Aus der Neurologischen Klinik

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DISSERTATION

White matter lesion severity in mild acute ischemic stroke patients and functional outcome after 1 year.

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Table of Contents

1. Abstract (Deutsch)

Hintergrund

Das funktionelle Ergebnis nach ischämischem Schlaganfall wird durch den Grad der Behinderung, durch Depression und durch weitere vaskuläre Ereignisse beeinflusst. Zuverlässige Prognosen würden es Klinikern ermöglichen, Patienten mit hohem Risiko zu identifizieren und vorbeugende Therapiekonzepte zu entwickeln. Wir wollten feststellen, ob das Ausmaß von Hyperintensitäten in der weißen Substanz (engl.: White Matter Hyperintensities; WMH) ein prognostischer Parameter für Patienten mit ischämischem Schlaganfall ist.

Methoden

Zerebrale WMH wurden bei Patienten mit ischämischem Schlaganfall mit Fluid-attenuated Inversion Recovery (FLAIR) und T2-gewichteter Magnetresonanztomographie (MRI) nach den beiden visuellen Bewertungsskalen nach Fazekas und Wahlund bewertet. Die Patienten nahmen an einem neuartigen kombinierten oralen Triglycerid- und Glukosetoleranztest teil, bekamen eine Ultraschalluntersuchung der Arteria carotis communis zur Bestimmung der Intima-Media-Dicke (CCA-IMT) und gaben Blutproben ab. Ein Telfoninterview wurde zur 1-Jahres-Nachverfolgung durchgeführt. Das funktionelle Ergebnis wurde mit Hilfe der modifizierten Rankin-Skala (mRS) bewertet. Eine Meta-Analyse wurde durchgeführt, um festzustellen, ob WMH mit Depression assoziiert sind.

Ergebnisse

Ausgeprägte WMH waren unabhängig sowohl mit mRS ≥ 2 ein Jahr nach dem ischämischen Schlaganfall (Wahlund ≥ 10: Odds Ratio 9,375, 95% Konfidenzintervall [CI] 1,957 bis 44,910, p = 0,005; Wahlund 5-9 : Odds Ratio 1,607, 95% CI 0,355-7,276, p = 0,538) als auch mit Depressionen (gepoolte Odds Ratio 1,22, 95% CI 1,06 bis 1,4, p <0,01) assoziiert. Erneute kardiovaskuläre Ereignisse waren ebenfalls unabhängig mit dem funktionellen Ergebnis nach einem Jahr assoziiert (Odds Ratio 6,137, 95% CI 1,388 bis 27,139, p = 0,017). Thyreoidea-stimulierendes Hormon (TSH) assoziierte mit WMH, CCA-IMT und Trigycleriden. Darüber hinaus waren vaskuläre Risikofaktoren -insbesondere Bluthochdruck, Glukose und eingeschränkter Nierenfunktion - mit WMH assoziiert.

Schlussfolgerungen

WMH sind ein prognostischer Parameter nach ischämische Schlaganfall, da sie mit einer Behinderung nach einem Schlaganfall und Depression assoziiert sind.

2. Abstract (English)

Background

Outcome following ischemic stroke is largely dependent on grade of disability, depression, and cardiovascular event recurrence. Reliable long-term outcome estimation would enable clinicians to identify high risk patients and develop preventative treatment paradigms. We sought to determine whether white matter hyperintensities (WMH) represent a prognostic marker for ischemic stroke patients.

Methods

Cerebral WMH severity was rated in first-ever ischemic stroke patients using fluid-attenuated inverse recovery (FLAIR) and T2-weighted magnetic resonance imaging (MRI) according to the Fazekas and Wahlund visual rating scales. Patients participated in a novel combined oral triglyceride and glucose tolerance test, underwent common carotid artery ultrasound to determine intima-media thickness (CCA-IMT), and provided both fasting and post-challenge blood samples. A 1 year follow-up telephone interview was performed to assess functional outcome via the modified Rankin Scale (mRS). A meta-analyses was performed to determine if WMH associated with depression.

Results

Severe WMH independently associated with disability (mRS ≥ 2) 1 year after ischemic stroke (Wahlund scores ≥ 10: odds ratio 9.375, 95% confidence interval [CI] 1.957–44.910, p = 0.005; Wahlund scores 5–9: odds ratio 1.607, 95% CI 0.355–7.276, p = 0.538) and also associated with depression (pooled odds ratio 1.22, 95% CI 1.06-1.4, p < 0.01). Recurrent cardiovascular events independently associated with 1 year functional outcome (odds ratio 6.137, 95% CI 1.388–27.139, p = 0.017). Thyroid-stimulating hormone associated with WMH, CCA-IMT, and trigyclerides. Additional vascular risk factors, specifically hypertension, glucose, and glomerular filtration rate associated with WMH.

Conclusions

WMH are a viable maker of poor outcome as they are associated with disability following stroke and depression.

3. Introduction

Stroke is one of the leading causes of disability worldwide and incurs a heavy social and financial burden.[1] Roughly 800,000 new or recurrent strokes occur annually (1 stroke every 4 minutes, 87% of which are ischemic strokes) and there are currently an estimated 4.8 million stroke survivors dependent on caregivers (family or professional).[1] In the United States alone, upwards of 66 million unpaid caregivers attended to the daily needs of stroke survivors in 2009 (for an average of 20 hours per week) yielding an estimated market value of roughly 375 billion dollars.[2,3] As the incidence of stroke is projected to rise with the aging population,[1] efforts to reduce the number of disabled patients following stroke is medically, ethically, and from a social perspective financially advisable.

The term disability is broad and is influenced by a wide variety of stroke sequelae. For instance, motor deficits,[4] depression,[5] and deficits arising from recurrent cardiovascular events[6] represent parameters that likely contribute to long-term disability in stroke survivors. Establishing a non-invasive, cheap, fast, and efficacious prognostic marker would help identify high risk patients who could benefit from preventative therapies and thus decrease the post-stroke disability burden. A reasonable prognostic maker would ideally independently and strongly associate with post-stroke outcome and be quickly and easily identifiable in the clinical setting using conventional techniques (ideally techniques already routinely performed).

The Berlin “Cream & Sugar” study (“C&S”; NCT 01378468 at clinicaltrial.gov) is an ongoing prospective cohort study with the purpose of determining whether triglyceride intolerance, as assessed using a novel oral triglyceride tolerance test, is associated with recurrent ischemic stroke. In reviewing the magnetic resonance images (MRI) of this cohort, it became apparent that many patients had incidental white matter hyperintensities (WMH) of presumed vascular origin.[7]

WMH are common radiological findings among the elderly (regardless of health status),[8] and can be visualized as hyperintensities on T2-weighted or fluid-attenuated inversion recovery (FLAIR) MRI and hypodensities on computed tomography (CT) images.[8] Historically, it was unclear if the presence of WMH represented any tangible risk to patients, though it is becoming increasingly clear that WMH associate with cognitive decline[9] and stroke.[10] The underlying pathophysiology leading to WMH development remains unclear, but it is hypothesized that they arise

from chronic cerebral ischemia as they have repeatedly been shown to associate strongly with vascular risk factors.[10]

Here, we sought to determine if WMH severity is an adequate prognostic marker of post-stroke disability. We specifically investigated the relationship between WMH and post-stroke functional outcome and depression. Moreover, we assessed triglycerides using a novel triglyceride tolerance test as a possible modifiable risk factor for intima-media thickness of the brain supplying vessels and possible pathophysiological contributor to WMH.

4. Methods

4.1. Studies 1 & 2: The Berlin "Cream & Sugar" Study

Studies 1 and 2 were retrospective sub-analyses of the Berlin "Cream & Sugar" Study (NCT 01378468). The Berlin "Cream & Sugar" Study is an ongoing prospective cohort study to determine whether triglyceride intolerance associates with recurrent stroke. The Berlin "Cream & Sugar" Study protocol has been published previously.[11]

4.1.1. Participants

Ischemic stroke patients who were admitted to one of the three Charité University campus hospitals in Berlin (i.e. Campus Charité Mitte, Campus Benjamin Franklin, and Campus Virchow Klinikum) were screened for "Cream & Sugar" eligibility 3-7 days after stroke onset.

Patients were eligible for Berlin "Cream & Sugar" study *inclusion* if: (1) they were at least 18 years of age, (2) had experienced their first ever ischemic stroke within (3) less than 1 week prior to testing, and (4) provided informed consent.

Patients were *excluded* from "Cream & Sugar" if: (1) they were unable to provide informed consent (for example due to severe aphasia), (2) were unable to swallow or drink high fat (32%) cream (dysphagia, pancreatitis, liver failure, malabsorption, kidney failure, cholescystolythiasis), (3) had a life expectancy of less than 1 year, (4) were pregnant, or (5) had severe cardiac disease (severe heart valve disorder, New York Heart Association grades III-IV, acute coronary syndrome).

All participants provided informed consent and the Berlin “Cream & Sugar” study was approved by the local ethics committee (EA4/100/08). For studies 1 and 2, patients who completed the Berlin “Cream & Sugar” study and had FLAIR or T2 images available for WMH assessment were eligible.

4.1.2 Definitions of Stroke and Disability

In the Berlin “Cream & Sugar” study, ischemic stroke was defined as a focal neurological deficit lasting for at least 24 hours with no signs of macroscopic hemorrhage on cerebral imaging. All suspected ischemic strokes were verified radiologically and categorized according to a mechanism-based classification scheme (Trial of ORG 10172 in Acute Stroke Treatment, or TOAST).[12]

Stroke severity was assessed at the time of testing using the National Institute of Health Stroke Scale (NIHSS). Functional assessment was perfomed according to the modified Rankin scale (mRS).[13]

Disability, or functional outcome, was assessed via telephone follow-up after 1 year also using the mRS and Barthel Index.

4.1.2. Blood samples & Definitions

Baseline data and venous blood samples were collected after participants had provided informed consent and fasted overnight for ≥12 hours. Fasting blood samples were drawn at 8 AM. Triglyceride and cholesterol concentrations were determined in freshly drawn venous blood samples enzymatically using a Cobas 6000 analyzer (Roche/Hitachi). Glomerular filtration rate (GFR) was estimated using the Modification of Diet in Renal Disease (MDRD)[14] formula: 186 x serum creatinine-1.154 x age-0.203 x 1.210 (if Black) x 0.742 (if female). Insulin resistance was assessed using the homeostasis model assessment (HOMA) formula.[15] Hyperlipidemia was defined as fasting total cholesterol ≥200 mg/dL and/or fasting triglycerides ≥150 mg/dL and/or use of lipid lowering medications prior to ischemic stroke.[16] Diabetes was defined as current use of antidiabetic medication, serum glycosylated hemoglobin (HbA1C) of >6.5% or 2h oral glucose tolerance test value of >200mg/dL. Hypertension was defined as current antihypertensive medication use.

4.1.3. Combined oral triglyceride & glucose tolerance test

Fasting blood samples were drawn at 8 AM. Upon completion of phlebotomy, patients drank 250 mL of 32% fat cream within 30 minutes in the presence of a Center for Stroke Research Berlin staff member to ensure cream ingestion. Three hours later (11 AM), a second blood draw was performed and immediately followed by a standard 75 g oral glucose tolerance test. Subsequent blood draws were then performed at 12 PM and 1 PM to further assess post-challenge glucose and triglyceride concentrations.

4.1.4. Image acquisition and white matter hyperintensities

MRI was performed using both 3T (Tim Trio; Siemens AG, Erlangen, Germany) and 1.5T (Avanto, Siemens Medical Solutions, Erlangen, Germany) scanners dependent on the admitting campus hospital. Those patients who underwent MRI (as opposed to CT), had a “Fast Track” stroke MRI that consisted of T1, T2*, and either FLAIR or T2 weighted images (depending on campus hospital)

WMH were assessed using FLAIR or T2 weighted images. Patients who only underwent CT were excluded from studies 1 and 2. WMH were scored according to both the (1) Wahlund or “Age-Related White Matter Changes” (ARWMC)[8] and (2) Fazekas[17] visual classification scales. Using the Wahlund scale, FLAIR or T2 image hyperintensities are rated from 0 to 3 based on size and confluence of the lesions on both the right and left sides of the brain in the following pre-specified regions: frontal, parieto-occipital, temporal, infratentorial/cerebellum, and basal ganglia. The final score is the sum of all regions and ranges from 0 (no WMH) to 30 (most severe WMH). When rating WMH according to the Fazekas scale, the image with the most severe WMH is rated on from 0 to 3 (0, no WMH; 1, punctate foci; 2, beginnings of confluent foci; 3 large confluent areas). A further subdivision of WMH into periventricular WMH (PVWMH) and deep WMH (DWMH) was also made using the same 0-3 scale yielding 3 Fazekas score variables—namely, (1) overall WMH (score 0-3), (2) PVWMH (score 0-3), and (3) DWMH (score 0-3).

4.1.5. Common carotid artery intima-media thickness

CCA-IMT measurements were determined from archived images for “Cream & Sugar” participants who were enrolled and tested at two campuses (CVK and CCM).

Original CCA ultrasounds were performed as part of the clinical routine using B-mode ultrasonography using 7.5 MHz high-resolution annular array scanners (CVK: Acuson X300, Siemens Healthcare, Germany; CCM: PowerVision 6000, Toshiba, Japan). Carotid ultrasounds were performed by the neurosonography laboratories in CVK and CCM under supervision of a DEGUM certified rater. Here, patients were evaluated in the supine position with their heads turned 45 degrees to the contralateral side of the artery being examined. Images were then saved and archived. CCA-IMT was thereafter determined from archived images using the ImageJ software package (National Institute of Health, Baltimore, Maryland).[18] Both the right and left CCA were evaluated and both maximum (maximum value of right and left CCA) and mean (average IMT of the right and left CCA) CCA-IMT were determined. The region of interest (ROI) for CCA-IMT assessment was defined as at least a 10 mm plaque-free portion of the CCA far-wall at least 10 mm before the bifurcation. CCA-IMT was determined by measuring the distance between the first hyperechogenic line (i.e. the border between vessel lumen and intima) and the second hyperechogenic line (i.e. media-adventitia border) of the CCA far wall.

4.1.6. Study 1: Statistical analyses

Wahlund scores were classified as (1) mild WMH (Wahlund score 0–4), (2) moderate WMH (Wahlund score 5–10), and (3) severe WMH (Wahlund score > 10).[19–21] For analyses taking into account TSH levels, patients were stratified into 3 groups — namely, (1) low TSH (0.1–µU/ml); (2) normal TSH (0.44–2.5 µU/ml), and (3) high TSH (2.5–20 µU/ml). The dependent variable, i.e. follow-up mRS scores, was also stratified into 3 groups for an ordinal logistical regression analysis (mRS: 0–1, 2–3, and 4–6). Continuous variables were tested for normal distribution graphically and using the Kolmogorov-Smirnov test. Mann-Whitney U and Kruskal-Wallis tests were performed to examine baseline parameters that were not normally distributed. Fisher's exact tests were performed for categorical variables. The level of significance was defined as $p<0.05$. If a significant association was identified in a Fisher's exact test, post hoc analysis using Sidak's method was performed at a significance level of 0.017. For multivariate models, variables that were not normally distributed (i.e. age and NIHSS) were stratified into quartiles. Ordinal logistical regression analysis models were adjusted for age, NIHSS scores, pre-stroke diabetes status, and stroke subtype (TOAST criteria). The potential association

between WMH and TSH (dependent variable) was evaluated using four regression models. Model 1 included stratified Wahlund scores as the independent variable. Model 2 included Fazekas scores as the independent variable. Model 3 included deep WMH as the independent variable, and model 4 included periventricular WHM as the independent variable. To determine whether an association existed between the follow-up functional outcome (mRS; independent variable) and TSH (stratified; dependent variable), an ordinal logistical regression analysis adjusted for age, NIHSS, and stroke subtype was performed. Retrospective analyses were performed to examine functional outcome in relation to L-thyroxin intake, recurrent cerebrovascular events (stroke, transient ischemic attack), recurrent cardiovascular events (myocardial infarction, angina pectoris). All statistical analyses in study 1 were two-tailed and conducted using SPSS 19.

4.1.5.2. Study 2 Statistics Specifics: CCA-IMT

Patients were stratified according to CCA-IMT (dependent variable) into three equal groups. To assess the relationship between continuous variables and CCA-IMT tertiles, individual ordinal logistical regression analyses were performed using CCA-IMT as the dependent variable. Non-normally distributed variables were log-transformed (log 10) to reduce skewness before analyses. Mann-Whitney U (2 categories) or Kruskal-Wallis-Tests (3 categories) were performed to assess the association between CCA-IMT (continuous variable) and categorical baseline characteristics (e.g. smoking, gender, etc.). Ordinal variables were examined using ordinal regression analyses (ordinal variable set as dependent variable, continuous CCA-IMT as independent variable). A backwards stepwise multiple linear regression analysis with log-transformed CCA-IMT (dependent variable) was performed that included variables significant (at the alpha-level of 0.10) in bivariate analysis. Collinearity was assessed by calculating tolerance (considered significant if < 0.2) and variance inflation factor (considered relevant collinearity if > 5). All statistical analyses were performed with SPSS 22 (SPSS Inc., Chicago, USA). All tests were 2-sided and, unless otherwise stated, relationships were considered significant at an a-level of 0.05. No adjustments for multiple testing were performed.

4.2. Study 3: Depression & White Matter Hyperintensities

To determine if an association existed between depression and WMH, a systematic literature review and meta-analysis was performed. Detailed methods for study 3[22] are presented in the attached document under section heading 10.4. Briefly, in study 3, original, human, epidemiological studies that evaluated the relationship between WMH and depression were identified using PubMed (MEDLINE) and Scopus (EMBASE). The following medical subject heading (MeSH) terms were used: "White matter lesions" OR "white matter diseases" OR "Cerebrovascular Disease" OR “Leukoencephalopathies” AND "Psychotic Disorders" OR "Depressive Disorder" AND "magnetic resonance imaging," and “Depression” AND “leukoaraiosis.” Hand searching was performed of all included studies and relevant review articles.

The review included any original human studies having to do either directly or indirectly with WMHs and depression published between January 1, 2002 and October 1, 2012. No selection was made based on language of publication. Studies were only eligible if MRI including T2-weighted or FLAIR sequences had been performed on patients to distinguish WMH. In instances where findings were reported from studies multiple times using the same participant/patient sample (i.e. overlapping publications), only the most recently published results were included.

4.2.4. Meta-Analysis procedure

Mix 2.0 Pro[23] software was used to perform two random effect meta-analyses which estimated pooled odds ratios and prediction intervals for studies that assessed the association between overall WMH, PVWMH, DWMH, and depression (endpoint). The first meta-analysis pooled results from “longitudinal studies.” Longitudinal studies were defined as studies that first evaluated baseline WMH and depression and then secondly performed follow-up depression assessment. The second random effect meta-analysis pooled results from “cross-sectional” studies—i.e. those studies that evaluated depression onset and white matter lesion load at one time-point. In cases where prospective cohort studies included both longitudinal and cross-sectional data, that study was included in both longitudinal and cross-sectional analyses.

Subgroup analyses were performed to determine whether volumetric vs. visual assessment of WMH and the clinical depression scale used for depression evaluation influenced the results.

Heterogeneity was tested using Cochrane's Q and the I^2 statistics.[24,25] If significant heterogeneity was present for a given analysis, we performed an Egger's regression test to test if small studies have larger effect sizes than would otherwise be expected and generated funnel plots to assess publication bias.[26]

5. Results

5.1. Study 1: White matter hyperntensities, Thyroid-stimulating hormone, & Outcome

5.1.1. Participants for Study 1

Of the 248 first-ever ischemic stroke patients that participated in the Berlin "Cream & Sugar" study between January 2009 and March 2013, 183 patients (median age 66, IQR 54–75; 33% females; median NIHSS 3, IQR 1–4, range 0–24) were eligible for sub-study analysis in Study 1.[27] Overall, 6 deaths, 2 myocardial infarctions, 20 cases of new onset angina pectoris, 13 recurrent strokes or transient ischemic attacks, 34 new neurological deficits (i.e. symptoms possibly associated with stroke), and 53 follow-up hospitalizations (10 for stroke, 43 for other causes) occurred prior to 1 year follow-up.

5.1.2. Functional outcome 1 year after ischemic stroke

A forward stepwise binary logistical regression analysis (outcome parameter 1 year follow-up mRS dichotomized 0-1; 2-6) performed in Study 1[27] revealed a significant, positive, independent association between severe WMH and disability following ischemic stroke (Wahlund scores ≥ 10: odds ratio 9.375, 95% CI 1.957–44.910, p = 0.005; Wahlund scores 5–9: odds ratio 1.607, 95% CI 0.355–7.276, p = 0.538; Wahlund scores 0-4: reference). This analysis adjusted for age (stratified into quartiles), new cardiovascular or cerebrovascular events (combined variable including myocardial infarction, angina pectoris, recurrent stroke, and TIA between the time of testing and follow-up), death, follow-up hospitalization (for stroke or other causes), new neurological deficit (where patients sought treatment), TSH levels (normal range = reference), and Wahlund scores. This analysis additionally revealed that recurrent cardiovascular and cerebrovascular events significantly associated with follow-up disability (odds ratio 6.137, 95% CI 1.388–27.139, p = 0.017).

5.1.3. Thyroid-stimulating hormone & White Matter Hyperintensities

TSH (specifically low TSH levels in asymptomatic stroke patients) was investigated as a potential modifiable risk factor for WMH and functional outcome following ischemic stroke. Univariate analysis from this sub-study showed that low TSH levels were not significantly associated with post-stroke disability (mRS 0-1 vs. 2-6: unadjusted odds ratio 2.0, 95% CI 0.423–9.199, p=0.446; mRS 0-2 vs. 3-6: unadjusted odds ratio 1.6, 95% CI 0.290–8.649, p=0.633; mRS as a semi-continuous variable: unadjusted odds ratio 1.3, 95% CI 0.654–2.529, p=0.466).

Additionally, the association between WMH and TSH was assessed using an ordinal logistical regression analysis. This analysis was adjusted for (significant univariate analysis variables at the p<0.10 level) age, NIHSS scores, diabetes, and stroke subtype (TOAST criteria) and showed an independent association between TSH and WMH severity (Wahlund scores: odds ratio 2.547, 95% CI 1.159–5.598, p = 0.020; Fazekas scores: odds ratio 2.530, 95% CI 1.115–5.741, p = 0.003).

5.1.4. Glucose, Triglycerides & Thyroid-Stimulating Hormone

High TSH levels had significantly lower 1-hour post-challenge glucose levels than those patients with normal TSH levels (high TSH: median glucose 125 mg/dl, IQR 92–170 mg/dl; normal TSH: median glucose 152 mg/dl, IQR 114–184 mg/dl; p = 0.016). Additionally, patients with high TSH levels had significantly higher 5 hour post-challenge triglycerides than patients with low TSH levels (high TSH: median 5-hour triglycerides 233 mg/dl, IQR 178–331 mg/dl; low TSH: median 5-hour triglycerides 133 mg/dl, IQR 120–186 mg/dl; p = 0.036). Patients with low TSH levels tended to have lower post-challenge triglycerides than patients with normal TSH levels (low TSH: median 5-hour triglycerides 133 mg/dl, IQR 120–186 mg/dl; normal TSH: median 5-hour triglycerides 213 mg/dl, IQR 160–300 mg/dl; p = 0.075). Neither fasting nor post-challenge triglycerides associated with WMH.

5.2. Study 2: Triglycerides & Intima-Media Thickness

5.2.1. Participants for Study 2

This Berlin “Cream & Sugar” sub-study included patients who underwent common carotid artery ultrasound in one of two Charité campus hospitals (CVK,

CCM). Between January 2009 and July 2014, 192 patients had participated in the "Berlin Cream & Sugar" study in CCM and CVK. Of those, 34 were excluded (4 no carotid imaging performed, 27 images not available in archive, 3 poor image quality). Thus, 158 patients were included (34% female, median age 65 years, IQR 51-73 years, median NIHSS 1, IQR 0-2). Here, patients had a median mean CCA-IMT (mean of left and right CCA) of 0.78 mm (IQR 0.65-0.93 mm), median maximum CCA-IMT (maximum value from right or left CCA-IMT) of 0.81 mm (IQR 0.65-1.00 mm), median right CCA-IMT of 0.73 (IQR 0.60-0.88 mm), and a median left CCA-IMT of 0.75mm (IQR 0.62-0.95 mm).

5.2.2. Triglyceride metabolism

A large proportion of patients (n = 64; 41%) had "medium triglyceride metabolism" and had peak triglyceride levels 4 hours post-challenge (Chi-square: $p = 0.017$). A larger proportion of "fast triglyceride metabolizers" (i.e. peak post-challenge triglyceride levels within 3 hours) had diabetes than medium and slow triglyceride metabolizers (fast: 38%, medium: 14%, slow: 18%; Chi squared: $p = 0.016$). No other significant differences were observed across triglyceride metabolism groups and baseline characteristics.

5.2.3. Common Carotid Intima-Media Thickness

A backwards stepwise multiple linear regression analysis included 127 patients (log-transformed maximum CCA-IMT values were the outcome variable). This analysis included variables significant at an alpha-level of 0.10 in bivariate analysis—namely, age, waist-to-hip ratio, c-reactive protein, GFR, TSH, insulin resistance (according to HOMA), and stroke subtype (according to TOAST classification). Here, we found that higher maximum CCA-IMT values were associated with older age (unstandardized $\beta = 0.004$, $p < 0.001$), higher waist-to-hip ratio (log-transformed, unstandardized $\beta = 0.475$, $p = 0.023$), and higher TSH (log-transformed, unstandardized $\beta = 0.082$, $p = 0.042$). Only the variables with reported regression coefficients remained in the final model. No evidence for collinearity was identified.

5.2.4. Carotid plaques

Carotid plaque presence (dichotomized present vs. not present) was associated with higher HbA1c (present: median HbA1c 5.7%, IQR 5.4 -6.3%; not present: median HbA1c 5.3%, IQR 5.0-5.6, Mann-Whitney U test, p = 0.001), waist-to-hip ratio (plaques: median 0.98, IQR 0.95-1.03; no plaques: median 0.94, IQR 0.88-0.99, Mann-Whitney U test, p = 0.027), 5-hour post-challenge triglycerides (plaques: median 246 mg/dL, IQR 166-356 mg/dl; no plaques: median 214 mg/dL, IQR 110-255 mg/dl, Mann-Whitney U test, p = 0.05), fasting glucose (plaques: median 98 mg/dL, IQR 88-113 mg/dl; no plaques: median 88 mg/dL, IQR 85-95 mg/dl, Mann-Whitney U test, p = 0.003). Additionally, plaques presence was significantly associated with a lower GFR (plaques: median 84 mL/min, IQR 71-97 mL/min; no plaques: median 103 mL/min, IQR 84-111 mL/min, Mann-Whitney U test, p = 0.009) and HDL/LDL ratio (plaques: median 0.40, IQR 0.31-0.53, no plaques: median 0.47, IQR 0.43-0.69, Mann-Whitney U test, p = 0.01).

5.3. Study 3: White Matter Hyperintensities & Depression

Pooled analysis with patients from cross-sectional studies revealed that overall WMH significantly associated with depression (N=6281, pooled odds ratio 1.2, interquartile range [IQR] 1.03-1.40, p = 0.02; Q = 17.83, p = 0.01; I^2 = 60.73%). Neither DWMH (N=3338, pooled odds ratio 1.04, 95% CI 0.97-1.1, p = 0.27; Q = 10.84, p = 0.01; I^2 = 72.33%) nor PVWMH (N=3813, pooled odds ratio 1.08, 95% CI 0.99-1.17, p = 0.07; Q = 5.97, p = 0.20; I^2 = 33.02%) associated with depression in cross-sectional analyses. Pooled analysis with patients from longitudinal studies revealed a significant association between overall WMH and depression (N=1882, pooled odds ratio 1.22, 95% CI 1.06-1.41, p = 0.01, Q = 0.527, p = 0.77; I^2 = 0.0%).

6. Discussion

The primary objective of this dissertation was to determine if WMH are prognostic markers of post-stroke disability. We found that WMH severity was (1) related to post-stroke disability and (2) weakly associated with depression. Though

preliminary reports have shown a relationship between stroke outcome and WMH, results from precursor publications[21,28] leading up to this dissertation and the results presented herein were some of the first to identify a strong independent association between WMH and global disability (as assessed by the mRS[29]) following ischemic stroke. Depression, along with physical disability, was also an outcome of interest here because depression is (1) common in stroke patients (upwards of 33% of patients within first year of stroke recovery)[30] and (2) adversely affects stroke recovery even if in a non-physical way (which is difficult to assess with conventional indices). It seems that overall WMH burden plays a role in depression, possibly by decreasing the brain's functional reserve capacity—which in turn could adversely affect functional outcome.

Of all of the serum parameters evaluated, HbA1c, TSH, and GFR seem to be the strongest modifiable risk factors for both vascular pathology (CCA-IMT and carotid plaques) and WMH. Additionally, our results implicate TSH alterations in both WMH and vascular pathology. We suspected TSH alterations of being a precursor to WMH and a marker for post-stroke disability because (1) subclinical hyperthyroidism has been shown to associate with functional outcome following ischemic stroke[31] and (2) it is well-established that central nervous system tissue utilizes thyroid hormones for embryonic differentiation, maintenance, and myelinization.[32] Though TSH levels were not associated with functional outcome in the "Berlin Cream & Sugar" cohort, they were associated with WMH[27] and carotid intima-media thickness.[33]

Surprisingly, the results presented herein did not support the hypothesis that triglycerides (either fasting or post-challenge) play a crucial role in WMH or CCA-IMT. As we previously reported a significant association between cavitating lacunes (an additional marker of small vessel disease along with WMH) and 5 hour post-challenge triglycerides,[28] one would expect that the area under the curve for post-challenge triglycerides or at least multiple absolute triglyceride measurements would have associated with WMH if the relationship between triglycerides and small vessel disease is truly robust. Interestingly, our group has also reported that, unlike fasting triglycerides, that post-challenge triglycerides were not associated with common carotid artery intima-media thickness.[33] Taken together, these findings suggest that either (1) triglyceride metabolism is not or at least not strongly related to small vessel disease or, perhaps more likely, (2) that the oral triglyceride tolerance test presented herein is not an adequate screening maker for such an association to be identified.

Although it has been postulated that the optimal time-point to assess post-challenge serum triglyceride concentrations is 3-4 hours following cream ingestion,[34] post-challenge triglycerides have been, in practice, shown to not be significantly associated with coronary artery atherosclerosis until at least 8 hours post-challenge.[35] As we previously found,[28] triglyceride levels assessed at the 5 hour post-challenge time-point were the only measure of triglycerides that associated with likely markers of cerebral ischemia—in this case cavitating lacunes (which have been postulated to arise from chronic cerebral ischemia due to "lipohyalinosis," or fatty hyalinization of the arterial wall).[36] It is possible, that the serial blood draws performed following the oral triglyceride tolerance test here were not carried out over a long enough timespan.

The studies presented herein have limitations. First, analyses from the "Berlin Cream & Sugar" study are largely retrospective. Second, the patients included in the "Berlin Cream & Sugar" study had mild ischemic strokes and so the findings presented herein may not be representative of the greater stroke population. Third, because TSH levels have been found to fluctuate during critical illness[37] and all of the "Berlin Cream & Sugar" patients were tested in the semi-acute setting (3-7 days after stroke), it is possible that the TSH data are not generalizable. Finally, though we found an association between WMH and depression, the mean estimated odds ratio was relatively small (1.22). It is possible that WMH mark a generalized decline in overall health (i.e. increased general morbidity), which itself may associate with depression.

Given the primary and secondary findings, the results presented herein suggest that WMH are a solid prognostic marker for long-term disability. Because WMH substantially increase the risk of both post-stroke functional disability and depression, patients with WMH may particularly benefit from early and enhanced screening and intervention. For instance, these results suggest that patients with incidental WMH may benefit from (1) tight long-term blood pressure management, (2) glucose control, and (3) depression screening and (if necessary) pharmacological intervention. Given the association presented in the precursor publication between reduced GFR and WMH,[21] angiotensin converting enzyme (ACE) inhibitors may effectively slow the progression of WMH and reduce the risk of stroke and disability in patients with WMH. Future research is warranted here.

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8 Affidavit

I, Christopher Olaf Leonards certify under penalty of perjury by my own signature that I have submitted the thesis on the topic “White matter lesion severity in mild acute ischemic stroke patients and functional outcome after 1 year” I wrote this thesis independently and without assistance from third parties, I used no other aids than the listed sources and resources.

All points based literally or in spirit on publications or presentations of other authors are, as such, in proper citations (see "uniform requirements for manuscripts (URM)" the ICMJE www.icmje.org) indicated. The sections on methodology (in particular practical work, laboratory requirements, statistical processing) and results (in particular images, graphics and tables) correspond to the URM (s.o) and are answered by me. My contributions in the selected publications for this dissertation correspond to those that are specified in the following joint declaration with the responsible person and supervisor. All publications resulting from this thesis and which I am author of correspond to the URM (see above) and I am solely responsible.

The importance of this affidavit and the criminal consequences of a false affidavit (section 156,161 of the Criminal Code) are known to me and I understand the rights and responsibilities stated therein.

_____ _____

Date Signature

9. Declaration of selected publications

Christopher Olaf Leonards had the following share in the following selected publications:

9.1. Selected Publication 1

Leonards, C.O., Schneider, H.J., Fiebach, J.B., Endres, M., Ebinger, M. (2014) Thyroid-stimulating hormone, white matter hyperintensities, and functional outcome after ischemic stroke. Cerebrovascular Diseases EXTRA. doi. 10.1159/000360217. PMID:24803914.

9.1.1. Contribution Selected Publication 1

Christopher O. Leonards actively participated in data collection (including "Cream & Sugar" testing), independently performed all statistical analyses, wrote the first and final drafts of the manuscript, and managed the submission, correspondence, and revision process.

9.2. Selected Publication 2

Batluk, J.,* Leonards, C.O.,* Grittner, U., Nolte, C., Endres, M., Ebinger, M. (2015) Triglycerides and carotid intima-media thickness in ischemic stroke patients. Atherosclerosis. doi: 10.1016/j.atherosclerosis.2015.09.003. Impact factor: 3.994, Eigenfactor 0.05. *Contributed equally, arbitrary order.

9.2.1. Contribution Selected Publication 2:

Chrisotpher O. Leonards actively participated in data collection (including Cream & Sugar testing and CCA-IMT measurement), sub-study analysis design, and performed the statistical analyses (under the tutelage of Dr. Grittner), wrote the final draft of the manuscript, and managed the submission, correspondence, and revisions process.

9.3. Selected Publication 3:

Wang, L.,* Leonards, C. O.,* Sterzer, P., Ebinger, M. (2014) The association of white matter hyperintensities with depression: a meta-analysis. Journal of Psychiatric

Research 2014. doi:10.1016/j.jpsychires.2014.05.005. Impact factor: 3.957, Eigenfactor: 0.02273. *Authors contributed equally, arbitrary order.

9.3.1. Contribution Selected Publication 3:

Christopher O. Leonards actively participated in the study design and data collection (including literature review and data extraction). He performed all statistical analyses, final manuscript preparation and revisions, and correspondence during the submission and publication process.

Signature, date and stamp of the supervising University teacher

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Signature of the doctoral candidate

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10. Selected Publications

10.1. Selected Publication 1.

EXTRA Cerebrovascular Diseases

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Original Paper

Thyroid-Stimulating Hormone, White Matter Hyperintensities, and Functional Outcome in Acute Ischemic Stroke Patients

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Key Words
Stroke · Ischemia · Hyperthyroidism · Hypothyroidism · Thyrotropin · White matter hyperintensities · Leukoaraiosis

Abstract
Background: Thyroid-stimulating hormone (TSH) concentrations are frequently altered in acute ischemic stroke patients. It is becoming increasingly apparent that various hormones in the hypothalamus-pituitary-thyroid axis may be associated with functional stroke outcome. We have previously shown that white matter hyperintensities (WMH) of presumed vascular origin are strong indicators of functional outcome. It is unclear whether an association exists between WMH and TSH. We therefore sought to determine whether TSH levels, measured in acute ischemic stroke patients, are associated with WMH and functional outcome. ***Methods:*** We analyzed all first ischemic stroke patients who participated in the Berlin 'Cream & Sugar' Study (NCT 01378468) and completed a 1-year follow-up assessment from January 2009 to March 2013. Patients were stratified into 3 groups: (1) low TSH (0.1–0.44 μU/ml); (2) normal TSH (0.44–2.5 μU/ml), and (3) high TSH (2.5–20 μU/ml). WMH were assessed using the Fazekas and Wahlund visual rating scales. Functional outcome was assessed using the modified Rankin Scale and was performed via telephone at 1 year by a certified rater. ***Results:*** 183 patients were included [median age 66, interquartile range (IQR) 54–75; 33% females; median National Institute of Health Stroke Scale 3, IQR 1–4, range 0–24]. Venous samples were collected a median of 4 days (IQR 3–5) following initial symptom onset between 8 and 9 a.m. following a 10-hour fast. Patients with normal TSH levels (n = 132; 72%) had significantly higher rates of prestroke diabetes than patients with high TSH levels (normal TSH 17%; high TSH 1%; p = 0.03). Additionally, patients with normal TSH levels tended to have higher estimated glomerular filtration rates than patients with high and low TSH concentrations (normal TSH median

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estimated glomerular filtration rates: 83 ml/min/1.73 m^2; high TSH median estimated glomerular filtration rates: 76 ml/min/1.73 m^2; low TSH median: 78 ml/min/1.73 m^2; p = 0.068). Logistical regression analysis force-adjusted for age (quartiles), NIHSS (quartiles), prestroke diabetes status, and stroke subtype revealed significant associations between WMH and TSH [Wahlund scores: odds ratio 2.547, 95% confidence interval (CI) 1.159–5.598, p = 0.020; Fazekas scores: odds ratio 2.530, 95% CI 1.115–5.741, p = 0.003]. Functional outcome was not significantly associated with TSH levels in univariate or multivariate models. ***Conclusion:*** TSH levels are independently associated with WMH in acute ischemic stroke patients. Based on our findings, we cannot recommend assessing TSH to estimate the 1-year functional outcome following ischemic stroke.

Introduction

Thyroid-stimulating hormone (TSH) concentrations are frequently altered in critically ill patients, specifically during the acute period of their illness [1]. During the early recovery period following ischemic stroke, thyroid dysfunction has been noted in more than 36% of patients [2]. It is becoming increasingly apparent that various hormones in the hypothalamus-pituitary-thyroid axis, specifically triiodothyronine, may be associated with functional stroke outcome [3–6]. Data regarding TSH levels are less clear. Some studies have found that thyroid dysfunction (either clinical or subclinical) is associated with functional outcome following ischemic stroke [3, 5, 7], whereas others have not found this [8, 9].

White matter hyperintensities (WMH) of presumed vascular origin [10] have been shown to be strong indicators of functional outcome [11, 12] and stroke recurrence [13]. It is unclear, however, whether an association exists between WMH and TSH in acute ischemic stroke patients. Because central nervous system tissue, oligodendrocytes in particular, is known to rely heavily on thyroid hormones for embryonic differentiation, maintenance, and myelinization [14], it is possible that altered TSH levels in the acute period of ischemic stroke represent a possible modulator of WMH and functional outcome.

We therefore sought to determine whether TSH levels are associated with WMH and functional outcome in acute ischemic stroke patients.

Methods and Methods

Participants

This was a retrospective subanalysis of the Berlin 'Cream & Sugar' Study (NCT 01378468). The Berlin 'Cream & Sugar' Study is an ongoing prospective cohort study seeking to determine whether elevated postchallenge triglycerides are associated with recurrent stroke. Detailed methods are presented elsewhere [15]. Briefly, we screened all male and female first-time acute ischemic stroke patients over 18 years of age (3–7 days after symptom onset), who were admitted to one of three university campus hospitals in Berlin. Because thyroid dysfunction was not an exclusion criterion for participating in the 'Cream & Sugar' Study, we excluded all 'Cream & Sugar' participants who were taking L-thyroxine for the purpose of this analysis. Ischemic stroke was defined as a focal neurological deficit lasting for at least 24 h with no signs of hemorrhage on cerebral imaging. All suspected ischemic strokes were verified radiologically using diffusion-weighted magnetic resonance imaging. Strokes were categorized according to a mechanism-based classification scheme (Trial of ORG 10172 in Acute Stroke Treatment or TOAST) [16]. Stroke severity was assessed on hospital admission and at the

EXTRA Cerebrovascular Diseases

Cerebrovasc Dis Extra 2014;4:61–68
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time of testing using the National Institute of Health Stroke Scale (NIHSS), the modified Rankin Scale (mRS), and the Barthel Index. Functional outcome was assessed via telephone follow-up after 1 year using the mRS and the Barthel Index and was performed by a certified rater. All patients provided informed consent, and the study was approved by the local ethics committee.

Blood Samples

Baseline data and venous blood samples were collected after participants had provided informed consent and fasted overnight for ≥12 h. Fasting blood samples were drawn at 8 a.m. Triglyceride and cholesterol concentrations were enzymatically determined in freshly drawn venous blood samples using a Cobas 6000 analyzer (Roche/Hitachi). The glomerular filtration rate was estimated using the Modification of Diet in Renal Disease [17] formula according to the relationship: $186 \times \text{serum creatinine}^{-1.154} \times \text{age}^{-0.203} \times 1.210$ (if black) × 0.742 (if female). Diabetes was defined as the current use of antidiabetic medication, a serum glycosylated hemoglobin of >6.5% or a 2-hour oral glucose tolerance test value of >200 mg/dl. Hypertension was defined as current antihypertensive medication use. Hyperlipidemia was defined as fasting total cholesterol ≥200 mg/dl and/or fasting triglycerides ≥150 mg/dl and/or the use of lipid-lowering medications prior to ischemic stroke [18].

Combined Oral Triglyceride and Glucose Tolerance Test

Fasting blood samples were drawn at 8 a.m. Upon completion of phlebotomy, patients drank 250 ml of 32% fat cream within 30 min in the presence of a Center for Stroke Research Berlin staff member to ensure that the cream was ingested. Three hours later (11 a.m.), a second blood draw was performed immediately followed by a standard 75-gram oral glucose tolerance test. Subsequent blood draws were then performed at 12 and 1 p.m.

Image Acquisition and WMH

MRI was performed using both 3-tesla (Tim Trio; Siemens, Erlangen, Germany) and 1.5-tesla (Avanto, Siemens Medical Solutions, Erlangen, Germany) scanners. FLAIR or T2-weighted images were used to assess WMH. WMH were scored according to both the age-related white matter changes [19] and Fazekas visual classification scales [20]. In the age-related white matter changes system, FLAIR and T2 image hyperintensities are rated from 0 to 3 based on size and confluence of the lesions on both the right and left sides of the brain in the following prespecified regions: frontal, parieto-occipital, temporal, infratentorial/cerebellum, and basal ganglia. The final score is the sum of all regions and ranges from 0 (no WMH) to 30 (most severe WMH). In the Fazekas classification system, the image with the most severe WMH is rated on a scale of 0–3 (0 = no WMH; 1 = punctate foci; 2 = beginnings of confluent foci, and 3 = large confluent areas).

Statistical Analysis

We stratified patients into 3 groups according to TSH levels [(1) low TSH (0.1–μU/ml); (2) normal TSH (0.44–2.5 μU/ml), and (3) high TSH (2.5–20 μU/ml)] and according to follow-up mRS scores (mRS: 0–1, 2–3, and 4–6). Continuous variables were tested for normality of distribution using the Kolmogorov-Smirnov test. Kruskal-Wallis tests were used to examine relationships between baseline and serum parameters (not normally distributed) and TSH groups. The relationship between categorical variables was assessed using two-sided Fisher exact tests. If a significant relationship was found, post hoc analyses using Sidak's method were performed (level of significance at 0.017). For multivariate models, variables that were not normally distributed (i.e. age and NIHSS) were stratified into quartiles. Wahlund scores were stratified into tertiles (Wahlund score 0–4 = low/no WMH; Wahlund score 5–10 = moderate WMH, and Wahlund >10 = severe WMH). Ordinal logistical regression analysis models were

EXTRA Cerebrovascular Diseases

Cerebrovasc Dis Extra 2014;4:61–68
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force-adjusted for age, NIHSS scores, prestroke diabetes status, and stroke subtype (TOAST criteria). To assess the potential relationship between WMH and TSH, we generated 4 models: namely, model 1 included stratified Wahlund scores as the independent variable, model 2 included Fazekas scores as the independent variable, model 3 included deep WMH as the independent variable, and model 4 included periventricular WHM as the independent variable. Stratified TSH concentrations were used as the dependent variable in models 1–4. To determine whether an association existed between the follow-up functional outcome and TSH concentrations, we performed an ordinal logistical regression analysis force-adjusted for age, NIHSS, and stroke subtype, using follow-up mRS scores as the independent variable and stratified TSH concentrations as the dependent variable. Thereafter, a retrospective analysis was conducted that included patients taking L-thyroxine. A further retrospective binary logistical regression analysis was performed to determine whether recurrent cerebrovascular events (stroke or TIA), new cardiovascular events/symptoms (myocardial infarction or angina pectoris), or the development of comorbidities between the time of initial testing and the 1-year follow-up influenced our results concerning functional outcome. This analysis was adjusted for age (quartiles), new cardiovascular, or cerebrovascular events (combined variable including myocardial infarction, angina pectoris, recurrent stroke, and TIA between the time of testing and follow-up), death, follow-up hospitalization (for stroke or other causes), new neurological deficit (where patients sought treatment), and included TSH levels (normal range = reference) and Wahlund scores. Unless otherwise specified, the significance was set at $p \leq 0.05$. All statistical analyses were 2-tailed and conducted using SPSS 19.

Results

The Berlin 'Cream & Sugar' Study tested 248 participants between January 2009 and March 2013. Of these, 197 patients completed the follow-up, and of these, 183 had complete datasets and were eligible for inclusion in this retrospective analysis. Baseline characteristics of the patient sample are presented in table 1. Six deaths (1 patient with low TSH levels, 2 patients with high TSH levels, and 3 patients with normal TSH levels) occurred before the 1-year follow-up. Patients with normal TSH levels had significantly higher rates of diabetes than patients with high TSH levels (Sidak's post hoc method, $p = 0.03$). Diastolic blood pressure significantly increased across the groups (table 1).

In the univariate analysis that excluded all patients taking L-thyroxine, low TSH levels (normal TSH levels = reference) were not significantly associated with outcome regardless of mRS stratification [mRS excellent: unadjusted odds ratio 2.0, 95% confidence interval (CI) 0.423–9.199, $p = 0.446$; mRS good: unadjusted odds ratio 1.6, 95% CI 0.290–8.649, $p = 0.633$; mRS not stratified: unadjusted odds ratio 1.3, 95% CI 0.654–2.529, $p = 0.466$]. Functional outcomes did not significantly differ between patients with normal (reference category) and high TSH levels (mRS excellent: unadjusted odds ratio 0.888, 95% CI 0.426–1.850, $p = 0.853$; mRS good: unadjusted odds ratio 0.990, 95% CI 0.406–2.12, $p = 1.00$; mRS not stratified: unadjusted odds ratio 0.944, 95% CI 0.499–1.789, $p = 0.861$). Including patients taking L-thyroxine did not change the results.

The ordinal logistical regression analysis force-adjusted for age, NIHSS scores, prestroke diabetes status, and stroke subtype (TOAST criteria) revealed that TSH levels independently associated with WMH severity (Wahlund scores: odds ratio 2.547, 95% CI 1.159–5.598, $p = 0.020$; Fazekas scores: odds ratio 2.530, 95% CI 1.115–5.741, $p = 0.003$). The ordinal logistical regression analysis force-adjusted for age, NIHSS scores, and stroke subtype (TOAST criteria) revealed that there was no significant association between the 1-year functional outcome and TSH (adjusted odds ratio 1.292, 95% CI 0.811–2.058, $p = 0.281$).

EXTRA Cerebrovascular Diseases

Cerebrovasc Dis Extra 2014;4:61–68
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Table 1. Demographic characteristics according to the TSH level

	High TSH	Normal TSH	Low TSH	p value
Total participants, n	41	132	10	
Females, n (%)[a]	13 (7)	45 (25)	6 (3)	0.239
Age, years[b]	62 (51–75)	67 (57–75)	68 (57–80)	0.613
L-Thyroxine, n (%)[a]	1 (0.5)[c]	8 (4.4)[c]	3 (1.6)[c]	0.021[c]
Atrial fibrillation, n (%)[a]	3 (2)	17 (12)	2 (1)	0.675
Hypertension, n (%)[a]	22 (13)	81 (48)	9 (67)	0.115
Hyperlipidemia, n (%)[a]	13 (8)	37 (23)	2 (1)	0.740
Current smokers, n (%)[a]	9 (5)	31 (19)	1 (0.6)	0.641
Diabetes, n (%)	2 (1)	28 (17)	1 (0.6)	0.019
Days since stroke[a]	5 (3–6)	4 (3–5)	4 (3–5)	0.167
NIHSS (admission)[b]	3 (2–7)	3 (2–4)	2 (0–4)	0.402
mRS (time of testing)	1 (0–2)	1 (1–2)	1 (0–2)	0.594
BI (time of testing)	100 (90–100)	100 (90–100)	100 (90–100)	0.903
eGFR[b]	76 (63–95)	83 (73–97)	78 (44–89)	0.068
Wahlund score[b]	2 (0–3)	3 (1–8)	3 (1–4)	0.037
Severe WMH, n (%)[a]	3 (2)	14 (9)	0 (0)	0.243
Severe DWMH, n (%)[a]	1 (0.7)	10 (7)	0 (0)	0.570
Severe PVWMH, n (%)[a]	3 (2)	13 (9)	0 (0)	0.836
Microbleeds, n (%)[a]	0 (0)	6 (4)	2 (1)	0.061
Creatinine, mg/dl[b]	0.98 (0.87–1.14)	0.89 (0.76–1.03)	0.94 (0.72–1.29)	0.076
Systolic BP[b]	130 (120–150)	137 (120–150)	149 (120–157)	0.427
Diastolic BP[b]	70 (69–80)	80 (70–85)	80 (75–91)	0.020
BMI[b]	27 (24–28)	26 (24–29)	29 (27–34)	0.145
Total cholesterol, mg/dl[b]	180 (153–215)	182 (157–210)	196 (135–238)	0.787
LDL, mg/dl[b]	106 (88–140)	109 (88–137)	123 (70–155)	0.950
CRP, mg/dl[b]	0.23 (0.1–0.69)	0.27 (0.12–0.65)	0.37 (0.09–0.72)	0.783
HbA_{1c}, %[b]	5.6 (5.2–6.0)	5.7 (5.3–6.2)	5.8 (5.3–6.5)	0.740
Waist-to-hip ratio[b]	0.97 (0.94–1.0)	0.97 (0.92–1.0)	0.96 (0.89–1.0)	0.777
Glucose, mg/dl[b]	94 (86–103)	97 (87–112)	99 (90–132)	0.135
Triglycerides, mg/dl[b]	115 (86–171)	118 (90–151)	97 (71–135)	0.394
Follow-up mRS[b]	1 (0–2)	1 (0–2)	2 (0–3)	0.816
mRS deterioration, n (%)[a]	14 (11)	33 (26)	4 (3)	0.649
Follow-up BI	100 (98–100)	100 (95–100)	100 (100–100)	0.774
Stroke subtype, n (%)[a]				0.112
Large artery	12 (7)	37 (21)	3 (2)	
Cardioembolic	7 (4)	25 (14)	2 (1)	
Small artery	5 (3)	39 (22)	1 (1)	
Other determined subtype	6 (3)	8 (5)	2 (1)	
Undetermined subtype	11 (6)	20 (11)	1 (1)	

Values in parentheses represent median (IQR) unless otherwise specified. BI = Barthel Index; BP = blood pressure; CRP = C-reactive protein; DWMH = deep WMH; eGFR = estimated glomerular filtration rate; HbA_{1c} = glycosylated hemoglobin; LDL = low-density lipoprotein; mRS deterioration = mRS score at the time of testing versus mRS score at the follow-up interview (classified as same, improvement, or deterioration); NIHSS = National Institute of Health Stroke Scale; PVWMH = periventricular WMH. [a] Fisher's exact test followed by Sidak's post hoc method (if significant). [b] Mann-Whitney U test. [c] All patients taking L-thyroxine were excluded from the initial analyses. Only the retrospective analysis included these patients.

The retrospective univariate analysis (Sidak's post hoc method) taking postchallenge glucose and triglyceride values into account revealed that the patients with high TSH levels had significantly lower 1-hour postchallenge glucose levels than those patients with normal TSH levels [high TSH: median glucose 125 mg/dl, interquartile range (IQR) 92–170; normal TSH: median glucose 152 mg/dl, IQR 114–184; p = 0.016]. No difference was observed across

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Cerebrovasc Dis Extra 2014;4:61–68
DOI: 10.1159/000360217

TSH groups and 2-hour postchallenge glucose values. Levels of mean plasma glucose (MPG) tended to be lower in patients with high TSH levels than in those patients with normal TSH levels (high TSH: median MPG 107 mg/dl, IQR 90–129; normal TSH: median MPG 119 mg/dl, IQR 102–140; p = 0.072). Additionally, MPG levels tended to be lower in patients with high TSH levels than in those with low TSH levels (high TSH: median MPG 107 mg/dl, IQR 90–129; low TSH: median MPG 135 mg/dl, IQR 119–138; p = 0.091). Of the 3 postchallenge time points where triglycerides were measured, only 5-hour postchallenge triglyceride levels significantly differed across the TSH groups. Patients with high TSH levels had significantly higher postchallenge triglycerides than patients with low TSH levels (high TSH: median 5-hour triglycerides 233 mg/dl, IQR 178–331; low TSH: median 5-hour triglycerides 133 mg/dl, IQR 120–186; p = 0.036). Finally, patients with low TSH levels tended to have lower postchallenge triglycerides than patients with normal TSH levels (low TSH: median 5-hour triglycerides 133 mg/dl, IQR 120–186; normal TSH: median 5-hour triglycerides 213 mg/dl, IQR 160–300; p = 0.075).

The retrospective forward-stepping binary logistical regression analysis adjusted for age (quartiles), new cardiovascular or cerebrovascular events [combined variable including myocardial infarction (n = 2), angina pectoris (n = 20), recurrent stroke and TIA (n = 13) between the time of testing and follow-up], death (n = 6), follow-up hospitalization [for stroke (n = 10) or other causes (n = 43)], and new neurological deficits [where patients sought treatment (n = 34)] revealed that severe WMH was independently associated with 1-year functional disability (mRS ≥2; Wahlund scores ≥10: odds ratio 9.375, 95% CI 1.957–44.910, p = 0.005; Wahlund scores 5–9: odds ratio 1.607, 95% CI 0.355–7.276, p = 0.538). Cardiovascular and cerebrovascular events (modeled as a combined group to decrease covariates) were also significantly and independently associated with the 1-year functional outcome (odds ratio 6.137, 95% CI 1.388–27.139, p = 0.017). The TSH levels were not associated with the 1-year functional outcome.

Discussion

The primary findings of this study were (1) that TSH levels assessed in acute ischemic stroke patients are independently associated with WMH severity and (2) that they were not associated with the 1-year functional outcome. In accordance with previous findings [2], roughly 28% of our ischemic stroke patients had TSH concentrations outside the reference range. Interestingly, patients with normal TSH values had both higher rates of prestroke diabetes and higher postchallenge glucose levels than those patients with high TSH levels. Conversely, patients with high TSH levels had higher postchallenge triglyceride levels than patients with low TSH levels.

These findings support a growing body of literature showing that thyroid hormones play a role in both lipid regulation and the metabolic syndrome [21–23]. The possible role that TSH plays in nonfasting glucose and triglyceride regulation may help to explain the relationship we observed between WMH and TSH levels. Although the underlying pathophysiological mechanism of WMH remains unclear, it seems likely that diabetes and glucose play a contributing role [24]. We have also previously reported that there was a significant positive relationship between glycosylated hemoglobin levels and WMH [11].

Our results concerning TSH levels and functional outcome must be interpreted very cautiously, as it is likely that we were underpowered to detect such relationships. Based on the effect sizes that we found here (low TSH vs. normal TSH levels, Cohen's d = 0.236, effect size r = –0.1176), we would have needed a total sample size of approximately 780 patients to achieve a power of 80%. A recent study [5] reported that subclinical hyperthyroidism was

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significantly associated with the 3-month functional outcome. However, the sample size in this study was similar to ours. Therefore, the conflicting results may be explained by more than simply power. First, the time to follow-up differed between the two studies (3 months vs. 1 year). Because subclinical hyperthyroid patients frequently (upwards of 50%) and spontaneously revert to the euthyroid state over time (1–4 years), it is possible that the conflicting results may be explained by compensatory changes that occurred after 3 months [25, 26]. Second, because we analyzed TSH levels in our acute ischemic stroke patients and had no data on free thyroid hormones (fT_3 and fT_4), we could not differentiate between patients with overt and subclinical thyroid dysfunction, and this is a limitation of our study.

Our study has further limitations. As a Berlin 'Cream & Sugar' substudy, inclusion criteria mandates [for inclusion criteria, see 15] resulted in a patient sample with relatively mild ischemic strokes (median NIHSS 3, IQR 0–4, range 0–24). Additionally, women were underrepresented in our patient sample (33%). Because TSH levels may vary in the acute period of critical illness [1], it is possible that data leveraged from our mild acute ischemic stroke patient sample are not representative, specifically in patients that have had more severe strokes. We had no data on thyroid hormones (fT_3 and fT_4) and therefore could not conclude whether high/low TSH levels are associated with thyroid dysfunction. Because we did not have information regarding TSH levels prior to stroke onset, it remains unclear whether the high/low TSH levels observed in our study were stroke-induced or feedback changes due to primary thyroid dysfunction. Further studies are needed to help determine whether TSH levels impact stroke outcome and WMH development/progression or vice versa. Finally, because we chose to follow patients for 1 year, it is possible that the development of comorbidities, such as recurrent stroke, has influenced our results concerning functional outcome. However, we observed no change from our initial findings following a retrospective analysis adjusted for these factors.

In conclusion, we found an independent association between WMH and TSH in acute ischemic stroke patients. Although WMH have been shown to be strongly associated with the 1-year functional outcome, we did not observe an association between TSH levels assessed in the acute ischemic stroke period and the 1-year functional outcome. We therefore cannot recommend assessing TSH to estimate the 1-year functional outcome following ischemic stroke.

Acknowledgements

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Disclosure Statement

C.O.L., M. Ebinger, H.J.S., and T.G.L. have no conflicts of interest to declare. J.B.F. has received consultant or lecture fees from Boehringer Ingelheim, Lundbeck, Siemens, Sygnis, and Synarc. M. Endres has received grant support from AstraZeneca and Sanofi, has participated in advisory board meetings of Bayer, Boehringer Ingelheim, Bristol-Myers Squibb, MSD, Pfizer, Sanofi and has received honoraria from AstraZeneca, Bayer, Berlin Chemie, Bristol-Myers Squibb, Boehringer Ingelheim, Desitin, Eisei, Ever, GlaxoSmithKline, MSD, Novartis, Pfizer, Sanofi, Takeda, and Trommsdorff.

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Cerebrovasc Dis Extra 2014;4:61–68
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10.2. Selected Publication 2.

Batluk, J.,* **Leonards, C.O.,*** Grittner, U., Nolte, C., Endres, M., Ebinger, M. (2015) Triglycerides and carotid intima-media thickness in ischemic stroke patients. *Atherosclerosis*. *Contributed equally, arbitrary order.

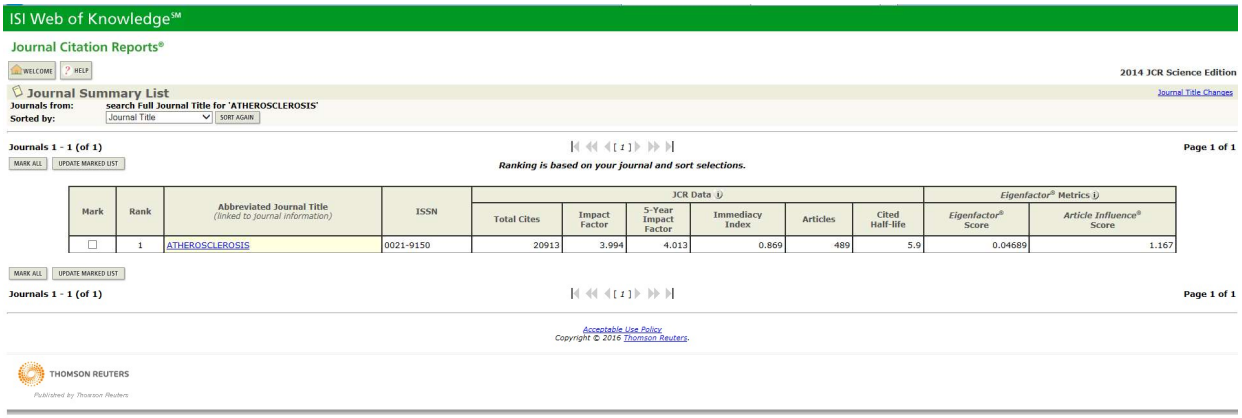
doi*:* http://dx.doi.org/10.1016/j.atherosclerosis.2015.09.003

10.2.1 Impact factor & Eigenfactor for Selected publication 2.

Atherosclerosis

Impact Factor: 3.994

Eigenfactor: 0.05



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10.3. Selected Publication 3.

Wang, L.,* **Leonards, C. O.,*** Sterzer, P., Ebinger, M. (2014) The association of white matter hyperintensities with depression: a meta-analysis. *Journal of Psychiatric Research* doi. 10.1016/j.jpsychires.2014.05.005. *Contributed equally, arbitrary order.

doi: https://doi.org/10.1016/j.jpsychires.2014.05.005

10.3.1. Impact factor & Eigenfactor for Selected Publication 3.

Journal of Psychiatric Research

Impact Factor: 3.957

Eigenfactor: 0.02273

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☐	2	NEUROPSYCHOPHARMACOL	0893-133X	22005	7.048	8.168	1.582	292	6.4	0.05016	2.642
☐	3	ARCH GEN PSYCHIAT	0003-990X	36976	14.480	15.560		0	>10.0	0.04879	6.131
☐	4	AM J PSYCHIAT	0002-953X	42476	12.295	14.644	3.368	95	>10.0	0.04850	5.421
☐	5	J AFFECT DISORDERS	0165-0327	19211	3.383	3.939	0.671	557	5.7	0.04465	1.144
☐	6	MOL PSYCHIATR	1359-4184	14510	14.496	13.834	3.152	151	5.4	0.04325	5.005
☐	7	PSYCHOL MED	0033-2917	19189	5.938	6.336	1.024	293	8.6	0.03708	2.223
☐	8	J NEUROL NEUROSUR PS	0022-3050	25650	6.807	5.550	2.129	202	>10.0	0.03499	1.970
☐	9	SCHIZOPHR RES	0920-9964	17683	3.923	4.644	0.668	391	6.6	0.03329	1.271
☐	10	ADDICTION	0965-2140	16193	4.738	5.781	2.125	192	7.6	0.03284	1.995
☐	11	PSYCHOPHARMACOLOGY	0033-3158	24703	3.875	3.974	0.952	399	9.5	0.03265	1.130
☐	12	DRUG ALCOHOL DEPEN	0376-8716	13688	3.423	3.903	0.530	400	6.7	0.03009	1.252
☐	13	BRIT J PSYCHIAT	0007-1250	22557	7.991	8.196	1.905	116	>10.0	0.02984	2.875
☐	14	SCHIZOPHRENIA BULL	0586-7614	13525	8.450	8.686	1.859	177	6.7	0.02809	2.678
☐	15	PSYCHONEUROENDOCRINO	0306-4530	11843	4.944	5.659	0.927	259	5.9	0.02740	1.686
☐	16	PSYCHIAT RES	0165-1781	13160	2.467	2.947	0.373	581	6.5	0.02733	0.848
☐	17	J CHILD PSYCHOL PSYC	0021-9630	15504	6.459	6.681	1.212	118	9.2	0.02601	2.495
☐	18	J CLIN PSYCHIAT	0160-6689	18227	5.498	5.818	0.772	171	9.2	0.02546	1.824
☐	19	J AM ACAD CHILD PSY	0890-8567	17723	7.260	8.459	1.667	96	>10.0	0.02486	3.155
☐	20	J PSYCHIATR RES	0022-3956	11803	3.957	4.542	0.796	226	7.9	0.02273	1.418

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11. Curriculum Vitae

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

For reasons of confidentiality my curriculum vitae has been removed from the electronic version of this dissertation.

12. Publications

12.1. Articles

1. **Leonards, C. O.,*** Ebinger, M.,* Batluk, J., Malzahn, U., Heuschmann, P., and Endres, M. (2010) The role of fasting versus nonfasting triglycerides in ischemic stroke: a systematic review. *Frontiers in Neurology*. doi:10.3389/fneur.2010.000133. **Impact factor: 3.184, Eigenfactor 0.00987.** *Contributed equally, arbitrary order.

2. Ebinger, M., Sievers, C., Klotsche, J., Schneider, H., **Leonards, C. O.,** Pieper, L., Wittchen, H., Stalla, G., and Endres, M. (2010) Triglycerides and stroke risk prediction: lessons from a prospective cohort study in German primary care patients. *Frontiers in Neurology.* doi:10.3389/fneur.2010.00148. **Impact factor: 3.184, Eigenfactor 0.00987.**

3. **Leonards, C. O.,** Ipsen, N., Malzahn, U., Fiebach, J. B., Endres, M., Ebinger, M. (2012) White matter lesion severity and functional outcome in mild acute ischemic stroke. *Stroke.* doi: 10.1161/STROKEAHA.111.646554**. Impact factor: 5.729, Eigenfactor 0.105.**

4. **Leonards, C. O.,*** Wang, L.,* Fiebach, J. B., Endres, M., Ebinger, M. (2013) Fasting versus post-challenge triglycerides and pre-existing cavitating lacunes: a Berlin “Cream & Sugar” substudy. *Frontiers in Neurology*. doi:10.3389/fneur.2013.00092. **Impact factor: 3.184, Eigenfactor 0.00987.** *Contributed equally, arbitrary order.

5. **Leonards, C.O.,** Schneider, H.J., Fiebach, J.B., Endres, M., Ebinger, M. (2014) Thyroid-stimulating hormone, white matter hyperintensities, and functional outcome after ischemic stroke. *Cerebrovascular Diseases EXTRA.* doi. 10.1159/000360217.

6. Wang, L.,* **Leonards, C. O.,*** Sterzer, P., Ebinger, M. (2014) The association of white matter hyperintensities with depression: a meta-analysis. *Journal of Psychiatric Research* doi. 10.1016/j.jpsychires.2014.05.005. **Impact factor: 4.066, Eigenfactor 0.0165**. *Contributed equally, arbitrary order.

7. Ebinger, M., Ipsen, N., **Leonards, C.O.**, Empl, L., Hanne, L., Liman, T., Mai, K., Strasburger, C.J., Spranger, J., Endres, M. (2015) Circulating Insulin-like Growth Factor Binding Protein-3 Predicts one-year Outcome after Ischemic Stroke. *Experimental and Clinical Endocrinology & Diabetes.* doi: 10.1055/s-0035-1554632. **Impact factor: 1.555, Eigenfactor 0.00358**.

8. Heinze, G., Christ, T., **Leonards, C.O.,** Konertz, W. (2015) Risk and outcome of aortic valve surgery in the transcatheter valve era: The gender aspect. *Annals of Thoracic and Cardiovascular Surgery* doi: 10.5761/atcs.oa.14-0029. **Impact factor: 0.69.**

9. Nave, A.H., Lange, K.S., **Leonards, C.O.**, Abo-Jasser, A., Döhner, W., Landmesser, U., Steinhagen-Thiessen, E., Endres, M., Ebinger, M. (2015) Lipoprotein a: an underestimated risk factor for ischemic stroke? An updated systematic review and meta-analysis. *Atherosclerosis.* doi: 10.1016/j.atherosclerosis.2015.08.021. **Impact factor: 3.994, Eigenfactor 0.05.**

10. Batluk, J.,* **Leonards, C.O.,*** Grittner, U., Nolte, C., Endres, M., Ebinger, M. (2015) Triglycerides and carotid intima-media thickness in ischemic stroke patients. *Atherosclerosis*. *doi:* 10.1016/j.atherosclerosis.2015.09.003. **Impact factor: 3.994, Eigenfactor 0.05**. *Contributed equally, arbitrary order.

11. Hong, J.B.,* **Leonards, C.O.,*** Endres, M, Siegerink, B., Liman, T.G. (2015) Ankle-brachial index and recurrent stroke risk: a meta-analysis. *Stroke (submitted, under review, R0)*. **Impact factor: 5.723, Eigenfactor 0.10311.** *Contributed equally, arbitrary order.

12.2. International Posters and Presentations

1. **Leonards, C.O.,** Müller-Jensen, L., Fiebach, J.B., Sobeskey, J., Ebinger, M., Endres, M. Recanalization versus reperfusion: a meta-analysis of revascularization scales and 3 month functional outcome in ischemic stroke patients. Oral presentation. *European Stroke Organization, Glasgow, UK*. April 16, 2015.

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