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Blood biomarkers of outcomes after ischemic stroke

Degree Project in Medicine

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ABSTRACT

Introduction: Today, knowledge about predictors of stroke outcomes is limited and few studies have investigated blood biomarkers as predictors of outcomes after ischemic stroke, especially in the long-term.

Aim: To investigate if there is an association between circulating concentrations of three novel biomarkers and long-term outcomes after ischemic stroke.

Methods: The study population was derived from the Sahlgrenska Academy Study on Ischemic Stroke (SAHLSIS), which includes patients with ischemic stroke aged 18-69 years. Blood was drawn 3 months after the index stroke. Concentrations of neurofilament light chain (NFL) were analysed using a single-molecule array (Simoa) method. Concentrations of glial cell-derived neurotrophic factor family receptor α1 (GFRα1) and mesencephalic astrocyte-derived neurotrophic factor (MANF) were analysed using a proximity extension assay (PEA) technique. Associations to post-stroke outcomes assessed as the National Institutes of Health Stroke Scale (NIHSS) and the modified Rankin Scale (mRS) at a 7-year follow-up were examined using linear and logistic regression, respectively.

Results: Concentrations of all three biomarkers were significantly higher in cases with poor (mRS 3-6) compared to good (mRS 0-2) outcome. All biomarkers were associated to NIHSS and mRS in the univariate analysis and the multivariate analysis adjusted for age and history of stroke. When additionally adjusting for stroke severity, i.e. baseline NIHSS, the association to mRS remained for all three biomarkers and the association to NIHSS remained for NFL and MANF. For GFRα1 and MANF, physical activity was also included in the model

together with the covariates indicated above. In this model, the association for MANF and NIHSS remained.

Conclusions: We identified three novel circulating biomarkers that were independently associated to neurological and functional outcomes after ischemic stroke. Further research is needed to design an assay with a set of biomarkers that effectively predicts post-stroke outcomes.

Key words: ischemic stroke, functional outcomes, prognosis, biomarkers

INTRODUCTION

Each year, approximately 10 million people suffer from stroke worldwide (1), of which 25 000 affects people in Sweden (2). Globally, stroke causes 6.5 million deaths every year (1), which makes it the second most common cause of mortality (3). There are currently about 25.7 million stroke survivors (1), of whom many are suffering from neurological and cognitive impairments (4). Thereby, stroke is the third most common cause of disability-adjusted life-years (DALYs) (5) and causes major socioeconomics consequences on both individual and society levels. The cost due to stroke in Sweden is approximately 18 billion Swedish crowns every year (6). In addition, the annual cost of spouses' informal support has been estimated to approximately 990 € for independent stroke survivors and 25 000 € for dependent stroke survivors (7).

Definition of stroke, pathophysiology and etiology

Stroke is a disruption of the blood supply to a brain area causing oxygen and nutrient deficiency, and accordingly neuronal death. Depending on which area that is affected, different neurological symptoms occur. A stroke is either caused by a bleeding, hemorrhagic stroke, or by a blood clot (i.e. thrombus or embolus), ischemic stroke (8). This study will focus on ischemic stroke.

In high-income countries, roughly 85% of all strokes is due to ischemic stroke (9). However, the thrombus or embolus can appear through different pathophysiological mechanisms and ischemic stroke is therefore divided into subtypes. One of the most frequently used protocols for this is the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification with 5

subtypes: 1) large vessel disease (LVD), 2) cardioembolism (CE), 3) small vessel disease (SVD), 4) stroke of other determined etiology and 5) cryptogenic stroke (10).

LVD accounts for approximately 15% of all ischemic stroke, but this proportion varies by ethnicity (11-13). This subtype of ischemic stroke occurs due to atherosclerosis (14) and is characterized by a significant stenosis (>50%) or a complete occlusion in a precerebral (e.g. the carotid artery) or cerebral artery. LVD typically causes infarcts >1.5 cm in cerebral cortex, brain stem or cerebellum. Accordingly, this subtype causes cerebral cortical dysfunction such as aphasia, neglect and paralysis or brainstem or cerebellar impairment. (10)

CE accounts for 25-30% of all ischemic stroke (11-13). The major cause of CE is atrial fibrillation, but it may also occur due to recent myocardial infarction, mechanical heart valve prosthesis, sick sinus syndrome, dilated cardiomyopathy and infective endocarditis. Symptoms and brain imaging results are often similar to those of LVD. (10)

SVD accounts for approximately 20% of all ischemic stroke (11-13) and is caused by an occlusion of a deeply located, perforating artery which derives from the circle of Willis or the basilar artery (15). Most commonly, SVD causes small infarcts <1.5 cm in basal ganglia, the internal capsule, thalamus or brain stem (16) and gives rise to pure motor, sensory or sensorimotor strokes (13).

Stroke of other determined etiology account for nearly 5% of all ischemic stroke (11-13), although it occurs more frequently among younger stroke patients along with cryptogenic stroke (17). The most common cause of stroke of other determined etiology is arterial

dissection. Other etiologies are hematologic diseases, vasculitis and coagulopathies (13). The clinical findings as well as location and size of the ischemic lesion vary (16).

A stroke is classified as cryptogenic if the etiology cannot be determined despite of an extensive diagnostic evaluation (16). About 25-30% of all ischemic stroke is classified as cryptogenic (18). Suggested causes of cryptogenic stroke are occult paroxysmal atrial fibrillation, paradoxical embolism trough persistent foramen ovale (PFO) and atherosclerosis with stenosis <50% (19). When two or more potential etiologies have been found or when the clinical work-up is incomplete, the stroke is classified as of undetermined etiology (10).

Risk factors for ischemic stroke

Several risk factors for ischemic stroke also apply for myocardial infarction because of their partly shared etiology (20, 21). The INTERSTROKE study, a case-control study including 3000 cases with acute first-ever stroke and 3000 age- and sex-matched controls, reports 10 risk factors that accounted for 90% of the population attributable risk (PAR) for all stroke: history of hypertension, current smoking, waist-to-hip ratio (i.e. abdominal obesity), unhealthy diet, physical inactivity, diabetes mellitus, high alcohol intake (>30 drinks per month or binge drinking), psychosocial stress/depression, cardiac causes (atrial fibrillation or flutter, previous myocardial infarction, rheumatic valvular disease, prosthetic heart valve) and high ratio of apolipoproteins B to A1. (21)

Trends in ischemic stroke incidence

Altogether, the incidence of all stroke (1) and ischemic stroke is decreasing (22). However, when looking at different ages the change in incidence differs. The average age for first-ever

ischemic stroke has decreased, mainly since the incidence of ischemic stroke among younger people has increased (23).

In Sweden between 1987 and 2010, the incidence decreased among people aged 45-64 years (0.4% for men and 0.6% for women per year) and 64-84 years (3.7% for men, 2.5% between 1987 and 2005 and 5.1% after 2005 for women per year) but increased among people 18-44 years of age (1.3% for men and 1.6% for women per year) (22). This might partly be the result of an increased prevalence of stroke risk factors such as hypertension, diabetes, obesity, hyperlipidemia and smoking among stroke patients aged 15-44 years (24). It has also been found that an increase in BMI through puberty and adolescence is associated with stroke in adult age (25).

Post-stroke outcomes

Mortality

The proportion of patients that die after stroke varies depending on, for instance, subtype of stroke and if treated in high- or low-/middle-income countries. According to a review, 13-35% of the patients have died 1 month after the stroke and at 1 year, 5 years and 10 years after stroke, 25-38%, 50-60% and 75%, respectively, have died (26). The FUTURE study, a prospective cohort study including stroke patients aged 18-50 years, reports a one-year mortality of 2.4%, 5-year mortality of 5.8%, 10-year mortality of 12.4% and 20-year mortality of 26.8% (27). Redfors et al. reported a 1-year mortality of 2.0%, 5-year mortality of 9.5% and 10-year mortality of 20.2% in patients <70 years of age at the index stroke (28).

The mortality rate is decreasing for both stroke in general (29) and ischemic stroke (30) in all ages. In a study of stroke patients aged 18-54 years, the standardized mortality ratio, i.e. the

ratio of the observed number of deaths in the study to the expected deaths in the general population, was 5.51 (95% CI 5.15-5.88) for men and 7.06 (95% CI 6.37-7.78) for women. Fortunately, the mortality risk decreased by 32% in men and 45% in women during the study period between 1987 and 2006. (30)

There are several causes of death among stroke patients which, to some extent, varies depending on studying short- or long-term mortality. Neurological complications such as brain edema, increased intracranial pressure, hemorrhagic transformation and recurrent stroke are common causes of death, especially in the acute and subacute phase of stroke (4, 31). Other causes of death early after stroke onset are medical complications: infections, mostly pneumonia and urinary tract infections, cardiac disease such as ischemic heart disease and cardiac arrhythmias (4) and vascular events other than recurrent stroke, i.e. pulmonary embolism. Vascular events remain common causes of death together with cardiac diseases among stroke survivors for years after the index event (31). The proportion of deaths due to the index or recurrent stroke decreases and the proportion of deaths due to cardiac disease increases as time passes after stroke onset (32). After a median follow-up time of almost 9 years, Redfors et al. found that 64.3% of stroke patients died from vascular causes (e.g. cardiac causes, ischemic stroke, hemorrhagic stroke) in comparison to 26.5% among controls (28).

Recurrent stroke

According to a review, the cumulative risk of recurrent stroke among stroke survivors, i.e. the proportion that experience another stroke after the first one, is 1-4% at 1 month, 7-13% at 1 year and nearly 40% at 10 years after the index stroke (26). The FUTURE study reports a 20-year cumulative risk of 19.4% (33). P. Redfors reports recurrent stroke in 9% of stroke

survivors 2 years after the index stroke in patients <70 years of age (34). A study of stroke patients aged 18-54 years, reported a cumulative four-year risk of recurrent stroke of 11.8% in men and 9.8% in women, which is a decrease of 55% in men and 59% in women from the first (1987-1991) to the last (2002-2006) four-year period studied (35).

Neurological and cognitive impairments after stroke

Approximately 50% of stroke survivors are suffering from neurological and/or cognitive disabilities and 20% of disabled survivors are institutionalized (4). Common neurological impairments after stroke are weakness or paralysis of extremities, spasticity, language difficulties, neglect and pain which often affect the ability to perform activities of daily living (ADLs) (36).

The modified Rankin Scale (mRS) (Table 1) describes the grade of disability and is used for evaluation of functional outcome after stroke. The scale is widely used in clinical trials. The original Rankin Scale scored 1-5, whereas the modified scale added a grade, 0, for no symptoms. In clinical trials, another grade, 6, for death is usually also used. (37)

Table 1. Description of mRS.

Grade	Description
0	No symptoms at all
1	No significant disability: despite symptoms, able to carry out all usual duties and activities
2	Slight disability: unable to perform all previous activities but able to look after own affairs without assistance
3	Moderate disability: requiring some help but able to walk without assistance
4	Moderately severe disability: unable to walk without assistance and unable to attend to own bodily needs without assistance
5	Severe disability: bedridden, incontinent and requiring constant nursing care and attention
6	Death

mRS, modified Rankin Scale.

The National Institutes of Health Stroke Scale (NIHSS) (Table 2) is a tool for evaluation of stroke severity and how the neurological symptoms change over time. It is most commonly used during the acute phase of stroke and hospitalization. It involves 11 categories testing major neurological functions. For each category, the examiner chooses the description that best represents the patient's result of the test and points are given accordingly. The minimum points of 0 is normal and maximum points of 42 represent the worst neurologic symptoms. (38)

Table 2. Description of NIHSS.

Neurological function	Description
1. Level of consciousness	a. Level of consciousness: alert – drowsy – stuporous – coma b. Questions: ask about the current month and the patient's age c. Commands: "close eyes" and "make fist"
2. Gaze	Normal – partial gaze palsy – deviation conjugée
3. Visual field	No visual loss – partial hemianopia – complete hemianopia – bilateral hemianopia
4. Facial palsy	Normal – minor – partial – complete
5. Motor arm	The patient's arm is lifted about 90 degrees and the patient is asked to keep the arm elevated
6. Motor leg	The patient's leg is lifted about 30 degrees and the patient is asked to keep the leg elevated
7. Limb ataxia	Finger-nose and heel down shin
8. Sensory	Normal – partial loss – severe loss
9. Language	The patient is asked to name items, describe a picture and read sentences
10. Dysarthria	Evaluated when the patient is asked to read listed words out loud
11. Extinction and attention	Identify neglect through previous testing and/or by double simultaneous stimuli testing with patient's eyes closed

NIHSS, National Institutes of Health Stroke Scale.

In addition to neurological deficits, "hidden" impairments such as depression, cognitive dysfunction (4, 36) and fatigue (4) are common post-stroke complications that also affect the

mRS score after stroke. Cognitive functions such as executive functions, attention and memory are often impaired among stroke survivors, even in patients with good motor recovery (39) and when tested several years after the stroke (40). The prevalence of fatigue varies, though it is clear that it is a common post-stroke complication that, along with the other cognitive impairments mentioned, affects quality of life and the ability to go back to work among other things (41).

Predictive factors of post-stroke outcomes

Older age, severe stroke, history of cardiac disease (e.g. ischemic heart disease, atrial fibrillation and congestive heart failure) and vascular disease (e.g. TIA and intermittent claudication) are all predictors of mortality among stroke patients (32, 42). Recurrent stroke (4), pneumonia and venous thromboembolism (31) also increase the risk of mortality. Stroke patients with diabetes mellitus has a higher short- and long-term mortality (43). Considering the etiologic subtypes of ischemic stroke, the mortality is highest in patients with CE stroke (13, 44).

The short-term risk of recurrent stroke is highest for LVD stroke (13, 44, 45). Diabetes mellitus (32, 43) and older age are also predictors of recurrent stroke (32). Similarly to the risk factors of mortality, predicting factors of post-stroke disability, most commonly measured using mRS, are older age, severe stroke (32), recurrent stroke (4, 32), cardiac complications, pneumonia, venous thromboembolism (31), diabetes mellitus (43) and CE stroke (13, 44).

Potential predictive biomarkers of post-stroke outcomes

According to the Biomarkers Definition Working Group, a biomarker is "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention". A biomarker can be used to diagnose a patient with a disease, to decide on classification of a disease, to predict prognosis as well as predict and evaluate response to treatment (46). Regarding post-stroke outcomes, there are a few biomarkers that have been suggested to have predicting value.

Inflammatory biomarkers

In patients with ischemic stroke, inflammatory responses are activated due to necrosis following hypoxia. Dying neurons release damage signals and activate damage-associated molecular patterns (DAMPs), which activate immune cells and enhances the secretion of pro-inflammatory cytokines. The powerful activation of the immune system during ischemic stroke results in a large-scale recruitment of mature and immature immune cells which accumulate in the ischemic lesion resulting in further secretion of cytokines and even greater brain damage as well as post-stroke immunosuppression with increased risk of complicating infections. The inflammatory processes also increase the permeability of the blood-brain-barrier (BBB) with increased risk of brain edema and hemorrhagic transformation. (47)

Considering the above, some pro-inflammatory proteins have been hypothesized to predict poor post-stroke outcomes and some anti-inflammatory proteins have been suggested to predict good post-stroke outcomes (Table 3). However, studies have reported somewhat contradictive results and it has yet not been clarified whether they add any predictive value in the clinical situation. (47-51)

Table 3. Summary of studies on pro- and anti-inflammatory proteins as predicting biomarkers of post-stroke outcomes.

Protein	Associations to post-stroke outcomes	Study
CRP	Higher levels have been associated to • Larger infarct volume • Severe stroke • Post-stroke edema and infections • Poor functional outcome • Death	• Bustamante et al. 2016 (reference 47) • Yu et al. 2017 (reference 48)
IL-6	Higher levels have been associated to • Larger infarct volume • Worse neurological symptoms • Post-stroke infections • Poor functional outcome • Death	• Bustamante et al. 2016 (reference 47) • Doll et al. 2014 (reference 49) • Whiteley et al. 2009 (reference 50)
TNF	Higher levels have been associated to • Larger infarct volume • Worse neurological symptoms • Poor outcome 3 months after stroke	• Bustamante et al. 2016 (reference 47) • Doll et al. 2014 (reference 49)
IL-1β	Higher levels have been associated to • Severe stroke • Poor functional outcome	• Bustamante et al. 2016 (reference 47) • Doll et al. 2014 (reference 49)
IL-33	Higher levels have been associated to • Smaller infarct volume • Mild stroke measured as NIHSS • Good functional outcome	• Qian et al. 2016 (reference 51)

NIHSS, National Institutes of Health Stroke Scale; CRP, C-reactive protein; IL-6, interleukin 6; TNF, tumor necrosis factor; IL-1β, interleukin 1β; IL-33, interleukin-33.

Genetics

Genetics can play a role in several aspects of stroke: genetic variations have been identified showing an association to risk of stroke in general, stroke subtypes and conditions associated with stroke such as hypertension and atrial fibrillation (52). Some studies have also suggested that post-stroke functional outcome might be influenced by genetics (53).

For instance, variations of the brain-derived neurotrophic factor (BDNF) gene have been associated with long-term neurological outcome, recurrent stroke and mortality as well as stroke recovery and response to rehabilitation treatment (53). Another example is genetic variations of Apo E that has been associated with stroke recovery (53). Other associations have been seen between genetic variations affecting serotonin signalling and post-stroke depression and variations in several different genes that affect the response to treatment with tissue plasminogen activator (tPA), warfarin and dabigatran (53).

Novel biomarkers in the present study

NFL

Neurofilament light chain (NFL) is one of three subunits of neurofilament, a central nervous system (CNS) cell-type specific structural protein in the cytoskeleton of axons. Neurofilament is released into the extracellular space in response to axonal damage and later reaches both the cerebrospinal fluid (CSF) and the blood stream. The neurofilament subunits are suggested as biomarkers of neurodegenerative diseases since higher levels have been detected in patients with amyotrophic lateral sclerosis (ALS), Parkinson's disease and Alzheimer's disease among others. (54)

Until recently, NFL has been measured in CSF only. Today, with the development of analyses with higher sensitivity for detection of the protein in blood it is possible to measure serum concentrations of NFL. For instance, one study found higher serum levels of NFL in patients with Alzheimer's disease, Guillain-Barré-syndrome and ALS compared to patients with neurological diseases without brain damage and healthy controls. Serum concentrations were highly correlated to CSF levels (55). Elevated serum concentrations of NFL have also been

observed in patients with multiple sclerosis (56) and mild traumatic brain injury (57) as well as TIA and ischemic stroke (58).

GFRα1

Glial cell-derived neurotrophic factor family ligands (GFLs) include 4 ligands (Table 4) which are members of the transforming growth factor-beta (TGF-β) superfamily. GFLs bind to glial cell-derived neurotrophic factor family receptor alpha (GFRα). Thereafter, the GFL-GFRα-complexes bind to receptor tyrosine (RET) kinases which subsequently initiate an intracellular signalling cascade. (59)

Table 4. Table of glial cell-derived neurotrophic factor family ligands (GFLs) and their receptors, glial cell-derived neurotrophic factor family receptor alpha (GFRα).

Glial cell-derived neurotrophic factor family ligands (GFLs)	Glial cell-derived neurotrophic factor family receptor alpha (GFRα)
Glial cell-derived neurotrophic factor (GDNF)	GFRα1
Neurturin (NRTN)	GFRα2
Artemin (ARTN)	GFRα3
Persephin (PSPN)	GFRα4

GDNF was first discovered in rat glioma, acting as a trophic factor for the embryonic development of midbrain dopamine neurons (59). Later on, GDNF has been found to play role in the development and functioning of other types of neuronal cells, in kidney development and differentiation of spermatogonia (59). In humans, mutations that interrupt the RET signalling pathway causes Hirschsprung's disease which is characterized by absence of enteric ganglion in the intestines (59). GDNF has also been suggested as a treatment of Parkinson's disease since several animal models has shown protective and facilitated recovery effects on dopamine neurons of this protein (59). Some experimental studies on animal

models of ischemic stroke have shown that the neuroprotective effect of GDNF is limited by the amount of GFRα1 available (60, 61), why it might be of greater interest to study the concentrations of the receptor than GDNF itself.

MANF

Mesencephalic astrocyte-derived neurotrophic factor (MANF) was originally discovered *in vitro* in a culture medium of type-1 astrocyte ventral mesencephalic cell line from rat as a neurotrophic factor protecting nigral dopaminergic neurons (62). It belongs to the novel neurotrophic family (NTF) (63) and was initially called arginine-rich mutated in early stage of tumours (ARMET) since mutations in the gene was found in early-stage tumours. However later on, the mutations were instead found to be common polymorphisms (64).

The expression of MANF is upregulated by endoplasmic reticulum (ER) stress (63, 65). Pharmacological effects, viral infections, increased protein expression and other factors disturbing the protein folding process result in accumulation and aggregation of un- or misfolded proteins in the ER, causing ER stress and ER stress-induced apoptosis if the unfolded proteins are not taken care of (66). ER stress triggers can activate unfolded protein response (UPR) which is processes that promote protein folding (66). MANF has been shown to protect cells from ER stress, probably through mechanisms such as inhibition of ER stress-induced apoptosis and involvement in the removal of un- or misfolded proteins (63, 65). In addition, MANF has shown neuroprotective effects in experimental studies of animal models of ischemic stroke (67, 68).

The importance of predictive biomarkers of post-stroke outcomes

The heterogeneity of stroke etiology makes it difficult to predict outcomes and response to treatment. Finding biomarkers that separates stroke patients into subgroups with more resembling pathophysiological molecular mechanisms will not only facilitate the identification of patients in the risk zone of poor outcomes, but also make it easier to stratify study populations in clinical trials which in turn makes it possible to use smaller study populations while keeping the same statistical power. (69)

The increasing incidence and decreasing mortality of stroke in young adults result in a greater number of young individuals living with various types of impairments and disabilities after stroke. Due to their longer life expectancy, these post-stroke consequences will affect their life for many years. At the same time, the social and economic consequences might be of greater extent since people in this period of life has other demands in their social as well as working lives. Therefore, it is of great interest to find biomarkers that contributes with additional predictive value and better identify individuals in the risk zone of poor post-stroke outcomes than the prognostic factors used today.

AIM

The aim of this study is to investigate if there is an association between circulating concentrations of three novel biomarkers and long-term outcomes after ischemic stroke.

MATERIAL AND METHODS

Study population

This study is based on the Sahlgrenska Academy Study on Ischemic Stroke (SAHLSIS), which has been described in greater detail elsewhere (34, 70, 71). Patients with ischemic stroke aged 18-69 years were consecutively recruited within 10 days after stroke onset. The first 600 patients were recruited from Stroke Units at Skaraborgs sjukhus Skövde, Södra Älvsborgs sjukhus, Sahlgrenska Universitetssjukhuset (SU)/Östra and SU/Sahlgrenska between 1998 and 2003.

The inclusion criteria were an episode of focal neurological deficits with acute onset and duration >24 hours or resulting in death indicating stroke and computed tomography (CT) scan or magnetic resonance imaging (MRI) of the brain excluding hemorrhage. Patients with etiologies other than ischemic stroke and/or diagnosis of advanced cancer, infectious hepatitis and HIV were excluded.

For all participants, information about cardiovascular risk factors and sociodemographic factors were collected through structured questionnaires and physical examinations, including routine blood analyses. Stroke severity was determined using the Scandinavian stroke scale (SSS) (Appendix). Nowadays, NIHSS is more commonly used for this purpose and therefore, SSS was converted to NIHSS using an algorithm (72). Finally, stroke subtypes were classified according to the TOAST system (10).

Blood was drawn after an overnight fast between 8.30 and 10.30 a.m. Serum and plasma was isolated within 2 hours by centrifugation at 2000 x *g* at 4°C for 20 minutes. All samples were aliquoted and stored at -80 °C.

Assessments of post-stroke outcomes

Cause of death and recurrent events

The Swedish Total Population Register (Folkbokföringen) was used for identification of non-survivors. Cause of death was found in the Swedish Cause of Death Register and from the National Hospital Discharge Register (NHDR), which contains almost complete data (99%) on International Classification of Diseases, 10th Revision (ICD10) codes for hospital discharge diagnoses and procedures in Sweden. Recurrent vascular events were also identified in the NHDR. For recurrent stroke events, the NHDR was screened for ICD10 codes 160.0-168.8. Diagnoses were confirmed by reviewing the corresponding medical record.

Functional and neurological outcomes

All cases were invited to a 3-month follow-up visit including SSS and mRS scoring as well as identification of recurrent strokes by a stroke neurologist. mRS scores of 0-2 were defined as good outcome and 3-6 as poor outcome. Cases recruited from the Stroke Unit at SU/Sahlgrenska were invited to an additional 7-year follow-up visit (n = 411) including a visit to the study nurse and a stroke neurologist. The study nurse performed mRS scoring and the neurologist performed NIHSS scoring to determine stroke-related neurological deficits. For cases included from Stroke Units other than SU/Sahlgrenska (n = 189), the study nurse performed mRS scoring through a telephone interview.

Substudy I

In substudy I, serum concentrations of NFL were investigated in 595 cases. The analysis was performed using a single-molecule array (Simoa) method, a kind of digital ELISA where the protein is caught between two antibodies. With magnetic beads, which one of the antibodies is connected to, the protein-antibodies complex is brought into the well where detection of the protein occurs. This method makes it possible to detect proteins at subfemtomolar concentrations, i.e. at concentrations of less than 10^{-15} moles/litres. (73)

Substudy II

In substudy II, we went on to perform a pilot study of 92 proteins suggested as potential markers of neurobiological processes and/or neurological conditions by the so called Neurology panel (Olink) (74). For this study, we selected a subgroup of 220 cases in SAHLSIS. Plasma concentrations of the biomarkers were analysed using a multiplex immunoassay called proximity extension assay (PEA) (75) (Figure 1) where each protein in the assay is detected with two antibodies labelled with one DNA oligonucleotide each (A). The two antibodies bind to their target protein, bringing the oligonucleotides close enough to hybridize. Thereafter, the hybridized DNA sequence is used as a template for extension by a DNA polymerase resulting in a double-stranded DNA sequence unique for each protein (B). The DNA sequence is amplified by polymerase chain reaction (PCR) (C) and the amount is quantified using quantitive PCR (D).

Figure 1. Illustration of the PEA technology. (A) A pair of antibodies labelled with DNA oligonucleotides is used to detect the proteins in the assay. When the antibodies bind to the target protein (B) the oligonucleotides hybridize and the sequence is elongated by a DNA polymerase. The unique DNA sequence for each protein is (C) amplified by PCR and (D) quantified by quantitive PCR. PEA, proximity extension assay; PCR, polymerase chain reaction. (75)

Values are presented as an arbitrary unit on Log2 scale, called normalized protein expression (NPX). NPX gives relative quantification and therefore, comparisons of values can only be performed for the same protein; values of two different proteins cannot be compared. High NPX values corresponds to high protein concentration. (76)

Through literature research, two proteins considered to be of greatest interest for post-stroke outcomes, GFRα1 and MANF, were selected from the Olink Neurology panel for further investigation of their association with post-stroke outcomes.

Statistical methods

Statistics software SPSS statistics 24 for PC and 25 for MAC were used for all statistical analyses. Biomarker concentrations at 3 months were used in all analyses. The reason that we did not select the acute phase concentrations was for practical reasons, since the time point for this blood draw had a large interval, i.e. from day 0-10. Thus, the acute phase concentrations are dependent on the day of the blood draw and are also influenced by factors other than ischemic stroke itself such as pharmacological treatments and complications. NFL concentrations were not normally distributed and therefore, log transformed values were used

in all statistical analyses.

Differences between the group with good outcome, mRS 0-2, and poor outcome, mRS 3-6, and between cases with high physical activity and sedentary lifestyle were examined using χ^2-test for proportions, Mann-Whitney *U*-test for continuous, non-normally distributed variables and Student's *t*-test for log transformed NFL values and GFRα1.

Associations between biomarkers and NIHSS and mRS were examined using linear and logistic regression, respectively. Univariate and multivariate analyses were performed, adjusting for age and previous history of stroke before the index stroke (model 1) and for age, previous history of stroke before the index stroke and NIHSS at baseline (model 2). For GFRα1 and MANF, an additional model adjusting for age, previous history of stroke before the index stroke, NIHSS at baseline and physical activity <4 hours per week was performed as well (model 3).

Age, previous history of stroke and NIHSS score at baseline were included as covariates in the multivariable analyses because they were significantly associated to outcomes and/or biomarker levels and could therefore influence the result. In addition, stroke severity (i.e. baseline NIHSS) in particular, but also age and previous history of stroke are well known predictors of post-stroke outcomes. Physical activity <4 h per week were included for GFRα1 and MANF only. The reason for this was that preliminary results from our research group have shown an association between physical inactivity and long-term outcome measured as mRS. When concentrations of the three biomarkers were compared between cases with high physical activity and cases with a sedentary lifestyle, biomarker levels differed significantly

for GFRα1 (p = 0.012) and MANF (p = 0.017), but not for NFL (p = 0.27). Therefore, we chose to include this variable for GFRα1 and MANF only.

The area under the receiver operating characteristic curve (AUC) was calculated for assessing the diagnostic accuracy of biomarker concentrations measured 3 months after the index stroke for good (mRS 0-2) versus poor (mRS 3-6) outcomes at 7 years.

ETHICS

The study was approved by the local Ethics Committee at the University of Gothenburg. Written informed consent was obtained from all participants prior to inclusion in the study. For patients who were unable to communicate, consent was obtained from their next of kin.

RESULTS

Substudy I

Serum concentrations of NFL at 3 months were available in 546 patients, among whom NIHSS and mRS scores at 7 years were available for 272 and 438 patients, respectively. Forty-five patients had died before the 7-year follow-up.

In the poor outcome group, cases were significantly older and had higher NIHSS scores at baseline and at 7 years (Table 5). Also, a larger proportion had diabetes mellitus and previous history of stroke before the index stroke. Serum concentrations of NFL was significantly higher in the poor outcome compared to the good outcome group (Table 5 and Figure 2).

Table 5. Baseline characteristics, serum concentrations of NFL and NIHSS at 7 years for the study participants in substudy I stratified in good and poor outcome according to mRS.

	Good outcome at 7 years mRS 0-2 (n = 293)	**Poor outcome at 7 years mRS 3-6 (n = 181)**
Age, median (IQR)	57.5 (50.4-61.9)	62.3 (54.7-66.6)***
Male gender, n (%)	183 (63)	117 (65)
Hypertension, n (%)	164 (56)	111 (61)
Diabetes mellitus, n (%)	38 (13)	51 (28)***
Current smoker, n (%)	102 (35)	78 (43)
Previous history of stroke, n (%)	44 (15)	48 (27)**
NIHSS score at baseline, median (IQR)	2.03 (0.7-4.1)	5.9 (2.5-13.6)***
NIHSS score at 7 years, median (IQR)	0 (0-1)	6.5 (2.0-10.5)***
NFL (pg/ml) 3 months, n (%)	278 (95)	160 (88)
median (IQR)	64.7 (34.8-135.6)	194.8 (69.8-411.4)***

Data are shown as median and interquartile range (IQR) or number (n) and percentage. Differences between the good (mRS 0-2) and poor (mRS 3-6) outcome groups were examined using χ^2-test for proportions, Mann-Whitney *U*-test for continuous, non-normally distributed variables and Student's *t*-for serum concentrations of NFL on log transformed values. NIHSS, National Institutes of Health stroke scale; mRS, modified Rankin Scale; NFL, neurofilament light chain. *p <0.05, **p <0.01, ***p <0.001.

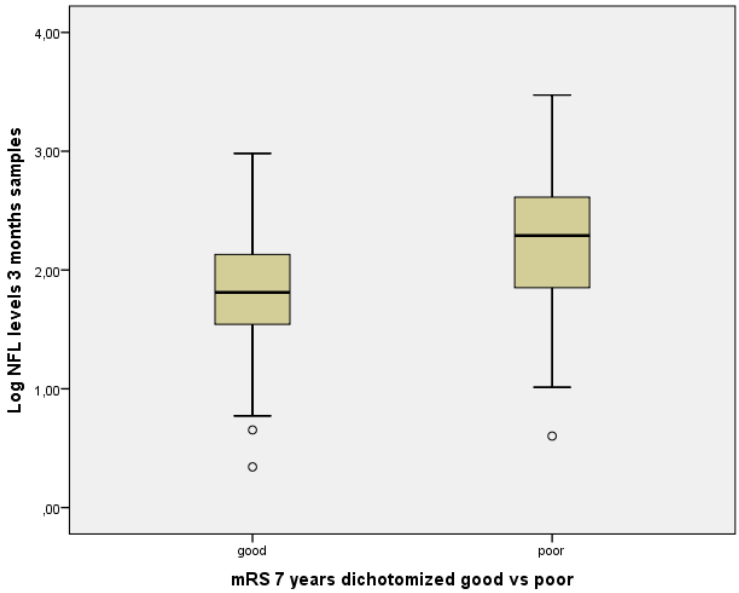

Figure 2. Boxplot of log transformed NFL levels 3 months after the index stroke for good outcome (mRS 0-2) and poor outcome (mRS 3-6). mRS, modified Rankin Scale; NFL, neurofilament light chain.

Three-month NFL was significantly associated to 7-year NIHSS both in univariate and multivariate analyses (Table 6). The sensitivity analysis excluding cases with recurrent stroke before the 7-year follow-up performed on model 2 confirmed an association (β_{std} = 0.22, 95% CI 0.10-0.35, p = 0.001).

Table 6. Linear regression analyses of associations for serum concentrations of NFL 3 months after the index stroke and NIHSS score 7 years post-stroke.

	β_{std}	95% CI for β_{std}	p
Log NFL	0.54	0.44-0.64	<0.001
Log NFL[1]	0.53	0.43-0.63	<0.001
Log NFL[2]	0.23	0.12-0.35	<0.001

Standardized β and 95% confidence intervals for NIHSS score. The betas represent an estimate of how many standard deviations (SD) the NIHSS score will change per 1 SD increase in log transformed NFL concentrations. [1]Model 1 including age and previous history of stroke before the index stroke. [2]Model 2 including age, previous history of stroke before the index stroke and baseline NIHSS. NIHSS, National Institutes of Health Stroke Scale; NFL, neurofilament light chain.

Three-month NFL was also significantly associated to mRS 7 years after the index stroke, both in the univariate and multivariate analyses (Table 7 and Figure 3). The sensitivity analysis excluding cases with recurrent stroke before the 7-year follow-up performed on model 2, confirmed an association (OR = 1.6, 95% CI 1.14-2.27, p = 0.007).

Table 7. Logistic regression of associations for serum concentrations of NFL 3 months after the index stroke and outcome measured as mRS 7 years post-stroke.

	OR	CI	p
Log NFL 3 months	2.9	2.2-3.9	<0.001
Log NFL 3 months[1]	2.9	2.2-3.9	<0.001
Log NFL 3 monhts[2]	1.8	1.3-2.6	0.001

Odds ratios and 95% confidence intervals for poor outcome (mRS 3-6) per 1 SD increase in log transformed NFL concentrations. [1]Model 1 including age and previous history of stroke before the index stroke. [2]Model 2 including age, previous history of stroke before the index stroke and baseline NIHSS. NIHSS, National Institutes of Health Stroke Scale; mRS, modified Rankin Scale; NFL, neurofilament light chain.

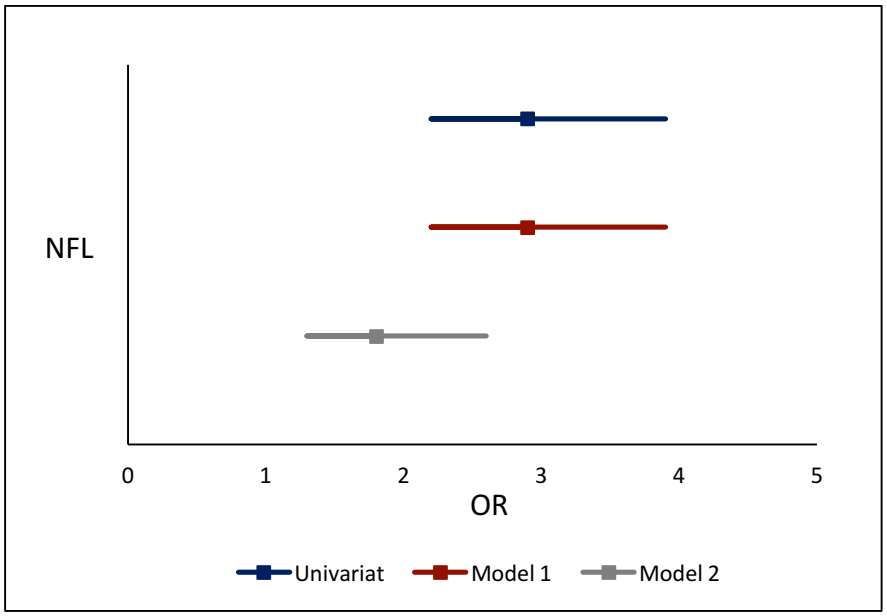

Figure 3. Forest plot of odds ratio and 95% confidence intervals for poor outcome (mRS 3-6) per 1 SD increase in log transformed NFL concentrations. Model 1 including age and previous history of stroke. Model 2 including age, previous history of stroke before the index stroke and baseline NIHSS. NIHSS, National Institutes of Health Stroke Scale; mRS, modified Rankin Scale; NFL, neurofilament light chain.

The diagnostic accuracy of 3-month NFL for good (mRS 0-2) versus poor (mRS 3-6) outcome at 7 years were examined by assessing the AUC (Figure 4). 3-month NFL generated an AUC of 0.72 (95% CI 0.67-0.77). For baseline NIHSS in the study population for substudy I, the AUC was 0.74 (95% CI 0.69-0.79).

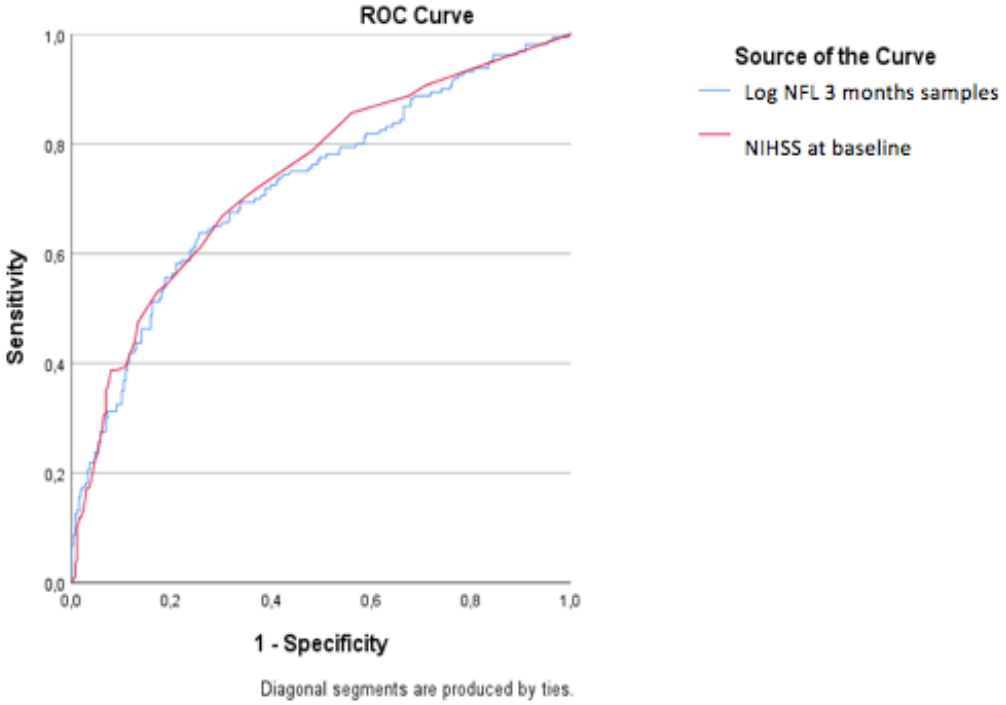

Figure 4. ROC-curves for log transformed NFL concentrations and baseline NIHSS for good (mRS 0-2) versus poor (mRS 3-6) outcome at 7 years post stroke. ROC, Receiver operating characteristics; NIHSS, National Institutes of Health Stroke Scale; mRS, modified Rankin Scale; NFL, neurofilament light chain.

Substudy II

Plasma concentrations of GFRα1 and MANF at 3 months were available for 215 patients, among whom NIHSS and mRS scores at 7 years were available for 212 and 215 patients, respectively.

In the poor outcome group, a larger proportion of cases had diabetes mellitus and previous history of stroke before the index stroke compared to the good outcome group (Table 8). In addition, they were less physically active. They also had higher NIHSS scores both at baseline and at 7 years. Plasma concentrations of GFRα1 (Table 8 and Figure 5) and MANF (Table 8 and Figure 6) were significantly higher than in the good outcome group.

Table 8. Baseline characteristics, serum concentrations of GFRα1 and MANF and NIHSS at 7 years for the study participants in substudy II stratified in good and poor outcome according to mRS.

	Good outcome at 7 years mRS 0-2 (n = 183)	**Poor outcome at 7 years mRS 3-6 (n = 37)**
Age, median (IQR)	55.2 (48.4-60.6)	57.8 (50.3-65.4)
Male gender, n (%)	117 (64)	24 (65)
Hypertension, n (%)	96 (53)	21 (57)
Diabetes mellitus, n (%)	22 (12)	10 (27)*
Current smoker, n (%)	62 (34)	16 (43)
Previous history of stroke, n (%)	24 (13)	11 (30)*
Moderate physical activity <4 hours per week = sedentary before stroke, n (%)	18 (10)	14 (38)***
NIHSS score at baseline, median (IQR)	2.0 (0.7-4.2)	6.3 (3.1-15.4)***
NIHSS score 7 years, median (IQR)	0 (0-1)	5 (2-9)***
GFRα1 3 months, n (%) median (IQR)	180 (98)	35 (95)
	5.9 (5.6-6.1)	6.1 (5.9-6.4)***
MANF 3 months, n (%) median (IQR)	180 (98)	35 (95)
	6.0 (5.1-7.0)	6.9 (5.5-7.7)*

Data are shown as median and interquartile range (IQR) or number (n) and percentage. Differences between the good (mRS 0-2) and poor (mRS 3-6) outcome groups were examined using χ^2-test for proportions, Mann-Whitney *U*-test for continuous, non-normally distributed variables and Student's *t*-for GFRα1 values. NIHSS, National Institutes of Health stroke scale; mRS, modified Rankin Scale; GFRα1, glial cell-derived neurotrophic factor family receptor-alpha-1; MANF, mesencephalic astrocyte-derived neurotrophic factor. *p <0.05, **p <0.01, ***p <0.001.

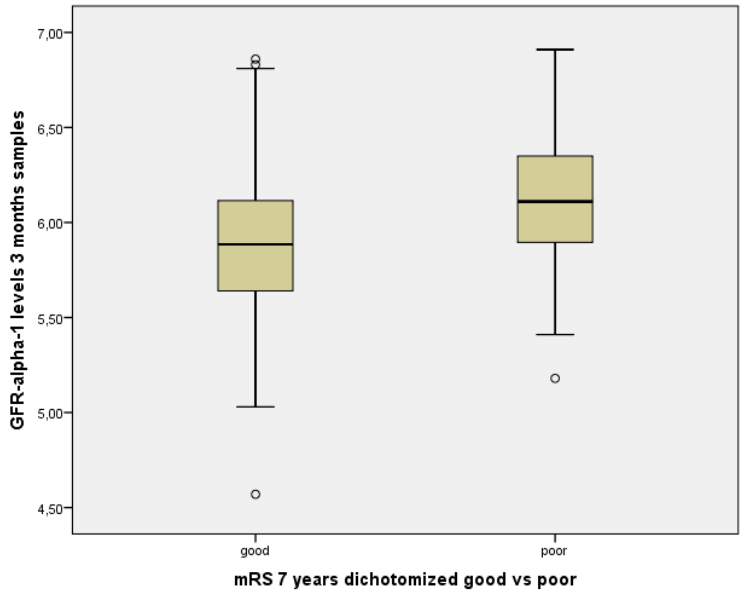

Figure 5. Boxplot of GFRα1 levels 3 months after the index stroke for good outcome (mRS 0-2) and poor outcome (mRS 3-6). mRS, modified Rankin Scale; GFRα1, glial cell-derived neurotrophic factor family receptor-alpha-1.

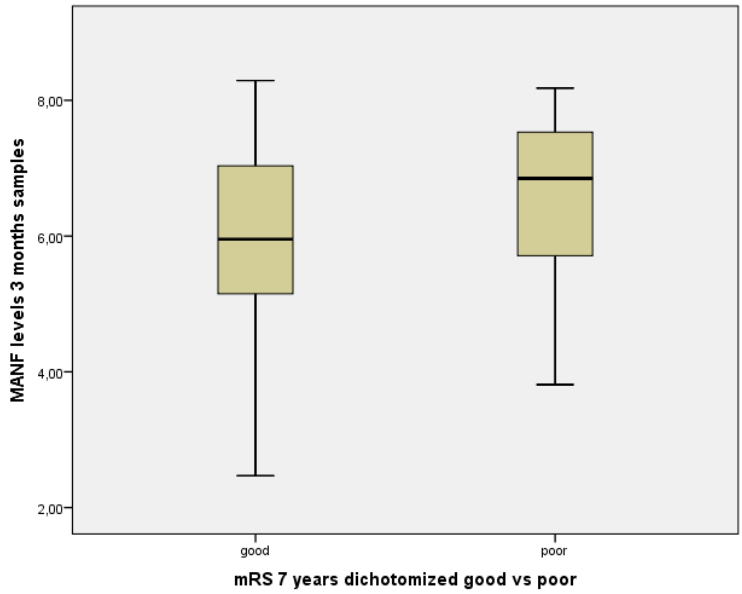

Figure 6. Boxplot of MANF levels 3 months after the index stroke for good outcome (mRS 0-2) and poor outcome (mRS 3-6). mRS, modified Rankin Scale; MANF, mesencephalic astrocyte-derived neurotrophic factor.

Three-month levels of both GFRα1 and MANF were significantly associated to 7-year NIHSS in univariate and multivariate analyses when adjusting for age and previous history of stroke (Table 9). When also adjusting for baseline NIHSS, the significant association remained for

MANF but not for GFRα1 (Table 9). The association for MANF remained when additionally adjusting for physical activity (Table 9).

The sensitivity analysis excluding cases with recurrent stroke before the 7-year follow-up was performed for model 2 and 3. For GFRα1, the association was still not significant for model 2 (β_{std} = 0.07, 95% CI (-0.05)-0.19, p = 0.24) or 3 (β_{std} = 0.05, 95% CI (-0.07)-0.17, p = 0.42). For MANF, the association was borderline significant for model 2 (β_{std} = 0.11, 95% CI (-0.002)-0.23, p = 0.05) and model 3 (β_{std} = 0.12, 95% CI ($-1.11E^{-05}$)-0.24, p = 0.05).

Table 9. Linear regression analyses of associations for serum concentrations of GFRα1 and MANF 3 months after the index stroke and outcome measured as NIHSS 7 years post-stroke.

	β_{std}	**95% CI for β_{std}**	**p**
GFRα1 3 months	0.17	0.03-0.30	0.015
MANF 3 months	0.14	0.0007-0.27	0.049
GFRα1 3 months[1]	0.15	0.01-0.28	0.031
MANF 3 months[1]	0.15	0.01-0.28	0.032
GFRα1 3 months[2]	0.07	(-0.04)-0.19	0.206
MANF 3 months[2]	0.12	0.008-0.23	0.036
GFRα1 3 months[3]	0.07	(-0.05)-0.19	0.259
MANF 3 months[3]	0.13	0.008-0.24	0.036

Standardized β and 95% CI for NIHSS score. The betas represent an estimate of how many standard deviations (SD) the NIHSS score will change per 1 SD increase in arbitrary units for GFRα1 and MANF concentrations. [1]Model 1 including age and previous history of stroke before the index stroke. [2]Model 2 including age, previous history of stroke before the index stroke and baseline NIHSS. [3]Model 3 including age, previous history of stroke before the index stroke, NIHSS acute and physical activity. NIHSS, National Institutes of Health Stroke Scale; mRS, modified Rankin Scale; GFRα1, glial cell-derived neurotrophic factor family receptor-alpha-1; MANF, mesencephalic astrocyte-derived neurotrophic factor.

Three-month levels of both GFRα1 and MANF were significantly associated to 7-year mRS in univariate and multivariate models including age, previous history of stroke and baseline

NIHSS (Table 10 and Figure 7). However, the association was not retained for either GFRα1 or MANF when additionally adjusting for physical activity (Table 10 and Figure 7).

The sensitivity analysis excluding cases with recurrent stroke before the 7-year follow-up was performed on model 2 and 3. For GFRα1, the significant association remained (OR = 1.8, 95% CI 1.10-3.09, p = 0.023) in model 2 but was still not significant in model 3 (OR = 1.5, 95% CI 0.80-2.85, p = 0.21). For MANF, the association remained significant in model 2 (OR = 1.72, 95% CI 1.11-2.68, p = 0.016) and became significant in model 3 (OR = 1.75, 95% CI 1.02-3.00, p = 0.041).

Table 10. Logistic regression of the associations for serum concentrations of GFRα1 and MANF 3 months after the index stroke and outcome measured as mRS 7 years post-stroke.

	OR	**95% CI for OR**	**p**
GFRα1 3 months	2.0	1.4-3.0	<0.001
MANF 3 months	1.7	1.1-2.5	0.015
GFRα1 3 months[1]	1.9	1.3-2.9	0.002
MANF 3 months[1]	1.7	1.1-2.6	0.014
GFRα1 3 months[2]	1.8	1.1-2.7	0.01
MANF 3 months[2]	1.7	1.1-2.7	0.02
GFRα1 3 months[3]	1.5	0.8-2.9	0.21
MANF 3 months[3]	1.5	0.9-2.6	0.12

Odds ratios and 95% confidence intervals for poor outcome (mRS 3-6) per 1 SD increase in arbitrary units for GFRα1 and MANF concentrations. [1]Model 1 including age and previous history of stroke before the index stroke. [2]Model 2 including age, previous history of stroke before the index stroke and baseline NIHSS. [3]Model 3 including age, previous history of stroke before the index stroke, baseline NIHSS and physical activity. NIHSS, National Institutes of Health Stroke Scale; mRS, modified Rankin Scale; GFRα1, glial cell-derived neurotrophic factor family receptor-alpha-1; MANF, mesencephalic astrocyte-derived neurotrophic factor.

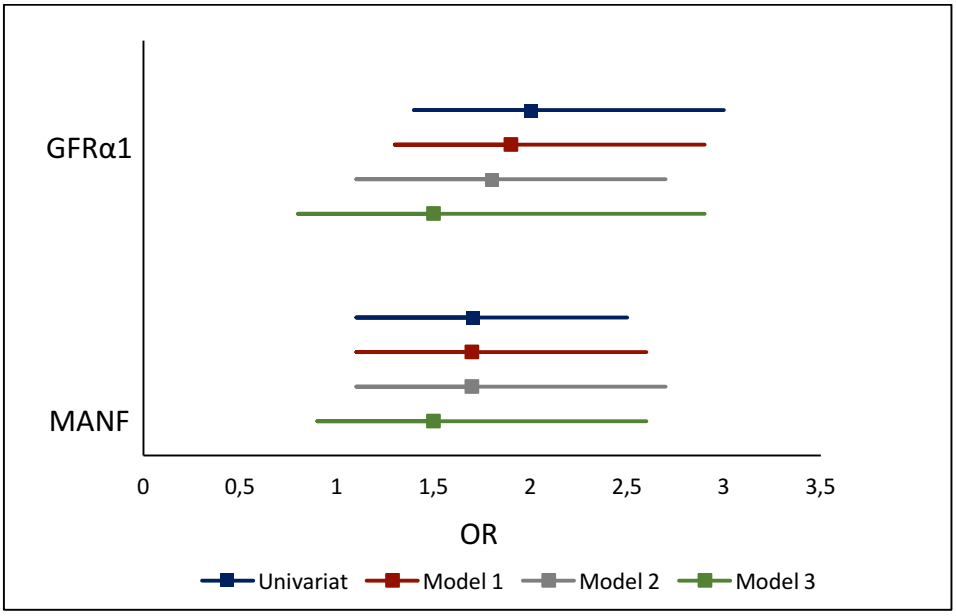

Figure 7. Forest plot of odds ratio and 95% confidence intervals for poor outcome (mRS 3-6) per 1 SD increase in arbitrary units for GFRα1 and MANF concentrations. Model 1 including age and previous history of stroke before the index stroke. Model 2 including age, previous history of stroke before the index stroke and baseline NIHSS. Model 3 including age, previous history of stroke before the index stroke and physical activity. NIHSS, National Institutes of Health Stroke Scale; mRS, modified Rankin Scale; GFRα1, glial cell-derived neurotrophic factor family receptor-alpha-1; MANF, mesencephalic astrocyte-derived neurotrophic factor.

The diagnostic accuracy of 3-month GFRα1 and MANF for good (mRS 0-2) versus poor (mRS 3-6) outcome at 7 years were examined by assessing the AUC (Figure 8). GFRα1 generated an AUC of 0.69 (95% CI 0.60-0.79) and MANF an AUC of 0.64 (95% CI 0.53-0.74). For baseline NIHSS in the study population of substudy II, the AUC was 0.76 (95% CI 0.67-0.85).

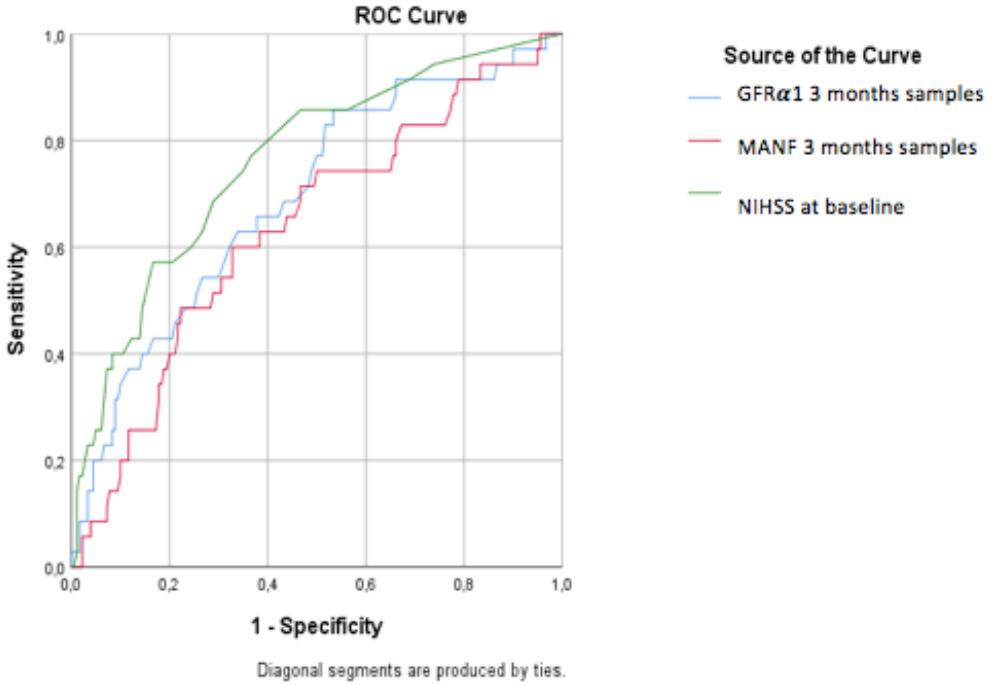

Figure 8. ROC-curves for GFRα1 and MANF concentrations and baseline NIHSS for good (mRS 0-2) versus poor (mRS 3-6) outcome at 7 years post stroke. ROC, Receiver operating characteristics; NIHSS, National Institutes of Health Stroke Scale; mRS, modified Rankin Scale; GFRα1, glial cell-derived neurotrophic factor family receptor-alpha-1; MANF, mesencephalic astrocyte-derived neurotrophic factor.

DISCUSSION

In the present study, we have investigated the association between circulating concentrations of three novel biomarkers and long-term outcomes after ischemic stroke. Firstly, we investigated 595 ischemic stroke patients and found that serum concentrations of NFL at 3 months after the index stroke were significantly higher in the group with poor outcome (mRS 3-6) compared to the group with good outcome (mRS 0-2) at a 7-year follow-up. Furthermore, 3-month NFL was significantly and independently associated to both 7-year NIHSS and mRS. Next, we analysed plasma concentrations of GFRα1 and MANF in a subgroup of 220 patients. Three-month concentrations of these two biomarkers were also

significantly higher in cases with poor compared to those with good outcome at 7 years and both biomarkers were significantly associated to 7-year NIHSS and mRS.

The finding that higher serum levels of NFL were associated to poor outcome was expected since it is a known marker of brain damage. However, the few previous studies that has been conducted on associations between NFL concentrations and stroke outcomes have been unsuccessful in showing an independent association. One study on 487 patients with ischemic stroke found an association between serum NFL and 3-month mRS in the univariate analysis that did not remain when adjusting for covariates (58). Reasons for this might be the use of the electrochemiluminescence immunoassay for measurement of NFL concentrations which is a less sensitive method compared to the Simoa method used in the present study (73) and that NFL concentrations were measured within 24 hours of stroke onset, which is too early to catch the peak in circulating concentrations of NFL after ischemic stroke according to previous studies (77, 78). Another study on 31 patients with ischemic stroke due to non-traumatic cervical artery dissection also found an association between serum NFL and poor outcome at 3 months that did not remain in multivariate analyses (78). Again, the less sensitive electrochemiluminescence immunoassay was used and also the sample size was small, which may influence the results.

As far as we are aware, this is the first study to investigate the association between plasma concentrations of GFRα1 and MANF and stroke outcomes. Previous experimental studies show that GDNF, the ligand of GFRα1, reduces the size of cerebral infarctions in rats. This is presumably mediated by inhibition of nitric oxide (NO) synthesis, which is believed to be involved in the neurotoxicity during brain ischemia (79). It has also been shown that the neuroprotective effect of GDNF is depending on the amount of GFRα1 available. In one

study, GFRα1 (+)/(+) and GFRα1 (+)/(-) mice with induced occlusion of arteria cerebri media were treated with GDNF administered into the cerebral cortex. GFRα1 (+)/(+) mice had better preserved motor function and smaller infarctions compared to the GFRα1 (+)/(-) and untreated mice, indicating that the amount of GFRα1 is a limiting factor of the effect of GDNF (60). In line with these results, another study has shown an increase in GFRα1 mRNA levels in response to both ischemia and reperfusion in rats and just a slight increase in GDNF mRNA levels, which suggests that levels of GFRα1 is a regulating factor of the GDNF neuroprotective effect (61).

With regards to MANF, cerebral ischemia causes endoplasmic reticulum (ER) stress which contributes to neuronal death. As previously mentioned, the expression of MANF is upregulated by ER stress and it has been shown that the expression of MANF is upregulated in both neurons and glial cells in response to ischemia in ischemic stroke rat models (65). From other experimental studies on ischemic stroke, it has been shown that treatment with MANF, both as injections into the ventricular system of the brain (67) and as adeno-associated virus serotype 7 encoding human MANF (68), reduces infarct volume and improves neurological recovery.

Thus, both GFRα1 and MANF have been shown to have neuroprotective effects in animal models of ischemic stroke. Consequently, elevated levels of these biomarkers would be anticipated to associate with good outcome, but we found associations in the opposite direction. The reasons for this remain elusive. To our knowledge, there are no previous clinical studies on circulating levels of GFRα1 and MANF. Thus, further studies are clearly warranted to be able to interpret our results.

Strengths of the present study include a large study sample of consecutive and well characterized cases that have been followed for a long period of time and outcomes have been assessed by both NIHSS and mRS. Blood sampling was strictly standardized and for measurement of NFL, we used the most sensitive method available. However, there are also several limitations. It can not be excluded that the results are influenced by confounders not included in our multivariable analyses. In addition, all cases were <70 years of age and of mainly European ancestry and therefore, our results might not be representative for stroke patients of older ages or for stroke patients of other ethnicities. Furthermore, the loss to follow-up might have influenced our results.

CONCLUSIONS

In this master thesis, associations between three novel biomarkers and outcomes after ischemic stroke were investigated. We identified independent associations to outcomes for all three biomarkers studied. As NFL is a known marker of brain damage, it was expected that cases with poor outcomes would have higher concentrations. We observed highest levels of NFL at 3 months after index stroke, but additional studies are needed in order to evaluate the most informative time point for NFL as a predictor of stroke outcomes. From what is known from previous experimental studies, GFRα1 and MANF both have neuroprotective effects in ischemic stroke and therefore it was unexpected that concentrations of these biomarkers were higher in cases with poor outcome. Thus, further studies are clearly needed in order to increase the knowledge about the role of these two biomarkers for stroke outcomes and to understand why high levels are associated with poor outcomes. The ultimate goal for future studies would be to find a combination of circulating biomarkers that predicts outcomes after ischemic stroke with high sensitivity and specificity.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Stroke innebär att hjärnan får brist på syre, antingen p.g.a. en blödning som trycker på hjärnan eller p.g.a. en blodpropp som täpper till ett blodkärl som går till hjärnan. När en del av hjärnan inte får tillräckligt mycket syre kommer dess nervceller att dö. Beroende på i vilken del av hjärnan som syrebristen uppstår kan olika symtom uppkomma såsom förlamning av ansikte, arm och/eller ben, försämrad känsel eller talsvårigheter. När en blodpropp orsakar syrebristen kallas det för hjärninfarkt, vilket utgör cirka 85% av all stroke i Sverige och är den form som är studerad i det här arbetet.

Patienter som överlever en hjärninfarkt återhämtar sig mycket olika. Vissa blir helt återställda medan andra har många kvarstående funktionsnedsättningar som kan resultera i att de inte kan återgå till sitt arbete och att de behöver mycket hjälp och stöd i sin vardag. De som återhämtar sig dåligt kan till exempel ha kvarstående förlamning, försämrad känsel eller talsvårigheter, men också andra symtom såsom kraftig trötthet, minnes- och koncentrationssvårigheter eller depression.

Idag vet man att patienter som får syrebrist i en stor del av hjärnan, som får många och svåra symtom av sin hjärninfarkt och som får upprepade hjärninfarkter oftast återhämtar sig sämre. Det finns dock en stor variation i återhämtning som inte bara kan förklaras av de prediktiva faktorer som vi känner till idag och det finns därför ett behov av att hitta så kallade biomarkörer, t. ex. proteiner som kan mätas genom ett blodprov, som kan hjälpa till att identifiera patienter som befinner sig i riskzonen för dålig återhämtning. I det här arbetet har vi tittat på tre proteiner som kan mätas genom ett blodprov för att se om de kan förutsäga vilka patienter som kommer att återhämta sig dåligt efter sin hjärninfarkt.

Vi har kunnat påvisa att högre nivåer av de studerade proteinerna i blodet hos patienter som genomgått en hjärninfarkt har en koppling till sämre återhämtning. Dessa resultat behöver dock verifieras i andra studier för att med större säkerhet kunna säga att resultatet i det här arbetet stämmer. Framtida studier behöver dels mäta de tre proteinerna som vi har undersökt i den här studien och dels undersöka fler proteiner för att kunna hitta den bästa kombinationen av proteiner som kan förutsäga vilka patienter som riskerar dålig återhämtning efter en hjärninfarkt.

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APPENDIX

SCANDINAVIAN STROKE SCALE

Patient Name: _____
Rater Name: _____
Date: _____

Function	Score	Prognostic Score	Long Term Score
Consciousness:			
-fully conscious	**6**	____	
-somnolent, can be awaked to full consciousness	**4**		
-reacts to verbal command, but is not fully conscious	**2**		
Eye movement:			
-no gaze palsy	**4**	____	
-gaze palsy present	**2**		
-conjugate eye deviation	**0**		
Arm, motor power *:			
-raises arm with normal strength	**6**	____	____
-raises arm with reduced strength	**5**		
-raises arm with flexion in elbow	**4**		
-can move, but not against gravity	**2**		
-paralysis	**0**		
Hand, motor power *:			
-normal strength	**6**		____
-reduced strength in full range	**4**		
-some movement, fingertips do not reach palm	**2**		
-paralysis	**0**		
Leg, motor power *:			
-normal strength	**6**	____	____
-raises straight leg with reduced strength	**5**		
-raises leg with flexion of knee	**4**		
-can move, but not against gravity	**2**		
-paralysis	**0**		

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Orientation:			
-correct for time, place and person	**6**		____
-two of these	**4**		
-one of these	**2**		
-completely disorientated	**0**		
Speech:			
-no aphasia	**10**		____
-limited vocabulary or incoherent speech	**6**		
-more than yes/no, but not longer sentences	**3**		
-only yes/no or less	**0**		
Facial palsy:			
-none/dubious	**2**		____
-present	**0**		
Gait:			
-walks 5 m without aids	**12**		____
-walks with aids	**9**		
-walks with help of another person	**6**		
-sits without support	**3**		
-bedridden/wheelchair	**0**		
Maximal Score	____	**22**	**48**

* Motor power is assessed only on the affected side.

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