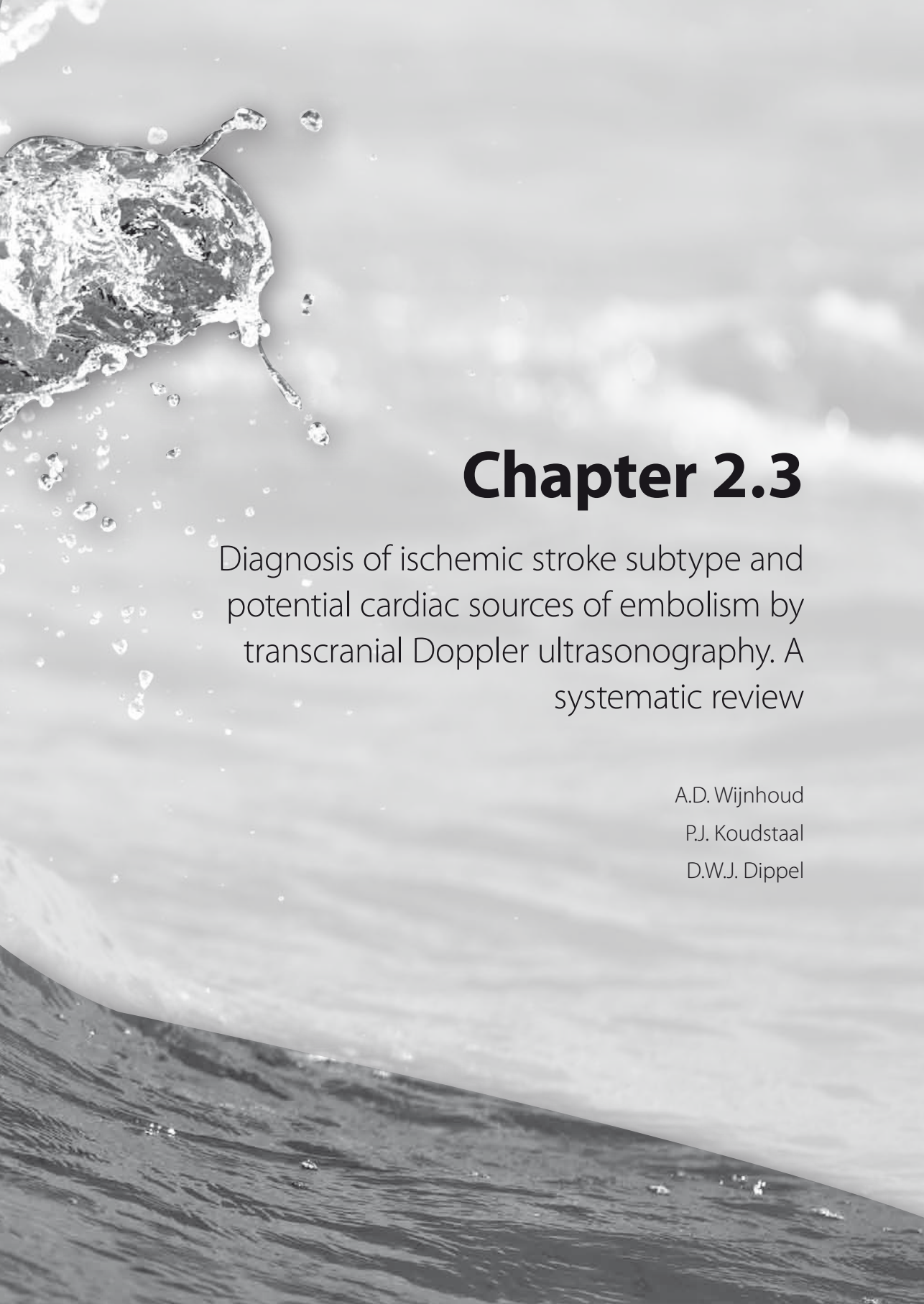

Chapter 2.3

Diagnosis of ischemic stroke subtype and potential cardiac sources of embolism by transcranial Doppler ultrasonography. A systematic review

A.D. Wijnhoud
P.J. Koudstaal
D.W.J. Dippel

ABSTRACT

Background: High Intensity Transient Signals (MES) in Transcranial Doppler ultrasonography (TCD) monitoring may indicate micro-thromboembolism in patients with recent ischemic stroke or TIA. However, it is unclear whether TCD monitoring for MES is helpful in the diagnosis of a potential cardiac source of embolism (PCSE) and of stroke subtype (i.e. small-vessel disease or not).

Methods: We searched NLM Medline, Embase and cross-references in articles for published studies on TCD MES monitoring in patients with a TIA or ischemic stroke. Of each study we computed the sensitivity and specificity of MES for stroke subtype and for a PCSE. Next, we combined data from each study into a summary receiver operating characteristic (sROC) curve. We took into account the clinical characteristics of each study population, method of MES detection and whether technical details of TCD examination were reported. We contrasted the results of the meta-analysis with the findings in our own study.

Results: Thirteen studies were identified. There was heterogeneity in study design and methods between the studies, and technical details were not always reported in detail. Nevertheless, our analysis showed that in the nine studies that used a well-defined reference test for ischemic stroke subtype the specificity of TCD for non-lacunar stroke was 0.90, and sensitivity was 0.76. Sensitivity for PCSE in the eight studies that used a well-defined reference test was 0.74 and specificity was 0.65 at the optimal point of the sROC curve. In our own study sensitivity for stroke subtype was 0, and specificity was 0.91. For PCSE sensitivity was 0, and specificity was 0.95. Sensitivity was low because this was not performed in the acute phase.

Conclusions: The sensitivity and specificity of MES detection with transcranial Doppler ultrasound in the diagnosis of PCSE is not high enough to recommend its use for screening prior to more invasive diagnostic investigations, such as transesophageal echocardiography. However, results in patients with a recent stroke, a confident diagnosis of non-lacunar stroke can be made when MES are detected with TCD.

INTRODUCTION

Deciding whether a patient had a lacunar or a non-lacunar stroke can be quite difficult. Many patients had a short attack, have residual symptoms that are not very helpful, and their CT or MRI may be normal.[1] Additional diagnostic information that helps to determine stroke subtype in such patients could be helpful. The "micro-embolic signals" (MES), detected by transcranial Doppler ultrasonography (TCD), may help in distinguishing non-lacunar (thromboembolic) from lacunar stroke. TCD could perhaps also be helpful in the detection of a potential cardiac source of embolism (PCSE). A truly non-invasive diagnostic investigation that could be used to identify patients, who will not benefit from transesophageal echocardiography (TEE), would be most welcome.

Monitoring for microembolic signals by means of TCD is not a standard investigation in stroke patients, and its potential clinical usefulness is unclear. In patients with a recent stroke or transient ischemic attack, MES are not uncommon.[2-4] Several studies have shown that MES are more often present in patients with a cortical stroke[2] and in patients with PCSE.[5] However, the sensitivity and specificity of TCD (i.e. MES monitoring) for non-lacunar stroke or PCSE in these studies varied greatly. Although most studies used similar criteria for MES, comparison of the data is made difficult by differences in important other variables such as detection threshold,[6-8] method of MES-detection (automated, human observer, or a combination),[7-9] or time window between stroke and TCD investigation.[10] This diversity makes it difficult to draw conclusions about the diagnostic value of TCD in patients who had an ischemic stroke or TIA.

The purpose of this study was to determine the diagnostic value of TCD MES monitoring in patients with transient ischemic attack or ischemic stroke regarding stroke subtype and the presence of a PCSE. We therefore compared the data in a summary receiver operating characteristic curve as suggested by Moses et al.[11] Finally, we compared the findings from our systematic review with the results of the Rotterdam TCD study, a cohort study of the prognostic and diagnostic value of TCD in 592 patients with a recent TIA or stroke.

METHODS

Selection of relevant studies

We searched NLM Medline, Embase, and cross references in articles for published studies on TCD MES monitoring in patients with a recent TIA or ischemic stroke. We used the following search keys: [(TIA OR stroke) AND (TCD OR transcranial Doppler ultrasonography)]. The articles were selected on the basis of their title and the information in the abstract. To be included in this review, the study should concern a series of patients with stroke or TIA who underwent TCD monitoring for MES. The results of the TCD investigation should be compared with a "gold standard" or "reference" diagnosis.

Definitions

Small vessel disease was defined according to the TOAST criteria:[12] a lacunar syndrome with a normal CT, or as a CT showing a relevant lacunar infarct sized 1.5 cm in diameter or less without another cause of the stroke.

We defined PCSE according to the TOAST criteria,[12] and included high risk sources of embolism, such as atrial fibrillation, recent (<4 weeks) myocardial infarction, sick sinus syndrome, prosthetic cardiac valves and left atrial appendage or ventricular thrombus, and medium risk sources, such as patent foramen ovale, left atrial or ventricular aneurysm, mitral valve prolaps, old (1-6 months) myocardial infarction and atrial or ventricular septal defect. Preferably, the diagnosis of PCSE should have been confirmed by echocardiography.

Criteria for inclusion of studies

We checked whether the study population and relevant clinical characteristics was described adequately to evaluate the diagnostic accuracy and reproducibility.[13] Relevant characteristics that were considered in this review were time since onset of stroke, its vascular distribution, the presumed mechanism, especially whether the infarct was considered to be due to small vessel occlusion or not, age, and sex distribution. These characteristics may affect the accuracy of TCD: the test may perform well in certain subgroups and poorly in others, despite reasonable average values of accuracy. Verification bias occurs when patients with positive (or negative) diagnostic tests preferentially receive verification by the reference test. For example, patients with MES may have a higher chance of being referred for a cardiologic consultation and echocardiography than patients without MES. We noted whether patients had undergone thransthoracic or transesophageal echocardiography. We tried to estimate from the studies the ratio of the verification rate in patients with MES and the verification rate in patients without MES. Review bias can be introduced when either the diagnostic test or the reference procedure is assessed without objectivity in the interpretation. We noted whether the TCD examination was carried out without knowledge of the results of the reference test or other investigations. In

addition, we checked whether the reference test (for example echocardiography or a CT scan) was interpreted without knowledge of the results of the TCD-investigation.

TCD investigation: Technical aspects.

MES were defined as transient Doppler microembolic signals, usually lasting less than 300 milliseconds. The amplitude of the Doppler microembolic signal should be at least 3 decibel (dB) higher than that of the background blood flow signal, within the appropriate dynamic range of bi-directional Doppler equipment. In addition, MES are unidirectional within the Doppler velocity spectrum and accompanied by a "snap," "chirp," or "moan" on the audible output.[8] We checked if a fixed detection threshold was used in each study.

We checked whether studies reported parameters that are likely to influence the sensitivity and specificity of TCD, as recommended by the International Consensus Committee on Microembolus Detection.[8] These parameters include ultrasound device, insonated artery, insonation depth, algorithms for signal intensity measurement, detection threshold, axial extension of sample volume, fast Fourier transform (FFT) size (number of points used), FFT length (time), FFT overlap, transmitted ultrasound frequency, and recording time.

Method of quantitative analysis

We computed for each study the sensitivity and specificity for non-lacunar stroke and PCSE after translation of the results in a simple 2´2 table. Sensitivity for non-lacunar stroke was computed by the number of MES-positive patients with a non-lacunar stroke divided by the total number of patients with a non-lacunar stroke. Sensitivity for PCSE was taken as the number of MES-positive patients with a PCSE divided by the total number of patients with a PCSE. Thus, sensitivity was estimated by taking the probability of a positive test in patients with the condition of interest; this will be referred to as the True Positive Rate (TPR). The specificity for non-lacunar stroke was defined as the number of MES-negative patients with a lacunar stroke divided by the total number of patients with a lacunar stroke. The specificity for PCSE was assessed as the number of MES-negative patients without a PCSE divided by the total number of patients without a PCSE. Similarly, 1-specificity represents the probability of a positive test in patients without the condition or disease of interest; this will be referred to as the False Positive Rate (FPR). The sensitivity and specificity for PCSE was determined after exclusion of patients with a significant carotid stenosis (>50% by duplex ultrasound), since carotid stenosis can be an important source of MES.[14-17]

TPR and FPR are not independent of each other since both may increase or decrease when the threshold for a positive test, i.e. dB threshold, is changed. TPR can be plotted against FPR in a receiver operating characteristic (ROC) curve. An ROC curve is a path in the unit square, rising from the lower left corner, where TPR and FPR are both zero, to the upper right corner, where TPR and FPR are both one. This ROC curve can be generated from one set of data with the same settings with only a varying threshold. An ROC curve cannot be generated from different

studies without an exact match of study population and analysis characteristics. To determine the effect of interstudy differences, we carried out a correlation analysis with parameter D (Appendix A), the natural logarithm of the odds ratio, which is a measure for how well the test discriminates between the population with and without a lacunar infarct, or with and without PCSE. This analytical method is described by Moses et al.[11] The resulting summary ROC curve is not based on individual data, but on sensitivity and specificity of each study separately, and takes into account the number of patients in each study. The calculations were performed using a standard spreadsheet.

Rotterdam TCD study

Our own data were derived from the Rotterdam TCD study.[18] This study was designed to evaluate the diagnostic and prognostic value of transcranial Doppler ultrasonography in a prospective cohort of unselected patients with a recent TIA or minor ischemic stroke. Patients were 18 years and older and had the index event within the preceding 6 months. They were independent (a score of 3 or less on the modified Rankin scale),[16] and the TIA or stroke was of presumed atherosclerotic origin; this implied that patients with a mechanical heart valve and a proven dissection were excluded. Patients with atrial fibrillation or a significant symptomatic carotid artery stenosis (>70% stenosis, NASCET criteria) were also excluded. The study population consisted of patients for whom no other treatment than risk factor modification and antiplatelet medication was available.

In our study, all patients underwent routine workup including neurological examination, a CT scan to rule out hemorrhage, and an ECG. These investigations were performed on admission or first visit to the outpatient clinic, always before the TCD investigation. Risk factors (diabetes mellitus, smoking, hypertension, increased serum cholesterol level) were assessed. A duplex-ultrasound scan of the carotid arteries was made in all patients with an anterior circulation stroke. Echocardiography was done in randomly selected patients, but also in patients with a history of cardiac disease, abnormalities on ECG, or a cardiac bruit on physical examination. To address the value of TCD in diagnosing stroke subtype, we included only patients with a recent infarct on the CT scan. To address the value of TCD in diagnosing PCSE, we included all patients in whom echocardiography was performed.

Our study included patients in the subacute phase of stroke. This differed significantly from the other studies, which were acute phase studies. We therefore chose not to directly compare the results of our study to the other studies.

RESULTS

We identified twelve studies[2-4,10,19-26] that addressed the value of MES in diagnosing stroke subtype, and twelve studies[3,5,10,19-27] that addressed the diagnostic value of MES in screening for a PCSE. Eleven of these studies were selected for both purposes. Their characteristics, quality aspects, technical aspects, and results are described in Tables 1-4.

Table 1: Characteristics of the selected studies

Author	Year	TIA (%)	Age (years)	Sex (% Male)	Time- since onset	
A: Stroke subtype						
Grosset et al.	1994	20	67 (32-96)	60	<48 hrs.	
Daffertshofer et al.	1996	26	63.0 ± 13.9	70	<4 weeks	
Kaposzta et al.	1999	0	69.6 ± 12.1	52	<72 hrs.	
Del Sette et al.	1997	Unknown	69.4 ± 13.9	68	<72 hrs	
Koennecke et al.	1997	15	62 ± 14	67	<48 hrs.	
Koennecke et al.	1998	26	60.6 ± 14	63	<48 hrs.	
Sliwka et al.	1997	Unknown	62.5 (29-90)	65	<1 week †	
Lund et al.	2000	12	73.0 ± 11	53	< 72 hrs. ‡	
Serena et al.	2000	29	65.1 ± 12.3	74.2	69.5 ± 44 hrs.	
Iguchi et al.	2007	0	71.6	54	<24 hrs.	
Poppert et al.	2006	Unknown	59.4	64	5.3 ± 3.5 days	
Idicula et al.	2010	5	70 ± 15.5	72.5	< 6 hrs.	
B: Potential cardiac source of embolism						% Mechanical heart valves
Daffertshofer et al.	1996	26	63.0 ± 13.9	70	<4 weeks	0
Kaposzta et al.	1999	0	69.6 ± 12.1	52	<72 hrs.	0
Del Sette et al.	1997	Unknown	69.4 ± 13.9	68	<72 hrs	0
Koennecke et al.	1997	15	62 ± 14	67	<48 hrs.	10
Koennecke et al.	1998	26	60.6 ± 14	62	<48 hrs.	2
Sliwka et al.	1997	Unknown	62.5 (29-90)	65	<1 week †	0
Tong et al.	1995	0	70 ± 14	58	<48 hrs.	7
Lund et al.	2000	12	73.0 ± 11	53	< 72 hrs. ‡	Unknown
Serena et al.	2000	29	65.1 ± 12.3	74.2	69.5 ± 44 hrs.	Unknown
Iguchi et al.	2007	0	71.6	54	<24 hrs.	0
Poppert et al.	2006	Unknown	59.4	64	5.3 ± 3.5 days	0
Idicula et al.	2010	5	70 ± 15.5	72.5	< 6 hrs.	Unknown

† A second TCD investigation was carried out 24 hrs later, and a third within one week
‡ A second TCD investigation was carried out 5 days later

Table 2: Quality aspects of the reviewed studies

Author	Year	Reference test	Spectrum reported	Consecutive patients	Verification rate (%)	Verification ratio	Blind assessment of TCD	Blind assessment of reference test?	Window failure reported
A. Stroke subtype									
Grosset et al.	1994	TOAST	Yes	Yes	100	1	+	-	Yes
Daffertshofer et al.	1996	2 CT's	Yes	Unknown	100	1	+	-	No
Kaposzta et al.	1999	TOAST	Yes	Yes	100	1	+	-	Yes
Del Sette et al.	1997	TOAST	Yes	Yes	100	1	+	-	Yes
Koennecke et al.	1997	1 CT	Yes	Yes	100	1	+	-	Yes
Koennecke et al.	1998	1 CT	Yes	Yes	100	1	+	-	Yes
Sliwka et al.	1997	1 CT	Yes	Unknown	100	1	+	-	Yes
Lund et al.	2000	TOAST	Yes	Yes	100	1	+	-	Yes
Serena et al.	2000	TOAST	Yes	Yes	100	1	+	-	Yes
Iguchi et al.	2007	TOAST	Yes	Yes	100	1	+	-	Yes
Poppert et al.	2006	TOAST	Yes	Yes	100	1	+	+	No
Idicula et al.	2010	TOAST	Yes	No	100	1	-	-	Yes
B: Potential cardiac source of embolism									
Daffertshofer et al.	1996	Ill defined	Yes	Unknown	100	1	+	-	No
Kaposzta et al.	1999	TOAST	Yes	Yes	62	Unknown	+	-	Yes
Del Sette et al.	1997	TOAST	Yes	Yes	100	1	+	-	Yes
Koennecke et al.	1997	TOAST	Yes	Yes	76	Unknown	+	-	Yes
Koennecke et al.	1998	TOAST	Yes	Yes	96.6	Unknown	+	-	Yes
Sliwka et al.	1997	Ill defined	Yes	Unknown	100	1	+	-	Yes
Tong et al.	1995	Ill defined	Yes	Unknown	100	1	+	-	No
Lund et al.	2000	TOAST	Yes	Yes	44.6	Unknown	+	+	Yes
Serena et al.	2000	Ill defined	Yes	Yes	Unknown	Unknown	+	-	Yes
Iguchi et al.	2007	TOAST	Yes	Yes	Unknown	Unknown	+	-	Yes
Poppert et al.	2006	TOAST	Yes	Yes	85.3	Unknown	+	-	No
Idicula et al.	2010	TOAST	Yes	No	Unknown	Unknown	-	-	Yes

Table 3 Technical aspects of the MES detection method in each study.

Author	Year	Ultrasound device	Insonated artery	Depth	Method	Decibel threshold	Sample volume	FFT size	FFT overlap	High pass filter setting	Recording time
Grosset et al.	1994	TC 2000, Nicolet	MCA	46-54 mm	A+H[†]	3 dB	-	-	-	-	30
Daffertshofer et al.	1996	TCD7/ Multidop, DWL	MCA	50-56 mm	H	4 dB	-	-	-	-	48 (30-60)
Kaposzta et al.	1999	TC 4040, Nicolet/EME	MCA	52±3.4 mm	H	7 dB	5 mm	128	>66%	-	60
Del Sette et al.	1997	Multidop X, DWL	MCA	-	A+H	8 dB	-	-	-	-	30
Koennecke et al.	1997	TC 2020, EME/Nicolet	PCA	60-70 mm	H	9 dB	10-12 mm	128	67-90%	-	20
Koennecke et al.	1998	TC 2020, EME/Nicolet	MCA	45-58 mm	H	9 dB	10-12 mm	128	25-81%	-	30
Sliwka et al.	1997	Multidop X, DWL	MCA	-	H	3 dB	6 mm	-	-	-	30
Tong et al.	1995	Medasonics	-	-	?	3 dB	-	-	-	-	30
Lund et al.	2000	TC 4040, EME/Nicolet	MCA	49.9±2.3mm	H	4 dB	10 mm	128	67%	120 Hz	45
Serena et al.	2000	Multidop X, DWL	MCA	45-60 mm	A	9 dB	5 mm	128	-	100 Hz	30
Iguchi et al.	2007	Multidop X, DWL Pioneer TC 8080	MCA	50-55 mm	H	?	-	-	-	-	20
Poppert et al.	2006	Multidop, DWL	MCA	-	H	11 dB	-	64 - 256	-	-	30
Idicula et al.	2010	Pioneer TC 8080	MCA	56 ± 4 mm	A	7dB	10 mm	-	-	-	60
Rotterdam TCD		Multidop X, DWL	MCA	45-60 mm	H	7 dB	5 mm	128	67%	-	30

[†]A=Automated detection program, H=Human observer

Table 4: Quantitative comparison of the reviewed studies

Author	Year	Number of patients	Number of patients with a positive ref. test	Number of MES-positive patients	Number of MES-positive patients with ref. Test	Sensitivity (=True Positive Rate)	1-specificity (=False Positive Rate)
A. Stroke subtype: non-lacunar stroke							
Grosset et al.	1994	41	33	29	29	0.88	0
Daffertshofer et al.	1996	280	187	26	26	0.14	0
Kaposzta et al.	1999	100	80	16	16	0.2	0
Del Sette et al.	1997	75	20	9	9	0.45	0
Koennecke et al.	1997	28	11	4	2	0.18	0.12
Koennecke et al.	1998	102	76	26	19	0.25	0.27
Sliwka et al.	1997	78	69	40	38	0.55	0.22
Lund et al.	2000	73	56	19	16	0.29	0.18
Serena et al.	2000	182	64	17	17	0.27	0
Iguchi et al.	2007	125	98	61	53	0.54	0.30
Poppert et al.	2006	653	506	37	37	0.07	0
Idicula et al.	2010	40	38	25	25	0.66	0
Rotterdam TCD	2011	120	64	5	0	0	0.09

B. Potential cardiac source of embolism							
Daffertshofer et al.	1996	143	65	4	4	0.06	0
Kaposzta et al.	1999	80	22	6	1	0.05	0.09
Del Sette et al.	1997	58	23	4	3	0.13	0.03
Koennecke et al.	1997	41	20	8	8	0.4	0
Koennecke et al.	1998	104	63	19	12	0.19	0.17
Sliwka et al.	1997	44	20	19	9	0.45	0.42
Tong et al.	1995	33	23	3	3	0.13	0
Lund et al.	2000	66	19	18	10	0.53	0.17
Serena et al.	2000	143	54	11	10	0.19	0.01
Iguchi et al.	2007	105	53	34	19	0.56	0.48
Poppert et al.	2006	550	17	143	5	0.03	0.03
Idicula et al.	2010	27	6	11	4	0.36	0.125
Rotterdam TCD	2011	149	5	7	0	0	0.05

Characteristics of the studies

The studies were published in the years 1994 through 2010. The studies were similar with respect to demographic characteristics of the study population, and the timing of the investigation relative to the onset of symptoms (24 to 72 hours in most, less than 4 weeks in one study).

Technical aspects

Four of the 13 studies described the method of intensity measurement of the signal in detail. Information about the algorithm for MES detection was lacking in most studies. The detection threshold that was employed in each study varied from 3 to 9 dB.

None of the studies reported all 14 parameters suggested by the International Consensus Group on Microembolus Detection.[8] Most of these parameters influence the intensity of a microembolic signal and may therefore affect test accuracy. Only five studies reported at least 7 of the 14 parameters, and the remaining five studies reported less than 7. The parameters reported by all thirteen studies were the insonated artery, the transmitted ultrasound frequency and the length of the recording time. All studies except two reported the depth of the insonated artery. Six studies reported the size of the sample volume. Only four studies reported the FFT-overlap. FFT length was never reported. Presumably, all studies used pulsed wave Doppler ultrasound, but only two reported this information. Scale settings were reported in just one study. In all studies ultrasound frequency was 2 MHz.

Diagnosis of stroke subtype

The sensitivity for non-lacunar stroke ranged from 0.14 to 0.88 and the specificity ranged from 0.73 to 1.0 (Table 4). In the nine studies with a robust reference test, based on the TOAST criteria,[12] specificity ranged from 0.82 to 1. In these studies, the mean D increased from 2.41 to 3.33, for a sensitivity of 0.76 and a specificity of 0.90 at the optimal point of the sROC (Fig.1). Sensitivity increased when TCD investigation was carried out sooner after stroke onset (Table 1 & 4, Figure 1).

Detection of likely sources of potential cardio-embolism

The sensitivity for PCSE ranged from 0.05 to 0.53 and the specificity from 0.58 to 1.0. The sensitivity in studies that had not included patients with mechanical prosthetic heart valves was lower than in the other studies. The mean D was 1.40, for a sensitivity of 0.22 and a specificity of 0.94. Since this heterogeneity may be partly caused by the noted differences in definition of the reference test, we made a distinction between eight studies where a PCSE was defined according to the TOAST criteria,[12] and the four remaining studies which simply described which kind of PCSE was observed. After this modification, sensitivity increased to 0.74 in the first group, but specificity decreased (0.65) at the optimal point on the sROC curve (Fig.2).

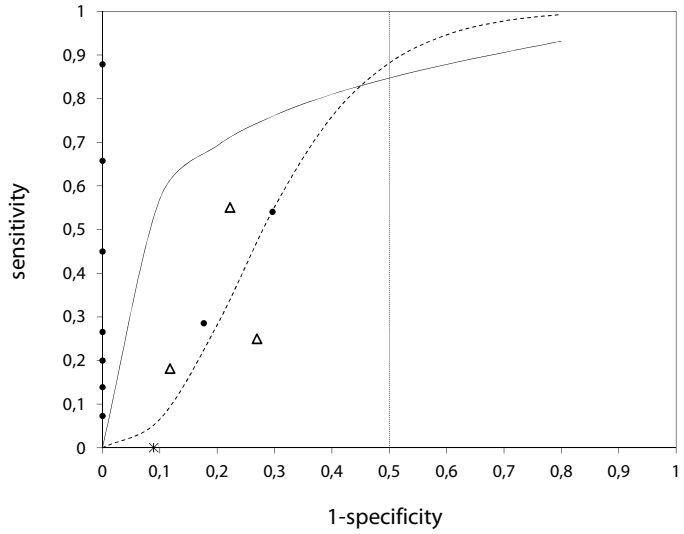

Figure 1. Summary ROC curve for diagnosis of stroke subtype. The dotted line in the figure indicates the predefined range of interest, no data are available beyond that line. Nine studies (dots) met our criteria; the summary ROC curve of these studies is the continuous line in the figure. Three studies were excluded (triangels) because in these studies the stroke subtype was based on just the admission CT scan; the summary ROC curve of those studies is the interrupted line in this figure. Results of the present study represented by the asterisk.

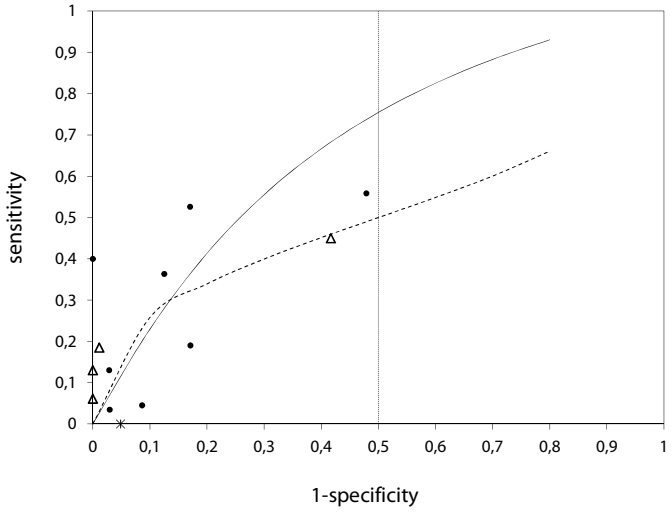

Figure 2. Summary ROC curve for diagnosis of a potential cardiac source of embolism. The dotted line in the figure indicates the predefined range of interest, no data are available beyond that line. Eight studies (dots) met our criteria; the summary ROC curve of these studies is the continuous line in the figure. Four studies were excluded (triangels) because in these studies the definition of PCSE was incomplete; the summary ROC curve of those studies is the interrupted line in this figure. Results of the present study are represented by the asterisk.

Characteristics of the Rotterdam TCD Study

Mean delay between stroke or TIA and TCD examination was 53 ± 49 days (range 1-207). To address the value of TCD in diagnosing stroke subtype, 120 patients were included, of which 56 had had a lacunar infarction on CT. None of the patients with a non-lacunar infarction had MES on TCD (Table 4). Thus, sensitivity for stroke subtype was 0, specificity was 0.91.

TO address the value of TCD in diagnosing PCSE, 149 patients were included. Five patients had a cardiac source of embolism, and five patients had MES, none of which had a PCSE. Sensitivity for PCSE was 0, specificity was 0.95 (Table 4). These data are indicated by the asterisk in Figure 1 and 2.

DISCUSSION

In this systematic review, we studied the value of MES monitoring in the diagnosis of non-lacunar infarcts or a PCSE in patients with a recent TIA or minor stroke. We used the summary ROC curve proposed by Moses et al.[11] The comparison of the studies was hampered by the differences in TCD settings, and by the differences in the criteria for stroke subtype and PCSE between the studies. However, our results indicate that TCD could be a useful test to distinguish non-small-vessel disease from small vessel disease. The sensitivity for non-small vessel disease was 0.76, and specificity was 0.90, in the subgroup of studies that used a robust reference test.

For PCSE, TCD does not seem to be able to adequately distinguish between the presence and the absence of a PCSE. Sensitivity was low in all studies described in this review. The sensitivity at the optimal point on the sROC curve in the studies with a robust reference test was reasonable (0.74), but outside the predefined range of interest. No data were available in that part of the sROC curve, and therefore any point on the sROC curve outside the range of interest is not meaningful. Moreover, in two of the five studies with a robust reference test it was not clear whether patients with mechanical prosthetic heart valves were included or not. These patients usually have more frequent MES and MES of higher intensity because their MES probably do not reflect solid particles but cavitation bubbles.[28,29] This may therefore influence the sensitivity and specificity of TCD for the presence of a PCSE.

In the Rotterdam TCD study no patients with a non-lacunar infarction had MES. Moreover, no patients with a PCSE had MES. The very low frequency of MES in our study could be related to the relatively long time interval between event and TCD investigation.

Our systematic review provides an estimate of the accuracy of TCD in the diagnosis of PCSE and ischemic stroke subtype. Data can be viewed in ROC space, and the trade-off between sensitivity and specificity is made clear.

Limitations

Study populations in this type of analyses are often highly selected, but in our review this is unlikely: most studies involved consecutive patients, and in most studies the percentage of PCSE was not abnormally high (range 3 to 70%). Because of the differences in design between the studies, we cannot determine or quantify the contribution of each separate factor on diagnostic accuracy.

Only few studies reported a detailed description of the technical aspects of the MES detection, which were recommended by the International Consensus Committee on Microembolus Detection. More than half of the studies were published before the publication of the consensus on microembolus detection.[8] Reporting all 14 parameters suggested by the Consensus Committee may lead to redundancy, but the parameters such as ultrasound device, insonated artery, insonated depth, method of intensity measurement, decibel threshold, sample volume, FFT size, and FFT overlap have a high face validity. For example, only four studies[3,10,19,20] gave a detailed description of intensity measurement of the ultrasound signal and this varied between those studies. Since intensity of MES is dependent on the method of intensity measurement, MES counted in one study might not have reached the decibel threshold in another study. This is especially important in patients in whom only one or two MES were counted. Moreover, because of these reported and unreported differences, the employed detection threshold is not directly comparable between the studies.

Our analysis did not include studies which used TCD and contrast agents to detect a patent formane ovale, since these studies were mainly different in study design. Direct comparison of these studies with the selected studies was not possible. Moreover, patent foramen ovale is a low risk PCSE, and medical and percutaneous treatment options are still under evaluation.

Other studies

As far as we know, no systematic review of the diagnostic value of TCD in patients with a TIA or minor stroke has been carried out. The direct influence of variation in TCD-parameters such as detection threshold, duration of the investigation, and parameters related to the sampling method on diagnostic accuracy, could not be analysed on the basis of the reported data. ROC analysis in a single large study would be the method of choice to assess the effect of changing the dB threshold for MES detection, and perhaps, the added value of a human observer versus automated detection.

One other application of TCD in patients with a minor stroke or TIA that has not been studied sufficiently, is its potential in predicting long term outcome and recurrent strokes. In our opinion, this has to be studied in a large cohort of patients with adequate follow up. This will have to be studied with unambiguous criteria that can be duplicated in other TCD laboratories.

Final conclusions and clinical relevance

The sensitivity and specificity for PCSE of MES detection with transcranial Doppler ultrasound is not high enough to recommend its use for screening prior to more invasive diagnostic investigations, such as transesophageal echocardiography.

The sensitivity and specificity for non-lacunar stroke of MES detection with transcranial Doppler ultrasound in the diagnosis of non-lacunar stroke is of a more acceptable level. Its high specificity suggests that in patients with a very recent stroke a confident diagnosis of non-lacunar stroke can be made, when MES are detected with TCD. Even though MES detection seems to be of limited diagnostic value in determining PCSE, it may be of use alongside other clinical information in determining stroke subtype in patients with TIAs, where history and other diagnostic information provides insufficient clues.

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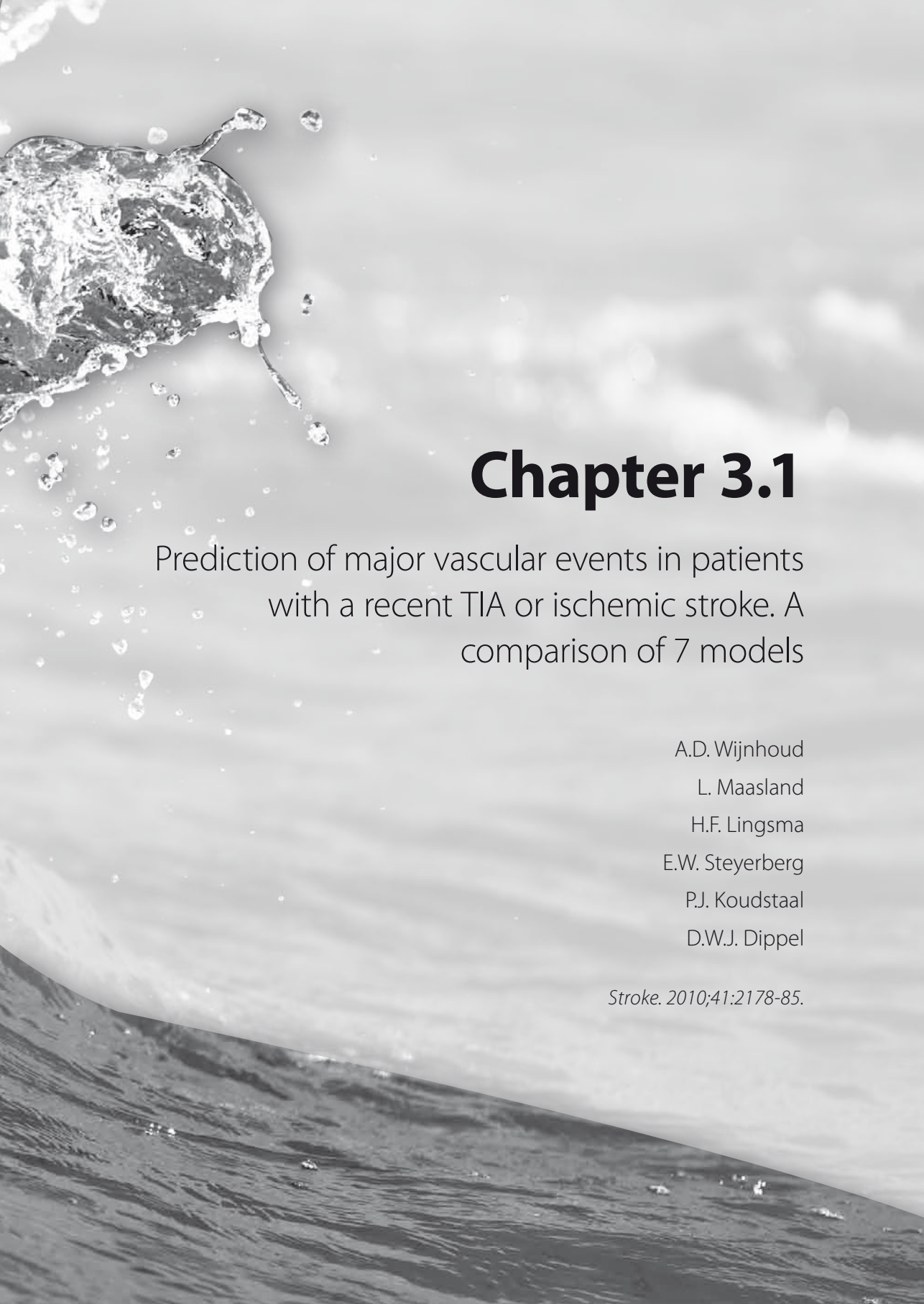

Chapter 3.1

Prediction of major vascular events in patients with a recent TIA or ischemic stroke. A comparison of 7 models

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Stroke. 2010;41:2178-85.

ABSTRACT

Background: In patients with a recent TIA or minor stroke, prediction of long term risk of major vascular events is important, but difficult. We aimed to study the external validity of currently available prediction models.

Methods: We validated predictions from 3 population-based models (Framingham, SCORE and INDIANA project) and 4 stroke-cohort-based models (SPI-II, Oxford TIA, Dutch TIA study, and the ABCD2 study) in an independent cohort of patients with a recent TIA or minor stroke.The validation cohort consisted of 592 patients with a TIA or minor stroke, with a mean follow-up of 2 years. The primary outcome was the two-year risk of the composite outcome event of non-fatal stroke, MI or vascular death. We used calibration graphs and c-statistics to evaluate the 7 models.

Results: The two-year risk of the primary outcome event was 12%. Calibration was adequate for stroke-population based studies. After adjustment for baseline risk and for prevalence of risk factors, calibration was adequate for the Dutch TIA, the ABCD2, and SPI-II models. Discrimination ranged from 0.61 to 0.68.

Conclusions: Discrimination was poor for all currently available risk prediction models for patients with a recent TIA or minor stroke, indicating the need for stronger predictors. Clinical usefulness may be best for the ABCD2 model which had a limited number of easily obtainable variables, a reasonable c-statistic (0.64), and good calibration.

INTRODUCTION

Patients with a recent stroke have an increased long-term risk of new cerebrovascular and cardiovascular events. Estimates of long term risk of cardiovascular and cerebrovascular events are important for patients who want to know their individual risk, and for treating physicians, because in patients with increased long-term risk, more expensive or more hazardous interventions) could be worthwhile. Long term risk seems to be dependent on the uynderlying risk factors.[1]

In an American Heart Association (AHA) statement it was pointed out that validated prediction models for long-term cardiovascular risk in patients with ischemic stroke or TIA were not available.[2] The use of a population-based prediction model, i.e. the Framingham risk score,[3] was recommended, however, this model has not been evaluated and compared with other prediction models to predict long-term cerebrovascular and cardiovascular risk in clinical practice. Treatment recommendations based on predictive models have been published and are currently used for patients with a significant carotid artery stenosis and for patients with atrial fibrillation.[4,5] For patients without AF or a carotid artery stenosis, more precise risk estimations are needed.[6,7] For this purpose, we need a reliable prognostic model with good discrimination between patients who will have new events and those who do not.

The aim of the present study is to determine which prediction models are adequate for long-term risk estimation for cardiovascular or cerebrovascular events in TIA and minor stroke patients. We primarily focused on major vascular events, i.e. non-fatal stroke, myocardial infarction and vascular death, because these affect health status most and are the focus of intervention studies. Secondarily, we considered fatal and non-fatal stroke.

METHODS

Selection of prediction models

We compared the external validity, i.e. calibration and discrimination, of 3 population-based models (the Framingham risk score for cardiovascular events,[3] the Framingham risk score for stroke,[8] SCORE,[9] and the INDIANA project cardiovascular risk score[10]), and 4 stroke-cohort-based models (SPI-II,[11] the Oxford TIA,[7] the Dutch TIA study[12], and the ABCD2 score[13]), in an independent cohort of patients with a recent TIA or minor stroke (Table 1). All models were designed to predict long term outcomes, except for the ABCD2 score which predicts 90-day risk of stroke. The stroke-population based models were designed to predict events after TIA or stroke, the population-based models were designed to predict first ever stroke or cardiovascular events. These models were based on well-described large cohorts of patients with TIA or minor stroke, and had the relative risks of the predictors estimated in a multiple (logistic or proportional hazards) regression model. The models predicted stroke and other cardiovascular events.

The Stroke Prognosis Instrument I (SPI-I) was developed in a small cohort of patients with a recent stroke. Kernan et al created SPI-II by incorporating new predictive variables identified in a cohort of patients from the WEST study, and validated it in three cohorts of patients from several secondary prevention trials.[11]

Hankey et al. developed a prediction equation for recurrent vascular events, based on eight clinical prognostic factors from a cohort of 469 hospital-referred TIA patients.[7] The prediction model was validated in cohorts of TIA patients from the Oxfordshire Community Stroke Project and the UK-TIA study.[14]

The Dutch TIA trial investigators developed a set of predictors of major vascular events and of stroke. Their prediction model was based on data of patients with a TIA or minor stroke, who entered a multicenter, randomized controlled clinical trial with a 2x2 factorial design, of high versus low dose aspirin and propanolol versus placebo.[12,15]

The ABCD2 score calculates the 2, 7, and 90 day stroke risk in TIA patients.[13] This prediction model was based on TIA patients presenting within 1 day of onset of symptoms. We used the 90-day risk score, since this was closest to the two-year risk calculated in the validation cohort.

The three other models were population based models which have been widely used to assess cardiovascular risk. For risk of major vascular events we used the Framingham risk score for cardiovascular events, published in 1998.[3] This model is based on categorical variables. For risk of stroke, we used the Framingham risk score for stroke, published in 1994.[8]

The SCORE prediction model methodology differs slightly from the other models. Annual, age and sex-dependent rates of coronary and vascular event were estimated from the data in an accelerated failure time model (Weibull). This model made use of age as a measure of exposure time to risk rather than a risk factor. Estimates of mortality rates were based on observations in age-categories.[9]

Table 1. Overview of the seven prediction models

Study	SPI-II	Oxford TIA	Dutch TIA	ABCD[2]	Framingham	SCORE	INDIANA project
Source population	Stroke trials	Hospital cohort	Stroke RCT	Hospital cohort	Population cohort	Prevention trials	Prevention trials
Outcomes	a. Stroke or death	a. Stroke, MI or vascular death b. Fatal or non-fatal stroke c. Coronary event	a. Stroke, MI or vascular death b. Fatal or non-fatal stroke	a. Stroke	a. Vascular events (cardiovascular risk and cerebrovascular risk separately)	a. Fatal coronary heart disease b. Fatal vascular (non-coronary) disease	a. Fatal coronary heart disease b. Fatal stroke c. Fatal cardiovascular disease
Time window	2 years	5 years	4 years	2, 7, 90 days	10 years	10 years	2.0 to 6.9 years
Predictors (n)	7	8	17	5	9	5	11
Demographics	Age Gender	Age Gender	Age Gender	Age	Age Gender	Age Gender	Age Gender
Clinical chart.	Stroke vs TIA	Amaurosis fugax only >1 attack residual signs	Amaurosis fugax only >1 attack persisting>6wks dysarthria vertigo	Duration of symptoms Clinical symptoms of TIA			
Risk factors	Prior stroke Severe hypertension Heart failure Diabetes Mellitus	Peripheral arterial disease Ischemic heart dis.	Diabetes Mellitus Angina pectoris Peripheral arterial disease	Systolic BP Diabetes	Total Chol/HDL-chol* Hypertension/SBP Diabetes Mellitus Smoking Atrial fibrillation† CVD†	Total cholesterol Syst blood pressure Smoking	Total cholesterol Syst blood pressure Smoking Previous myoc. infarction Previous stroke Diabetes Mellitus
Imaging			Borderzone infarct Any other infarct WML				
Other		Left ventricular hypertrophy	Left ventricular hypertrophy Incr term P wave Anteroseptal inf ST depression		Left ventricular hypertrophy‡		Left ventricular hypertrophy Antihypertensive treatment Serum creatinin

* cardiovascular risk only

† cerebrovascular risk only

Pocock et al. published a prediction instrument based on data from 8 randomized controlled trials of antihypertensive treatment in asymptomatic individuals, the INDIANA project. They developed three separate models for overall cardiovascular mortality, fatal coronary heart disease and fatal stroke.[10]

Validation cohort

The validation cohort consisted of 592 consecutive patients included in the Rotterdam Transcranial Doppler study.[16] Patients were recruited in our hospital. This study was designed to evaluate the diagnostic and prognostic value of transcranial Doppler ultrasonography in patients with a recent TIA or minor ischemic stroke. Patients were 18 years and older and had the index event within the preceding 6 months. They were independent (a score of 3 or less on the modified Rankin scale),[17] and the TIA or stroke was of presumed atherosclerotic origin; this implied that patients with a mechanical heart valve and a proven dissection were excluded. Patients with atrial fibrillation or a significant symptomatic carotid artery stenosis (>70% stenosis, NASCET criteria) were also excluded. Follow-up started at time of recruitment. The mean time between index event and recruitment was 51 ± 45 days. The mean follow-up in this study was 755 days or 2.1 years (interquartile range 0.9 to 3.1). The study population consisted of patients for whom no other treatment than risk factor modification and antiplatelet medication was available.

Procedure and definitions

The primary outcome was the composite end point of stroke, myocardial infarction and vascular death. The secondary outcome was the composite end point of fatal or non-fatal stroke. In the validation cohort, stroke was defined as a focal neurological deficit, resulting in disability for more than 24 hours, confirmed by a neurologist, with CT scan, if possible.

Myocardial infarction was defined as an episode of precordial chest pain accompanied by ECG evidence of recent infarction, and/or release of cardiac enzymes. Vascular death was death within 4 weeks after stroke or MI, or sudden death. Follow-up was done annually by telephone survey by an experienced nurse. If patients reported any hospital visits or any symptoms suggesting a vascular event, their treating physician was contacted for further information or discharge letters. In the validation cohort Kaplan-Meier analysis of survival was used to estimate the two-year risk of the primary and secondary outcome.

The validation procedure was similar for each prediction model. First we estimated the actual two-year risk of primary and secondary outcome events for each patient in the validation cohort using Kaplan-Meier survival analysis. For missing values, the mean value of a variable was imputed. We then calculated the two-year risk as predicted by the seven models. To do so we used the original prediction equations of each model. Each equation was adjusted for the mean overall risk and for the prevalence of risk factors in the validation cohort, as recommended by several authors.[18,19]

Calibration and discrimination

Two aspects of validity were examined: calibration and discrimination. Calibration, or reliability, measures how closely predicted outcomes agree with actual outcomes. Discrimination refers to the ability to distinguish patients with different outcomes.[20] The predicted probabilities of vascular events should be trustworthy (calibration) and extreme (discrimination).

Calibration-in–the-large reflects whether the overall outcome of the study cohort was close to the average predicted two-year risk from a model. We constructed calibration plots, in which observed two-year outcome was plotted against predicted two-year risk (using a Kaplan-Meier survival curve). We indicate outcome by quintiles of predicted probabilities. Calibration curves can be approximated by a regression line (or calibration line) with intercept (α) and slope (β). Well-calibrated models have α=0 and β=1. We calculated slopes for each prediction model. Discrimination was quantified with the concordance (c)-statistic.The c-statistic resembles the area under the ROC curve. A c-statistic of 1 implies a test with perfect sensitivity and specificity, while a value of 0.5 implies that the model predictions are no better than chance.[20]

RESULTS

Comparability of the cohorts

We first compared the distribution of prognostic factors in the seven derivation cohorts (Table 2). People in the population-based studies from the INDIANA project and Framingham were younger than patients in the stroke cohorts and by definition did not have a history of stroke or myocardial infarction. For the SCORE population, the mean age or age distribution was not stated. In this study, relatively young subjects had participated, and subjects aged 40 to 60 were particularly well represented. In the Oxford TIA cohort, all patients had a TIA, and about one third had amaurosis fugax (Table 2). In the validation cohort 57 major vascular events occurred during follow-up, for a two-year risk of 12 %, and 41 fatal or non-fatal strokes, for a two year-risk of 9%.

Table 4 shows the observed the predicted two-year risk of stroke, myocardial infarction or vascular death, as well as fatal or non-fatal stroke. The SPI-II overestimated the risk for both outcome events in patients with low risk and underestimated in patients with high risk, whereas Oxford TIA, the Dutch TIA trial, and ABCD2 score overestimated the risk for both outcome events (Figure 1).

Discrimination

Discrimination varied between 0.61 and 0.68 for the risk of stroke, myocardial infarction or vascular death, and between 0.58 and 0.65 for the risk of fatal or non-fatal stroke (Table 4). Discrimination was higher in the stroke-cohort-based studies than in the population-based studies, but remained below 0.70.

Table 2. Baseline characteristics of the seven prediction model cohorts and the validation cohort. Mean values are given, unless indicated otherwise. - indicates data not available; n.a. indicates not applicable.

	Validation cohort	SPI-II	Oxford TIA	Dutch TIA	ABCD[2]	Framing-ham	SCORE	INDIANA project
N in study	592	525	469	3127	4809	5345	205178	47008
Age (years)	62	-	62	65	70	49	-	55
Age over 60	-	-	-	-	77%	-	-	-
Age over 65	44%	73%	-	53%	-	-	-	
Age over 70	29%	57%	25%	34%	-	5%	-	
Male sex	60%	0%	68%	65%	47%	47%	57%	57%
Stroke characteristics								
TIA (not stroke)	54%	23%	100%	32%	100%	n.a.	n.a.	n.a.
Multiple attacks	20%	-	-	32%	-	n.a.	n.a.	n.a.
Amaurosis fugax only	6%	-	34%	6%	-	n.a.	n.a.	n.a.
Risk factors								
Previous stroke	21%	20%	0%	0%	-	0%	n.a.	1%
Previous MI[1]	24%	24%	21%	10%	-	0%	n.a.	5%
Diabetes mellitus	11%	31%	5%	8%	17%	4.6%	-	3%
Angina pectoris	9%	6%	-	9%	-	0%		
Current smoker	26%	-	47%	44%	-	39%	40%	
Total chol. (mmol/l)	5.7	-	6.7	-	-	5.4	6.1	
LDL-chol. (mmol/l)	3.8	-	-	-	-	3.5	-	-
HDL-chol. (mmol/l)	1.3	-	-	-	-	1.3	1.4	-
SBP (mmHg)	148	-[2]	-	158	-	146[3]	133	162
DBP (mmHg)	86	-	-	91	-	82	-	-
SBP > 140 mm Hg	-	-	-	-	69%	-	-	-
DBP > 90 mm Hg	-	-	-	-	30%	-	-	-
BMI (kg/m^2)	26.2	-	-	-	-	25.8	-	-

* Any MI, by history or ECG.

† Severe hypertension (systolic BP>180 or diastolic BP>100 mHg) in 31 patients (6%).

‡ Median systolic and diastolic BP instead of means were reported in the Framingham study

Table 3: Estimated two-year risk in the validation and derivation cohorts*

	Validation cohort	SPI-II	Oxford TIA	Dutch TIA	ABCD^{2} *	Framingham	SCORE	INDIANA project
Stroke	9%	-	9%	7%	7%	0.5%	- †	-
Stroke or death	13%	20%	-	-	9%	3%	- †	-
MI	4%	-	6%	-	-	3%	3.5%‡	0.5%
Stroke, MI or vasc death	12%	-	14%	12%	-	3%	5.7%	

* estimated 90-day risk in the ABCD^{2} study

† Product limit estimates were available for all studies but SCORE.

‡ Cumulative risk at age 65

Table 4. Calibration and discrimination of the seven prediction models.

A . Major vascular events

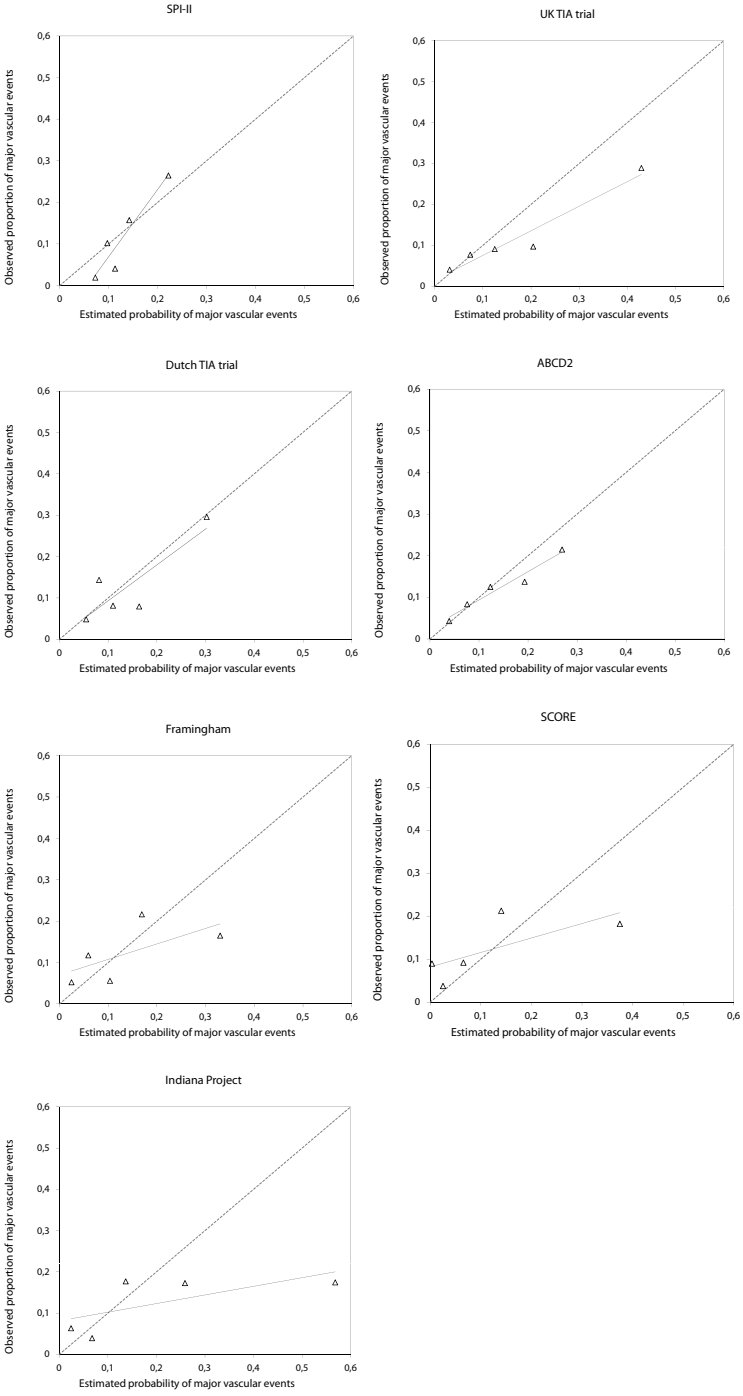
	Calibration			Discrimination*
	Predicted two-year risk	Observed two-year risk	Slope [95% CI]	c-statistic [95% CI]
SPI-II	13 %	12 %	1.62 [0.57 – 2.66]	0.68 [0.61 - 0.75]
Oxford TIA	17 %	12 %	0.60 [0.32 – 0.88]	0.66 [0.58 - 0.74]
Dutch TIA	14 %	12 %	0.92 [-0.27 – 2.11]	0.64 [0.56 - 0.72]
ABCD^{2}	14%	12%	0.68 [0.40 – 0.96]	0.64 [0.57 - 0.70]
Framingham	14 %	12 %	0.37 [-0.46 – 1.21]	0.64 [0.57 - 0.72]
SCORE	12 %	12 %	0.34 [-0.29 – 0.97]	0.61 [0.53 - 0.69]
INDIANA project	21 %	12 %	0.21 [-0.22 – 0.63]	0.61 [0.54 - 0.69]

* The nullhypothesis that all values were equal was rejected (p=0.02, χ^2 =15,27, 6 d.f.)

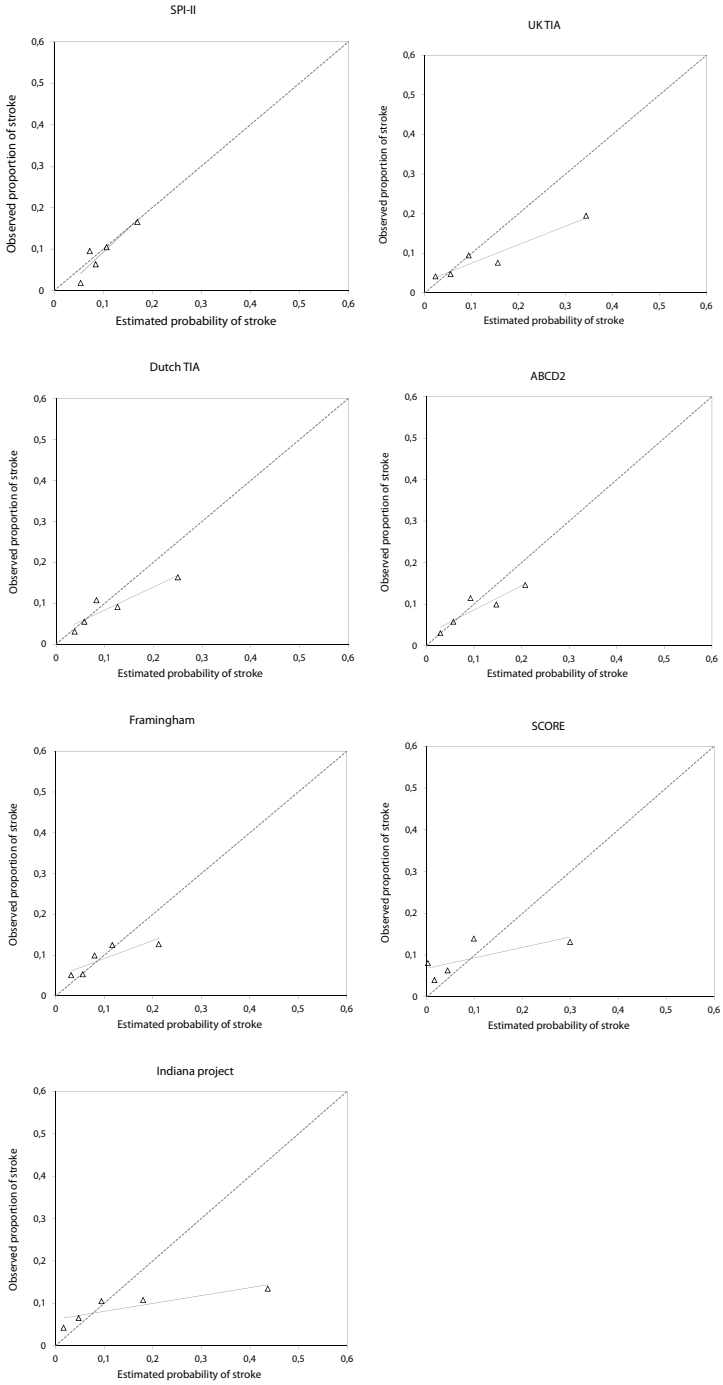
B . For stroke

	Calibration			Discrimination*
	Predicted two-year risk	Observed two-year risk	Slope [95% CI]	c-statistic [95% CI]
SPI-II	10 %	9 %	1.12 [0.24 – 1.99]	0.64 [0.56 - 0.72]
Oxford TIA	13 %	9 %	0.47 [0.22 – 0.72]	0.63 [0.54 - 0.72]
Dutch TIA	11 %	9 %	0.56 [0.13 – 0.99]	0.64 [0.57 - 0.72]
ABCD^{2}	11 %	9 %	0.58 [0.09 – 1.07]	0.61 [0.53 – 0.69]
Framingham	10 %	9 %	0.44 [-0.08 – 0.97]	0.58 [0.50 - 0.68]
SCORE	9 %	9 %	0.25 [-0.21 – 0.71]	0.58 [0.49 - 0.68]
INDIANA project	16 %	9 %	0.19 [-0.02 – 0.39]	0.61 [0.52 - 0.69]

* The 0 hypothesis that all areas were equal was not rejected (p=0.55, χ^2 =4.93, 6 d.f.)


SPI-II
UK TIA trial
Dutch TIA trial
ABCD2
Framingham
SCORE
Indiana Project
Observed proportion of major vascular events
Estimated probability of major vascular events
0
0,1
0,2
0,3
0,4
0,5
0,6

(A)



(B)

Figure 1. Calibration graph. Mean predicted probabilities versus observed frequencies of the composite outcome event of A. non-fatal stroke, non-fatal myocardial infarction or vascular death, and B. Stroke (in quintiles).

DISCUSSION

This is the first study comparing the external validity of long term prediction models. In a scientific statement for healthcare professionals from the Stroke Council and the Council on Clinical Cardiology of the American Heart Association/American Stroke Association, the Framingham study[3] was recommended for long term cardiovascular risk in patients with a TIA or ischemic stroke.[2] The purpose of our study was to find out whether stroke-cohort based studies indeed perform better and should be used in the future to estimate long term risk of major vascular events in patients with a TIA or ischemic stroke.

Calibration was fairly good in most models, but in the SPI-II study, the Dutch TIA study, and the ABCD2 study calibration was best. All models were adjusted for baseline risk and prevalence of risk factors as suggested by several authors.[18,19] Without these adjustments calibration was poor. Overall, stroke-based prediction models from the Dutch TIA trial, the SPI-II study, and the ABCD2 study showed best calibration and discrimination. A clinically useful prediction model shows good calibration, discrimination and uses a limited number of clear-cut, easily available variables.

The ABCD2 score and the SCORE prediction model used the smallest number of variables in their prediction rule for long term cardiovascular risk, 5 and 6 respectively, and risk estimation derived from these studies is therefore easy to obtain. The Framingham prediction model for cerebrovascular risk included 8 factors, which are all easy to obtain except for left ventricular hypertrophy for which an ECG is needed. The Dutch TIA study used 17 variables in the prediction rule. The Oxford TIA prediction rule and the INDIANA project prediction rule included ECG variables, which are more difficult to ascertain. The Dutch TIA prediction rule used ECG variables that require some expertise, such as left ventricular hyperthrophy by the Casale criteria,[21] and anteroseptal infarction (Table 1), and included also CT scan variables. This makes these models less attractive to use.

Limitations of the study

Some limitations of this study have to be discussed. First, although the validation cohort was of reasonable size (592 patients), the effective sample size was not large, with 57 major events, and 41 fatal or non-fatal strokes. Second, predictions from all models, except for the ABCD2 study, exceeded the two- year risk observed in our validation cohort. We have overcome this miscalibration by adjusting for this difference in follow-up in our analysis.

We excluded patients with AF or a severe carotid artery stenosis, and focussed on patients with TIA or minor ischemic stroke. This reduces the heterogeneity of the validation cohort, and may reduce the discriminatory ability of the models. Risk estimates are more readily available for carotid artery stenosis as well as for patients with AF. For the remaining group of patients with TIA or minor stroke there is a particular need for a prediction model.

In the validation cohort as well as in two of the four stroke cohorts, the mean age was rather low (Table 2). These cohorts consisted of a hospital population. Hospital populations tend to be of lower age. They may therefore not be representative of patients with TIAs and minor strokes in the community.

The time between index event and recruitment in the validation cohort was 51 days, which differs substantially from the ABCD2 study, not the remaining stroke-based population studies. We have overcome this problem by adjusting for the baseline risk in all validation studies. In this way, we adjusted for the low number of outcome events compared to the ABCD2 study.

Validity

Although this was a study on long term risk, we also included the ABCD2 model, which was developed for estimation of short term risk. Calibration and discrimination in the ABCD2 study, however, did not differ substantially from the long term prediction models. Comparisons with other studies cannot be made, because similar studies of long term risk assessment in patients with TIA or ischemic are not available.

Possible improvements

The seven models used dichotomized variables instead of continuous variables. Perhaps with continuous variables estimations of risk would have been more precise and discrimination may improve.[22] Furthermore, the stroke based prediction models were derived from trial populations with certain in- and exclusion criteria. The best fitting model for everyday practice will probably be derived from a representative cohort; this means a consecutive stroke population without exclusion criteria.

Conclusion

Current prediction models for long term prognosis in TIA and stroke patients have limited validity. Calibration seems to be adequate for most models, but especially discrimination between high risk patients and low risk patients is relatively poor. Improvements such as implementation of continuous variables are needed, as well as extension with stronger predictors of outcome. For now, the SPI-II and the ABCD2 prediction models seem to be most adequate. Of these two, the ABCD2 may be preferred because of its easy applicability in clinical practice.

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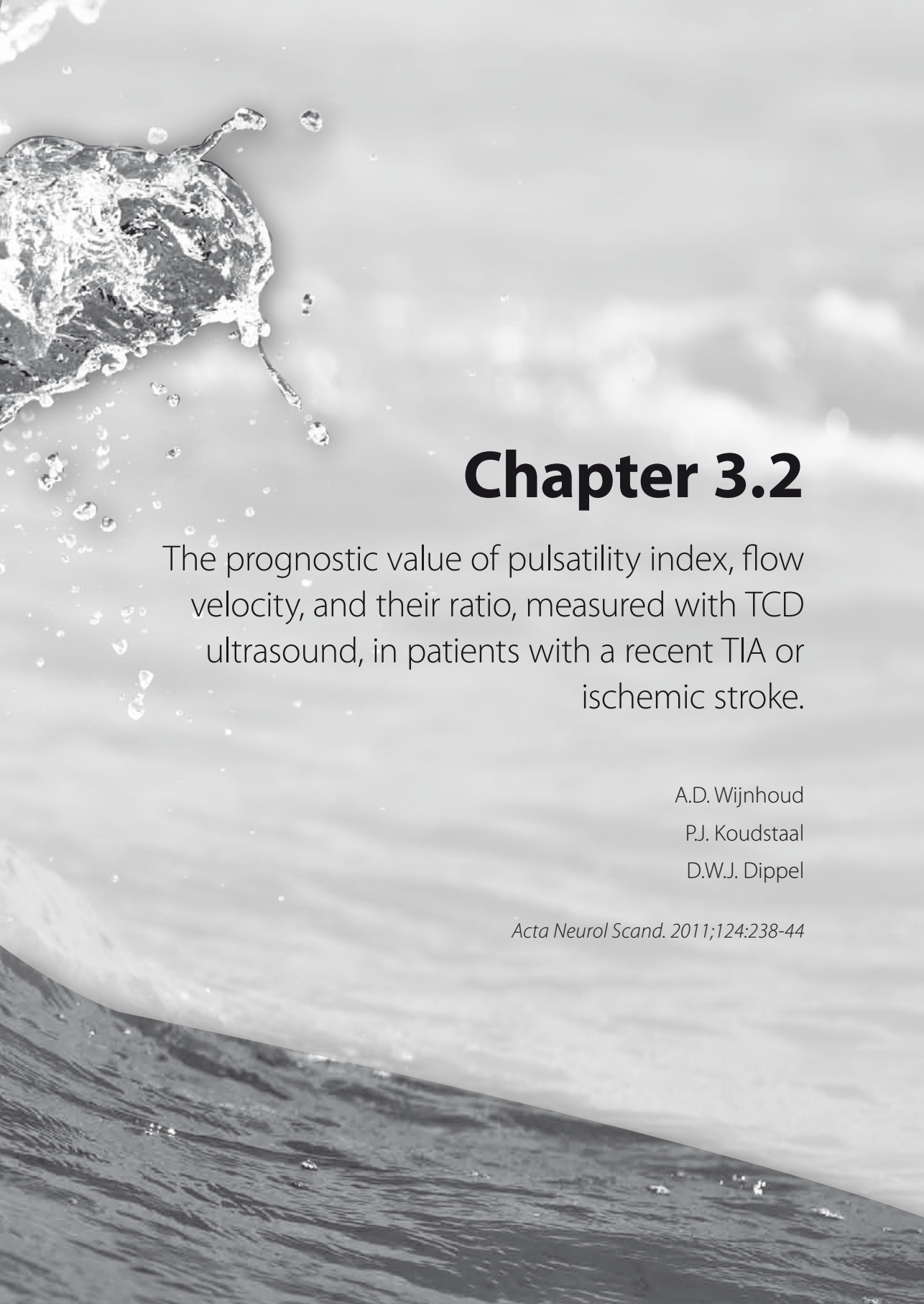

Chapter 3.2

The prognostic value of pulsatility index, flow velocity, and their ratio, measured with TCD ultrasound, in patients with a recent TIA or ischemic stroke.

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P.J. Koudstaal
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Acta Neurol Scand. 2011;124:238-44

ABSTRACT

Background: Increased flow velocities, and combinations of low mean flow velocity (MFV) and a high pulsatility index (PI) are associated with intracranial arterial disease. We investigated the association of MFV and the ratio of PI and MFV (PI-MFV ratio) in the middle cerebral artery with recurrence of vascular events in patients with a TIA or minor ischemic stroke.

Methods: 598 consecutive patients underwent TCD-investigation. Outcome events were fatal or non-fatal stroke, and the composite of stroke, myocardial infarction or vascular death (major vascular events). Hazard ratio's (HR) were estimated with Cox proportional hazards multiple regression method, adjusted for age, gender and vascular risk factors.

Results: TCD registration was successful in 489 patients. Mean follow-up was 2.1 years. Cumulative incidence was 9% for all stroke, and 12% for major vascular events. MFV over 60.5 cm/s increased the risk both for stroke (HR 2.8; 95%CI: 1.3-6.0) and major vascular events (HR 2.6; 95%CI: 1.3-5.0). Each unit increase in PI-MFV ratio was associated with a HR 2.8 (95%CI: 1.7-4.8) for stroke and HR 2.2 (95%CI: 1.3-3.6) for major vascular events.

Conclusion: In patients with a TIA or non-disabling ischemic stroke, MFV and the PI-MFV ratio in the middle cerebral artery are independent prognostic factors for recurrent vascular events.

INTRODUCTION

Transcranial Doppler ultrasound investigation of the intracranial arteries in patients with a recent stroke or TIA may provide prognostic information in addition to conventional vascular risk factors. Increased flow velocities in the middle cerebral artery (MCA) may indicate a local stenosis.[1] Furthermore, increased flow velocities in the MCA have been shown to be strongly related to vascular risk factors: flow velocities increase with hypertension, hypercholesterolemia, and in patients with diabetes mellitus.[2] This suggests that increased flow velocities may be related to extra- and intracranial vascular disease. On the other hand, the combination of low mean flow velocity (MFV) and a high pulsatility index (PI) has been associated with diffuse stenosis of intracranial arteries in patients with stroke.[3] Flow velocities however, decrease with increasing age and male gender.[4,5] Because of these associations, unadjusted flow-velocities are difficult to interpret. We analysed the prognostic value of TCD- flow-parameters for recurrent stroke and other vascular events in a consecutive cohort of patients with a recent TIA or minor stroke.

METHODS

Patients

We studied patients from a prospective cohort study that investigated the diagnostic and prognostic value of TCD in patients with a recent TIA or ischemic non-disabling stroke.[2] In brief, inclusion criteria were a recent (<6 months) TIA or non-disabling ischemic stroke of presumed arterial origin. Patients had to be over 18 years of age, and had to give informed consent. Patients had to be independent in most activities of daily living, corresponding with grade 3 or better on the modified Rankin scale.[6,7] We excluded patients with planned carotid surgery or stenting, because both TCD parameters and risk of recurrent events would be affected by the treatment. We also excluded patients with mechanical heart valves.

The study was approved by the Medical Ethics Committee and Review Board of the Erasmus MC in Rotterdam, the Netherlands.

TCD recordings

All patients underwent TCD examination (Multidop X-4, DWL, Sipplingen, Germany). All TCD recordings were carried out by one investigator (A.W.). TCD examination was performed within 6 months after TIA or non-disabling ischemic stroke. In all patients, peak systolic flow velocity (PSV) and end-diastolic flow velocity (EDV) was measured with a handheld 2 MHz probe in both middle cerebral arteries (MCA). Mean flow velocity (MFV) was calculated as EDV + 1/3(PSV-EDV). The pulsatility index (PI) was calculated as (PSV-EDV)/MFV. Because of the expected interaction between PI and MFV, we created a new parameter the PI-MFV ratio, calculated as 100xPI/MFV.

In a note we added that the multpiplication by 100 is just for practical reasons. The mean of the above mentioned TCD parameters was calculated as the mean of both left and right sided measurements. In case of unilateral window failure, the contralateral parameter was taken as the mean.

Blood pressure was measured automatically with a self-inflating cuff (Dynamap, Datascope, The Netherlands) during TCD investigation. Pulse pressure was calculated as systolic blood pressure minus diastolic pressure.

Ancillary investigations

All patients underwent routine workup including neurological examination, a CT scan to rule out hemorrhage, and an ECG. These investigations were performed on admission or first visit to the outpatient clinic, always before the TCD investigation. Risk factors (diabetes mellitus, smoking, hypertension, increased serum cholesterol level) were assessed as described previously.[2] A duplex-ultrasound scan of the carotid arteries was made in all patients with an anterior circulation stroke. Echocardiography was done in patients with a history of cardiac disease, abnormalities on ECG, or a cardiac bruit on physical examination.

Outcome

Primary outcome event was all stroke, both fatal and non-fatal. Secondary outcome event was the composite of either non-fatal stroke, non-fatal myocardial infarction and death from all vascular causes, whichever occurred first. Tertiary outcome events were fatal or non-fatal ischemic stroke, or TIA. The definition of death from vascular causes included sudden death (unexpected death ocurring within one hour after onset of symptoms, or within 24 hours given circumstantial evidence) or death from stroke, ischemic heart disease, or peripheral vascular disease.

Follow-up was done annually by telephone survey by an experienced nurse. If patients reported any hospital visits or any symptoms suggesting a vascular event, their treating physician was contacted for further information or discharge letters. All outcome events were anonimized and next adjudicated by an experienced panel (A.W. and D.D.) without knowledge of the TCD findings.

Statistical analysis

The cumulative probability of all outcome events was estimated with Kaplan-Meier survival analysis. The prognostic value of flow velocity parameters was analysed with Cox proportional hazards regression, and expressed as a hazard ratio (HR). Quintiles of TCD flow-parameters were used, because of non-normal distributions, and the lowest quintile was set als reference. For exploration of the relationship between the outcome events and the TCD flow velocity parameters, we used the upper quintiles of the TCD flow velocity parameters. Since PI-MFV ratio is an interaction factor it was analysed in a model including MFV and PI. In a note we added that

the multiplication by 100 is just for practical reasons. For exploration of the interaction factor PI-MFV ratio we included the variables MFV, PI, and PI-MFV ratio in the proportional hazards multipe regression model. Further adjustments for other vascular risk factors were also made with this method.

RESULTS

Clinical characteristics

We included 598 consecutive patients in our study. In 4 patients, the diagnosis was revised shortly after inclusion (1 patient had multiple sclerosis, 2 had focal epileptic seizures and 1 had migraine with aura). In 105 of the remaining 594 patients (17.7%), we failed to obtain adequate Doppler data due to two-sided window failure or other limitations.[8] Therefore 489 patients were included in the present analysis. Mean age was 60 years, and two-thirds of the patients were men. Approximately half of the patients had transient symptoms; more than 75% had focal ischemia in the anterior circulation. Almost one third of the patients were current smokers, nearly half had hypertension and almost one sixth had diabetes mellitus (Table 1).

Results of TCD examination

Mean time between TIA or stroke and TCD examination was 51 ± 45 days (range 1-207). In 22% of the patients TCD was performed within 2 weeks. Insonation depth varied from 48 to 56 mm. Mean MFV was 51.1 ± 14.4 cm/sec, mean PSV was 83.8 ± 22.1 cm/s, and mean EDV was 34.7 ± 12.1 cm/s. Only four patients had a PSV higher than 155 cm/s, only one had a PSV higher than 220 cm/s (223 cm/s).

The PI was 0.93 (SD 0.22). Mean PI-MFV ratio was 2.02 (SD 0.95). Nearly 25% of the patients had one-sided or double-sided window failure. Patients with window failure were older (71 ± 10 vs. 60 ± 13, $p<0.001$), were more often female (31% vs. 9%, $p<0.001$), and smoked less often (9% vs. 31%, $p<0.001$) than patients without window failure. MFV was lower in men, and MFV was negatively related to age, whereas diabetes and total serum cholesterol was positively related to MFV in this study, as described in a previous study of the same study population.[2] MFV was linearly related to PI, for every 10 cm/s increase in MFV, PI decreased with 0.05.

Outcome events

Mean follow-up was two years and one month (range 17 days to 5.7 years). A recurrent TIA or stroke occurred in 64 patients. A major vascular event (stroke, myocardial infarction and vascular death) occurred in 60 patients (Table 2). The cumulative incidence was 9% (95% CI: 7 to 12%) for fatal or non-fatal stroke, 12% (95% CI: 9 to 15%) for stroke, myocardial infarction or vascular death, and 14% (95% CI: 11 to 17%) for TIA, fatal or non-fatal ischemic stroke.

Table 1: Baseline patient characteristics.

	Overall N=489	MFV£60.5cm/s N=393	MFV>60.5cm/s N=96	
Demographics				
Age, years (mean ± SD)	60 ± 13	62 ± 12	52 ± 13	p<0.0001
Male gender	323 (66%)	278 (71%)	45 (47%)	p<0.0001
Caucasian race	410 (87%)	332 (87%)	78 (85%)	NS
Stroke characteristics				
TIA	261 (53%)	206 (52%)	55 (57%)	NS
Anterior circulation	391 (80%)	318 (81%)	73 (76%)	NS
Lacunar syndrome	180 (37%)	152 (39%)	28 (29%)	P=0.083
CT characteristics				
Any infarct on CT scan	225 (46%)	190 (48%)	35 (36%)	P=0.036
White matter lesions on CT scan	62 (13%)	57 (15%)	5 (5%)	P=0.014
Risk factors				
Smoking, current	140 (29%)	107 (27%)	33 (34%)	NS
Hypertension	259 (53%)	216 (55%)	43 (45%)	P=0.073
Diabetes	77 (16%)	58 (15%)	19 (20%)	NS
Previous TIA or stroke	106 (22%)	83 (21%)	23 (24%)	NS
Previous myocardial infarction	53 (11%)	45 (12%)	8 (8%)	NS
Carotid artery stenosis (≥70%)	37 (8%)	29 (7%)	8 (8%)	NS
Body-mass-index, kg/m2 (mean ± SD)	26.1 ± 3.9	26.2 ± 3.8	25.9 ± 4.3	NS
Use of cholesterol lowering medication	123 (26%)	102 (27%)	21 (23%)	NS
Cholesterol (mean ± SD)				
Total cholesterol,mmol/l	5.7 ± 1.2	5.6 ± 1.1	6.1 ± 1.3	p<0.0001
HDL-cholesterol, mmol/l	1.3 ± 0.4	1.3 ± 0.4	1.4 ± 0.5	NS
LDL-cholesterol, mmol/l	3.8 ± 1.1	3.7 ± 1.0	4.1 ± 1.2	P=0.013
Cholesterol-HDL ratio	4.7 ± 1.6	4.7 ± 1.6	4.8 ± 1.7	NS

Table 2: Occurrence of first outcome events

Outcome event	N=489
Fatal or non-fatal stroke	39 (9%)
Stroke, myocardial infarction, or vascular death	57 (11.5%)
TIA or ischemic stroke	62 (12.5%)
TIA	31 (6%)
Non-fatal stroke	33 (7%)
Non-fatal myocardial infarction	6 (1%)
Vascular death	21 (4%)
Fatal stroke	8 (1.5%)
Fatal myocardial infarction	2 (0.5%)
Sudden death	10 (2%)

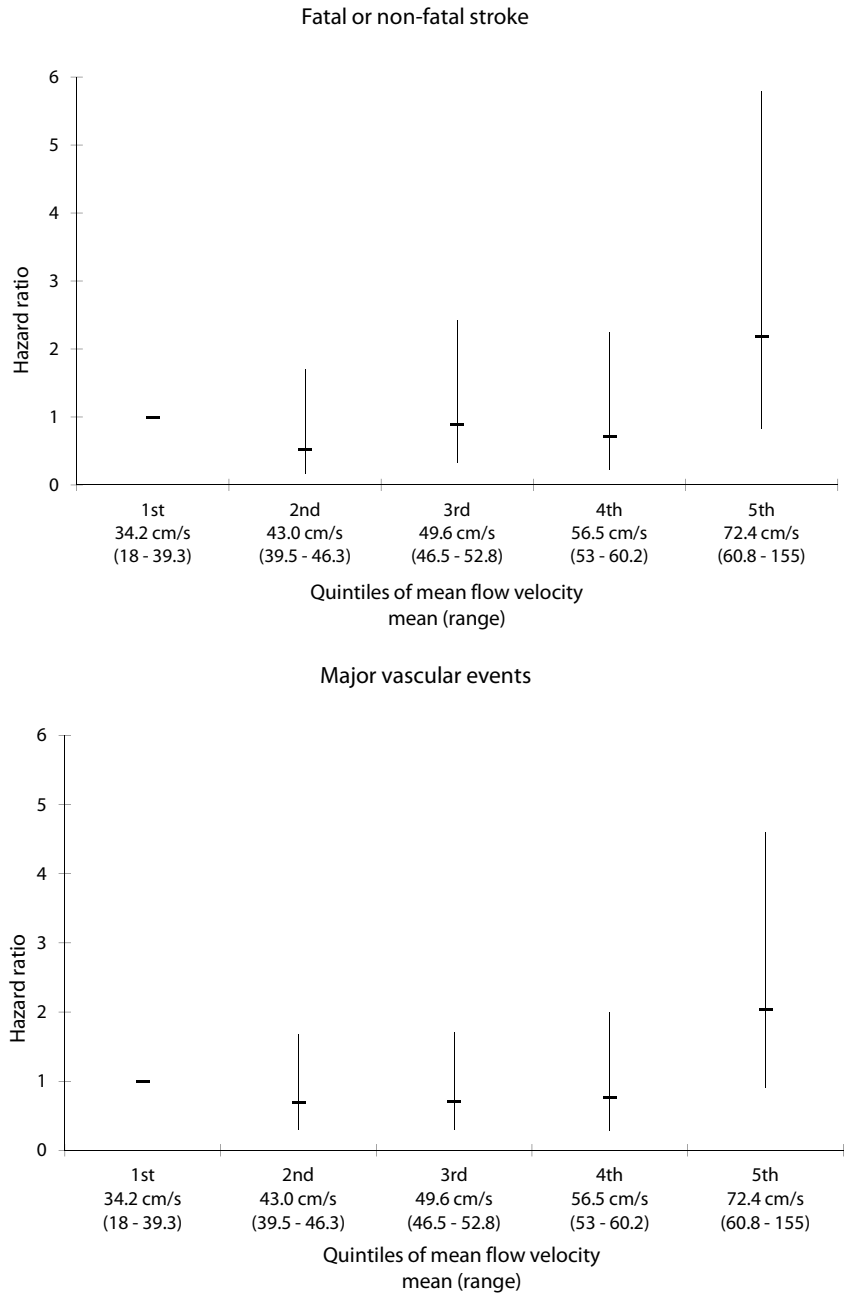

Figure 1: Hazard ratio's for quintiles of mean flow velocity for major vascular events (stroke, myocardial infarction, or vascular death) (upper graph), and for fatal or non-fatal stroke (lower graph), adjusted for age, gender, and vascular risk factors. Values on the x-axis are mean and range in each quintile.

Table 3: Relationship between TCD flow parameters, occurrence of fatal or non-fatal stroke, major vascular events and stroke or TIA in a cohort of 489 patients with a TIA or non-disabling ischemic stroke. For EDV, MFV, PSV and PI HRs with 95% confidence intervals (95% CI) of the highest quintiles compared to the risk in the lower four quintiles combined are reported, adjusted for age, gender, and major vascular risk factors.

	Fatal or non-fatal stroke	Major vascular events	Stroke or TIA
Mean flow velocity > 60.5 cm/s	2.8 (1.3 – 6.0)	2.6 (1.3 – 5.0)	1.7 (0.9 – 3.2)
Peak systolic flow velocity > 98 cm/s	2.3 (1.1 – 4.9)	2.1 (1.1 – 3.9)	1.7 (0.9 – 3.1)
End-diastolic flow velocity >43 cm/s	2.1 (0.9 – 4.8)	2.3 (1.1 – 4.8)	1.2 (0.6 – 2.5)
Pulsatility Index >1.09	1.5 (0.7 – 3.4)	1.4 (0.7 – 2.7)	1.6 (0.8 – 3.0)

Table 4: Relationship between TCD flow parameters, occurrence of fatal or non-fatal stroke, major vascular events and stroke or TIA in a cohort of 489 patients with a TIA or non-disabling ischemic stroke. For PI-MFV ratio, MFV, and PI HRs with 95% confidence intervals (95% CI) of the highest quintiles compared to the risk in the lower four quintiles combined are reported, adjusted for age, gender, and major vascular risk factors.

	Fatal or non-fatal stroke	Major vascular events	Stroke or TIA
PI-MFV ratio	2.5 (1.4 – 4.3)	1.9 (1.2 – 3.2)	2.3 (1.4 – 3.6)
Mean flow velocity	1.7 (1.3 – 2.0)	1.6 (1.3 – 1.9)	1.4 (1.2 – 1.7)
Pulsatility Index	0.8 (0.6 – 1.0)	0.8 (0.7 – 1.0)	0.8 (0.7 – 1.0)

Mean flow velocity

The HR of MFV for fatal or non-fatal stroke, adjusted for age and gender, did not differ between the first four quintiles, but in the upper quintile the risk for major vascular events nearly doubled with a HR of 1.8 (95% CI 0.7 to 4.5) although this was not statistically significant. After adjustment for vascular risk factors (diabetes, hypertension, cholesterol) the HR for fatal or non-fatal stroke did not change appreciably (HR 2.2, 95% CI 0.8 to 5.8) (Figure 1). Analyses with the MFV at the symptomatic side showed the same relationship, but this relationship was less strong.

Because the four lower quintiles of MFV showed similar risk, these were combined and a single cut point for the MFV was set at 60.5 cm/sec. Patients with an MFV>60.5 cm/s had a HR for fatal or non-fatal stroke of 2.8 (95% CI 1.3 to 6.0) after adjustment for age, gender, and vascular risk factors. HR for stroke, myocardial infarction or vascular death, was 2.6 (95% CI 1.3 to 5.0) for patients with an MFV>60.5 cm/s after adjustment for age, gender, and vascular risk factors (Table 3). MFV and PI did not show a statistically significant relationship with the outcome event TIA, fatal and non-fatal ischemic stroke.

Because of the possibility of increased risk with low flow, we did a more detailed analysis of the MFV in deciles. In the lowest decile of MFV the HR for fatal or non-fatal stroke was 10.9 (95% CI: 1.3 to 88) compared to the decile with the lowest HR for fatal or non-fatal stroke (i.e. the second decile), adjusted for age, gender, and vascular risk factors. The median velocity in this decile was 33 cm/s (range: 18 to 36 cm/s). The HR in the 9th and 10th decile was 9.7 (95%

CI: 1.0 to 92) and 16.1 (95% CI: 1.9 to 140) respectively, again compared to the lowest decile. The lowest decile of MFV also showed an increased risk of major vascular events (HR 2.6, 95% CI: 0.9 to 8.0), but this was not statistically significant.

PI-MFV ratio

The mean PI-MFV ratio was 2.01 (SD 0.95) and ranged from 0.46 to 8.25. Patients with a high PI-MFV ratio were older (67 ± 11 vs 55 ± 12 years, p<0.001), were more often male (75% vs 61%, p=0.001), and had more often hypertension (59% vs 49%, p=0.035). The HR for fatal or non-fatal stroke was 2.5 (95% CI: 1.4 to 4.3) for 1 unit increase in PI-MFV ratio, 1.7 (95% CI: 1.3 to 2.0) per 10 m/s MFV, and 0.8 (95% CI: 0.6 to 1.0) per 0.1 unit of PI (Table 4) after adjustment for vascular risk factors. The HR for major vascular events was 1.9 (95% CI: 1.2 to 3.2) for 1 unit increase in PI-MFV ratio, 1.6 (95% CI: 1.3 to 1.9) per 10 m/s MFV, and 0.8 (95% CI: 0.7 to 1.0) per 0.1 unit of PI (Table 4) after adjustment for vascular risk factors. The HR for TIA, fatal and non-fatal ischemic stroke was 2.3 (95% CI: 1.4 to 3.6) for 1 unit increase in PI-MFV, 1.4 (95% CI: 1.2 to 1.7) per 10 m/s MFV, and 0.8 (95% CI: 0.7 to 1.0) per 0.1 unit of PI (Table 4) after adjustment for vascular risk factors. Pulse pressure was not related to any of the outcome events, but it was associated with PI and PI-MFV ratio (correlation coefficient 0.51, p<0.00 and 0.34, p<0.00 respectively). Adjustment for pulse pressure did not change the hazard ratios for MFV, PI, and PI-MFV ratio.

DISCUSSION

This cohort study of 489 patients with recent TIA or minor stroke shows that MFV and PI-MFV ratio, measured in the MCA by TCD are independent risk factors for recurrent stroke. Interestingly, MFV and PI-MFV ratio were also associated with the occurrence of major vascular events (combined outcome measure "stroke, MI, and vascular death"). This suggests that the MFV and PI-MFV ratio reflect not only the condition of the major cerebral blood vessels, but also that of coronary and peripheral arteries.

Some methodological issues need to be discussed. In our study, one experienced investigator carried out all the TCD registrations, and all audiotapes were analysed by the same investigator. This excludes interobserver variability.[9] We did not assess intra-observer variability, but previous studies have shown that intra-observer variability regarding Doppler ultrasound measurements is reasonable.[10,11] Second, the MFV was a statistically significant risk factor only after adjustment for age, gender, and other vascular risk factors (hypertension, diabetes, and total serum cholesterol). This is probably caused by complex associations between these factors and outcome. For example, stroke risk increases with age, but when age increases, MFV decreases.[2,4,12] This was also the case in our study. Third, the association between MFV and the occurrence of fatal and non-fatal stroke and major vascular events became statistically significant after a dichotomization at the level of the fifth quintile. This observation was therefore

partly data-driven. Nevertheless, the association remained significant after alternative dichotomization at the more usual cut point of 65 cm/sec. This dichotomization seems justified since the quintiles showed a pathophysiologically plausible J-shaped relationship with the outcome events. We did not adjust for several other vascular risk factors, most notably smoking and presence of vascular lesions on CT, because our previous study[2] did not suggest a relationship between these factors and TCD parameters. Nevertheless, additional adjustment for these factors increased the risk ratios, and statistical significance. Fifth, in nearly 18% of our patients no adequate acoustical window could be obtained. These patients were predominantly elderly female patients. Although this proportion matches that of other studies,[13,14] it implies that our findings are not representative for the entire hospital population of patients with recent TIA or stroke. Sixth, the majority of our patients underwent TCD several weeks after the index event, when the period of very high instantaneous risk of recurrent stroke has passed. However, time since onset of symptoms did not affect the HR for recurrent stroke or major vascular events in our study. Finally, we excluded patients with planned carotid surgery in our study, because after desobstruction of a symptomatic carotid stenosis, TCD findings will have changed and the risk for vascular events will be diminished.

In our study, the risk for fatal or non-fatal stroke nearly tripled at MFV > 60.5 cm/s, suggesting that the elevated risk may perhaps be due to local MCA stenoses.[15] MCA stenosis of more than 50% has been reported to be related to PSV[1] and MFV.[16,17]. The risk of fatal or non-fatal stroke and major vascular events was most strongly related to MFV. However, intracranial atherosclerotic stenosis is an uncommon condition in Western Europe.[16-19] Indeed, in only one patient (0.2%) MFV exceeded the 100 cm/sec cut point for an MCA stenosis of more than 50%. Because the negative predictive value of TCD in detecting intracranial arterial stenosis is high, 94-100%,[16,17] we can safely assume that the increased risk in the upper quintile of MFV is not caused by the presence of an MCA stenosis of more than 50%. Increased flow velocity may also be caused by hypertension, but we adjusted for this risk factor in our analyses.

The most likely explanation, however, is that the upper quintiles of MFV in our study represent a slight but uniform narrowing of the intracranial arteries. Bos et al., who reported similar findings in an elderly population, have argued that according to Poiseuille's law small decreases in diameter of the arteries caused by atherosclerotic disease have to be compensated by relatively strong increases in blood flow.[20] Increased flow itself may induce further endothelial damage of the smaller calibre intracranial arteries, followed by inflammatory response, lipid deposition and atherosclerotic plaque formation,[21,22] especially in areas of low shear stress.[23] MFV may be a more reliable indicator of this process than PSV or systolic blood pressure. Therefore, increased flow velocity could be a subtle marker for atherosclerosis.

The prognostic value of the PI-MFV ratio is a novel finding. Previous reports have shown a strong relationship between intracranial atheromatous disease and the combination of a high PI and a low MFV or PSV in intracerebral vessels measured by TCD.[3,24] The PI, calculated as (PSV-EDV)/MFV, is a complex parameter thought to reflect cerebrovascular resistance. Interestingly,

PI alone was not a prognostic factor for recurrent stroke. This may suggest that cerebrovascular resistance is only important when high cerebral blood flow cannot be maintained. A higher PI-MFV ratio is thought to reflect diffuse intracranial stenotic disease, especially when MFV is lower than 30 cm/s, and PI is higher than 1.2.[3] Prognosis of acute stroke (<12 hours) seems to be poor when MFV is lower than 30 cm/s.[25] In our study the PI-MFV ratio was related to an increased risk of major vascular events (combined outcome measure "stroke, MI, and vascular death"). This may be explained by the presence of diffuse intracranial and extracranial atherosclerotic disease. A high PI-MFV ratio may indicate higher vulnerabiltiy of the brain in case of a thrombo-embolic event with less or lower quality collateral flow. These explanations fall beyond the scope of the present analysis, but certainly deserve further study. Pulse pressure was associated with PI and also with PI-MFV ratio in our study. Nevertheless, even after adjustment for pulse pressure, the association between PI-MFV ratio and recurrent vascular events was not affected.

We further subdivided the population according to deciles of the MFV was divided in deciles. Our results then suggested a J or U-shaped relationship of MFV with the risk for recurrent stroke. Other physiological parameters are also known to have a U or J-shaped relationship with stroke and cardiovascular mortality. For example, low blood pressure as well as high blood pressure increase risk of stroke and cardiovascular mortality.[26,27]

We conclude that MFV and the PI-MFV ratio, measured with TCD is an independent prognostic factor for fatal or non-fatal stroke and major vascular events in patients with a recent TIA or minor stroke. These parameters may not only reflect the degree of local atherosclerosis of the cerebral arteries, but also of the general cardiovascular system. Our findings may help to improve the prediction of recurrent stroke and other vascular events in clinical practice.

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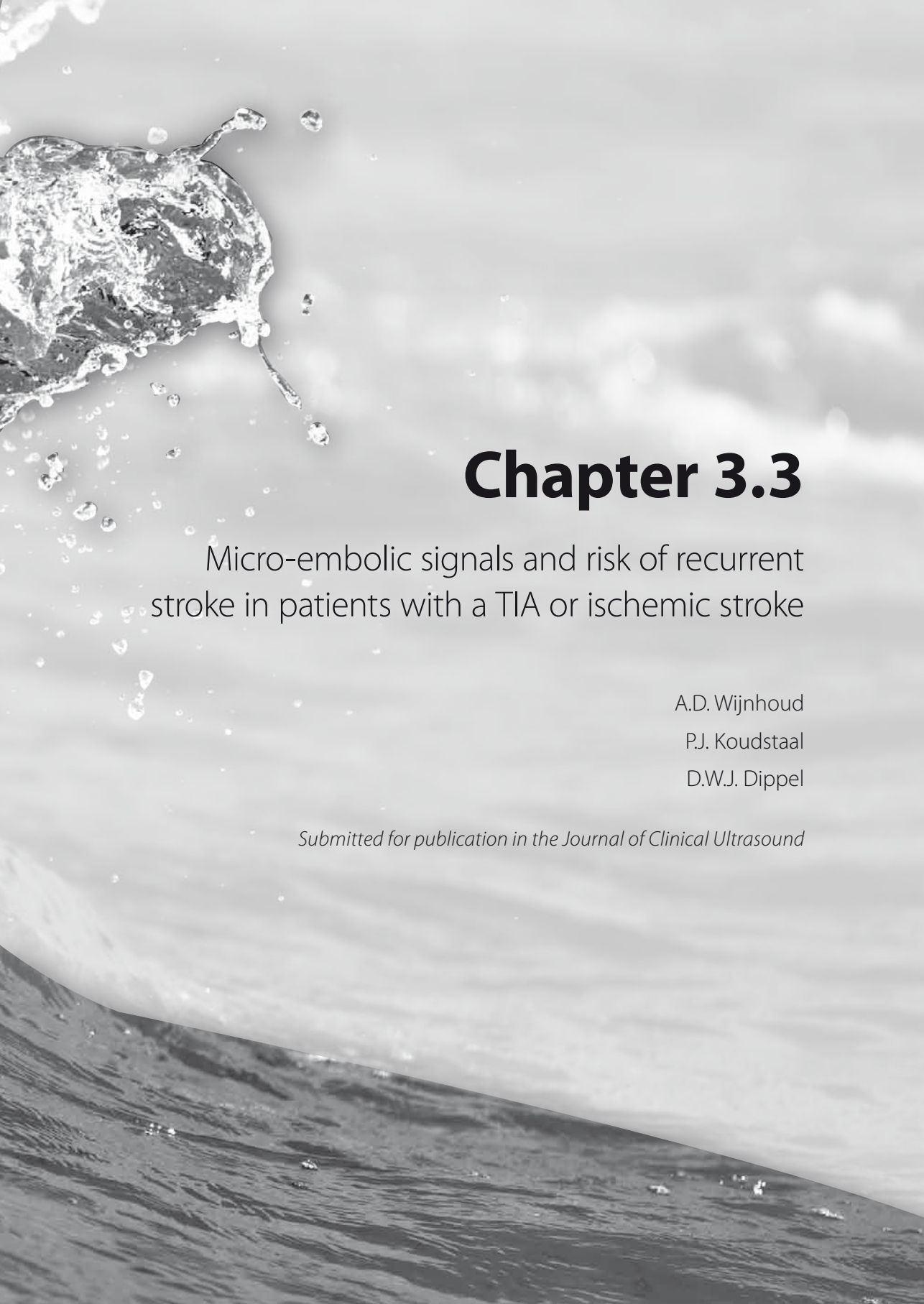

Chapter 3.3

Micro-embolic signals and risk of recurrent stroke in patients with a TIA or ischemic stroke

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Submitted for publication in the Journal of Clinical Ultrasound

ABSTRACT

Purpose: The prognostic value of micro-embolic signals (MES) on transcranial Doppler (TCD) ultrasound in patients with previous TIA or ischemic stroke and without significant carotid stenosis is unknown.

Methods**:** TCD-investigation was performed in a cohort of 365 patients with a recent TIA or ischemic stroke. The signal of the left and right middle cerebral artery (MCA) was recorded for 30 minutes and was stored on digital audio tape for off-line analysis. MES were defined according to the "Consensus on microembolus detection by TCD". Outcome events were stroke and major vascular events (non-fatal stroke, non-fatal myocardial infarction or vascular death). Hazard ratio's (HR) were estimated with Cox proportional hazards multiple regression method.

Results**:** Mean follow-up was 2.1 years. MES were observed in 16 (4%) patients. The occurrence of MES increased the risk of stroke, HR 3.8 (95% CI: 1.5 to 9.9), and the risk of ischemic stroke or TIA (HR: 2.6; 95% CI: 1.1 – 6.1). The HR for risk for major vascular events was 2.3; 95% CI: 0.9 –6.0).

Conclusion**:** In patients with a TIA or non-disabling ischemic stroke without severe carotid disease, MES in the MCA are rare. Nevertheless, in these patients, MES constitute a risk factor for recurrent stroke.

INTRODUCTION

Detection of microembolic signals (MES) with transcranial Doppler ultrasound may provide prognostic information in addition to the conventional risk factors in patients with a TIA or minor stroke.[1] In patients with symptomatic or asymptomatic carotid stenosis, MES are associated with an increased short-term risk of stroke or TIA.[2,3] It is unknown whether these results apply to stroke patients without carotid artery stenosis.

We assessed the prognostic value of MES for recurrent stroke and other vascular events in a consecutive cohort of patients with a previous TIA or minor stroke.

METHODS

Patients

We studied the diagnostic and prognostic value of TCD in consecutive patients with a recent TIA or ischemic non-disabling stroke prospectively, from April 1999 until March 2003. Nearly all patients were included in our hospital, 22 were included in Meander Medisch centrum in Amersfoort. Inclusion criteria were a TIA or non-disabling ischemic stroke in the preceding 6 months, corresponding with grade 3 or better on the modified Rankin scale.[4] Patients had to be over 18 years of age, and had to give informed consent. We excluded patients in whom carotid surgery or stenting was planned. These patients were excluded because carotid artery stenosis is related to the presence of MES.{Molloy, 1999 25 /id} Patients with known atrial fibrillation, and patients with mechanical heart valves were also excluded, since in these patients MES occur frequently, and are thought to be gaseous, not solid.{Georgiadis, 1998 208 /id} The study was approved by the Medical Ethics Committee and Review Board of the Erasmus MC in Rotterdam, the Netherlands.

TCD recordings

All patients underwent TCD examination (Multidop X-4, DWL, Sipplingen, Germany). TCD examination was performed within 6 months after TIA or non-disabling ischemic stroke by one investigator (A.W.). The middle cerebral artery (MCA) was insonated bilaterally. For MES detection, the signal was recorded for 30 minutes and stored on digital audio tape (ADAT-LX20, Alesis, Santa Monica, California) for off-line analysis. This analysis was also performed by one investigator (A.W.), who was blinded to other clinical data and to the occurrence of outcome events, during off-line analysis. The intensity, depth and side of the MES were registered. MES were defined according to the Consensus on Microembolus Detection by TCD.[5]

Ancillary investigations

All patients underwent routine workup including neurological examination, a CT scan to rule out hemorrhage, an ECG, and a duplex-ultrasound scan of the carotid arteries in case of an anterior circulation stroke. Risk factors (diabetes, smoking, hypertension, serum cholesterol level) were assessed as described previously.[6]

Outcomes

The primary outcome event was fatal or non-fatal stroke ("stroke"). Secondary outcome events were the composite of non-fatal stroke, non-fatal myocardial infarction and death from all vascular causes ("major vascular events"), whichever occurred first.

Follow-up was done annually by telephone survey by an experienced nurse who was blinded for the TCD results. All possible events were adjudicated by two experienced vascular neurologists.

Statistical analysis

The cumulative probability of composite outcome events was estimated with Kaplan-Meier survival analysis. Hazard ratios (HR) with 95% confidence intervals (CI) for MES were estimated with Cox proportional hazards regression.

RESULTS

Clinical characteristics

We included 598 consecutive patients in our study. In 4 patients, the diagnosis was revised shortly after inclusion. Exactly 105 of the remaining 594 patients had two-sided window failure. Because of technical problems or because they did not tolerate the headband, 124 patients did not complete the 30 minute registration. The remaining 365 patients were included in the present analysis (Table 1).

Results of TCD examination

Mean time between index event and TCD examination was 53 ± 49 days (range 1-207), and did not differ between patients with MES and patients without MES (38 ± 43 vs 53 ± 48 days, p=0.21). MES occurred in 16 patients (4 %). The mean number of MES was 7 (range 1 to 37).

Outcome events

Mean follow-up was 2.3 years (range 17 days to 5.7 years). The two-year cumulative incidence was 10% for stroke and 13% for major vascular events.

Table 1: Patient characteristics.

	Overall N=365	No MES N=349	MES N=16	
Demographics				
Age, years (mean ± SD)	59 ± 13	59 ± 13	59 ± 10	NS
Male gender	243 (67%)	229 (66%)	14 (88%)	P=0.07
Caucasian	313 (87%)	298 (87%)	15 (94%)	NS
Stroke characteristics				
TIA	187 (51%)	179 (51%)	8 (50%)	NS
Anterior circulation	286 (78%)	271 (78%)	15 (94%)	NS
Lacunar syndrome	134 (36%)	124 (36%)	10 (63%)	P=0.029
CT characteristics				
Any infarct on CT scan	171 (47%)	163 (47%)	8 (50%)	NS
White matter lesions on CT scan	52 (14%)	50 (14%)	2 (13%)	NS
Risk factors				
Smoking, current	100 (27%)	95 (27%)	5 (31%)	NS
Hypertension	189 (52%)	182 (52%)	7 (44%)	NS
Diabetes	53 (15%)	52 (15%)	1 (6%)	NS
Previous TIA or stroke	83 (23%)	78 (23%)	5 (31%)	NS
Previous myocardial infarction	45 (12%)	45 (13%)	0 (0%)	NS
Carotid artery stenosis (≥70%)	25 (7%)	24 (7%)	1 (6%)	NS

Table 2: Relationship between MES and relative risk of fatal or non-fatal stroke, major vascular events, and stroke or TIA in a cohort of 365 patients with a TIA or non-disabling ischemic stroke.

	Hazard ratio (95% Confidence Interval)
Fatal or non-fatal stroke	3.8 (1.5 – 9.7)
Major vascular events	2.3 (0.9 – 6.0)
Stroke or TIA	2.6 (1.1 – 6.1)

Micro-embolic signals

The risk for stroke was increased in patients with MES, HR 3.8 (95% CI: 1.5 to 9.9). The HR for major vascular events was 2.3 (95% CI: 0.9 to 6.0). The occurrence of MES was not related to time between TIA or stroke and TCD examination (Figure 1).

The risk for stroke was increased with the number of observed MES per patient, HR 1.11 (95% CI: 1.01 to 1.22 per single observed MES). Adjustment for age, gender, and vascular risk factors did not attenuate this association. One patient with a symptomatic carotid artery occlusion and an asymptomatic artery stenosis had 37 MES in 30 minutes on the asymptomatic side. Because this patient was initially not intended to undergo a carotid endarterectomy, we included him in the study. Exclusion of this patient did not change our results.

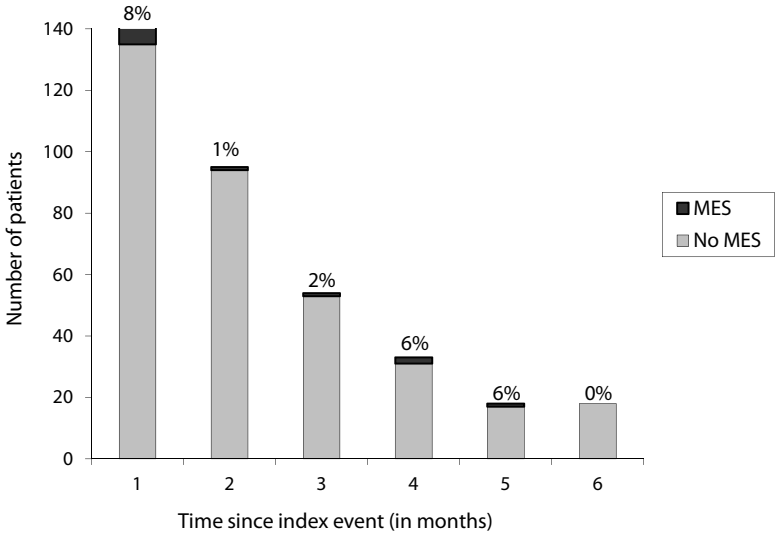

Figure 1: Number of patients with MES in relation to time from index event to TCD examination in a series of 365 patients with a TIA or ischemic stroke.

DISCUSSION

This study indicates that MES in the MCA measured by TCD increase the risk of stroke almost fourfold. MES were present in only 4% of the patients.

Some methodological issues need to be discussed. In our study, one experienced investigator carried out all the TCD registrations, and all tapes were analysed by the same investigator. This excludes interobserver variability. Second, 17.7% of our patients had a bilateral inadequate acoustical window. These patients were predominantly elderly female patients. Although this proportion matches that of other studies,[7] it implies that our findings are not representative for the entire hospital population of patients with previous TIA or stroke. The majority of our patients underwent TCD several weeks after their TIA or stroke, that is, beyond the period with the highest risk of recurrent stroke. However, time since onset of symptoms did not affect the HR for recurrent stroke or major vascular events.

In the patients without a carotid artery stenosis or atrial fibrillation it is not clear what the origin of the MES is. However, MES constitute a risk factor for stroke and major vascular events, regardless of the origin.

Only 4% of our patients had MES. This low prevalence was not surprising since MES most frequently occur in patients with a carotid artery stenosis[8] and we had excluded patients with a planned carotid surgery in our study, because after surgery for a symptomatic carotid stenosis, the risk of ischemic stroke diminishes, which would make our results more difficult to interpret.

MES most often occur in the acute phase after stroke,[9] whereas our patients were predominantly examined in the subacute or chronic phase. Nevertheless, MES were still related to a

recurrent fatal or non-fatal stroke. Idicula et al.[10] showed that MES are frequent in acute stroke, with MES present in 25% of the patients. MES were, however, in the acute phase not related to a recurrent vascular event. It is therefore likely that the mechanism of MES is in the acute phase different from the chronic phase.

In patients with a carotid artery stenosis, either symptomatic or asymptomatic, MES were related to increased risk of ipsilateral stroke. As far as we know, this has not been investigated in an unselected series of patients with stroke until now. In our study, only one of the patients with MES had carotid artery stenosis, and leaving that patient out of the statistical analysis did not change the results.

We conclude that in patients with a TIA or non-disabling ischemic stroke, even in the absence of a carotid artery stenosis, MES constitute a risk factor for recurrent stroke.

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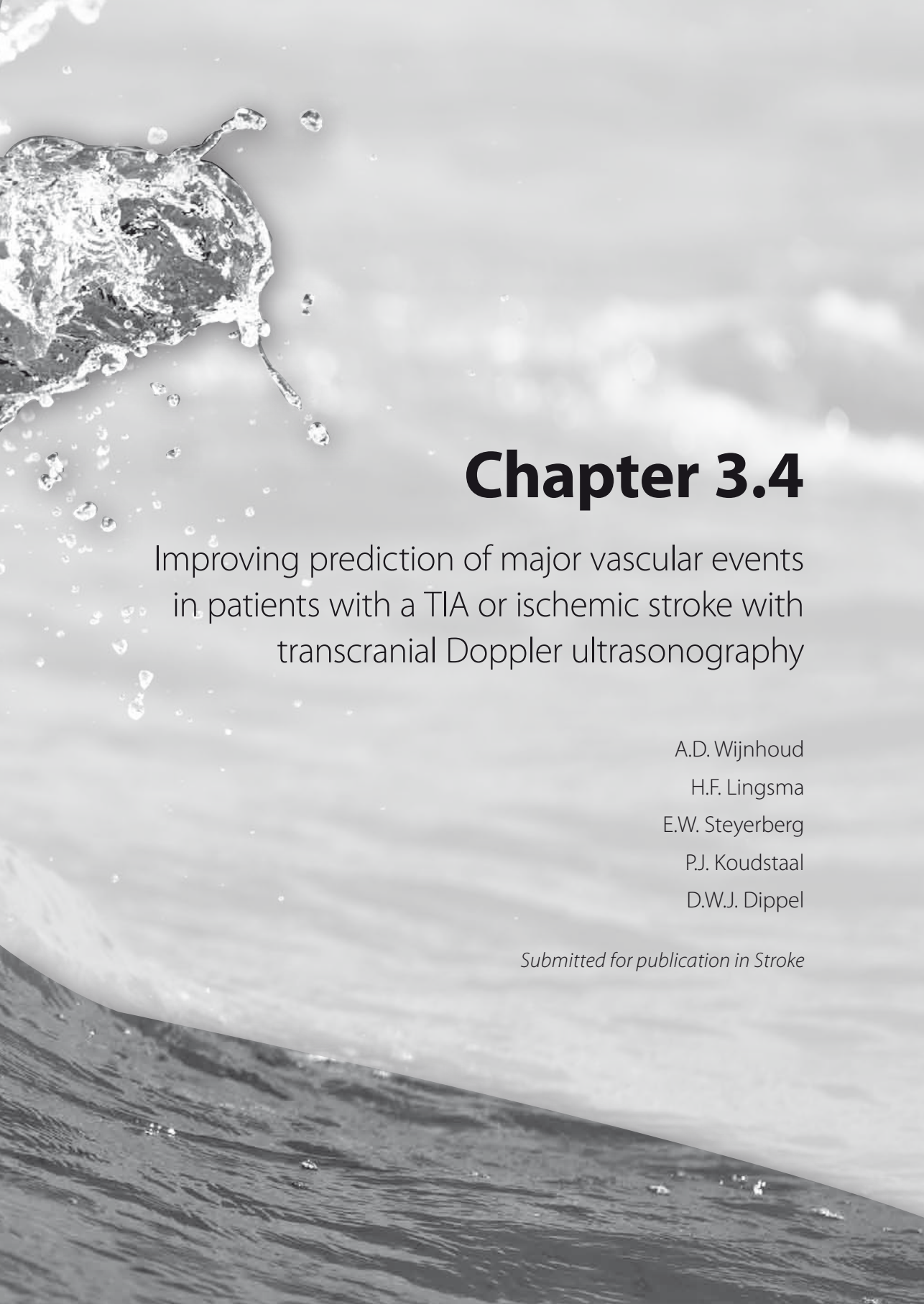

Chapter 3.4

Improving prediction of major vascular events in patients with a TIA or ischemic stroke with transcranial Doppler ultrasonography

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Submitted for publication in Stroke

ABSTRACT

Background: Prediction models are important for long-term vascular events in patients with a recent TIA or ischemic stroke. We aimed to study the incremental value of micro-embolic signals (MES) and flow velocity parameters measured with transcranial Doppler (TCD) over clinical variables alone in predicting recurrent vascular events.

Methods: The cohort consisted of 592 patients with a TIA or minor stroke, with a mean follow-up of 2 years. Mean flow velocity (MFV) could be measured in 489 of these patients in both middle cerebral arteries. Micro-embolic signals were recorded for 30 minutes and were analysed off-line in 365 patients. Outcomes were the composite of non-fatal stroke, myocardial infarction or vascular death (Major vascular events) with a two-year risk of 13%, and the composite of fatal or non-fatal stroke (Stroke) with a two-year risk of 10%. We used calibration graphs, the c-statistic, and the Net Reclassification Index (NRI) to evaluate the effect of adding TCD parameters to the ABCD2 clinical prediction model.

Results: Adding MES and high mean flow velocity to the ABCD2 score improved the discrimination for both models, with increasing c-statistic from 0.62 to 0.69 ($p<001$) for major vascular events, and from 0.61 to 0.71 ($p<0.01$) for fatal or non-fatal stroke. The NRI was 0.31 (95% CI 0.01 to 0.60) for prediction of major vascular events, and 0.42 (0.07 to 0.77) for prediction of fatal or non-fatal stroke.

Conclusion: Information concerning micro-embolic signals and mean flow velocity on TCD modestly improves the prediction of models for the recurrence of fatal or non-fatal stroke. Further studies are necessary on the validity and clinical application of these models.

INTRODUCTION

Patients with a recent stroke have an increased long-term risk of new cerebrovascular and cardiovascular events. In a previous study, we showed that the ABCD2 prediction model is most useful compared to other available models for prediction of long term risk in these patients. However, the discrimination between patients with high and low risk of recurrent stroke could be much improved.[1] Micro-embolic signals and flow velocity parameters measured with transcranial Doppler ultrasonography are prognostic factors for cerebrovascular and cardiovascular events in patients with a recent TIA or ischemic stroke with[2] and without[3] carotid artery stenosis. The prognostic value of MES or the flow velocity parameters measured with TCD have not yet been studied in a long-term prediction model.

METHODS

We studied patients from a prospective cohort study of the diagnostic and prognostic value of TCD in patients with a recent TIA or ischemic non-disabling stroke as described in a previous study.[2] In brief, inclusion criteria were a recent (<6 months) TIA or non-disabling ischemic stroke of presumed arterial origin. Patients had to be over 18 years of age, and had to give informed consent. Patients had to be independent in most activities of daily living, corresponding with grade 3 or better on the modified Rankin scale.[4] We excluded patients with planned carotid surgery or stenting, because the risk of recurrent events would be affected by the treatment. For similar reasons, we excluded patients with mechanical heart valves. The study was approved by our Medical Ethics Committee and Review Board.

Procedure and definitions

The primary outcome was the composite end point of stroke, myocardial infarction and vascular death. The secondary outcome was the composite end point of fatal or non-fatal stroke. In the validation cohort, stroke was defined as a focal neurological deficit, resulting in disability for more than 24 hours, confirmed by a neurologist, with CT scan, if possible.

Myocardial infarction was defined as an episode of precordial chest pain accompanied by ECG evidence of recent infarction, and/or release of cardiac enzymes. Vascular death was death within 4 weeks after stroke or MI, or sudden death. Follow-up was done annually by telephone survey by an experienced research nurse. If patients reported any hospital visits or any symptoms suggesting a vascular event, their treating physician was contacted for further information or discharge letters. In the validation cohort Kaplan-Meier analysis of survival was used to estimate the two-year risk of the primary and secondary outcome.

TCD recordings

All patients underwent TCD examination (Multidop X-4, DWL, Sipplingen, Germany). All TCD recordings were carried out by one investigator (A.W.). TCD examination was performed within 6 months after TIA or non-disabling ischemic stroke. In all patients, peak systolic flow velocity (PSV) and end-diastolic flow velocity (EDV) was measured with a handheld 2 MHz probe in both middle cerebral arteries (MCA). Mean flow velocity (MFV) was calculated as EDV + 1/3(PSV-EDV). For MES detection, the signal was recorded for 30 minutes and stored on digital audio tape (ADAT-LX20, Alesis, Santa Monica, California) for off-line analysis. This analysis was also performed by one investigator (A.W.), who was blinded to other clinical data and to the occurrence of outcome events. During off-line analysis, the intensity, depth and side of the MES were registered. MES had to meet the following criteria: MES are transient, last less than 300 milliseconds, are unidirectional within the Doppler velocity spectrum, are accompanied by a "snap", "chirp", or "moan" on the audible output and their amplitude is at least 3 dB higher than that of the background blood flow velocity spectrum.[5]

Statistical analysis

MFV was dichotomized at 60.5 cm/s since MFV > 60.5 cm/s increases risk of major vascular events and stroke in patients with a TIA or ischemic stroke.[3] We estimated the actual two-year risk of primary and secondary outcome events for each patient in the validation cohort using Kaplan-Meier survival analysis. For missing values, the mean value of a variable was imputed. We then calculated the two-year risk as predicted by the ABCD2 prediction model in a Cox's proportional hazard model The equation was adjusted for the mean cumulative hazard and for the prevalence of risk factors in the validation cohort, as recommended by several authors.[6,7] We then added the TCD parameters MES and MFV>60.5 cm/s to this model.

Calibration and discrimination

Two aspects of validity were examined: calibration and discrimination. Calibration, measures how closely predicted outcomes agree with actual outcomes. Discrimination refers to the ability to distinguish patients who will have an outcome event and those who will not.[8] The predicted probabilities of vascular events should be trustworthy (calibration) and extreme (discrimination).

We constructed calibration plots, in which observed two-year outcome was plotted against predicted two-year risk, using a Kaplan-Meier survival curve and the ABCD2 prediction rule, with and without the TCD parameters. We indicate outcome by quintiles of predicted probabilities. Calibration curves can be approximated by a regression line (or calibration line) with intercept (α) and slope (β). Well-calibrated models have α=0 and β=1. We calculated slopes for each prediction model. Discrimination was quantified with the concordance (c)-statistic, taking censored observations into account. The c-statistic resembles the area under the ROC curve. A c-statistic of 1 implies a test with perfect sensitivity and specificity, while a value of 0.5 implies

that the model predictions are no better than chance.[8] Statistical significance of improvements of the model was analysed by chi square analysis of the log likelihood ratio, with appropriate degrees of freedom. We also calculated the Net Reclassification Index (NRI). The NRI measures the proportion of patients with an improved prediction minus the proportion of patients with a worse prediction after adding the TCD parameters to the ABCD2 model. A positive NRI means improvement of the model.

RESULTS

Clinical characteristics

We included 598 consecutive patients in our study. The study population consisted of patients for whom no other treatment than risk factor modification and antiplatelet medication was available. In 4 patients, the diagnosis was revised shortly after inclusion (1 patient had multiple sclerosis, 2 had focal epileptic seizures and 1 had migraine with aura). In 105 (17.8%) of the remaining 594 patients, we failed to obtain adequate Doppler data due to two-sided window failure. Therefore, in 489 patients (82.2%) we obtained adequate data for flow velocity measurements (i.e. mean flow velocity). A total of 114 (19%) did not complete the 30 minute registration because of technical problems or because they did not tolerate the headband. The remaining 365 patients (61.5%) were included in the analysis of MES. The baseline characteristics are described in Table 1.

Results of TCD examination

Mean time between index event and TCD examination was 53 ± 49 days (range 1-207), and did not differ between patients with MES and patients without MES (38 ± 43 vs 53 ± 48 days, p=0.21). MES occurred in 16 patients (4 %). The mean number of MES was 7 (range 1 to 37). Mean MFV was 51.1 ± 14.4 cm/sec. MFV was negatively related to age, and was lower in men, whereas diabetes and total serum cholesterol was positively related to MFV in this study, as described in a previous study of the same study population.[9]

Outcome events

Mean follow-up was two years and one month (range 17 days to 5.7 years). Two-year-risk was 13% for major vascular events and 10% for fatal or non-fatal stroke.

Calibration and discrimination

The ABCD2 prediction model overestimated the risk of recurrent vascular events (Table 2 and Fig 1). Adding MES improved calibration of the model (p=0.036), but not discrimination for major vascular events (Table 2). Adding MES to the prediction model for stroke improved the model (p=0.03), and also improved discrimination (delta c=0.04, p<0.02). Adding MFV>60.5

Table 1: Patient characteristics.

Patients	All N=594	With successful TCD registration N=365
Demographics		
Age, years (mean ± SD)	62 ± 13	59 ± 13
Male gender	353 (59%)	243 (67%)
Caucasian	495 (83)	313 (87%)
Stroke characteristics		
TIA	322 (54%)	187 (51%)
Anterior circulation	477 (80%)	286 (78%)
Lacunar syndrome	214 (36%)	134 (36%)
CT characteristics		
Any infarct on CT scan	276 (46%)	171 (47%)
White matter lesions (CT)	91 (15%)	52 (14%)
Risk factors		
Smoking, current	155 (26%)	100 (27%)
Hypertension	312 (53%)	189 (52%)
Diabetes	99 (17%)	53 (15%)
Previous TIA or stroke	123 (21%)	83 (23%)
Previous myocardial infarction	62 (10%)	45 (12%)
Carotid artery stenosis (≥70%)	44 (7%)	25 (7%)

Table 2. Effect of adding MES, MFV>60.5 cm/s, and both to ABCD2 prediction model in patients with successful TCD registration (n=365).

	Calibration	Discrimination	NRI
	Slope [95% CI]	c-statistic [95% CI]	
Major vascular events			
ABCD2 alone	0.88 [0.04–1.17]	0.62 [0.54–0.70]	
Model including MES	1.16 [0.5–1.76]	0.66 [0.58–0.74]	0.12 [-0.17–0.42]
Model including MFV>60.5 cm/s	1.15 [0.60–1.70]	0.64 [0.57–0.72]	0.21 [-0.08–0.51]
Model including MES and MFV>60.5 cm/s	1.25 [0.57–1.94]	0.69 [0.61–0.76]	0.31 [0.01–0.60]
Stroke			
ABCD2 alone	0.82 [-0.43–2.07]	0.61 [0.52–0.71]	
Model including MES	1.26 [0.67–1.86]	0.66 [0.57–0.75]	0.21 [-0.13–0.59]
Model including MFV>60.5 cm/s	1.03 [0.36–1.70]	0.65 [0.57–0.73]	0.25 [-0.10–0.60]
Model including MES and MFV>60.5 cm/s	1.04 [0.29–1.78]	0.71 [0.62–0.80]	0.42 [0.07–0.77]

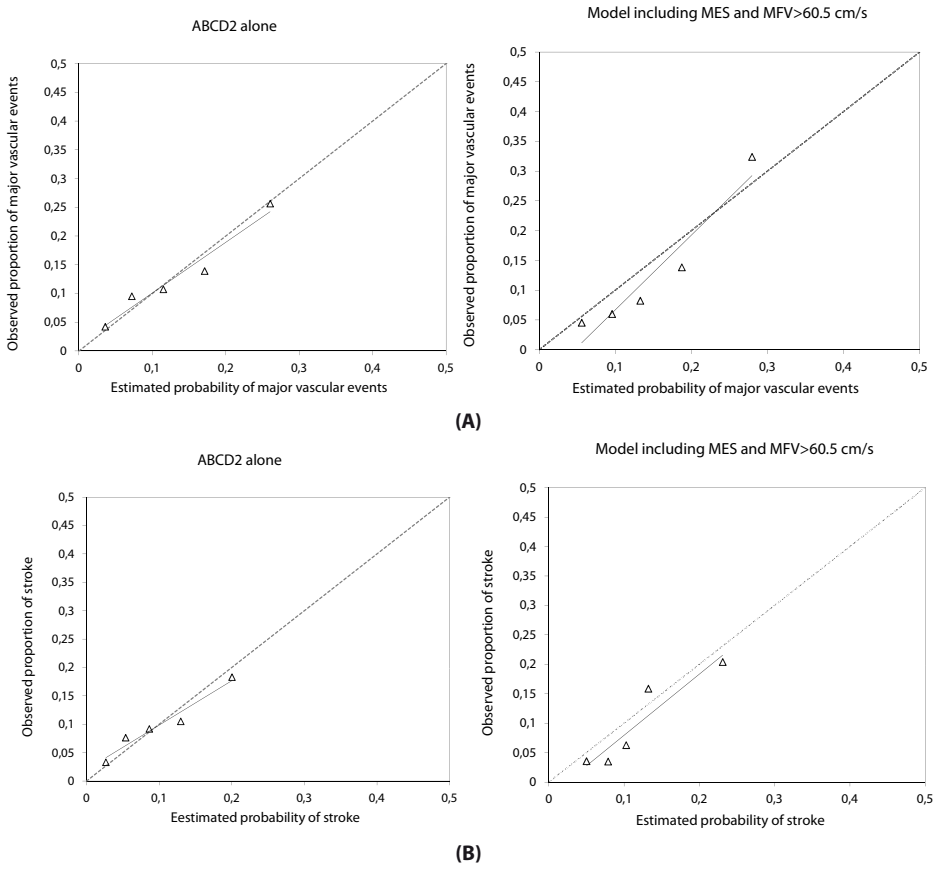

Figure 1. Calibration graph: Mean predicted probabilities (X-axis) versus observed frequencies (Y-axis) of the composite outcome event of non-fatal stroke, non-fatal myocardial infarction or vascular death (A) based on ABCD2 score with and without TCD parameters, of the composite outcome event fatal or non-fatal stroke (B) based on the ABCD2 score with and without MES and MFV>60.5 cm/s.

cm/s to the ABCD2 improved the model, but just not significantly (p=0.054) for major vascular events. Discrimination however, improved (p<0.02). Adding MFV>60 cm/s improved the model for stroke, but not significantly (p=0.079).

When both MES and MFV>60.5 cm/s were added to the ABCD2 prediction model using the prognostic sum score, both parameters improved of the model for major vascular events (p=0.037 for MES, and p=0.021 for MFV>60.5 cm/s. HR for the presence of MES in this model was 2.67 (95% CI: 1.06 to 6.73), and HR for MFV>60.5 cm/s was 2.04 (95% CI: 1.11 to 3.74) for the outcome event major vascular events. Discrimination improved from 0.62 to 0.69 (p<0.01). The NRI was positive, 0.31 (0.01-0.60), which means a significiant improvement of the model. Both MES and MFV improved the model for stroke (p=0.003 for MES, and p=0.029 for MFV>60.5 cm/s). For the outcome event fatal or non-fatal stroke, the HR for MES was 4.21 (95% CI: 1.63 to

10.87), the HR for MFV>60.5 cm/s was 2.23 (95% CI: 1.08 to 4.57). Discrimination improved from 0.61 to 0.71 (p<0.01). The NRI was again positive, 0.42 (0.07-0.77).

DISCUSSION

This study shows that MES and a MFV> 60.5 cm/s in the MCA are independent prognostic factors for long term prognosis in patients with a recent TIA or minor ischemic stroke when added to the ABCD2 prediction model. Moreover, adding these two factors to the ABCD2 prediction model improves calibration as well as discrimination. For a 70-yr old patient with hypertension but no diabetes, and a hemiparesis lasting longer than 60 minutes, the two-year risk would be 14%, for the same patient with MES and a MFV>60 cm/s in the MCA the two-year risk would be 28%, without MES and increased MFV, his two-year risk would amount to 19%.

Some limitations of this study have to be discussed. First, although the validation cohort was of reasonable size (598 patients), the effective sample size was not large, with 57 major vascular events, and 41 fatal or non-fatal strokes. Because of window failure and intolerance to the headband, not all patients were included in the final analyses which made the effective sample size even smaller. Our validation cohort was also our derivation cohort. This influences calibration, which is usually better based on the derivation cohort instead of an independent sample. It will be very interesting to see if our results can be replicated in other studies.

Although the ABCD2 prediction model was intended for short-term prognosis, external validation of the ABCD2 score shows reasonable calibration and discrimination.[1] Holzer et al. demonstrated that an ABCD2 score > 3 was associated with an increased risk for vascular events for medium- to long-term prognosis.[10]

TCD parameters have not been added to the ABCD2 score to improve prediction of stroke or other vascular events before. Adding brain infarction or carotid imaging to the ABCD2 model improves the prediction of stroke after TIA in the acute phase.[11,12] However, for long-term prognosis of the ABCD2 score no attempts of improvement have yet been made.

Conclusion: MES and MFV>60.5 cm/s improve prediction of recurrent vascular events in patients with a TIA or minor ischemic stroke when added to the ABCD2 prediction model.

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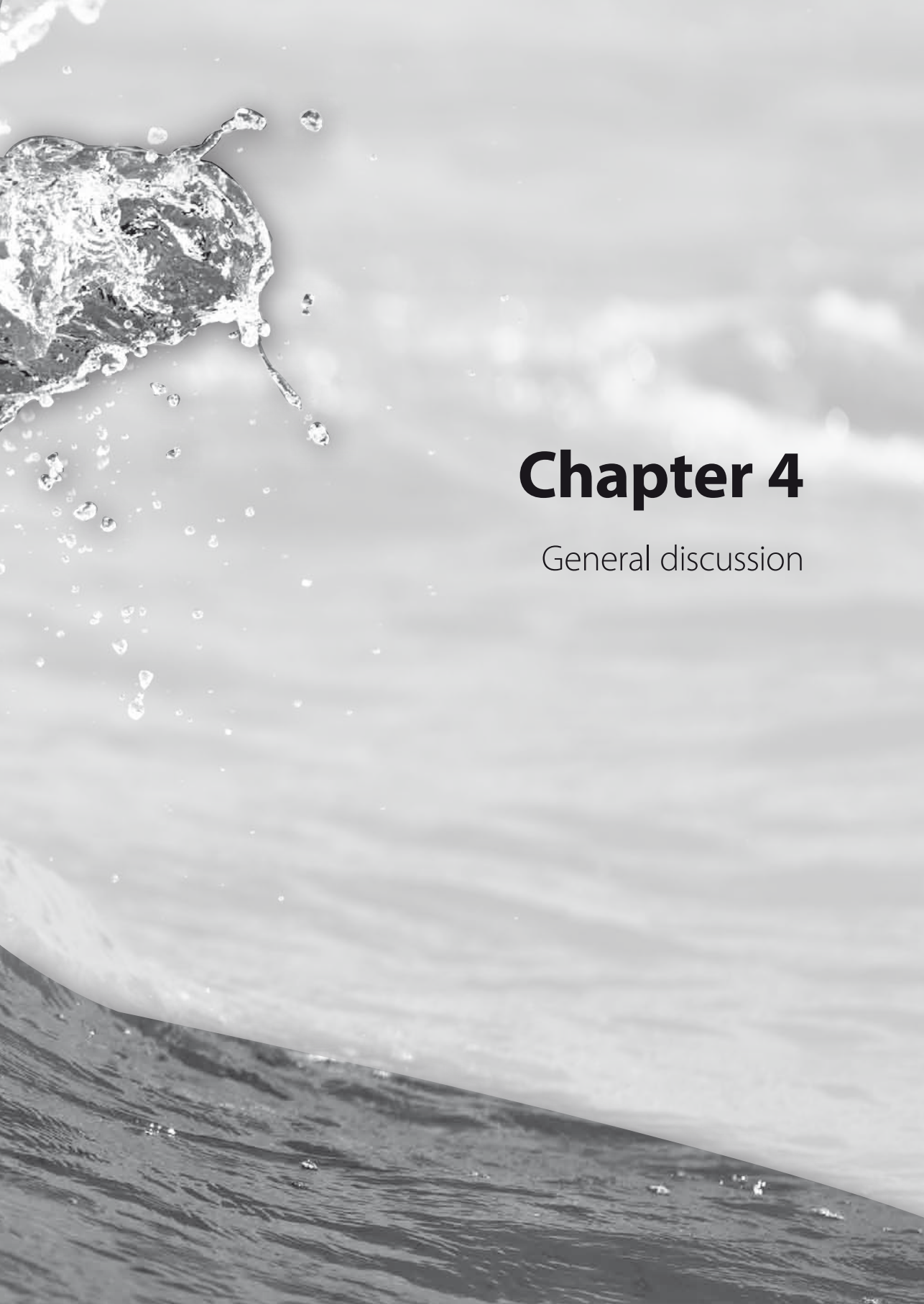

Chapter 4

General discussion

In this thesis, I have studied the value of transcranial Doppler ultrasonography (TCD) in patients with a recent TIA or ischemic stroke. In this Chapter, I will summarize the main findings, discuss methodological issues, review the results in the broader perspective of their clinical application and discuss their clinical relevance. Finally, I will provide suggestions for further research.

My research questions were:

1. Can TCD be of diagnostic value in detetecting the presence of a potential cardiac source of embolism and small vessel disease in patients with a TIA or minor ischemic stroke?
2. Is it possible to identify patients with a high risk of recurrent vascular events after TIA or minor ischemic stroke by means of TCD investigation? If so, can TCD improve prediction models?

MAIN FINDINGS

Diagnostic value of transcranial Doppler ultrasonography

I found that TCD parameters have a complex relationship with vascular risk factors such as age, hypertension and diabetes. I investigated these relationships, because these vascular risk factors may confound the association between TCD results and recurrent vascular events. [1-3] One of our main goals was to investigate if TCD parameters such as flow velocities can be of more diagnostic and prognostic value in predicting vascular outcome events than these vascular risk factors alone. In the patients participating in the study, blood flow velocity in the middle cerebral artery (MCA) decreased with age, and was lower in men than in women. Blood flow velocity in the MCA increased with total serum cholesterol, and was increased in diabetics compared to non-diabetics. Cerebrovascular CO_2-reactivity was lower in hypertensive patients. Cerebral CO_2-reactivity decreased with increasing total serum cholesterol (Chapter 2.1).

I found that micro-embolic signals (MES) were more often present in patients with a non-lacunar stroke than in patients with a lacunar stroke. MES were more often present in the acute phase of TIA or ischemic stroke than in the chronic phase. MES could not discriminate between patients with and without a potential cardiac source of embolism (PCSE) (Chapter 2.3) which was one of the main questions of this thesis. In our large cohort of patients, MES were present in only 4% of the patients.

Prognostic value of transcranial Doppler ultrasonography

Mean Flow velocity

Mean flow velocity (MFV) in the MCA is an independent risk factor for recurrent stroke. I found that patients with an MFV of more than 60.5 cm/s (the upper quintile) had 3 times increased

risk of recurrent stroke or other major vascular events compared with the other patients. This relationship was not influenced by other vascular risk factors (Chapter 3.2).

PI-MFV ratio

MFV was related to pulsatility index (PI), which is a measure of peripheral vascular resistance. The PI is calculated as PSV-EDV/MFV. Our results showed that PI decreased strongly when MFV increased, and both were associated with recurrent stroke. Because of this interaction, I created a new factor, the PI-MFV ratio which is the PI divided by the MFV, and therefore ((PSV-EDV)/MFV^2). The PI-MFV ratio was clearly related to other vascular risk factors. Patients with a high PI-MFV ratio were older, were more often male, and had more often hypertension. But even after adjustment for these risk factors, which are potential confounders, the PI-MFV ratio was associated with a higher risk of recurrent stroke or other vascular events, even stronger than the PI (Chapter 3.2).

Micro-embolic signals

Patients with MES had an almost fourfold increased risk of stroke in my study population. This risk applied to patients without a carotid artery stenosis. More surprisingly, I found that patients with MES also had an increased risk of other major vascular events such as myocardial infarction or vascular death (Chapter 3.3). The prevalence of MES was low however, compared to the prevalence in patients with symptomatic carotid stenosis; MES were only present in 4% of the patients.

Improvement of prediction

In order to improve prediction in patients with stroke and TIA, we first compared 7 prediction models to determine which provided the most adequate estimation of the long-term risk for cardiovascular or cerebrovascular events. I compared external validity, i.e. calibration and discrimination, of population based models and of stroke-cohort based models (Chapter 3.1). Calibration, or reliability, measures how closely predicted outcomes agree with actual outcomes. Discrimination refers to the ability to distinguish patients with and without outcome events. Overall, we found that the ABCD2 study performed best and was most easily clinically applicable (Chapter 3.1). I therefore chose the ABCD2 study to evaluate whether prediction of long-term risk of patients with a TIA or minor stroke could be improved by adding TCD parameters.

The ABCD2 prediction model includes 5 parameters: Age, Blood pressure, Clinical features (i.e. aphasia, unilateral weakness, or other symptoms), Duration of the symptoms, and Diabetes. These parameters are easy to assess and this model is therefore very easy to use. This model was originally designed to predict short term risk: 2 days, 7 days, and 30 days. I found that this model was also applicable to predict recurrent stroke or other major vascular events in 2

years. This model performed better when the before mentioned TCD parameters were added (Chapter 3.4).

Adding the presence of MES as well as the upper quintile of MFV separately to the ABCD2 prediction model improved both its calibration and discrimination, though not significantly. If both were added simultaneously calibration and discrimination for prediction of recurrent stroke and other vascular events were significantly improved (Chapter 3.4).

Discrimination measured by c-statistic improved from 0.62 to 0.69 ($p<0.01$) for major vascular events, and from 0.61 to 0.71 ($p<0.01$) for recurrent stroke. The Net Reclassification Index (NRI) was 0.31 (95% CI: 0.01 to 0.60) for the prediction of major vascular events. The NRI was 0.42 (95% CI: 0.07 to 0.77) for the prediction of recurrent stroke (Chapter 3.4). The NRI measures the proportion of patients with an improved prediction minus the proportion of patients with a worse prediction after adding the TCD parameters to the ABCD2 model. A positive NRI means improvement of the prediction model.

Window failure

In my study, TCD registration was not successful in all patients. Nearly 18% of the patients had two-sided window failure (WF), e.g. the failure of the ultrasound signal to pass through the temporal bone. I found that age and female gender were strongly related to WF. The skull thickness at the level of the temporal window was related to inadequacy of the ultrasound signal causing WF. A decreased radiodensity of the bone at the temporal window was also related to WF, but not significantly so, after adjustment for age and gender (Chapter 2.2).

METHODOLOGICAL CONSIDERATIONS

Study population and selection bias

We prospectively studied a large cohort of consecutive patients with a recent TIA or minor ischemic stroke corresponding with grade 3 or less on the modified Rankin scale.[4] We excluded patients with a carotid artery stenosis who were planned for carotid surgery. These patients were excluded because carotid artery stenosis is related to the presence of MES,[5] diminished cerebral CO_2-reactivity,[6] and altered flow velocities in the middle cerebral artery.[7] Both TCD parameters and risk of recurrent events would be affected by the carotid surgery, which would make our results more difficult to interpret. We also excluded patients with mechanical heart valves, since in these patients MES occur frequently, and are thought to be gaseous, not solid.[8] It may seem that exclusion of these patients can lead to selection bias, but in these particular patients subgroup, the value of TCD was already clear. These subgroups were therefore not of interest. Our study was designed to explore the value of TCD in the rest of the stroke population. We aimed for 800 patients, but inclusion was slower than expected. Therefore, only 598

patients were included, but we prolonged the duration for follow-up, which resulted in more outcome events and hence sufficient statistical power.

Although our proportion window failure of 18% matches that of other studies,[9,10] it implies that our findings are not representative for the entire hospital population of patients with recent TIA or stroke. This kind of selection bias is unavoidable. Of the patients with successful TCD registration, only 4 were lost to follow-up.

Patients were included in the study from 1999 to 2002. Risk of recurrent vascular events has declined over the past 50 years.[11] Risk of recurrent vascular events in patients with a TIA or ischemic stroke today is therefore very likely to be lower than 10 year ago at the time of our study period. The declined risk of recurrent vascular events can be attributed to a better treatment of hypertension and hypercholesterolemia.[11] In the past ten years the preventive effect of statins[12] and antihypertensive drugs[13] in patients with vascular disease has been sufficiently proven. The prevalence of these risk factors has not decreased, but treatment has improved. Patients with a TIA or ischemic stroke are currently more often treated with statins and antihypertensive drugs according to current guidelines. More recently, the ESPRIT study showed that adding dipyridamole to aspirin for secondary prevention led to a further reduction of recurrent vascular events of 1% per year.[14] It is not likely that my conclusions about the role of MES and flow velocity will be affected by these developments, because it concerns relative risk estimates. However, the calibration of our prediction model may be affected by the lower overall risk of recurrent vascular events, and it may have to be adapted before applying it to new populations.

TCD recordings

In our study, one experienced investigator carried out all the TCD registrations, and all audiotapes were analysed by the same investigator. This excludes interobserver variability.[15] We did not assess intra-observer variability, but previous studies have shown that intra-observer variability regarding Doppler ultrasound measurements is reasonable.[16,17] One investigator was involved in analysis of TCD registrations and in analysis of outcome events. In order to maintain blinded assessment the outcome events were anonimized.

Our results showed that window failure was related to bone thickness and bone density (Chapter 2.2). Our definition of window failure was the failure to develop en envelop around the TCD signal when using the headband. One could argue that this is still subjective, but to our opinion, no clearer definition is present.

TCD validation

Confounding is present when a factor is associated with both the determinant and the outcome. We have not performed any other tests to evaluate the cerebral circulation. There may

be unobserved confounding in our study. A stenosis of the middle cerebral artery (MCA) is such a potential confounder. Blood flow velocity is related to vessel diameter, it increases when the vessel diameter decreases. However, only one patient met the criteria for an MCA stenosis of more than 50%.[18] Excluding this patient from the analysis did not change our results. It is therefore very unlikely that our results were influenced by the presence of an MCA stenosis.

Prognostic value

The identification of prognostic factors in a multivariable model is essentially not the same as prediction. These two terms can be confusing. When a factor is related to outcome events, and therefore occurs more often in patients with an outcome event than in patients without the outcome event, it means that this factor is a prognostic factor in patients. It does not mean, however, that one can estimate the individual risk of an outcome event more precisely. The estimation of individual risks in patients is called prediction. We have first investigated whether TCD parameters were prognostic factors (Chapter 3.2), and after that, we investigated whether these factors could contribute to the prediction of recurrent events by adding the TCD parameters to existing prediction models (Chapter 3.4).

Prediction

We validated 7 prediction models in our cohort of patients with a recent TA or ischemic stroke. Our cohort consisted of 598 patients, which may seem rather small to perform external validation of these prediction models. Nevertheless, the results showed clear distinction between population based models and models based in patients with a recent TIA or stroke (Chapter 3.1).

We validated two-year risk using survival analysis which was not easy because of the censoring. Censoring occurs when patients are withdrawn from survival analysis after a certain time point because they have either already reached the outcome event, or when the end of the follow-up period is reached. In our study some patients only had a few months follow-up because the end of the study period was reached. Of these patients it is unknown whether they would experience an outcome event in the near future or not. Thus, they do not contribute the same amount of information to the analysis as patients with long follow-up. Usually, external validation concerns diagnostic test, not prognostic tests.

The discrimination was calculated using the c-statistic and the Net Reclassification Index (NRI). The c-statistic is derived from the sensitivity and specificity of a model, the NRI calculates the proportion of patients who were reclassified correctly after adding a parameter to the prediction model versus the proportion of patients who were reclassified incorrectly. The c-statistic was adjusted for censoring, the NRI was not adjusted for censoring. The statistical analysis using the NRI is new and has not been used in survival analysis before. It would be logical to adjust for duration of follow-up, or at least stratify for this duration since risk of recurrent events increases

with time of exposure. This method has however not yet been developed. This is a methodological restriction which cannot be overcome for now. However, in our study, the c-statistic as well as the NRI showed improvement of the ABCD2 prediction model when both the fifth quintile of MFV and PI-MFV ratio were added (Chapter 3.4). Moreover, as the comparison of NRI's was based on the same dataset, most of the bias because of censoring would be canceled out.

Finally, we used calibration graphs with only 5 groups because of the low number of patients and therefore outcome events. Fortunately, only few prediction models showed outliers.

CLINICAL IMPLICATIONS AND FUTURE RESEARCH

Diagnostic value of Transcranial Doppler ultrasonography

Mean Flow velocity

The finding that MFV is related to several vascular risk factors such as age, male sex,diabetes, cholesterol, and hypertension is not surprising (Chapter 2.1). These vascular risk factors lead to arterial stiffness[19] and MFV increases with narrowing of the arterial vessels. Thus, MFV can be regarded as a general measure of systemic disease, atherosclerosis. In this thesis I have focused on one measurement, but it would be very interesting to investigate whether MFV changes with aggressive treatment of the vascular risk factors such as diabetes, hypercholesterolemia, and hypertension, as well as cessation of smoking. Future research could include serial TCD investigation to answer that question.

Cerebrovascular CO_2-reactivity

In my study, cerebrovascular CO_2-reactivity was related to cholesterol: the cerebrovascular CO_2-reactivity decreased when total serum cholesterol increased chapter 2.1). This is in accordance with results of studies in the healthy population.[20] In the healthy population CO_2-reactivity is related to white matter lesions,[21,22] but this could not be reproduced in our patients with a TIA or minor stroke. The cerebrovascular CO_2-reactivity was not related to the other vascular risk factors in this study. Silvestrini et al. however, showed that smoking has a short-term, direct effect on the cerebrovascular CO_2-reactivity.[23] This provides suggestions for future research: it would be interesting to investigate the cerebrovascular CO_2-reactivity before and after cessation of smoking.

Micro-embolic signals

The occurrence of MES can clearly discriminate between non-lacunar and lacunar stroke, since MES are not present in lacunar stroke. These findings apply only to the acute phase of stroke. The sensitivity of MES is low, which indicates that MES are not a frequent finding in stroke

patients without carotid artery disease. Specificity, on the contrary, is very high which means that when MES are present, one may safely assume that the stroke was non-lacunar. Usually, non-lacunar stroke is a clinical diagnosis, but in some patients without a visible infarction on the first CT-scan of the head cortical symptoms may be mild or transient and therefore overlooked. The occurrence of MES is higher in the acute phase of stroke[24] then in a later phase of stroke.[25]

For screening of a PCSE, the presence of MES is not discriminatory (Chapter 2.3). Both sensitivity and specificity are not high. This has not been investigated in a cohort of unselected stroke patients before. In future, very early MES detection in a population of unselected stroke patients could possibly answer that question.

For MES detection, patients have to wear a metal headband for at least 30 minutes. This is very inconvenient, especially for older patients in the early phase of stroke. Some newer TCD systems do not work with these unfriendly headbands, but with recording systems where the transducer is held in place on a glasses frame.[26] With these systems patients can be ambulatory and even 24 hr registrations can be possible. Especially in patients with an asymptomatic carotid artery stenosis longer duration of TCD registration increases the probability of finding MES.[27]

Prognostic value of transcranial Doppler ultrasonography

Mean Flow velocity

Mean flow velocity is a prognostic factor for recurrent vascular events after TIA or minor ischemic stroke (Chapter 3.2). In the general population this is a linear relationship.[28] In our study, this increased risk of recurrent vascular events only occurred in patients in the highest quintile of MFV. The PI-MFV ratio also showed an increased risk of recurrent vascular events implying that high peripheral vascular resistance is an important factor in the occurrence of vascular events. There are strong indications that PI is related to small vessel disease,[29] and MFV is related to the vessel diameter. It is therefore not surprising that the PI-MFV ratio shows an increased risk of recurrent vascular events. To further evaluate this new factor TC-Duplex would be more helpful since it can also measure vessel diameter as well as flow velocities in the major cerebral arteries. These measurements should preferably be performed in the acute phase of stroke.

Cerebral CO_2 reactivity

The prognostic value of cerebrovascular CO_2-reactivity has not yet been elucidated. In our study in patients without a carotid artery stenosis, the cerebrovascular CO_2-reactivity was not related to recurrent major vascular events or recurrent stroke (Chapter 3.2). We investigated patients without a carotid artery stenosis since the presence of MES has already been extensively investigated in patients with a carotid artery stenosis; in these patients, cerebrovascular CO_2-reactivity is a prognostic factor for recurrent stroke, both in symptomatic patients,[30-33] and

asymptomatic patients.[30,34] Molina et al. have found that in the normal population an impaired cerebrovascular CO_2-reactivity was a marker for first-ever lacunar infarction.[35] In our study, we have not made the distinction between lacunar infarction and non-lacunar infarction in our outcome events. This may be promising to investigate in future.

Micro-Embolic Signals

The occurrence of MES in our population was low, but when MES were present, this was a strong prognostic factor for recurrent vascular outcome events (Chapter 3.3). We excluded patients with a carotid artery stenosis who were planned for carotid surgery. These patients were excluded because carotid artery stenosis is related to the presence of MES,[5] and in these patients risk of recurrent events would be affected by the carotid surgery, as mentioned before. In patients with a carotid artery stenosis presence of MES is higher, varying from 6% in patients with an asymptomatic carotid artery stenosis[36] to 45% in patients with a symptomatic carotid artery stenosis.[37] It would be interesting to evaluate the presence of MES in patients with mild carotid artery stenosis (30 to 70%). In these patients, the prognostic value of MES still has to be determined to evaluate if MES detection is worthwhile in these patients. In our study, we performed off-line analysis of audio tapes to detect MES. This is time consuming, especially when prevalence of MES is low. Newer automatic detection show better classification of signals into MES or artefacts and seem therefore promising.[38]

Prediction

External validation of several population based models and stroke based models showed that the ABCD2 prediction model performed best; both calibration and discrimination were fairly good (Chapter 3.1). Adding MFV and PI-MFV ratio improved this prediction model (Chapter 3.4). Our study was one of the first to externally validate this model; ours is the only study which has looked at improvement of this model by adding TCD parameters. Adding the presence of brain infarction in CT improves the ABCD2 model, which resulted in the ABCD2I model.[39] It would be interesting to evaluate the improvement of the ABCD2 model with both TCD parameters and the presence of brain infarction on CT for the long-term prediction of recurrent vascular events after stroke.

Final conclusion

Our results indicate that TCD is a promising technology in patients with a recent TIA or ischemic stroke, especially MES detection and measurement of flow velocities. MES detection, however, seems more promising in the early after stroke onset since MES are more often present in the acute phase. [24] The prognostic value of MES in the acute phase of stroke for the long-term prediction of recurrent stroke or other vascular events has not been elucidated and should be studied further.

Most importantly, MFV measured in the middle cerebral artery is an independent prognostic factor for recurrent vascular events such as ischemic stroke, non-fatal myocardial infarction and vascular death, as well as the combined PI-MFV ratio. The MFV improves the prediction of recurrent vascular events, when added to clinical prognostic variables. This finding addresses one major question of this thesis. The challenge for the future is to further improve the diagnostic and prognostic impact of transcranial ultrasound, by using more sophisticated techniques and at the same time improving patient-friendliness.

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Summary

Stroke is the third leading cause of death in developed countries, after heart disease and cancer. Most strokes are ischemic and caused by occlusion of a cerebral artery. This leads to dysfunction and eventually death of brain tissue through lack of oxygen. This may result in typical symptoms such as unilateral weakness, language disturbances, unilateral sensory disturbances, hemianopia, ataxia, or impaired speech. In many patients, cerebral ischemia is only transient and does not result in persistent symptoms and disability. When disability is persistent, the event is called an ischemic stroke. In the acute phase of tissue dysfunction, at most 25% of the patients can be treated with thrombolytic agents, but even when patients can be treated, treatment is not always successful. Secondary prevention is therefore one of the main objectives of stroke management. Risk of recurrent stroke is approximately 9-16% annually after ischemic stroke. Intensive secondary prevention by use of antiplatelet medication and treatment of high blood pressure and high cholesterol has reduced the annual risk of recurrent events to approximately 4.5%. Well-known risk factors for stroke or other major vascular events such as myocardial infarction or vascular death are age, male sex, hypertension, diabetes, and smoking. Unfortunately, it is still difficult to discriminate between patients with a high risk of recurrent stroke and patients with a low risk of recurrent stroke.

Transcranial Doppler ultrasonography, the main topic of this thesis, offers noninvasive information on the large cerebral arteries. Low frequency (2 MHz) ultrasound can pass through the bone at the level of the temporal window, which is located just above the ear where the temporal bone is the thinnest. With this technique, mean flow velocity (MFV), peak systolic flow velocity (PSV), and enddiastolic flow velocity (EDV) in the middle cerebral artery can be measured. With these parameters, the pulsatility index (PI) can be calculated: PI=(PSV-EDV)/MFV. The PI is thought to reflect the peripheral resistance in a large artery. Through inhalation of 5% carbon dioxide cerebral CO_2-reactivity can be measured, a functional TCD test which is thought to reflect elasticity of the smaller cerebral blood vessels. Micro-embolic signals (MES) can be measured during a continuous TCD registration.

To evaluate the clinical value of these TCD tests, we have conducted the Rotterdam TCD study. In this study we have performed TCD registration in 598 consecutive patients with a recent TIA or minor ischemic stroke. We measured flow velocities, cerebral CO_2-reactivity, and presence of MES during a 30 minute registration. The research questions were:

1. Can TCD be of diagnostic value in detetecting the presence of a potential cardiac source of embolism and the presence of small vessel disease in patients with a TIA or minor ischemic stroke?
2. Is it possible to identify patients with a high risk of recurrent vascular events after TIA or minor ischemic stroke by means of TCD investigation?

To answer these questions we have firstly evaluated the diagnostic value of TCD in **chapter 2**. **Chapter 2.1** describes the relationship between TCD parameters and vascular risk factors. Blood flow velocity in the MCA decreases with age, and is lower in men than in women. Blood flow velocity in the MCA increases with total serum cholesterol, and is increased in diabetics compared to non-diabetics. Cerebrovascular CO_2-reactivity is lower in hypertensive patients and decreases with increasing total serum cholesterol. Our results indicate that the cerebral flow velocity in the MCA is influenced by multiple interacting factors.

In **chapter 2.2** we show the relationship between skull thickness, bone density, and window failure. Window failure (WF) means that no adequate TCD signal can be obtained. Window failure is more common in older women. We conclude that both thickness and density of the temporal bone are related to the presence of WF, but that skull density does not independently predict WF. Absence of WF can be predicted accurately in nearly all patients with ischemic stroke or TIA by means of three simple parameters: skull thickness, age and gender.

In **chapter 2.3** we review studies on the diagnostic value of TCD in patients with a TIA or minor ischemic stroke. We give an overview of studies which evaluated whether the presence of MES can distinguish between patients with a lacunar infarction (small vessel disease) and patients with a cortical infarction (non-small vessel disease). We also give an overview of studies which evaluated whether TCD can be helpful in detection of a potential cardiac source of embolism (PCSE). We have combined these results with our own findings. Our results indicate that TCD could be a useful test to distinguish non-small vessel disease from small vessel disease. For a PCSE, TCD alone does not seem able to adequately distinguish between the presence and the absence of a PCSE.

In **chapter 3** we describe the prognostic value of TCD. In **chapter 3.1** we validate predictions from seven well-known prediction models in our own cohort of patients. Prediction models derived from stroke cohorts perform better than population-based prediction models in prediction of 2-year risk of recurrent vascular events. Of these prediction models, the ABCD2 prediction model seems to be most adequate and most easily applicable in clinical practice.

In **chapter 3.2** we investigate the prognostic value of the individual TCD parameters. Because of the expected interaction between MFV and PI, we created a new parameter, the PI-MFV ratio that MFV and PI-MFV ratio, calculated as PI/MFV*100. MFV and PI-MFV ratio, measured in the MCA by TCD, are independent risk factors for recurrent stroke. Interestingly, MFV and PI-MFV ratio were also associated with the occurrence of major vascular events (combined outcome measure "stroke, MI, and vascular death"). This suggests that the MFV and PI-MFV ratio reflect not only the condition of the major cerebral blood vessels, but also that of coronary and peripheral arteries.

Chapter 3.3 shows that MES are not often present in patients with a recent TIA or ischemic stroke without a carotid artery stenosis, only in 4%. However, MES do indicate increased risk for recurrent stroke in these patients.

In **chapter 3.4** we show that adding MFV and MES to the ABCD2 prediction model improves calibration as well as discrimination of this model. Calibration measures how closely predicted outcomes agree with actual outcomes. Discrimination refers to the ability to distinguish between patients who will have an outcome event and those who will not. This chapter shows that MES and a MFV>60.5 cm/s in the MCA are independent prognostic factors for long term prognosis in patients with a recent TIA or minor ischemic stroke when added to the ABCD2 prediction model. Moreover, adding these two factors to the ABCD2 prediction model improves calibration as well as discrimination.

In **chapter 4** I reflect on the main findings in the context of current knowledge and give suggestions for future research.

On the basis of our studies and current literature we conclude that TCD could be a useful test to distinguish non-small vessel disease from small vessel disease, but not for the detection of a PCSE. TCD parameters such as MFV and cerebral CO_2-reactivity are related to vascular risk factors such as age, gender, diabetes, cholesterol, and hypertension. This implies that in future studies of TCD parameters as prognostic or diagnostic factors in patients with a TIA or stroke, ultrasound findings will have to be adjusted for these vascular risk factors.

The presence of MES is a prognostic factor in patients with a recent TIA or ischemic stroke, even in patients without a carotid artery stenosis. Detection of MES is, however, time-consuming; occurrence of MES is low in patients without a carotid artery stenosis. MFV and PI-MFV ratio seem to reflect atherosclerosis. These parameters are prognostic factors after a TIA or minor ischemic stroke. Adding MES and MFV to the ABCD2 prediction model improves the calibration and discrimination of this model. These findings will have to be further investigated in larger studies.

Samenvatting

Een beroerte is de derde doodsoorzaak in Westerse landen na hartziekten en kanker. Het merendeel van de beroertes wordt gevormd door herseninfarcten. Hierbij wordt een bloedvat in de hersenen afgesloten. Dit leidt tot zuurstofgebrek en sterfte van hersencellen waardoor er functiestoornissen van de hersenen ontstaan. Deze functiestoornissen uiten zich onder andere als éénzijdig krachtsverlies, spraakstoornissen, coördinatiestoornissen, gezichtsvelduitval of een taalstoornis. Soms verdwijnen de verschijnselen weer en spreekt men achteraf van een tijdelijke ischemische aanval oftewel transient ischemic attack: een TIA. Als er blijvende schade optreedt gaat het om een herseninfarct. In de acute fase kan momenteel hooguit 25% van de patiënten behandeld worden met aggressieve bloedverdunners (trombolytica), en deze behandeling is ook niet altijd succesvol. Om die reden is preventie van toekomstige herseninfarcten of andere uitingen van hart- en vaatziekten bij patiënten met TIA's of herseninfarcten uitermate belangrijk. Het risico op nieuwe beroertes of andere uitingen van hart- en vaatziektes bij deze patiënten is ongeveer 9 tot 16% per jaar. Dit risico wordt teruggebracht tot ongeveer 4.5% na starten van intensieve medicameneuze preventie zoals bloedplaatjes-aggregatieremmers, antihypertensiva en statines. De belangrijke risicofactoren zijn hoge leeftijd, mannelijk geslacht, hoge bloeddruk, roken en diabetes mellitus. Helaas is het nog steeds niet mogelijk om te voorspellen wie wel of wie juist opnieuw een herseninfarct of andere uiting van hart- en vaatziekten zal krijgen.

Met transcranieel Doppler ultrageluid (TCD) kan men non-invasief informatie krijgen over de grote bloedvaten in de hersenen. Net boven het oor is het "temporale bot window" oftewel "temporal bone window" gelokaliseerd. Hier is het bot iets dunner waardoor ultrageluid (2 MHz) door het bot heen dringt. Hiermee kan in de arteria cerebri media de gemiddelde snelheid (MFV), de pieksystolische snelheid (PSV) en de einddiastolische snelheid (EDV) van het bloed worden gemeten. Aan de hand van deze waarden kan de pulsatility index (PI) worden berekend: PI=(PSV-EDV)/MFV. Dit is een maat voor de perifere weerstand in een slagader. Met toediening van kooldioxide kan de cerebrale CO_2-reactiviteit worden gemeten, een test waarmee de elasticiteit van de kleine bloedvaten in de hersenen wordt gemeten. Als derde kan tijdens een langdurige registratie het aantal micro-embolische signalen (MES) worden gemeten. Dit zijn luidere signalen die suggereren dat er een partikel in de bloedbaan passeert, dit kan onder andere een luchtbelletje, of een stolsel zijn.

Om meer inzicht te krijgen in de klinische waarde van deze tests hebben we de Rotterdam TCD Studie uitgevoerd. Hierbij hebben we bij 598 opeenvolgende patiënten met een TIA of een licht herseninfarct TCD metingen gedaan waarbij, indien mogelijk, de snelheden in de arteria cerebri media en de cerebrale CO_2-reactiviteit gemeten, en de aanwezigheid van MES geregistreerd gedurende een half uur. In deze studie stonden twee vraagstellingen centraal:

1. Heeft TCD diagnostische waarde in het detecteren van een cardiale emboliebron en het detecteren van subtype van ee herseninfarct bij patiënten met een TIA of licht herseninfarct?

2. Is het mogelijk om patiënten met een hoog risico op nieuwe vasculaire events (gebeurtenissen) na een TIA of licht herseninfarct te identificeren met TCD? Zo ja, kan TCD huidige predictiemodellen verbeteren?

Om deze vragen te kunnen beantwoorden hebben we in **hoofdstuk 2** allereerst gekeken naar de diagnostisch waarde van TCD. **Hoofdstuk 2.1** beschrijft allereerst welke risicofactoren van invloed zijn op de TCD metingen. Gebleken is dat oudere leeftijd en het mannelijk geslacht gerelateerd zijn aan lagere snelheden in de arteria cerebri media. De aanwezigheid van diabetes en de aanwezigheid van hypercholesterolemie waren juist gerelateerd aan hogere snelheden in de arteria cerebri media. De cerebrale CO_2-reactiviteit is gemiddeld lager bij patiënten met hypertensie of hypercholesterolemie. Dit betekent dat de TCD parameters door multipele factoren beinvloed worden en dat hier voor gecorrigeerd moet worden bij analyses naar de diagnostische of prognostische waarde van deze TCD parameters.

In **hoofdstuk 2.2** laten we de relatie zien tussen schedeldikte, densiteit van het schedelbot en de kans op window failure. Window failure betekent dat er geen goed TCD signaal verkregen kan worden. Dit treedt vaker op bij met name oudere vrouwen. In dit hoofdstuk tonen we aan dat zowel de schedeldikte als densiteit ook gerelateerd zijn aan het optreden van window failure. Wij hebben ook een predictiemodel ontwikkeld met als variabelen leeftijd, geslacht, schedeldikte en schedeldensiteit, waarmee vrij accuraat kan worden voorspeld of er sprake zal zijn van window failure of niet.

In **hoofdstuk 2.3** wordt gekeken naar de diagnostische waarde van TCD bij patiënten met een TIA of een licht herseninfarct. Wij geven allereerst een overzicht van studies die onderzoek hebben gedaan of de aanwezigheid van MES kan differentiëren tussen patiënten met een lacunair of corticaal herseninfarct. Hierna hebben we ook onderzocht of de aanwezigheid van MES een cardiale emboliebron kan detecteren. Deze bevindingen hebben we gecombineerd met onze eigen resultaten. Onze resultaten laten zien dat TCD nuttig kan zijn om onderscheid te maken tussen een corticaal en een lacunair infarct. TCD heeft echter geen diagnostische waarde in het detecteren van een cardiale emboliebron.

In **hoofdstuk 3** beschrijven we in op de prognostische waarde van TCD. Allereerst hebben we in **hoofdstuk 3.1** zeven gerenommeerde predictiemodellen met elkaar vergeleken door ze te valideren op onze eigen dataset. Hierbij kwam naar voren dat predictiemodellen die afgeleid zijn van grote groepen patiënten met een eerdere TIA of herseninfarct beter het 2-jaars risico op nieuwe vasculaire events voorspellen dan predictiemodellen die op de algemene populatie gebaseerd zijn. Van deze predictiemodellen is het ABCD2 predictiemodel vrij betrouwbaar en makkelijk te hanteren aangezien er weinig variabelen gebruikt worden in dit predictiemodel.

Hoofdstuk 3.2 toont aan dat een hoge snelheid in de arteria cerebri media (meer dan 60.5 cm/seconde) een verhoogde kans geeft op zowel nieuwe beroertes als nieuwe vasculaire events. Omdat er ook een interactie werd verwacht tussen de PI en de MFV hebben we een nieuwe factor gecreëerd: de PI-MFV ratio (berekend als PI/MFV*100). Deze interactiefactor heeft een lineaire relatie met het risico op nieuwe beroertes en nieuwe vasculaire events. MFV en de PI-MFV ratio zijn prognostische factoren na een TIA of een licht herseninfarct, onafhankelijk van andere risicofactoren. Mogelijk reflecteren deze parameters niet alleen de mate van atherosclerose in de hersenslagaders, maar ook in de rest van het cardiovasculaire systeem.

In **hoofdstuk 3.3** hebben we de prognostische waarde van MES onderzocht bij patiënten zonder een carotisstenose. Deze MES komen slechts bij 4% van de patiënten met een TIA of licht herseninfarct zonder carotisstenose in onze studie voor. Bij aanwezigheid van deze MES is er echter een verhoogd risico op nieuwe beroertes.

In **hoofdstuk 3.4** onderzoeken we of toevoeging van TCD parameters aan het ABCD2 predictiemodel voorspelling van nieuwe vasculaire events verbetert. Hierbij hebben we gekeken naar de calibratie en de discriminatie. De calibratie meet hoe goed voorspelde vasculaire events overeenkomen met daadwerkelijk opgetreden vasculaire events. Discriminatie verwijst naar het vermogen om onderscheid te kunnen maken tussen patiënten met een hoog risico op nieuwe vasculaire events en patiënten met een laag risico op nieuwe events. Toevoeging van de aanwezigheid van MES en MFV > 60.5 cm/seconde verbetert zowel de calibratie als de discriminatie van het ABCD2 predictiemodel.

In **hoofdstuk 4** worden de belangrijkste bevindingen in de context van de bestaande literatuur besproken en worden suggesties voor verder onderzoek gedaan.

Op basis van onze studies en de literatuur concluderen wij dat TCD geen waarde heeft bij het onderscheid maken tussen een corticaal of een lacunair infarct. Ook helpt TCD niet bij het detecteren van een cardiale emboliebron. Wel zien wij een duidelijke relatie tussen cardiovasculaire risicofactoren en TCD parameters zoals de MFV en de cerebrale CO_2-reactiviteit. Dit betekent dat bij onderzoeken naar deze parameters altijd voor cardiovasculaire risicofactoren gecorrigeerd moet worden.

De aanwezigheid van MES is prognostisch van belang, ook voor patiënten zonder carotisstenose. Het detecteren van MES is echter nog tijdrovend en MES komen weinig voor bij patiënten zonder carotisstenose. De MFV en de PI-MFV lijken een mate van atherosclerose te weerspiegelen; deze parameters hebben naast de eenvoudige klinische parameters een duidelijke prognostische waarde na een TIA of licht herseninfarct. Wanneer MFV and MES toegevoegd worden aan het ABCD2 predictiemodel verbeteren zowel de calibratie als de discriminatie van dit model. Deze bevindingen zullen echter in een groter onderzoek gerepliceerd moeten worden.

List of abbreviations

TIA	Transient ischemic attack
MES	Micro-embolic signals
TCD	Transcranial Doppler ultrasonography
MFV	Mean flow velocity
PSV	Peak systolic flow velocity
EDV	Enddiastolic flow velocity
PI	Pulsatility index
PCSE	Potential cardiac source of embolism
MCA	Middle cerebral artery
CT	Computed tomography
ROI	Region of interest
WF	Window failure
AF	Atrial fibrillation
PI/MFV ratio	100xPI/MFV
NRI	Net reclassification index

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Annemarie Dagmar Wijnhoud was born on the 7th of August 1972 in Zwolle, The Netherlands. She attended secondary school at the "Gymnasium Haganum" in The Hague. She graduated in 1990. She started her training in Medicine in 1991 at the Erasmus University of Rotterdam. From 1995 to 1996 she worked as student assistant under the supervision of Dr. R. Saxena and Prof. Dr. P.J. Koudstaal on the DIAS trial, a controlled safety study of a hemoglobin DCLHb (Diaspirin Cross-linked Hemoglobin) in acute ischemic stroke. She graduated in April 1999 from medical school. In that month she started working on the Rotterdam TCD Study, the study underlying this thesis. In February 2003 she started her training as a neurologist at the department of Neurology at the Erasmus Medical Centre Rotterdam (Prof. Dr. P.A.E. Sillevis Smitt). Since 2010 she has been working as a neurologist in the IJsselland ziekenhuis in Capelle aan den IJssel.

Dankwoord

Na een lange periode is nu het moment gekomen om als laatste onderdeel van dit proefschrift het dankwoord te mogen schrijven. Ik wil graag iedereen bedanken die geholpen heeft bij de tot standkoming van dit proefschrift op welke wijze dan ook. Graag noem ik een aantal met name.

Als eerste prof. dr. D.W.J. Dippel. Beste Diederik, ik heb zo veel van je geleerd, met name hoe je alles zelf moet kunnen! Gek werd ik van de stata problemen waarbij jij maar bleef beweren dat het kon. Uiteindelijk kreeg ik ook wel eens gelijk. Jouw onaflatend positivisme nadat onze eerste review volledig de grond in werd geboord door meerdere reviewers kan ik alleen maar waarderen. Ik kan me niet meer herinneren hoe vaak jij wel niet de beroemde woorden hebt uitgesproken: "Mijn eerste artikel werd pas door het 7e tijdschrift geaccepteerd en kreeg lovende kritieken". Uiteindelijk werd ons hoofdartikel ook pas bij het 7e tijdschrift geaccepteerd, een lovend voorwood was echter nergens te bekennen. Mijn hartelijke dank dat je mijn leermeester bent geweest en bovenal dank voor jouw geduld met mij.

En natuurlijk de tweede promotor prof. dr. P.J. Koudstaal. Beste Peter, enorm bedankt voor de geboden kans. Het is eigenlijk allemaal begonnen toen wij samen in een kwaliteitscommissie voor het onderwijs zaten; ik als student, jij als voorzitter. Hierna ben ik als student onderzoek gaan doen bij jou en van het één kwam het ander en nu is dit proefschrift eindelijk afgerond. Het meest contact liep via Diederik, maar als ik dan met jou een afspraak had was het altijd weer leuk en gezellig als vanouds. Nogmaals hartelijk dank voor de geboden kans.

De overige leden van de beoordelingscommissie, prof. dr. A. van der Lugt, prof. dr. E.W. Steyerberg en prof. dr. J. Stam wil ik graag bedanken voor het beoordelen van dit proefschrift. Prof dr. W. Mess, prof. dr. E. Boersma en dr. F.E. de Leeuw wil ik bedanken voor het zitting nemen in de commissie.

Alle co-auteurs wil ik danken voor zijn of haar bijdrage bij het aanscherpen van de manuscripten waardoor uiteindelijk mooie artikelen gepubliceerd konden worden.

Uiteraard wil ik Hanneke Hilkemeijer, Naziha El Ghanouti en Esther van der Heijden bedanken voor het bellen van patiënten en het invoeren van alle data. Zonder jullie fanatieke gebel hadden we de follow-up data niet zo uitgebreid gehad. Enorm bedankt!

De mede-onderzoekers met wie ik tegelijkertijd op de 22e verdieping heb gebivakkeerd wil ik graag bedanken voor de gezelligheid: Beste Sonia, Rinske, en de rest: het was leuk! Mary-Lou en Lisette: leuk dat we nog steeds bekend staan als Diederik's Angels. Dank voor de geestelijke steun.

Alle medeonderzoekers van de neurovasculaire groep wil ik bedanken voor de morele steun en de gezelligheid. Heleen, bedankt voor je stimulerende adviezen en positivisme. Maaike, jij bent uiteindelijk eerder gepromoveerd, bedankt voor de leuke tijd.

Prof. Dr. P.S. Sillevis Smitt, beste Peter, bedankt voor de leuke opleidingstijd! Ik waardeer jouw vertrouwen in mij enorm.

Marcel, Huib en Pieter, bedankt voor het enthousiasme in het mee bedenken van de stellingen. Jammer genoeg zijn de door ons in Stockholm bedachte stellingen allemaal afgekeurd.

Lieve Matthé, bedankt voor je hulp! Ik wist niet dat jij zo'n goede editor was! Dank voor je welwillendheid en je nuttige tips.

Uiteraard wil ik mijn paranimfen bedanken voor alle hulp de afgelopen maanden. Heerlijk dat ik met jullie allebei een heel goede klik heb gehad tijdens de opleiding. Beste Sarah en Janet, wat mij vooral bij zal blijven is het enthousiasme waarmee jullie mijn paranimf wilden zijn! Ik voel me vereerd! Ik ben erg blij geweest met alle praktische tips en opjutmomenten van de laatste tijd. Bedankt voor jullie vriendschap.

Uiteindelijk zijn het de patiënten geweest die ervoor hebben gezorgd dat dit proefschrift tot stand kon komen. Hartelijk dank voor de deelname aan mijn onderzoek.

Lieve Stef, Thomas en Lucas: bedankt dat julie er voor mij zijn en het leven mooier maken.