Aus der Klinik und Hochschulambulanz für Neurologie
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DISSERTATION

Associations between cerebral microbleeds and lipids in first time ischemic stroke patients

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Abstract

Background: Cerebral microbleeds (CMBs) are magnetic resonance imaging (MRI) markers of cerebral small vessel disease (SVD) and are a risk factor for ischemic stroke, hemorrhagic stroke, and poor functional outcome after stroke. Although dyslipidemia is associated with ischemic stroke, its relationship to CMBs is unclear. In this study, which is a substudy of the Berlin Cream&Sugar study, we sought to determine whether CMBs in first-time ischemic stroke patients were associated with various measures of dyslipidemia. **Methods:** This substudy included all patients enrolled in the Berlin Cream&Sugar study between January 2009 and October 2015, who had received necessary imaging for evaluation of CMBs. 3 – 7 days after ischemic stroke, baseline serum lipid parameters (total cholesterol [TC], low-density lipoprotein cholesterol [LDL-C], high-density lipoprotein cholesterol [HDL-C], and TG levels) were measured, and patients were administered oral TG tolerance tests (OTTT) and oral glucose tolerance tests (OGTT). **Results:** A total of 291 subjects were included in this substudy (median age 64.5 years, standard deviation [SD] ± 13 years; median National Institutes of Health Stroke Scale [NIHSS] 1, interquartile range [IQR] 0-2). Of these, 28 (9.6%) were found to have one or more CMB. Compared to patients with low TC (<165 mg/dl), patients with midrange TC (165-198 mg/dl) had an adjusted odds ratio [OR] of 0.20 (95% confidence interval [CI] 0.10 - 0.76) for CMBs (p=0.018); for patients with the highest tertile of TC (>198 mg/dl) the adjusted OR was 0.32 (95% CI 0.09 – 1.10, p=0.070). Additionally, in post hoc analysis, CMBs were independently associated with increasing severity of white matter hyperintensities (WMHs), based on Wahlund score (Wahlund 0-3: reference; Wahlund 4-10: adjusted OR 6.1; 95% CI 1.8 - 21.2; p= 0.004; Wahlund >10: adjusted OR 10.8; 95% CI 2.9 - 39.4; p<0.01) and with the lowest tertial of glomerular filtration rate (GFR), (GFR >92.2 ml/min/1.73 m^2: reference; m^2GFR<75.5 ml/min/1.73 m^2: adjusted OR 7.4; 95% CI 1.6 - 33.9; p=0.01) . **Conclusion**: Low TC, WMH severity, and poorer renal function were associated with CMBs in our cohort of first time ischemic stroke patients. Neither fasting TGs nor any measure of TG metabolism based on the OTTT were significantly associated with CMBs. Further studies may investigate the association between CMBs and renal function and TC level. Our results do not, however, suggest that further investigation of TG or TG metabolism would be a fruitful field for further research.

Zusammenfassung

Einleitung: Zerebrale Mikroblutungen (engl.: cerebral microbleeds, CMBs) sind ein Kernspintomographie Marker für eine Erkrankung der kleinen Hirngefäße (engl.: small vessel disease, SVD) und stellen ein Risikofaktor für einen hämorrhagischen Schlaganfall, einen ischämischen Schlaganfall, sowie für ein schlechtes funktionelles Endergebnis nach Schlaganfall dar. Obwohl eine Dyslipidämie mit dem Risiko eines ischämischen Schlaganfalls assoziiert ist, bleibt die Assoziation mit CMBs umstritten. Das primäre Ziel der vorliegenden Substudie der Berliner Cream&Sugar Studie, war es, den Zusammenhang zwischen CMBs und Dyslipidämie bei Patienten mit einem ersten ischämischen Schlaganfall zu untersuchen. **Methoden**: In diese Substudie wurden alle Patienten einbezogen, die in die Berliner Cream&Sugar Studie zwischen Januar 2009 und Oktober 2015 eingeschlossen wurden und für die kernspintomographischer T2*-gewichteter Gradientenechosequenzen vorlagen. 3 – 7 Tagen nach dem Schlaganfall wurden zirkulierende Lipidparameter (Gesamtcholesterin [engl.: total cholesterin, TC], Lipoprotein niederer Dichte [engl.: low density lipoprotein, LDL-C], Lipoprotein hoher Dichte [engl., high density lipoprotein, HDL-C] und TG) bestimmt und ein oraler Triglyzerid-Toleranz-Test (OTTT) und ein oraler Glukose-Toleranz-Test (OGTT) wurden durchgeführt. **Ergebnisse**: 291 Patienten konnten in dieser Substudie berücksichtigt werden (Durchschnittsalter 64.5 Jahre, SD ± 13; Durchschnitts-National Institutes of Health Stroke Scale [NIHSS] 1, IQR 0-2). CMBs wurden bei insgesamt 28 Patienten festgestellt (9.6%). Das Vorliegen von CMBs war signifikant seltener bei Patienten mit mittleren TC-Spiegeln (165-198 mg/dl), verglichen mit Patienten mit den niedrigsten TC-Spiegeln (<165 mg/dl) (adjustiertes Odds Ratio [OR] 0.20; 95% Konfidenzinterval [engl. confidence interval, CI] 0.10 - 0.76; p=0.018); bei Patienten mit den höchsten TC-Spiegeln (>198 mg/dl) war das adjustiertes OR 0.32 (95% CI 0.09 – 1.10, p=0.070). Zusätzlich zeigten sich statistisch signifikante Zusammenhänge zwischen CMBs und Ausprägung der Hyperintensitäten der weißen Hirnsubstanz (engl. White matter hyperintensities, WMHs) (Wahlund 0-3: Referenz; Wahlund 4-10: adjustiertes OR 6.1; 95% CI 1.8 - 21.2; p= 0.004; Wahlund >10: adjustiertes OR 10.8; 95% CI 2.9 - 39.4; p<0.01) und dem untersten Tertial von glomerulären Filtrationsrate (GFR >92.2 ml/min/1.73 m²: Referenz; GFR<75.5 ml/min/1.73 m²: adjustiertes OR 7.4; 95% CI 1.6 - 33.9; p=0.01). **Schlussfolgerung**: Zusammenfassend stellten wir fest, dass niedrige TC-Spiegeln, WMH Schwere sowie reduzierte Nierenfunktion mit CMBs signifikant

assoziieren. Weder nüchterne TC-Spiegeln noch die von uns untersuchten Parameter des TG Stoffwechsels waren mit dem Vorliegen von CMBs assoziiert. Weitere Studien könnten die Rollen von TC-Spiegeln und Nierenfunktion in der Entstehung von CMBs untersuchen. Anhand unserer Ergebnisse können wir jedoch keine weiteren Untersuchungen des Zusammenhangs zwischen CMBs und TG-Spiegeln / TG Metabolismus empfehlen.

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1. Introduction

1.1. Stroke definition and epidemiology

Stroke is one of the most common causes of death worldwide and is a leading cause of death and permanent disability in the industrialized world [1]. In Germany and other industrialized nations the mortality rate from stroke has declined, but survivors are often left with permanent and serious morbidities [2]. Stroke is generally (although not exclusively) a disease of advanced age, and approximately half of all stroke sufferers in Germany are over the age of 75. There are some 200,000 first time strokes in Germany every year [2], and as Germany's population continues to age, the incidence – as well as the associated human and economic costs – can only be expected to increase.
The definition of stroke, as established by the World Health Organization (WHO) in 1970 and still widely used, is a complex of "rapidly developing clinical signs of focal (or global) disturbance of cerebral function, lasting more than 24 hours or leading to death, with no apparent cause other than that of vascular origin" [3]. In order to account for post-1970 diagnostic advances, the American Heart Association and the American Stroke Association propose an updated definition that includes "brain, spinal cord, or retinal cell death attributable to ischemia" that is "based on neuropathological, neuroimaging, and/or clinical evidence of permanent injury" [4]. The WHO definition of stroke, however, is still widely applied and includes cerebral ischemia, cerebral hemorrhagic infarct, and subarachnoid hemorrhage. Ischemic stroke is the most common stroke form and accounts for at least 80% of strokes in Caucasian populations (up to 15% hemorrhagic stroke, 5% subarachnoid hemorrhage) [5].

1.2. Definition and diagnosis of cerebral microbleeds

Although stroke has been recognized and studied for decades, cerebral microbleeds (CMBs) only began appearing in the medical literature during the mid-1990s as magnetic resonance imaging (MRI) technologies became part of widespread clinical practice. As improvements in MRI software and hardware in recent years have improved imaging sensitivity, CMBs have become an increasingly common finding, both in apparently healthy elderly individuals [6–9] and at higher rates in subjects with known cerebrovascular diseases, including stroke [10]. Although the precise definition of CMB used varies according to the author, they are generally recognized as small (<5 mm, maximum 10

mm) round to ovaloid areas of hypointensity on T2*-weighted gradient echo or other susceptibility weighted MRI sequences [10,11]. See Figure 2 (E) below. Although macrobleeds produce similar hypointensities they can generally be differentiated on the basis of size: the two entities typically follow a bimodal size distribution (rather than a continuum), so that some variation in the precise size criteria used for CMBs should not present too much of a source of diagnostic error [12]. In histopathological studies, most (though not all) signal voids on T2*-weighted sequences have corresponded to hemosiderin deposits, often in macrophages, and (though there is some disagreement) these are generally thought to be the result of small localized hemorrhages [13–15]. For practical purposes, however, the term "CMB" in this study will refer specifically to the MRI phenomenon.

1.3. Cerebral microbleeds as a subform of small vessel disease

CMBs are generally considered part of a more generalized microangiopathy of the brain known as small vessel disease (SVD) [11,16]. SVD refers to a complex of clinical, radiological, and neuropathological findings thought to result from a pathology of the perforating cerebral arterioles, capillaries, and venules of the brain [17]. These vessels are critical for ensuring that the brain's most metabolically demanding nuclei and complex networks of white matter are sufficiently perfused [18]. The cerebrovascular endothelium has an area equivalent to that of a tennis court (around 260 square meters), and at rest the brain consumes some 20% of total cardiac output [19]. Although the pathophysiology has not been fully elucidated, the dysfunction of this massive endothelial system, which defines SVD, is the most common cause of vascular dementia, a key cause of mixed dementia and functional loss in the elderly, and is also blamed for some 20-30% of all strokes worldwide [17,20].

Apart from CMBs, SVD markers that can be identified with imaging include (again with some variation according to author) lacunar (or small subcortical) infarcts or hemorrhages, (cavitating) lacunes (thought to represent old fluid-filled infarcts), white matter hyperintensities (WMHs), perivascular spaces, and brain atrophy [10,21]. See Figure 1 below for a visual overview.

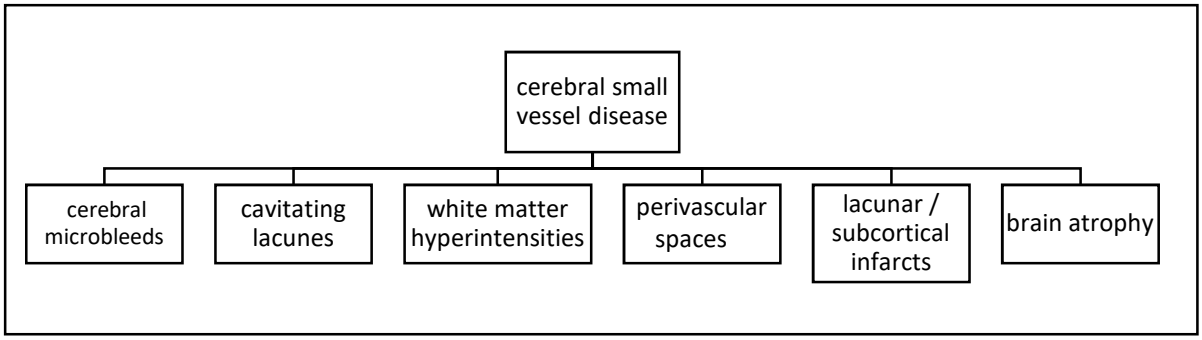


Figure 1. MRI-markers of cerebral small vessel disease

Several studies report associations between CMBs and these other indicators of SVD [22–24]. The different manifestations of SVD occur at different rates, however, both in healthy populations as well as in patients with known cerebrovascular diseases. Some differences in risk factors have also been identified. While smoking, for example, seems to associate with WMH [25–28], the findings regarding smoking and CMBs are much more tenuous [9,10,29–32]. This may be due to some variation in underlying pathology or may suggest different stages of the disease. All manifestations of SVD, however, are thought to ultimately derive, at least in part, from damage to the small vessels of the brain. This is likely associated with age, but the observed changes may also appear at an accelerated rate in patients with certain risk factors such as hypertension and diabetes mellitus [33].

1.3.1. Imaging features of small vessel disease

Lacunar / small subcortical strokes

A lacunar stroke is usually a small infarct that appears within the territory of a perforating arteriole. A lacunar infarct manifests itself as a round to oval, or sometimes tubular, hyperintensity on diffusion weighted imaging (DWI) or fluid-attenuated inversion recovery (FLAIR) MRI sequences. See Figure 2 (A) below. Lacunar infarcts are generally between 5 mm and 10 mm in diameter, although some authors suggest an upper range of 15 mm or even 20 mm [34,35]. Although computer tomography (CT) can sometimes be used in diagnosis, only about 50% of lacunar infarcts appear on CT, while some 70% or more can be seen with MRI. Small lacunar infarcts are often clinically silent, and although they sometimes disappear completely, they often leave a scar that can be visualized radiologically as a cavitating lacune or a WMH [36,37].

Cavitating lacunes

Cavitating lacunes are thought to represent fluid-filled cavities, occurring in the white matter or the deep grey matter of the brain and resulting from old lacunar infarcts [21]. They appear as small (between 3 mm and 20 mm depending on the author) areas of hypointensity, often with rims of hyperintensity, on FLAIR or T1-weighted MRIs or as hyperintensities on T2-weighted MRI sequences [35]. See Figure 2 (C) below. They may be preceded by symptoms but often appear silently and represent an incidental finding [21]. Of the acute lacunar infarcts that are confirmed by DWI rates of between 28% and 94% are reported to ultimately become cavitating lacunes, a broad range that is due in part to variations in the definition of cavitation applied [36].

White matter hyperintensities (WMHs)

WMHs or "leukoaraiosis" appear in the deep white matter of the brain and especially in the periventricular spaces as variously sized patches of increased signal intensity on T2-weighted, positron density, T2*-weighted, and FLAIR MRI sequences. FLAIR is generally considered the most sensitive means of detecting WMHs. See Figure 2 (B) below [35]. While histopathological studies of WMHs have focused on demyelination and axonal degeneration as correlates, these changes may be preceded by pathological accumulations of extracellular fluid that can only be visualized on MRI. They may stem from acute lacunar infarcts but are generally clinically silent [38].

Perivascular spaces or Virchow-Robin spaces

Perivascular spaces (PVSs) are thought to represent fluid that has extravasated due to small vessel damage [34]. They generally appear in the tissue surrounding the small deep arterioles that connect the deep grey matter and white matter of the brain [19]. Perivascular spaces tend to appear as linear (except in cross-section) hypointensities of ≤3 mm (without a rim of hyperintensity) on FLAIR or T1-weighted MRI sequences or as hyperintensities on T2-weighted MRI sequences. They often appear following a vein [39]. See Figure 2 (D) below. A small number of PVSs may appear normally at any age [40], and in postmortem studies they were long dismissed as artifacts of tissue preparation [41]. They appear, however, in significantly greater numbers in subjects with other features of SVD [42] as well as in acute ischemic stroke patients and are now considered a definite indication of cerebral small vessel pathology [43].

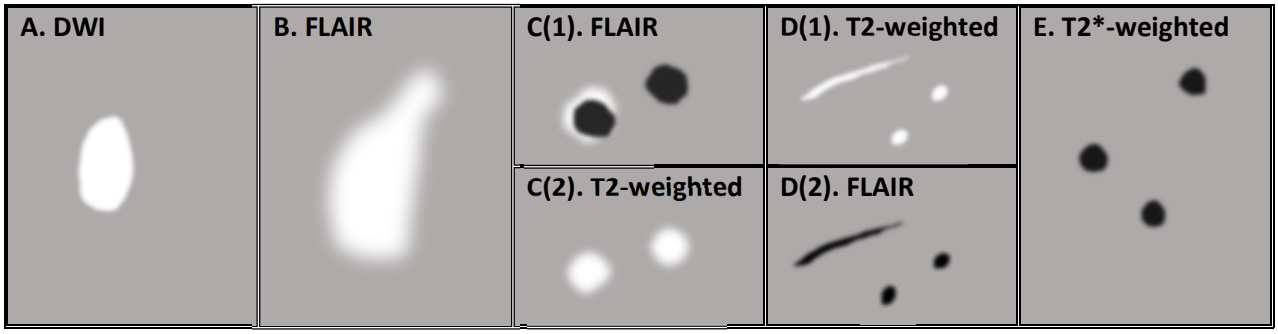


Figure 2. Schematic view of small vessel disease associated imaging findings: A. Recent subcortical infarct as ≤20 mm ovaloid hyperintensity on diffusion-weighted imaging (DWI) MRI, **B.** White matter hyperintensity as hyperintesity of varying shape/size on fluid-attenuated inversion recovery (FLAIR) MRI, **C.** Cavitating lacunes as (1) hypointensities, possibly with hyperintense rims, on FLAIR MRI (2) as hyperintensities on T2-weighted MRI, **D.** Perivascular spaces as linear or round (1) hyperintensities on T2-weighted MRI or as (2) hypointensities on FLAIR MRI, **E.** Cererbral microbleeds as hypointensities on T2*-weighted or other susceptibility weighted MRI sequences. (Figure adapted from Wardlaw, J. M. *et al.* Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. *Lancet. Neurol.* **12,** 822–38 (2013)).

Brain atrophy

Brain atrophy may be generalized or may only affect a specific brain region. It occurs with normal aging and is not necessarily indicative of neuronal loss [44]. In SVD, however, the pathological correlates of arteriolosclerosis, venous collagenosis, and white matter rarefication, can be observed in postmortem studies [45,46]. In the context of SVD, brain atrophy is defined as reduced brain volume that cannot be attributed to a focal injury such as infarct or trauma. It can be estimated on the basis of enlarged peripheral and central (ventricular) spaces. Observation of changes in brain volume over time also supports the diagnosis of brain atrophy. FLAIR, T1-weighted, T2-weighted, T2*-weighted, and other susceptibility-weighted MRI sequences can all be used to detect it [35].

1.3.2. General pathology of cerebral small vessel disease

Although the underlying mechanisms of cerebral SVD are not fully understood, the most commonly described histopathological finding is a diffuse arteriolosclerosis, lipohyalinosis or fibrinioid necrosis of the cerebral small vessels [19,47]. Three subforms of SVD are recognized. These include (1) atherosclerosis/arteriosclerosis, usually seen in the small intracerebral and leptomeningeal arteries (200–800 µm in diameter), (2) lipofibrohyalinosis, seen in smaller arteries (40 – 300 µm), and (3) arteriolosclerosis, also seen in smaller arteries (40 – 300 µm) [34,48]. The first vessels affected by SVD are usually

the arteries of the basal ganglia, which may exhibit signs of atherosclerosis or lipofibrohyalinosis. These changes are generally followed by arteriolosclerotic or lipofibrohyalinotic damage to the small arteries of the white matter. The leptomeningeal arteries of the cerebellum may exhibit signs of atherosclerosis as well, and the vessels of the brain stem may also develop lipofibrohyalinosis or arteriolosclerosis. This, however, generally only occurs in the advanced stages of SVD [34].

Pathology studies of small vessel atherosclerosis demonstrate proliferation of the endothelial cells, damage to the lamina elastic interna, and luminal microatheroma, containing plasma proteins, lipids, lymphocytes, and macrophages. Just as in large vessel atherosclerosis these microplaques can rupture and result in thrombotic occlusion [34,49].

Arterioloscleroisis of the small arteries is thought to result in large part from hypertension, which mechanically forces plasma components and lipids into the vascular walls and perivascular spaces. The high pressure also results in a reactive hyaline thickening of the vascular walls and subsequent concentric stenosis [34].

Lipofibrohylinosis, also observed in the small cerebral arteries, appears to be related to a dysfunction of the endothelium. Histopathologically it is characterized by fibrinoid necrosis and the presence of foam cells in the vascular walls [50]. Although the reasons for this endothelial dysfunction are not fully understood, it seems to play a key role in SVD. Tight junctions consisting of occludins and claudins join the endothelial cells lining the small vessels of the brain and form a key component of the blood-brain-barrier [51]. As this barrier breaks down and the endothelium becomes more permeable, lipids and other plasma contents diffuse and collect in the vascular walls. As the vascular walls thicken, inflammatory cells also migrate in, beginning a cycle of increased endothelial permeability, further deposition of lipids and fibrin, and further inflammation. The vascular lumen becomes smaller and can even become occluded, resulting in an infarct [52]. If atherosclerotic plaques have accumulated on the vascular walls these can also rupture and cause a thrombotic occlusion (generally in somewhat larger vessels) [53].

Even when complete occlusion does not occur, the collection of debris in the vascular wall and the associated inflammation damange the vascular smooth muscle, which is critical for autoregulation of blood flow in the arterioles [54]. As the vessels stiffen and become unable to respond to increased blood flow requirements (during exertion for example), matrix metalloproteinases become activated, resulting in further endothelial damage [55].

In the capillaries where smooth muscle is lacking, endothelial failure results in the leakage of plasma into the surrounding tissue. This resultant edema is likely one source of the WMHs which can be seen on MRI even at early stages of SVD when there is no histopathological correlate [19,34]. Perivascular fluid build-up is ultimately toxic to brain cells, and as the edema persists cells die, eventually resulting in the rarefaction, demyelination, and glial scarring that can be seen both histopathologically and radiologically [56].

1.3.3. Pathophysiology of cerebral microbleeds

Radiologically observed CMBs often (though not always) correlate with focal hemosiderin deposits [13,15], and in histology based investigations lipofibrohyalinosis and other indications of small vessel pathology are frequently observed in the small vessels proximate to these hemosiderin deposits [13,49]. One explanation for this is that SVD related damage to the cerebral arterioles renders them unable to adequately regulate blood flow so that – especially in the context of hypertension – already damaged vessels become more prone to rupture [19,34].

Histology based studies of CMBs specifically, however, are scarce, and their underlying pathophysiology remains the subject of debate among neuropathologists [13,14]. Janeway et al. have even proposed that CMBs are not always "bleeds" at all, but result instead from small areas of SVD related ischemia, which in turn results in oligodendrocyte death and the subsequent release of their iron stores [14]. CMBs associate with a reduction in resting state cerebral perfusion [57], and in the context of exertion the loss of adequate autoregulation could indeed result in areas of hypoperfusion [34]. Ultimately, however, it is currently not possible to determine whether the observed hemosiderin deposits (or in the case of MRI hypointensities) are due to hemorrhages, to local oligodendrocyte death, or to another cause [14]. "Cerebral microbleeds" remains the accepted label for areas of signal loss on T2*-weighted MRI sequences, and the vast majority of studies on the topic are imaging based [35]. Should further (especially histology based) investigations provide sufficient evidence for non-hemorrhagic causes, however, then the term "CMB" may eventually need to be revised.

1.3.4. Cerebral microbleeds not associated with small vessel disease

Although CMBs can appear in lobar regions of the brain they occur first [34] and most frequently [29,58] in deeper, subcortical brain regions. CMBs occurring exclusively in the

lobar cortices are generally attributed not to SVD, however, but to cerebral amyloid angiopathy (CAA) instead [14,22]. CAA refers to the damage that occurs when beta-amyloid deposits accumulate in the vascular walls. CMBs due to CAA are not associated with the same risk factors as SVD-related CMBs but are instead associated with Alzheimer's dementia in genetically susceptible individuals [59]. Although a definitive differentiation between SVD- and CAA-related causes is not possible in vivo, the Boston criteria use clinical history and anatomical location to make the CAA diagnosis. CMBs appearing only in the lobar cortices are attributed to CAA, while those occurring in subcortical regions, are attributed to SVD [60]. The Boston criteria also consider whether fitting clinical symptoms (dementia) are present and whether an alternative explanation (such as trauma, ischemic stroke or tumor) for the observed CMBs exists [61,62]. Moreover, there does seem to be an association between strictly lobar CMBs, APOE ε4 (which is associated with Alzheimer's disease), and manifest dementia [9], associations which do not apply to CMBs in non-lobar locations [9,31].

1.4. Epidemiology of cerebral microbleeds

Population based studies have reported CMB prevalence ranging between 6% and 19% [7,31,63,64]. MRI studies restricted to older populations have reported higher rates of up to 38%, a span that may be due to differences in MRI magnet strength as well as definitions applied [9,64,65]. Average rates of CMBs are even higher in patients with known cerebrovascular diseases. In ischemic stroke patients the reported rates range between 12% and 71%, with higher average rates in patients experiencing a recurrent stroke compared to those with a first time stroke [10]. CMBs have been reported to occur in some 60% of patients with intracerebral hemorrhage (ICH). Here, however, there seem to be some ethnic differences, with a recent meta-analysis reporting that CMBs were present in 47% of Caucasian ICH patients, compared to 67.5% of Asian ICH patients [10]. This may be related to higher rates of hypertension among Asians [66]. Although far fewer postmortem studies exist, focal hemosiderin deposits too small to be seen on MRI appear at much higher rates than radiologically diagnosed CMBs [14]. Both MRI and histology based studies have report significant correlations between CMBs and advanced age [7,14,29].

1.5. Clinical significance of cerebral microbleeds

CMBs seem to associate with increased mortality both in healthy populations and in stroke patients [67]. They appear at higher rates in patients with recently diagnosed ischemic stroke [10,68] and develop at an accelerated rate in the months and years following ischemic stroke [69]. Patients with CMBs also have worse functional outcomes after ischemic stroke [70]. Furthermore, CMBs are associated with an increased risk of recurrent ischemic stroke in Western cohorts [71], and in asymptomatic patients they increase the risk of both first time ischemic stroke and transient ischemic attack (TIA) [6,72].

CMBs increase the risk of hemorrhagic stroke as well, both in healthy populations and in ischemic stroke patients, a risk that is significantly higher in Asian populations [11,29,71,73,74]. After controlling for confounders, patients taking antiplatelet or anticoagulant drugs do not have higher rates of CMBs [10,75,76], but when CMBs are already present these drugs may further increase the (already elevated) ICH risk [77,78]. When incidental CMBs are discovered in acute ischemic stroke patients, clinicians are confronted with the question of whether or not to give thrombolysis. There is a growing body of evidence to suggest that patients with CMBs are at even greater risk of ICH when receiving thrombolysis [79,80] and may have worse functional outcomes after thrombolytic therapy [81]. Not all studies, however, have corroborated this finding [82,83], and in those that do it is difficult to rule out bias and confounders [79]. For these reasons, although the recommendations may eventually change, the presence of CMBs is not currently considered an absolute contraindication for thrombolysis [84].

CMBs have a clear function as a prognostic marker. The question remains, however, which risk factors exist and whether any of them are modifiable. Although some studies have identified a relationship between CMBs and potentially modifiable vascular risk factors such as hypertension [9,65] the influences of other lifestyle-oriented and potentially modifiable risk factors such as excessive body mass index (BMI), metabolic syndrome, and pathological lipid levels remain unclear [31]. Identification of modifiable risk factors could mean potential targets for intervention to prevent the development of CMBs and their associated complications, including a second stroke.

1.6. Lipid profiles associated with cerebral microbleeds and other forms of small vessel disease

Plasma lipid levels are one of the more pharmacologically modifiable potential risk factors [85]. Dyslipidemia has long been implicated in cardiovascular disease [86], and atherosclerotic micro-occlusions seem to play a role in cerebral small vessel pathology as well [34]. Fat-filled macrophages surrounding affected cerebral vessels have additionally been observed [49], and the affected vascular walls often contain fatty hyaline build-up (lipohyalinosis) [9,15,34]. This raises the question of whether elevated plasma lipid levels may play a role in the pathophysiology of SVD and CMBs too. So far, however, the clinical and epidemiological evidence on lipid levels and their relationship to CMBs and other makers of SVD has not provided any clear answers.

1.6.1. Cholesterol

Most population based studies have reported that total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) do not affect CMB risk [6,29,30,65,87], while a few have even reported that very low TC or LDL-C increase CMB risk [9,31,87]. Most [9,31,65], though not all [30] population based studies, have found that high-density lipoprotein cholesterol (HDL-C) plays no role in CMB risk. The role of statins in CMB risk is also unclear, with one larger [31] and one smaller [88] study having found that they increased CMB risk and another smaller study having found that they did not [89].

The relationship between cholesterol and CMBs in ischemic stroke patients has been less well studied. In one investigation high HDL-C associated with greater CMB prevalence [90], in another study (albeit of TIA patients) high LDL-C associated with fewer CMBs [69], and in the remaining studies of ischemic stroke patients only TC was considered and was not significant [91,92].

The evidence regarding cholesterol and other manifestations of SVD is also mixed. While some studies have found that lower LDL-C levels associated with a greater WMH burden [25,93] and lacune presence [93], others reported a non-association [94–96]. HDL-C has been infrequently studied, but a recent population based study with 2,608 participants found no association between HDL-C and WMHs or lacunes [93].

1.6.2. Triglycerides

Triglyceride (TG) levels have been less well investigated, but they present a particularly interesting parameter in the study of CMBs and other forms of SVD. In addition to their

established role in large-vessel atherosclerosis [97], they may play role in cerebral SVD, possibly by way of a pro-inflammatory release of free fatty acids during the lipolysis of TG-rich lipoproteins [98]. TG levels have been shown to associate with plasma biomarkers of inflammation [99], and these in turn to associate with radiological markers of cerebrovascular disease such as WMHs and lacunar infarcts [100]. Elevated TG levels are also associated with a dysfunction of the blood-brain barrier [98], and in light of the key role that endothelial (blood-brain barrier) dysfunction seems to play in SVD [34] the potential role of TGs is particularly interesting. Moreover, there is evidence that high TG levels compromise small-artery compliance, also contributing to chronic hypoperfusion, ischemia, and vascular fragility [25,54].

The clinical and epidemiological evidence, however, is once again both limited and conflicting. A population based study found an association between CMBs and the lowest level of serum TGs [30], while a study of ICH patients found that CMBs were associated with higher TG levels [101]. Yet another study of ICH patients found that TGs had no effect on CMB risk whatsoever [102]. Both patient-based studies, however, were limited by small sample sizes, and none involved ischemic stroke patients. Regarding other manifestations of SVD, some studies found that increased TG levels associated with severity or progression of WMHs or cavitating lacunes [19,39,93,103,104], while others reported non-associations [95,99].

1.7. Lipids and stroke

Regarding ischemic stroke, many – but not all – observational studies have identified higher TC [105–111], higher LDL-C [108,110,112] and lower HDL-C [107,108,112–117] as risk factors. The results regarding plasma TGs and ischemic stroke are mixed [114,118–122], but this may be at least partially due to heterogeneity in terms of fasting vs. non-fasting TG levels [123], and the cumulative tendency suggests that elevated TG levels increase the risk of ischemic stroke. TC and TG levels that are too low, however, may increase the risk of hemorrhagic stroke [106 111,121,124–126], as sufficient lipid levels may be necessary to maintain the fitness of arterial smooth muscle [30].

1.8. Investigation of cerebral microbleeds in this study

1.8.1. Study purpose and approach

Lipid levels are one of the pharmacologically more modifiable risk factors, and although lipid reduction has an established role in the treatment of ischemic stroke patients [127], it is unclear what role circulating lipid levels play in the development of CMBs. The bulk of the literature on CMBs draws from population based studies, but due to the strong association between CMBs and ischemic stroke we propose an investigation of lipid profiles (TC, LDL-C, HDL-C, and TG) and CMBs in first time ischemic stroke patients. Although population based studies allow for much larger sample sizes, their investigations of lipid parameters are often limited by heterogeneity in terms of fasting vs. non-fasting states as these are more difficult to control in the non-clinical setting. The clinical setting, on the other hand, allows for stricter control to ensure the accuracy of measured serum lipid values.

Although all main lipid fractions are of interest, TGs have been least well researched, particularly as they relate to CMBs, and their potentially pro-inflammatory effects make them of particular interest. Fasting TG levels were once considered the gold standard for avoiding artificial variability among study participants. Some larger epidemiological investigations, however, have suggested that measurements of TG levels after consumption of a given amount of fat (postchallenge TGs) may be a better indicator of TG-associated risk for cerebrovascular disease [118,119]. Since humans in industrialized countries spend a significant portion of their lives in non-fasting states, peak TG levels, as well as speed of TG metabolism may more accurately reflect the overall TG burden. For this reason an oral TG tolerance test (OTTT) has been recommended for the clinical practice [128]. Our study takes this approach as well: We consider not only fasting TGs but postchallenge and peak TGs, too. In this way we investigate TG metabolism with a precision not yet attempted in existing CMB studies and seek to determine whether more differentiated TG indicators associate with CMBs.

In summary, the purpose of this study is to:

1. Investigate the relationship between CMBs and different lipid fractions (TC, LDL-C, HDL-C, TG) in the fasting state in first time ischemic stroke patients.
2. Investigate the relationship between CMBs, postchallenge TGs, and TG metabolism in first time ischemic stroke patients (details under "Methods").
3. Investigate possible associations between CMBs and other risk factors in first time ischemic stroke patients.

1.8.2. Study hypothesis

We hypothesize that the presence of CMBs in first-time ischemic stroke patients is associated with fasting plasma markers of dyslipidemia, as well as with greater overall TG burden, indicated by slower TG metabolism and higher peak TG levels.

2. Methods

This is a retrospective substudy of the Berlin Cream&Sugar study (NCT 01378468). The Cream&Sugar study is a prospective cohort study of first time ischemic stroke patients, which seeks to determine whether postchallenge TG levels, measured as part of a standardized OTTT, associate with the risk of recurrent stroke, TIA, or other cardiovascular events in the year following the first ischemic stroke [129]. The Cream&Sugar study was designed in accordance with the principles of the Declaration of Helsiniki, and the study protocol, patient information, and patient informed consent were submitted to and approved by the ethics committees for all recruiting sites (EA4/100/08). Additionally, the study was registered with the European Union Drug Regulating Authorities Clinical Trials (EudraCT number 2009-010356-97) and at ClinicalTrials.gov (NCT 01378468).

2.1. Study participants

All first-time ischemic stroke patients over the age of 18 years, who were admitted to one of the three Charité university hospitals in Berlin between January 2009 and October 2015, were screened for inclusion in this study. Patients were admitted on the basis of clinical stroke symptoms, and the diagnosis of stroke was confirmed with MRI (details under "Image acquisition"). For the purpose of this study the diagnosis of ischemic stroke was established on the basis of the WHO definition of a focal neurological deficit lasting for at least 24 hours with no indication of acute cerebral hemorrhage on imaging. Here, the strokes were considered first time events if there was no known previous stroke in the patients' medical histories. Any radiological indications of previous “silent strokes” were disregarded. Patients were screened for study inclusion 3-7 days after the onset of stroke symptoms. Before giving their consent all patients were informed in detail of the purposes and expected benefits and risks of this study. The inclusion and exclusion criteria are given below in Table 2.1. For inclusion in this retrospective substudy patients were additionally required to have received a T2*-weighted gradient echo MRI to permit detection (or exclusion) of possible CMBs.

Table 1. Inclusion / exclusion criteria for Cream&Sugar study

Inclusion criteria	Exclusion criteria
First ischemic stroke	Life expectancy under 12 months
Onset of stroke symptoms at least 3 days and no more than 7 days before study inclusion	Second stroke before oral tolerance tests
Age at least 18 years	Dysphagia
Patient able to give informed consent (self)	Inability to give (own) informed consent
*Received T2*gradient-echo MRI**	Lactose intolerance
	Malabsorption syndrome
	Pregnancy
	Renal or hepatic failure
	Pancreatitis
	Cholecystolithiasis
	Severe drug addiction (including alcoholism)
	Aphasia, psychosis, dementia

For inclusion in this substudy

2.2. Stroke severity and classification

Stroke severity was evaluated 3-7 days after onset of symptoms on the basis of the National Institutes of Health Stroke Scale (NIHSS) [130]. See the Appendix for the NIHSS evaluation form used.

2.3. Analysis of cerebral MRI

In this substudy we investigated the prevalence of CMBs, WMHs, and cavitating lacunes within this cohort. We chose these additional manifestations of SVD as they are easily recognizable on MRI [35]. Additionally, WMHs are thought to represent a more mild form of SVD, while cavitating lacunes, like CMBs, are thought to represent a more severe form [131].

2.3.1. Image acquisition and analysis

Stroke patients that participated in the Cream&Sugar study at one of the three Charité university hospital locations and who received an MRI evaluation, which included (1) T2*-weighted gradient echo sequences and (2) FLAIR or T2-weighted MRIs, were included

in this substudy. Either a 3-Tesla scanner (Tim Trio; Siemens, Erlangen, Germany) at Campus Benjamin Franklin or a 1.5-Tesla scanner (Avanto; Siemens, Erlangen, Germany) at Campus Mitte and Campus Virchow were used. T2*-weighted images were used to identify or exclude the presence of CMBs. FLAIR images were used to evaluate for possible WMHs and lacunes. If no FLAIR but T2-weighted imaging was available, the latter was used for this purpose. Patients who did not receive the necessary imaging (i.e. stroke diagnosis via CT) were excluded from this substudy. All MRIs were evaluated by a professional radiologist.

2.3.2. Definition of cerebral microbleeds

Our target in this substudy was to identify either the presence or absence of any (≥1) CMB, which we defined as a rounded or oval hypointensity <10 mm on a T2*-weighted sequence, surrounded by cerebral parenchyma that measures at least half the diameter of the hypointensity [11]. See Figure 3 below. The presence or absence of CMBs was determined by a professional radiologist, blinded to the intentions of this study.

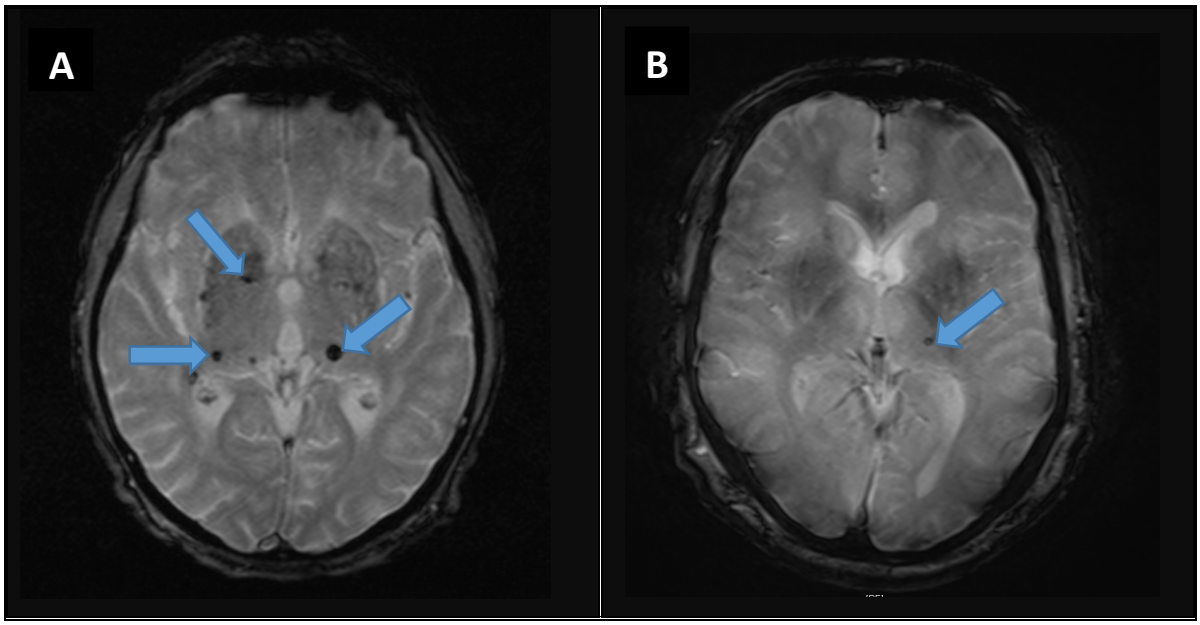


Figure 3. T2*-weighted MRI images depicting cerebral microbleeds in two patients from our study: A. A 76 year old male patient with multiple cerebral microbleeds in the basal ganglia and thalami (marked with arrows). **B.** A 72 year old male patient with a microbleed in the left thalamus (marked with an arrow).

2.3.3. Definition of white matter hyperintensities

WMHs were defined as bright lesions ≥5mm on FLAIR or T2-weighted images [38]. All MRIs were initially reviewed by a professional radiologist. Images were then evaluated by a

Cream&Sugar investigator and rated according to the Age-Related White Matter Change classification system (Wahlund score) [132]. All hyperintensities were given a score of between 0 and 3, based on size, lesion confluence, and location. The frontal, parieto-occipital, temporal, infratentoraial regions, and the basal ganglia on either side were all considered. The final score was the composite of scores for each region, ranging from 0 for no WMHs to 30 for maximum WMH severity [132]. This data was drawn retrospectively from the Cream & Sugar archive. See Figure 4 below.

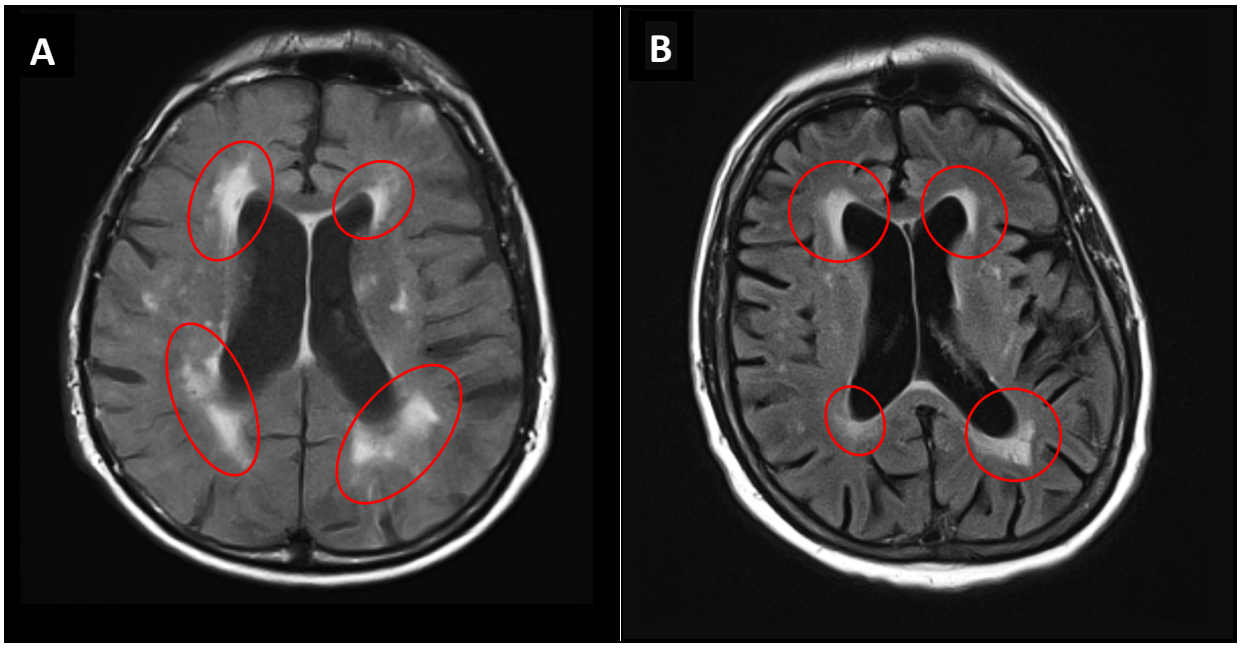


Figure 4. MRI images depicting white matter hyperintensities in two patients from our study (circled): A. Fluid-attenuated inversion recovery (FLAIR) MRI from an 85 year old male patient with a Wahlund score of 8. **B.** FLAIR MRI from a 67 year old male patient with a Wahlund score of 6.

2.3.4. Definition of cavitating lacunes

Cavitating lacunes were defined as small ovoid (3-20 mm) hypointensities, possibly surrounded by a rim of hyperintensity, on FLAIR MRI sequences, or as hyperintensities on T2-weighted images [131]. Perivascular spaces may display similar signal intensities but are smaller, so that all questionable lesions smaller than 3 mm were assumed to be perivascular spaces [94]. After initial evaluation by a professional radiologist, the presence or absence of cavitating lacunes was determined according to the above given definition by a member of our research group, investigating them as part of another project [103]. The data was then drawn retrospectively from the Cream&Sugar archive. See Figure 5.

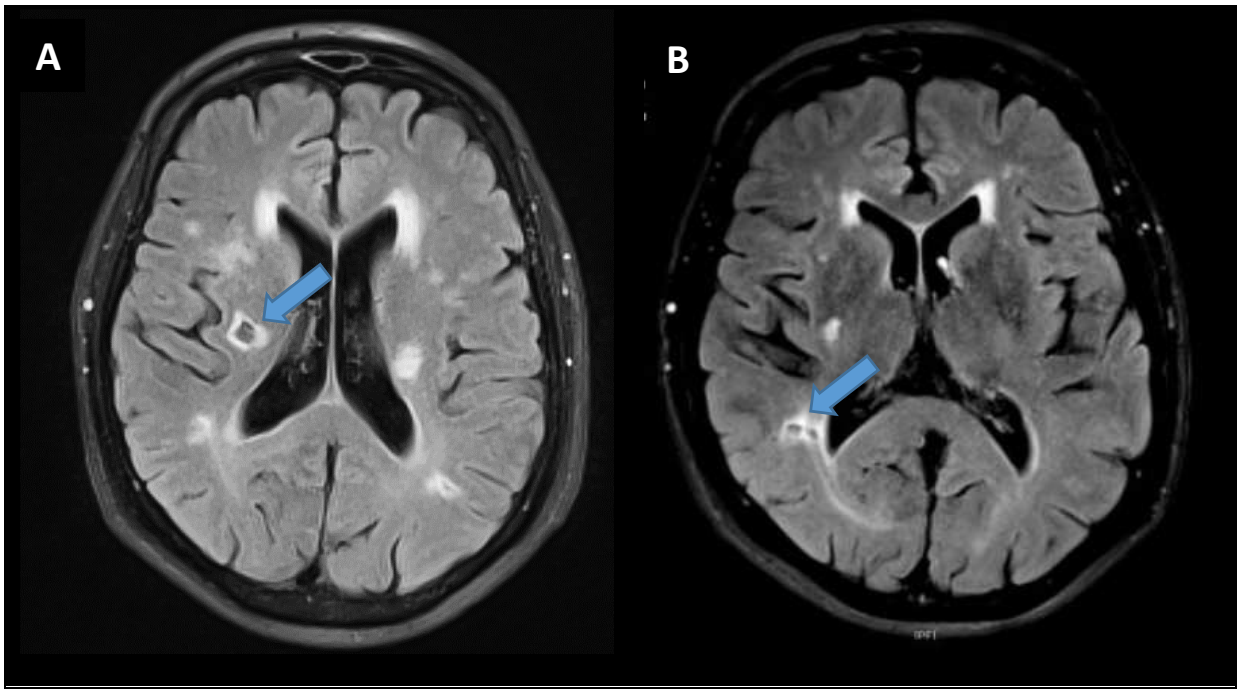


Figure 5. MRI images depicting cavitating lacunes (arrows) in two patients from our study: **A.** Fluid-attenuated inversion recovery (FLAIR) MRI from a 62 year old male patient. **B.** FLAIR MRI from a 63 year old male patient.

2.4. Compilation of clinical data on study participants

2.4.1. Medical history and physical examination in hospital

Patients who gave their informed consent to participate in the Cream&Sugar study provided a medical history, which was documented in a case report form (See Appendix). A physical examination was performed by a trained Cream&Sugar investigator, and patients were questioned regarding known preexisting conditions such as diabetes mellitus, hypertension, hyperlipidemia, and coronary heart disease, and whether they were currently taking medications for these conditions. Patients were also questioned regarding smoking and were classified as never smokers, former smokers, or current smokers.

Hypertension was considered present in all patients who had previously been diagnosed with hypertension and were on antihypertensive medications at time of hospital admission. Additionally, systolic and diastolic blood pressures were measured during the hospital stay and analyzed as continuous variables.

BMI was calculated as weight in kilograms divided by height in meters squared (kg/m^2) [133]. Waist circumference was measured at the umbilicus and hip circumference at the

largest circumference by trained clinical staff. Waist-to-hip ratio (WHR) was subsequently calculated.

2.4.2. Baseline serum parameters

Unless otherwise indicated all baseline serum parameters indicate measurements taken before intervention at ca. 8 a.m., after fasting overnight for ≥ 12 h. Serum parameters were assessed using freshly drawn samples of venous blood, analyzed in a Cobas 6000 analyzer (Roche/Hitachi) at the laboratories of Charité university hospital. LDL/HDL ratio was also calculated. Hypercholesteremia was defined as fasting TC ≥ 190 mg/dL. Prior Statin use was ascertained by questionnaire at time of study inclusion.

Additionally, we measured (fasting) levels of glucose, insulin, glycosylated hemoglobin (HbA1c), C-reactive protein (CRP), alanine transaminase (ALAT), aspartate transaminase (ASAT), creatinine, and thyroid stimulating hormone (TSH).

Diabetes mellitus was considered present in patients receiving antidiabetic medications at time of hospital admission or who had HbA1c levels ≥ 6.5% [134].

The glomerular filtration rate (GFR) was estimated using the Modified Diet and Renal Diseases (MDRD) formula: $GFR = 186 \times (\text{serum creatinine})^{-1.154} \times (\text{Age})^{-0.203} \times (0.742 \text{ if female}) \times (1.212 \text{ if black})$ [135].

The Homeostasis Model Assessment Insulin Resistance Index (HOMA-IR) was calculated using the formula: fasting insulin (mIU/l) x fasting glucose (mg/dl) / 405 [136].

2.4.3. Oral tolerance tests

Within 30 minutes of acquiring fasting venous blood samples (ca. 8 a.m.), study participants were required to drink 250 ml of 32% fat cream. A staff member from the Center for Stroke Research Berlin was present to ensure that the cream was consumed. Three hours after cream consumption (ca. 11 a.m.) a second venous blood sample was obtained. Immediately after the second blood draw a standard 75 gram oral glucose tolerance test (OGTT) was performed in all patients without currently known diabetes. In this way, baseline TGs (fasting) as well as TG levels at 3, 4, and 5 hours after cream consumption were recorded and analyzed as continuous variables. Additionally, we obtained and analyzed levels of glucose and insulin, both in the fasting states and at 3, 4, and 5 hours. See Figure 6 for a visual representation of the testing procedure.

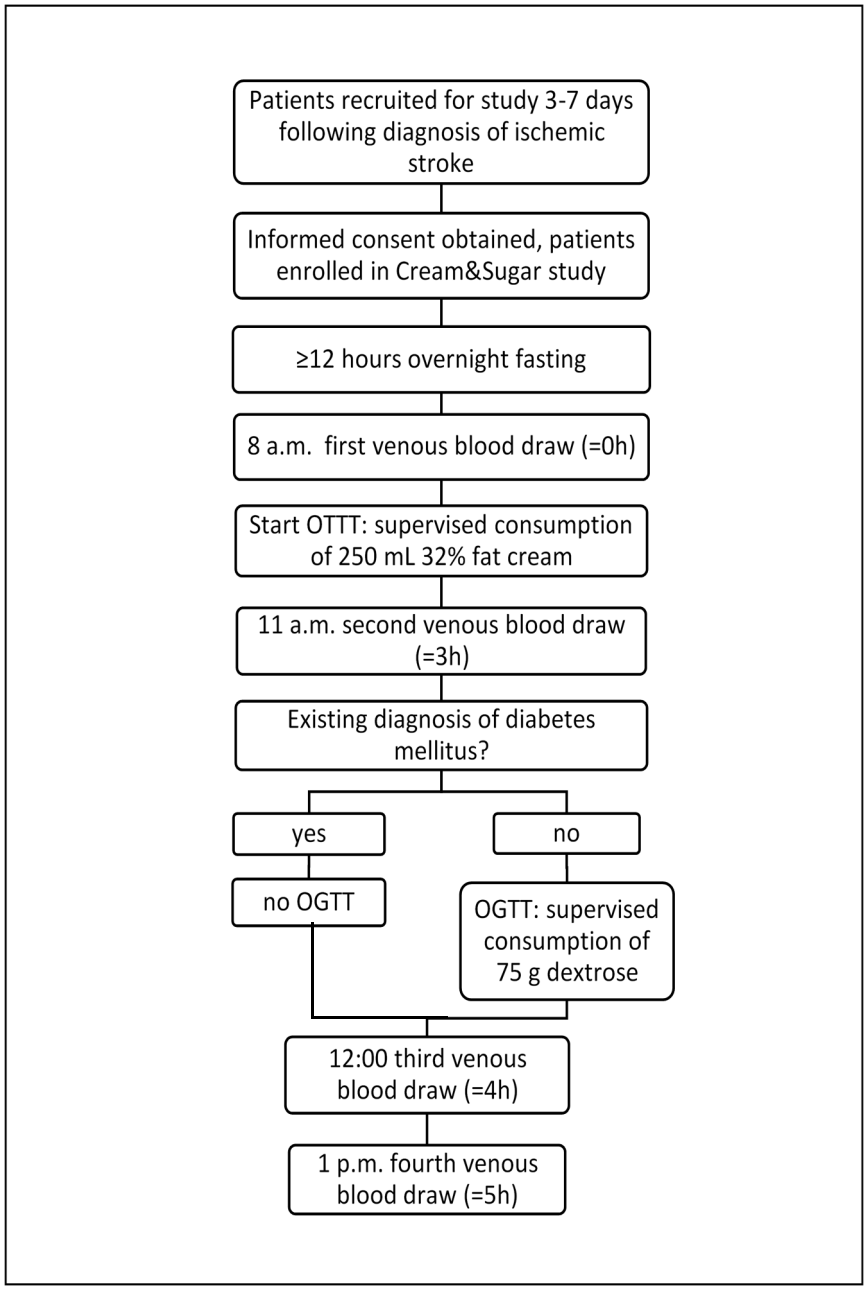


Figure 6. Visual representation of testing procedure. OTTT indicates oral triglyceride tolerance test; OGTT indicates oral glucose tolerance test.

Additionally, we calculated the area under the TG curve (TG AUC), as the integral of TG levels between baseline and 5 hours. We also considered TG variance by subtracting the peak TG measurement from the baseline TG measurement. We then grouped patients on the basis of peak TGs and fasting TGs, as described by Schmidt-Trucksäss et al. [137]. Patients in whom both fasting and peak TGs < 200 mg/dL were grouped in one category. Patients with fasting TGs < 200 mg/dL but peak TGs ≥200 mg/dL were placed in a second

category. Patients with both fasting and peak TGs ≥200 mg/dL were placed in a third category for analysis.

Finally, we classified patients as either slow or fast TG metabolizers as follows:

1. “Fast metabolizer”: TG peak at 3h
2. “Medium metabolizer” TG peak at 4h
3. “Slow metabolizer”: TG peak at 5h (or later).

2.5. Statistical analysis

To determine whether laboratory and clinical parameters were associated with the presence of CMBs, we performed statistical analyses in a two-step process. First, we conducted a univariate analysis, and second we performed a logistical regression analysis as outlined below. All statistical analyses were performed using SPSS software for windows (IBM, SPSS Statistics, version 21). Unless otherwise specified a 2-sided p-value of <0.05 was considered statistically significant.

2.5.1. Univariate analysis

Continuous variables were assessed for normal distribution visually (graphically/bell-shaped curve) and by skew. Variables with a skew from -1 to 1 were considered normally distributed. Relationships between normally distributed continuous variables (i.e. age, GFR, TC) and CMBs were assessed in bivariate analyses using t-tests. Relationships between non-normally distributed continuous variables (i.e. BMI, WMH, CRP) and CMBs were assessed using Mann-Whitney-U test. For normally distributed continuous variables, we report the mean and standard deviation (SD). For non-normally distributed continuous variables, we report the median and interquartile range (IQR).

Bivariate comparisons across categorical variables and CMBs were performed using Chi-squared and Fischer’s exact tests. Here the observed frequency of the individual cells were compared with the expected frequency. In the case of small groups (expected cell counts of less than 5), Fischer´s exact test was used. If a significant association was identified, Sidak’s post hoc analysis (level of significance 0.017) was performed.

2.5.2. Logistical regression analysis

All variables that had an association of p≤0.10 in the univariate analysis were incorporated into a forward stepwise binary logistical regression analysis using the Hosmer-Lemeshow goodness of fit test. CMBs (present vs. not present) was set as our

dependent variable. Continuous variables that did not exhibit a linear relationship were stratified into tertiles. WMHs and cavitating lacunes were both incorporated into the initial logistical regression analysis. We then performed a second logistical regression analysis in which WMHs and cavitating lacunes were excluded.

3. Results

3.1. Study population

Between January 2009 and October 2015 a total of 777 first time ischemic stroke patients were recruited for the Berlin Cream&Sugar study. 232 patients, after initially giving their consent, chose not to or were unable to complete the testing procedures, leaving 545 patients. For this retrospective substudy we considered all patients who had received the appropriate imaging (T2*-weighted MRI and T2-weighted or FLAIR MRI) to permit the diagnosis or exclusion of CMBs, as well as to evaluate for the presence of WMHs and cavitating lacunes. This provided a total of 291 patients for analysis. FLAIR was available for 242 patients; T2-weighted MRI sequences were used in 49 cases. This information is summarized in Figure 7 below.

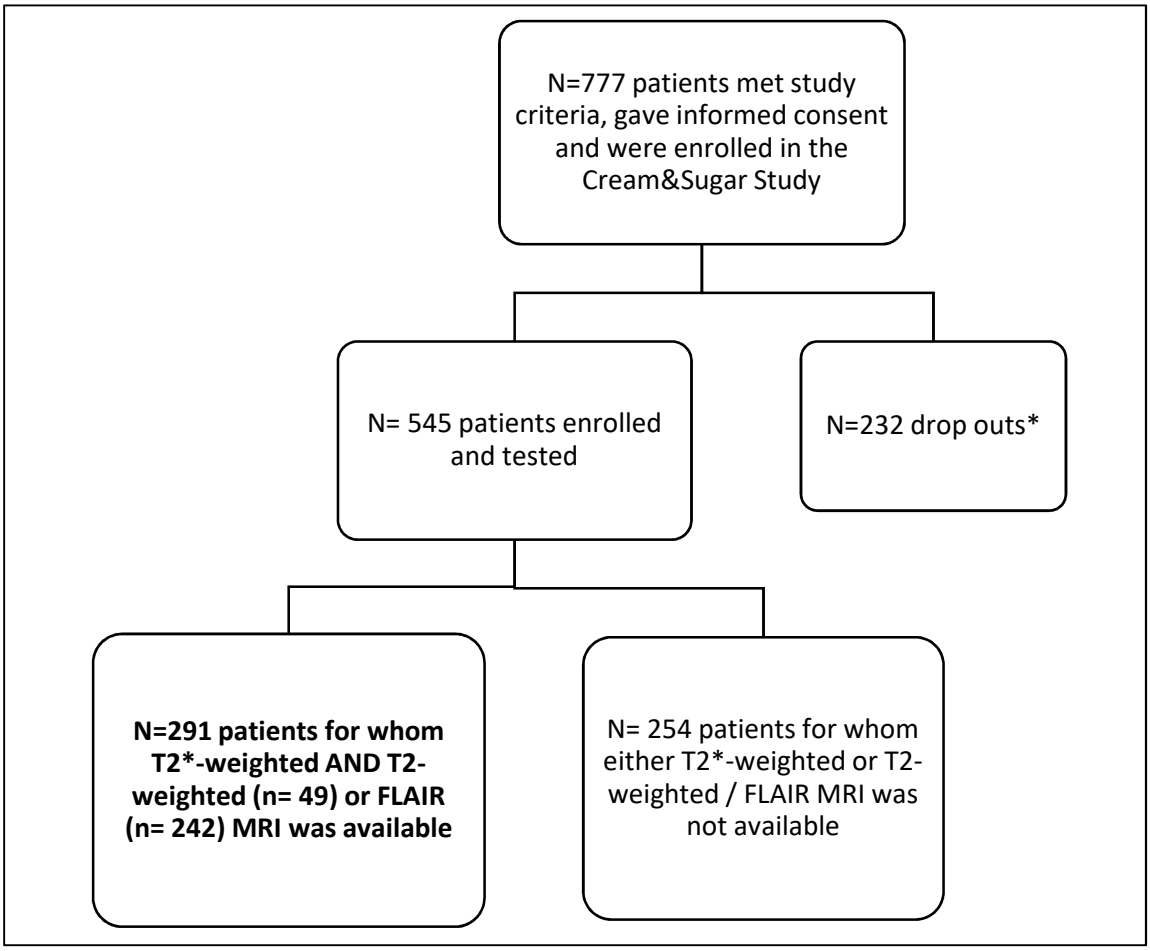


Figure 7. Organogram of study population

FLAIR indicates fluid-attenuated recovery inversion
*Patients who initially gave informed consent, then decided against or could not complete testing for this study, e.g. refused repeat blood draws, could not consume cream for oral glucose tolerance test (OTTT), were not in fasting state on morning of testing, were scheduled for other clinical tests during their hospital stay

3.2. Baseline patient data and indicators of cerebral small vessel disease

This substudy of 291 patients included more than twice as many men (n=202) as women (n=89). The mean patient age was 64.5 years (±13 years). The median NIHSS score at time of testing was 1 (IQR 0-2). Diabetes mellitus was present in 20% of the study population. Hypertension was known in 59%. Hypercholesteremia was present in 33%, and 55% of patients were on statins before the index stroke. Approximately half the study population had never smoked (49%), while 20% were former smokers and 31% were current smokers. The median WHR was 0.97 (IQR 0.92-1.02). The median BMI was 26.4 kg/m² (IQR 24.2-29.4). 64% of study participants were overweight (BMI 25 - < 30) or obese (BMI ≥ 30). This data is summarized in Table 2 below.

Table 2. Baseline characteristics of study population

Age, mean in years, (±SD)	64.5 (±14)
Women, n (%)	89 (31%)
NIHSS, median score (IQR)	1 (0-2)
Known diabetes mellitus [a], n (%)	58 (20%)
Known hypertension [b], n (%)	169 (59%)
Hypercholesteremia [c], n (%)	94 (33%)
Prior statin use, n (%)	158 (55%)
Smoking (n=287)	
Never smoker, n (%)	140 (49%)
Ex-smoker, n (%)	58 (20%)
Current smoker, n (%)	89 (31%)
WHR (n=276), median (IQR)	0.97 (0.92-1.02)
BMI (n=290), median, (IQR) (kg/m²)	26.4 (24.2-29.4)
Underweight (BMI < 18.5), n (%)	2 (< 0.01%)
Normal weight (BMI 18.5 - < 25), n (%)	101 (35%)
Overweight (BMI 25 - < 30), n (%)	122 (42%)
Obese (BMI ≥ 30), n (%)	65 (22%)

NIHSS indicate National Institutes of Health Stroke Score, BMI indicates body mass index, WHR indicates waist to hip ratio.

[a] based on antidiabetic medications at time of hospital admission or HbA1c ≥6.5% in hosptial

[b] based on antihypertensive medications at time of hospital admission

[c] based on in hospital fasting value

Within the overall study population the mean fasting TC level was 184.4 mg/dl (±39.7). The overall mean fasting TG level was 125.7 mg/dl (±54.0). Additional laboratory parameters for the overall study population are presented in Table 3 below.

Table 3. Laboratory parameters of overall patient population

Laboratory parameter	Mean (±SD)
CRP† (mg/dl) (n=276)	2.82 (±10.0)
GFR* (ml/min/1.73 m²) (n=275)	85.0 (±22.0)
TC* (mg/dl) (n=277)	184.4 (±39.7)
HDL-C† (mg/dl) (n=282)	49.6 (±16.7)
LDL-C* (mg/dl) (n=282)	111.9 (±36.6)
HDL/LDL† (mg/dl) (n=282)	0.52 (±0.41)
ALAT† (U/l) (n=275)	35.0 (±37.4)
ASAT † (U/l) (n=276)	32.3 (±28.3)
TSH† (µU/ml) (n=291)	2.0 (±2.0)
HbA1c† (%) (n=281)	6.1 (±3.0)
Glucose 0h† (mg/dl) (n=280)	103.0 (±27.6)
Glucose 3h† (mg/dl) (n=275)	101.9 (±26.6)
Glucose 4h* (mg/dl) (n=265)	150.0 (±43.0)
Glucose 5h* (mg/dl) (n=271)	138.8 (±45.1)
Insulin 0h† (mU/l) (n=265)	10.3 (±12.6)
Insulin 3h† (mU/l) (n=273)	14.7 (±17.0)
Insulin 4h† (mU/l) (n=258)	47.8 (±40.3)
Insulin 5h† (mU/l) (n=257)	53.2 (±51.5)
HOMA† (n=261)	2.8 (±5.0)
pTG† (mg/dl) (n=289)	259.0 (±123.6)
TG 0h† (mg/dl) (n=284)	125.7 (±54.0)
TG 3h† (mg/dl) (n=277)	209.2 (±97.4)
TG 4h† (mg/dl) (n=268)	47.8 (±40.3)
TG 5h† (mg/dl) (n=268)	235.2 (±119.4)

TG var † (mg/dl) (n=284)	133.5 (±87.8)
TG AUC† (n=287)	938.2 (±28.3)

CPR indicates C-reactive protein, GFR indicates glomerular filtration rate, TC indicates total cholesterol, HDL-C indicates high density lipoprotein cholesterol, LDL-C indicates low density lipoprotein cholesterol, ALAT indicates alanine transaminase, ASAT indicates aspartat aminotransferase, TSH indicates thyroid stimulating hormone, HbA1c indicates glycated hemoglobin, HOMA indicates homeostasis model assessment of insulin resistance, pTG indicates peak triglycerides, TG var indicates triglyceride variance (peak triglycerides – fasting triglycerides), TG AUC indicates triglyceride area under the curve.

Of the 291 patients included in this substudy, CMBs were present in 28 (9.6%). An acute ischemic lesion could be confirmed via MRI in all patients included. CMBs had a prevalence of 11.6% in men and 3.2% in women (p=0.016). Cavitating lacunes were present in 40 patients (13.2%). WMHs were present (Wahlund score ≥1) in 234 patients (80.4%). This data is summarized in Table 4 below.

Table 4. Prevalence of indicators of small vessel disease

	Number positive	Total number of patients included	Prevalence
Cerebral microbleeds (≥1)	28	291	9.6%
cavitating lacunes (≥1)	40	291	13.2%
White matter hyperintensites (Wahlund score ≥ 1)	234	291	80.4%

As shown in Table 4 WMHs occurred more frequently than cavitating lacunes or CMBs. Every patient who had ≥1 CMB had some indication of WMHs (Wahlund score ≥1) as well. In the univariate analysis both WMHs and cavitating lacunes associated with CMBs. See Table 5.

Table 5. CMBs and their association with WMHs and cavitating lacunes

	No CMBs	CMBs	Total	P-Value*
WMH (Wahlund score ≥ 1)	206	28	234	<0.01
≥ 1 cavitating lacunes	31	9	40	<0.01

WMH indicates white matter hyperintensities, CMBs indicates cerebral microbleeds,
* P-value from Chi-squared test

In the retrospective analysis of Wahlund score as a continuous variable we found that the association between CMBs (≥1 present / none present) and WMHs (quantified according to Wahlund score) remained (P <0.01, included in Table 7).

3.3. Results from the univariate analysis

3.3.1. Associations

All other results from the univariate analysis are summarized in Table 6 (categorical variables) and in Table 7 (continuous variables). We found that CMBs were more prevalent in older ischemic stroke patients (p<0.01) and in male ischemic stroke patients (p=0.016). NIHSS throughout the cohort was low (median 1, IQR 0-2), but we did observe a possible association between higher NIHSS and presence of CMBs (p=0.05). Additionally, we observed associations between CMBs and higher TSH (p=0.07) and higher CRP (p=0.06) levels, for which the p-values permitted inclusion in the logistic regression analysis.

We observed no statistically significant association between CMBs and previously diagnosed hypertension (antihypertensive medication at time of study inclusion); however, our in-hospital measurements suggest a possible association between CMBs and higher systolic (p=0.08) and diastolic (p=0.05) blood pressures.

Poorer kidney function was also found to associate with CMBs (p<0.01). See Figure 8.

Of the fasting lipid parameters investigated only TC was found to have an association with CMBs with a p-value low enough for inclusion in the logistic regression analysis (p=0.10). See Figure 9.

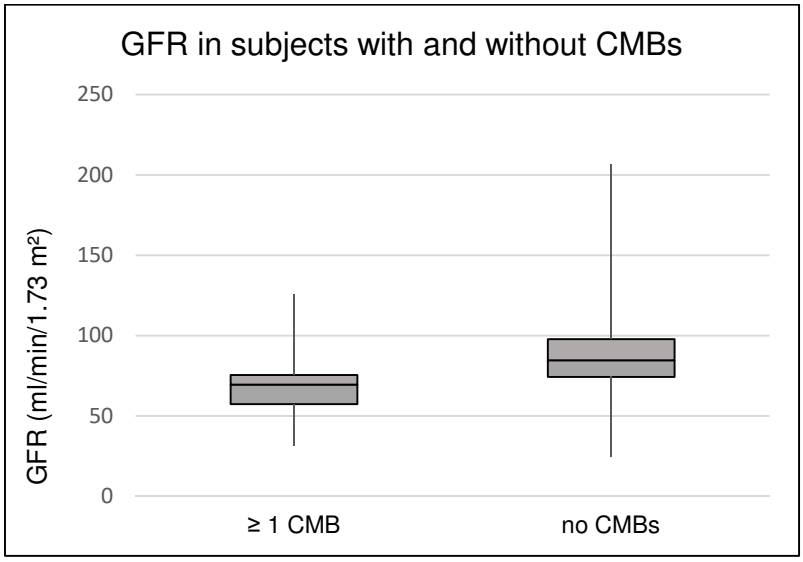


Figure 8. Box plot representing maximum, minimum, median, and IQR of glomerular filtration rate (GFR) in study subjects with and without cererbral microbleeds (CMBs)

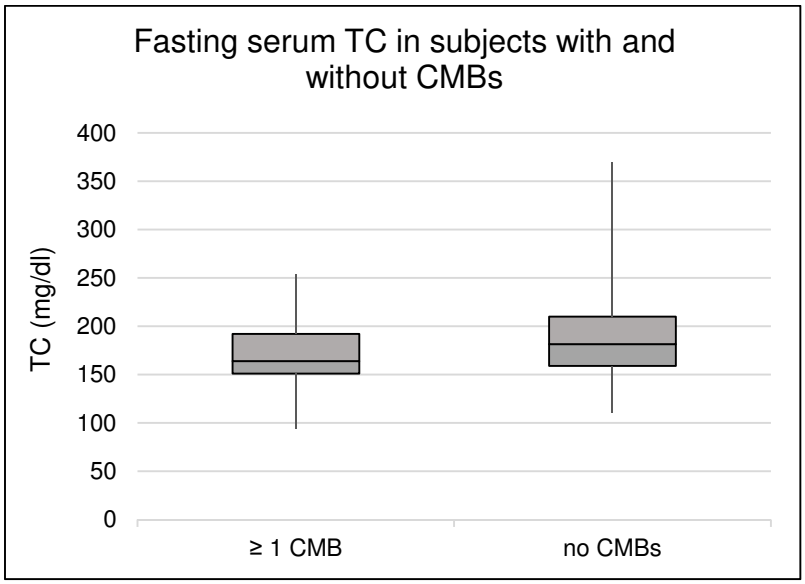


Figure 9. Box plot representing maximum, minimum, median, and IQR of fasting serum total cholesterol (TC) in study subjects with and without cererbral microbleeds (CMBs).

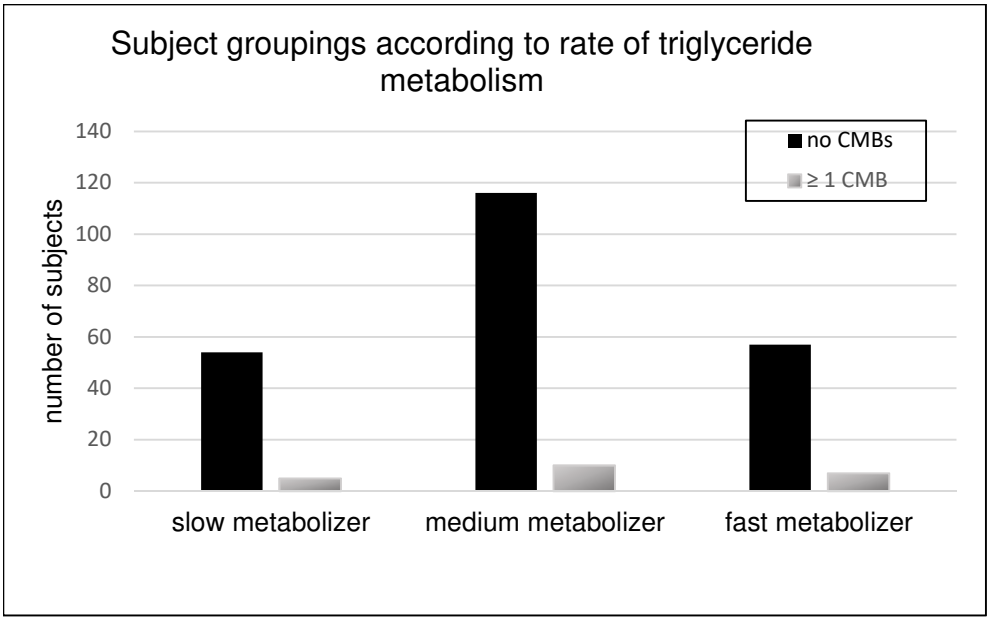
3.3.2. Non-associations

For the remaining fasting lipid parameters (LDL-C, HDL-C, LDL-C/HDL-C, TG) no significant associations with CMBs were observed. We found no statistical significance for hypercholesterolemia as a categorical variable nor for pre-stroke statin use. We

found no statistical significance for groupings of study subjects based on rate of TG metabolism or based on the categorization according to Schmidt-Trucksäss et al. (see "Methods"), as is represented in Figure 10.

We found no statistically significant associations between CMBs and peak TGs, TG variance, or TG AUC. Although study subjects with CMBs had on average lower overall TG levels, following the OTTT, this finding was of no statistical significance. The post-OGTT levels of insulin and glucose were also not significantly associated with CMBs. See Figure 11.

The univariate analysis also failed to demonstrate a significant association between CMBs and the remaining variables investigated: smoker status, BMI, WHR, liver function enzymes (ALAT and ASAT levels), CRP, diabetes mellitus, and HOMA. See Tables 6 and 7.



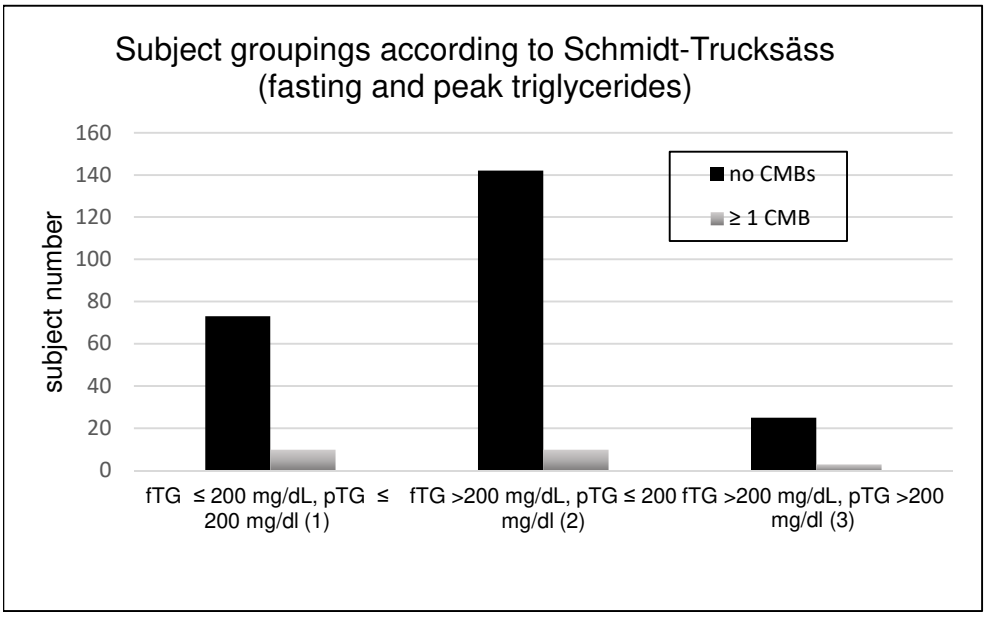
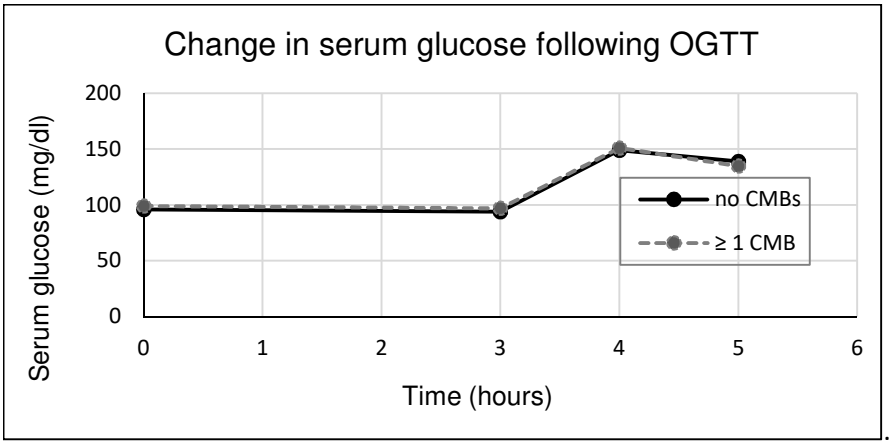
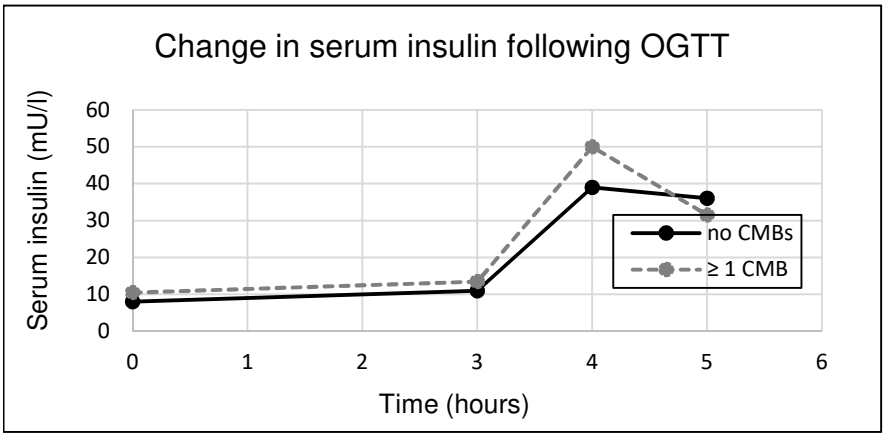


Figure 10. Subjects with and without cererbral microbleeds (CMBs) grouped according to markers of triglyceride metabolism
fTG indicates fasting triglycerides, pTG indicates peak triglycerides





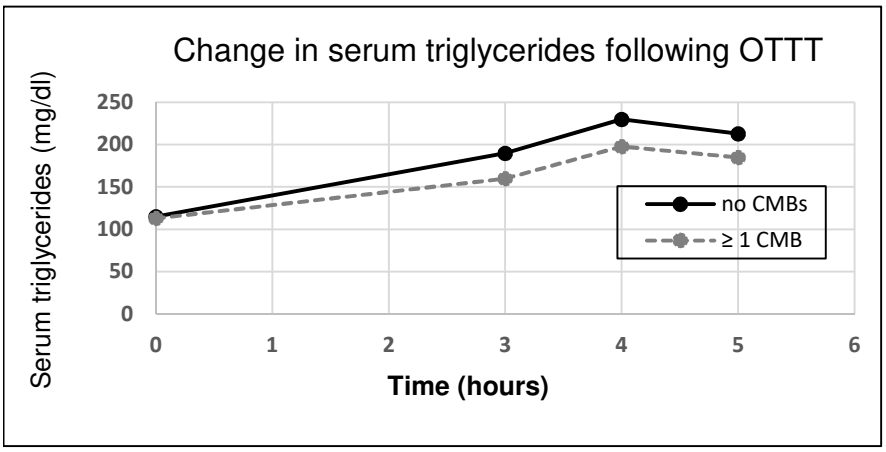


Figure 11. Changes in serum glucose, insulin and triglycerides, following oral glucose toelerance test (OGTT) and oral triglyceride tolerance test (OTTT)

CMBs indicates cerebral microbleeds

Table 6. Associations between categorical variables and CMB presence

	No CMBs	≥1 CMB	P-value*
Gender (n=291)	263	28	.016
Male, n	177	25	
Female, n	86	3	
TG metabolism (n=249)	227	22	.784
Slow metabolizer	54	5	
Medium metabolizer	116	10	
Fast metabolizer	57	7	
TG levels fasting and peak TG (n=263)			.339
fTG ≤ 200 mg/dL, pTG ≤ 200 mg/dl (1)	73	10	
fTG >200 mg/dL, pTG ≤ 200 mg/dl (2)	142	10	
fTG >200 mg/dL, pTG >200 mg/dl (3)	25	3	
Known diabetes mellitus [a] (n=290)	53	5	.760
Known hypertension [b] (n=287)	153	16	.844
Hypercholesteremia [c] (n=283)	85	9	.899
Prior statin usage (n=291)	142	16	.750
Smoking (n=287)			.500
Never smoker	124	16	
Ex-smoker	52	6	
Current smoker	83	6	

CMBs indicates cerebral microbleeds, TG indicates triglycerides, fTG indicates fasting triglycerides, pTG indicates peak triglycerides.
[a] based on antidiabetic medications at time of hospital admission or HbA1c ≥6.5% in hospital
[b] based on antihypertensive medications at time of hospital admission
[c] based on in hospital fasting value
P-values from Chi-squared test

Table 7. Associations between continuous variables and CMB presence

	No CMBs	≥1 CMB	P-Value
Age in years* (n=291)	63 (14)	71 (9)	<0.01
NIHSS† (n=276)	1 (0-2)	2 (1-3)	0.05
BMI† (kg/m^2) (n=290)	26.2 (24.2-29.3)	27.3 (24.0-30.6)	0.52

WHR† (n=276)	0.97 (0.92-1.0)	0.98 (0.93-1.0)	0.30
sBP* (mmHg) (n=284)	137 (22)	145 (22)	0.08
dBP* (mmHg) (n=284)	79 (13)	84 (13)	0.05
CRP† (mg/dl) (n=276)	0.40 (0.14-1.3)	1 (0.2-3.1)	0.06
GFR* (ml/min/1.73 m²) (n=275)	86.8 (21.5)	67.8 (20.0)	<0.01
TC* (mg/dl) (n=277)	186 (39.9)	172 (36)	0.10
HDL-C† (mg/dl) (n=282)	46 (40-57)	45 (35-51)	0.22
LDL-C* (mg/dl) (n=282)	113 (37.3)	103 (27.1)	0.17
HDL/LDL† (mg/dl) (n=282)	0.43 (0.32-0.57)	0.42 (0.34-0.56)	0.91
ALAT† (U/l) (n=275)	25 (18-38)	28 (17-38)	0.91
ASAT † (U/l) (n=276)	26 (21-33)	27 (21-47)	0.61
TSH† (µU/ml) (n=291)111	1.7 (1.1-2.3)	1.2 (0.9-2.0)	0.07
HbA1c† (%) (n=281)	5.6 (5.3-6.1)	5.8 (5.4-6)	0.48
Glucose 0h† (mg/dl) (n=280)	96 (87-110)	99 (88-111)	0.68
Glucose 3h† (mg/dl) (n=275)	94 (87-106)	97 (91-103)	0.32
Glucose 4h* (mg/dl) (n=265)	149 (44)	151 (38)	0.88
Glucose 5h* (mg/dl) (n=271)	139 (45)	135 (48)	0.70
Insulin 0h† (mU/l) (n=265)	8 (5-13)	10.5 (5.3-12)	0.55
Insulin 3h† (mU/l) (n=273)	11 (7-16)	13.5 (8-18.3)	0.16
Insulin 4h† (mU/l) (n=258)	39 (16-60)	50 (21-95)	0.13
Insulin 5h† (mU/l) (n=257)	36 (17-71)	31.5 (15.8-87.8)	0.71
HOMA† (n=261)	2.0 (1.2-3.3)	2.6 (1.1-3.2)	0.68
pTG † (mg/dl) (n=289)	237 (170-319)	204 (145-292)	0.19
TG 0h† (mg/dl) (n=284)	115 (88-146)	113 (89.5-154)	0.79
TG 3h† (mg/dl) (n=277)	190 (143-254)	160 (130-247)	0.39
TG 4h† (mg/dl) (n=268)	230 (160-313)	198 (130-278)	0.12
TG 5h† (mg/dl) (n=268)	218 (159-294)	185 (138-265)	0.19
TG var † (mg/dl) (n=284)	119 (70-187)	85 (55-182)	0.11
TG AUC† (n=287)	863 (651-1135)	781 (549-1051)	0.24
WMH† (Wahlund score) (n=291)	3 (1-6)	7.5 (5-14)	<0.01

* Normally distributed: values indicate mean (standard deviation), P-values from T-test.

† Non-normally distributed: values indicate median (interquartile range), P-values from Mann-Whitney-U test

CMBs indicates cerebral microbleeds, NIHSS indicates National Institutes of Health Stroke Score, BMI indicates body mass index, WHR indicates waist to hip ratio, dBP indicates diastolic blood pressure, sBP indicates systolic blood pressure, CPR indicates C-reactive protein, GFR indicates glomerular filtration rate, TC indicates total cholesterol, HDL-C indicates high density lipoprotein cholesterol, LDL-C indicates low density lipoprotein cholesterol, ALAT indicates alanine transaminase, ASAT indicates aspartat aminotransferase, TSH indicates thyroid stimulating hormone, HbA1c indicates glycated hemoglobin, HOMA indicates homeostasis model assessment of insulin resistance, pTG indicates peak triglycerides, TG var indicates triglyceride variance (peak triglycerides – fasting triglycerides), TG AUC indicates triglyceride area under the curve, WMH indicates white matter hyperintensity.

3.4. Results from the multivariate analysis

The first forward stepwise logistical regression analysis (dependent variable CMBs: categorized yes/no) included gender, age (grouped as tertiles), NIHSS (grouped as tertiles), systolic BP (grouped as tertiles), diastolic BP (grouped as tertiles), GFR (grouped as tertiles), CRP (grouped as tertiles), TSH (grouped as tertiles), WMHs (0-4; 5-10; >10), and cavitating lacunes (categorized yes/no). The cutoff points used for variables grouped as tertiles are summarized in Table 8 below.

Table 8. Input variables for multivariate analysis stratified by tertiles

	N (total)	Tertile group 1	Tertile group 2	Tertile group 3
Age (years)	291	18-58	58-71	>71
sBP (mmHg)	284	<129	129-146	>146
dBP (mmHg)	284	<74	74-82	>82
GFR (ml/min/1.73 m²)	275	>92.2	92.2-75.5	<75.5
CRP (mg/dl)	276	<0.2	0.2-0.9	>0.9
TSH (µU/ml)	270	<1.28	1.28-2.06	>2.06
TC (mg/dl)	277	<165	165-198	>198
NIHSS	276	0-1	1-2	2-10

sBP indicates systolic blood pressure, dBP indicates diastolic blood pressure, CRP indicates C reactive protein, GFR indicates glomerular filtration rate, TSH indicates thyroid stimulating hormone, TC indicates total cholesterol, NIHSS indicates National Institutes of Health Stroke Score.

This first analysis demonstrated a direct relationship between CMBs and greater severity of WMHs based on Wahlund scores (Wahlund 0-3: reference; Wahlund 4-10: adjusted odds ratio (OR) 6.1; 95% confidence interval (CI) 1.8 - 21.2; p= 0.004); Wahlund >10: adjusted OR 10.8; 95% confidence interval CI 2.9 - 39.4; p=0.000). The analysis also revealed that CMBs were significantly less likely to occur in patients with mid-range TC levels, compared to patients with the lowest TC levels (reference: TC < 165 mg/dl; TC 165-198 mg/dl: adjusted OR 0.20; 95% CI 0.10 - 0.76; p=0.018). The highest tertial of TC (>198 mg/dl), compared to the lowest tertial of TC, was not significantly associated with a higher or lower risk of CMBs (p=0.070). Additionally, we noted a trend toward association between CMBs and cavitating lacunes (p=.054). In the second forward step-wise binary logistical regression in which WMHs and cavitating lacunes were excluded (due to the expectation that they would strongly associate) mid-range TC was still associated with a lower risk of CMBs (adjusted OR:

0.24; 95% CI: 0.06 – 0.91; p=0.035). Additionally, we observed an independent association between CMBs and the lowest tertile of renal function (GFR >92.2 ml/min/1.73 m²: reference; GFR<75.5 ml/min/1.73 m²: adjusted OR: 7.4; 95% CI 1.6 - 33.9; p=0.01). Mid-range GFR (75.5 – 92.2 ml/min/1.73 m²) was not a significant risk factor.

Table 9. Results from logistical regression analysis based on risk factors for CMBs according to univariate analysis

Predictor	Beta	SE	P-Value	OR	95% CI
Step 1 (a)					
WMH (Wahlund <4)					reference
WMH (Wahlund 4-10)	1.812	.635	.004	6.1	1.8 – 21.2
WMH (Wahlund >10)	2.372	.664	.000	10.8	2.9 – 39.4
Constant	-3.604	.507	.000	.03	
Step 2 (b)					
WMH (Wahlund <4)					reference
WMH (Wahlund 4-10)	1.947	.649	.003	7.0	2.0 – 25.0
WMH (Wahlund >10)	2.368	.680	.000	10.7	2.8 – 40.5
TC <165mg/dl					reference
TC 165-198mg/dl	-1.632	.691	.018	.20	0.10 – 0.76
TC >198mg/dl	-1.135	.627	.070	.32	0.09 – 1.10
Constant	-2.923	.544	.000	.05	
Step 3 (c)					
WMH (Wahlund <4)					reference
WMH (Wahlund 4-10)	1.710	.667	.010	5.5	1.5 – 20.4
WMH (Wahlund >10)	2.096	.700	.003	8.1	2.1 – 32.1
Cavitating lacunes (yes/no)	1.076	.559	.054	2.9	1.0 – 8.8
TC <165 mg/dl					reference
TC 165-198 mg/dl	-1.558	.698	.026	.21	0.05 – 0.83
TC >198 mg/dl	-1.141	.637	.073	.32	0.09 – 1.1
Constant	-3.065	.556	.000	.05	

CMBs indicates cerebral microbleeds, SE indicates standard error, OR indicates odds ratio, CI indicates confidence interval, WMH indicates white matter hyperintensity, TC indicates total cholesterol.
a. Input variable for step 1: WMH stratified
b. Input variable for step 2: Total cholesterol (tertiles)
c. Input variable for step 3: Cavitating lacunes (yes/no)

Table 10. Results from logistical regression analysis based on risk factors for CMBs, excluding white matter hyperintensities and cavitating lacunes

Predictor	Beta	SE	P-Value	OR	95% CI
Step 1 (a)					
GFR >92.2 ml/min/1.73 m²					reference
GFR 75.5 - 92.2 ml/min/1.73 m²	0.69	.88	.43	2.0	0.36 – 11.2
GFR <75.5 ml/min/1.73 m²	2.0	.78	.01	7.4	1.6 – 33.9
Constant	-3.6	.72	.00	.03	
Step 2 (b)					
GFR >92.2 ml/min/1.73 m²					reference
GFR 75.5 - 92.2 ml/min/1.73 m²	.521	.890	.558	1.68	0.3 - 9.6
GFR <75.5 ml/min/1.73 m²	1.889	.785	.016	6.61	1.4 - 30.8
TC <165 mg/dl					reference
TC 165-198mg/dl	-1.432	.680	.035	.239	0.06 - 0.91
TC >198 mg/dl	-1.123	.617	.069	.325	0.1 - 1.1
Constant	-2.865	.760	.000	.057	

CMBs indicates cerebral microbleeds, GFR indicates glomerular filtration rate, OR indicates odds ratio, CI indicates confidence interval, TC indicates total cholesterol.
a. Input variable for step 1: GFR (as tertiles)
b. Input variable for step 2: Total cholesterol (tertiles)

4. Discussion

The main findings of this substudy of the Berlin Cream&Sugar study were as follows: (1) a prevalence of roughly 10% for CMBs in first time ischemic stroke patients, (2) an association between lower TC levels and CMB risk (3) a lack of association between CMBs and any other lipid parameter investigated, and (4) independent associations between CMBs and (a) lower renal function and (b) greater WMH severity.

4.1. Baseline findings

The CMB prevalence of 9.6% in our cohort is in line with reports in existing population based studies of CMBs. In the updated population based Framingham study Romero et al. reported a prevalence of 8.8% [31]. The AGES Reykjavik study reported a prevalence of 11.1% [7]. The 2015 Rotterdam study reported a much higher prevalence of 18.7% [63]. Roob et al. reported a prevalence of only 6.4% [64].

Studies on CMBs in ischemic stroke patients, in contrast, have reported prevalence rates of 12%-71% (33.5% [CI 30.7%-36.4%] in a pooled cohort study) [72]. In investigations restricted to first-time ischemic stroke patients the reported rates are a bit lower, ranging between 18% and 29% [72]. The strict inclusion criteria for the Cream&Sugar study may be one reason for the relatively low prevalence of CMBs in our study: a previous stroke for example, which is known to associate with CMBs, was an exclusion criterion for our study. Additionally, CMBs appear to be generally more prevalent in Asians than in Caucasians [72], and a number of the existing studies on CMBs drew from largely Asian cohorts. Although we did not systematically document patient ethnicity or background, our study was conducted entirely in Berlin, Germany, where the Asian population of the city (including residents from India, Pakistan, and Central Asia, as well as from East Asian countries) was at most 2-3% in 2014 [138].

Although increasing age was significantly associated with CMBs in our univariate analysis, it did not emerge as one of the strongest predictors in the multivariate analysis. Several large population based studies [7,9,29–31,65,91,92] corroborate our initially observed association between age and CMBs. This loss of significance in our study, however, may be due to the effect of age being masked by factors such as kidney function and WMHs – signs of direct vascular damage, which are also associated with increasing age [39] [103]. Male gender, too, associated with CMBs in the univariate analysis but in the multivariate analysis was no longer significant. While some studies reported an association between CMBs and male gender [7,30,31,65], those restricted to CMBs in

ischemic stroke patients reported no significant association for gender [32,72,90–92]. To our knowledge, no study has identified female gender as a risk factor for CMBs. The known sexual dimorphism of ischemic stroke risk is suspected to be related at least in part to the vasoprotective and vasodilative properties of estrogen [139]. Estrogen (and other vasoactive sex hormones), which may protect against stroke, may play a role in protecting women against CMBs as well. It is important to note, however, that our study included more than twice as many men as women, which may have influenced our findings.

4.2. Primary findings: cholesterol and triglycerides

The primary findings with regards to lipids were that (1) patients with lower TC (<165 mg/dl) were at greater risk for CMBs than patients with mid-range TC (165-198 mg/dl), but (2) that CMBs did not associate with any other serum cholesterol parameter investigated (LDL-C, HDL-C, LDL-C/HDL-C ratio) or (3) with fasting TGs or any marker of TG metabolism derived from the results of the OTTT. While some population based studies found no significant association between CMBs and TC [30,65,125] those that did reported values similar to ours. Romero et al. reported an association between CMBs and TC < 162 mg/dl [31]. Vernooj reported an association between CMBs and TC < 171 mg/dl [9]. In a study of patients receiving MRI for "neurological abnormalities" – excluding stroke – Lee et al. found that the lowest quartile of TC levels (<165 mg/dl) associated with CMBs [87]. It is important to note that patients in our study with high TC (>198 mg/dl) were neither more nor less likely to have CMBs (p=0.070), and to our knowledge there are no reports in the existing literature suggesting that high TC associates with CMBs. The existing studies of CMBs in ischemic stroke patients, all of which reported a non-association between TC and CMBs, were mostly small and drew from largely Asian cohorts [69,70,90–92]. As discussed previously there seem to be significant differences between CMBs in Asian and Western populations, making comparisons with our study difficult. One of these studies also considered CMBs as part of a follow-up investigation ≥12 months after an ischemic stroke or TIA patients. Since CMBs are known to develop at an accelerated rate in the months following ischemic stroke [69], this further complicates comparison with our study.

In concordance with our findings, several larger population based studies also reported a non-association between CMBs and LDL-C [6,30,31,65,87]. In the above mentioned follow-up study of ischemic stroke / TIA patients, the highest quartile of LDL-C was associated

with less risk of CMBs [69], but again this was determined 12 or more months after the ischemic event. Igase meanwhile found no association [90]. In Kim's investigation of ischemic stroke patients CMBs were not associated with LDL-C [70]. The other two studies of CMBs in ischemic stroke patients mentioned did not investigate LDL-C.

Regarding HDL-C, several population based studies reported a non-association with CMBs[9,31,65]. Ding's population based study of CMBs, meanwhile, reported an inverse association with HDL-C levels but only for CMBs in lobar regions [30]. We did not, however, differentiate on the basis of anatomical location, making a comparison here difficult. Additionally, CMBs that are exclusively in the lobar cortices are generally associated amyloid deposits rather than SVD [32], and although Ding did not discuss possible dementia within the cohort, for our study dementia was a clear exclusion criterion. In the study of patients with neurological abnormalities excluding stroke Lee et al., found that CMBs associated with the highest quartile of HDL-C levels (>56.8 mg/dl) [87]. We did not attempt a quartile-based analysis, but the initial univariate analysis of HDL-C as a continuous variable suggested that a significant association in our study was highly unlikely (p=0.22). Regarding HDL-C levels in ischemic stroke patients Igase found that deep (but not lobar) CMBs were associated with lower HDL-C levels [90]. In the follow-up study of ischemic stroke and TIA patients Lee reported a non-association for HDL-C [69]. The remaining studies of CMBs in ischemic stroke patients did not investigate HDL-C [91,92].

In summary, our findings regarding cholesterol are mixed. As discussed in the introduction, excessively low TC levels appear to increase the risk of hemorrhagic stroke [140]. Although hemorrhagic stroke and CMBs represent separate entities [12], CMBs are currently understood to derive at least in part from ruptures of the small vessels of the brain. Thus, the lipid dependent loss of smooth muscle integrity in the arteries of the brain, which is postulated to promote hemorrhagic stroke [141], may play some role in the pathogenesis of CMBs as well. This could help explain the association between CMBs and low TC observed in our study and the others mentioned above. Our observed non-associations for HDL-C and LDL-C do not, however, fit the risk profile for hemorrhagic stroke [125]. The low prevalence of CMBs within our cohort could be one reason for the lack of further associations, but as other studies – even those with much greater patient numbers and CMB rates – have shown little concordance, it seems likely that the relationship between cholesterol and CMBs is more complicated than between cholesterol and hemorrhagic stroke.

Regarding statins, although they have anti-inflammatory properties, which would tend to protect against CMBs, they also hinder platelet aggregation, which conversely would tend to promote CMBs [142]. This could help explain our non-association. It is important to note, however, that we were not able to ascertain how long our study participants had been on statins, which may have influenced results: patients who had only been on statins for a short time may have presented with normal range cholesterol values, despite having suffered the vascular effects of pathological cholesterol levels for many years previously. Our observed lack of association between CMBs and statin use was corroborated by Day et al. in their study of patients with ischemic stroke or TIA [89]. The population based Framingham Study, in contrast, reported a positive association, but here the authors made clear that bias by indication could not be excluded [31]. A much small study (n=163) also found that statin use associated with CMBs, but this was restricted to patients with ICH – a significant confounder [88]. To our knowledge, no other studies have investigated CMBs and statin use in ischemic stroke patients.

Additionally, CMBs in our cohort did not significantly associate with fasting TGs, post-OTTT TGs, or any of our employed measurements of TG metabolism. One study of ICH patients also found no association between CMBs and TGs (or any cholesterol parameter either) [102]. The population based Rekjavik study identified the lowest quartile of TG levels as a risk factor for CMBs [30], but the overall prevalence of CMBs was much higher (18.4% compared to 9.6% in our study). The patients were also older (mean age 74.6 compared to our 64.5 years), and some had had previous strokes (an exclusion criterion for our study). In sum, although our cohort comprised ischemic stroke patients, our participants were on average younger and perhaps comparatively healthier.

Although too low TG levels may increase the risk of hemorrhagic stroke [124,125], high TGs are also associated with inflammation [99,100], atherosclerotic processes [97] and ultimately ischemic processes as well [118,143]. The implications for cholesterol and especially LDL-C are similar [144]. The pathogenesis of CMBs and other SVD related vascular damage appears to be complex, and thus the protective, anti-hemorrhagic properties of lipids may be somewhat offset by their pro-atherosclerotic effects, which would explain the mixed findings in our study and so many others.

Finally, our key finding, that low TC (<165 mg/dl) is associated with greater CMB risk, must be viewed critically. Despite its significance in the multivariate analysis (p=.018), TC when analyzed as a continuous variable in the univariate analysis only barely

reached the level of significance (p=0.10) to permit inclusion in the multivariate analysis. Although Romero [31], Vernooij [9], and Lee [87] also reported associations between TC below ca. 160-170 mg/dl and CMBs, none of these studies considered CMBs specifically in ischemic stroke patients – for whom the benefits of pharmacological lipid reduction have been well established [127]. For ischemic stroke patients, who also present with CMBs, less aggressive cholesterol reduction may eventually prove to be appropriate. This would need, however, to be verified in larger independent cohorts before any sort of modification to the current recommendations on lipid reduction could be considered.

4.3. Secondary findings: significant associations

Secondary findings of this study include independent associations between CMBs and (1) increasing WMH severity and (2) reduced renal function. Male gender and increasing age, which were significantly associated with CMBs in the univariate analysis but not the multivariate analysis, are discussed under "Baseline findings." All other significant and non-significant variables are discussed in the following sections.

4.3.1. Cerebral microbleeds and kidney function

Our observed association between lower GFR and CMBs has been corroborated by population based studies [63,145], studies of ischemic stroke patients [91,92], and a study of patients with acute ICH [146]. Although there is some disagreement [146], poorer kidney function has been reported to associate with WMHs as well [63,147]. Another member of our research group, had previously determined that lower GFR associated with WMHs in this cohort and that both factors were predictive of poorer functional outcome one year after the initial ischemic stroke [148]. CMBs have been observed at higher rates in dialysis patients, but severe kidney failure was an exclusion criterion for our study [63]. We therefore have no data on patients with severely impaired kidney function.

The association between low GFR and CMBs is likely due to the anatomical similarities of the cerebral and renal small vessels [149], which predispose them to developing similar forms of endothelial dysfunction. Both brain and kidney are low-resistance end organs, subject to high volume blood-flow and thus particularly susceptible to damage from hypertension [150]. In the kidney, damage to microvascular structures leads to glomerular sclerosis, with increased capillary permeability and clinically measurable proteinuria. In the brain, damage to small vessels results in the radiologically visible signs of SVD. Even in subacute forms, chronic kidney disease results in an activation of the renin-

angiotensin system with subsequent sodium retention and blood pressure elevation [151]. Pharmacological modulation of the renin-angiotensin system, however, has been demonstrated to prevent both proteinuria and increase cerebral blood flow in patients with cerebral SVD [152,153].

Additionally, kidney disease has been independently associated with systemic inflammation and endothelial dysfunction [154,155]. Kidney disease may independently promote vascular damage to susceptible vessels in other parts of the body, or it may simply be one more manifestation of the damages of systemic inflammation as it affects highly permeable small vessels.

Interestingly, although both diastolic blood pressure and kidney function were significantly associated with CMBs in our univariate analysis, kidney function ultimately proved to be a stronger predictor of CMBs. While our study only considered GFR, other studies that have investigated microalbuminuria as well have found it to be a better predictor of CMB presence [156,157]. Although microalbuminuia does not necessarily represent a more advanced stage of kidney disease it does suggest a form of the disease that is particularly marked by increased glomerular permeability. This corresponds with the increased vascular permeability associated with cerebral SVD and would help explain why kidney function (as a marker for hypertension that has existed long enough to cause serious vascular damage) was a stronger predictor of CMBs than a single pathological blood pressure reading, hypertension that has only existed for a short time, or hypertension that is pharmacologically well-controlled.

4.3.2. White matter hyperintensities and cavitating lacunes

Our observed association between CMBs and increasing WMH severity is corroborated by a number of studies [6,9,10,29,87]. The wide CI (1.8 – 21.2) in our study suggests that we had a relatively low number of patients for this analysis and that our OR may therefore be artificially high. However, even with the relatively low number of patients included in the analysis, an association was present ($p<0.01$). Although it failed to reach the level of significance in the multivariate analysis we also observed a trend toward association between CMBs and cavitating lacunes ($p=0.054$). These findings are unsurprising, considering that postmortem studies have demonstrated that increased hemosiderin deposits associated not only with WMH but also with other histologically verifiable indicators of SVD such as lacunes, perivascular spaces [14], and microinfarcts [158]. Clinical studies have demonstrated associations between CMBs and lacunes as well [159,160]. Both, in our study and in others, CMBs and cavitating lacunes

appeared at much lower rates than WMHs. Additionally, they seemed to rarely occur independently of WMHs, suggesting perhaps a more advanced or severe form of SVD [131]. The overall low number of cavitating lacunes in our study may also help explain the loss of significance in the multivariate analysis, where the effect of WMHs was stronger.

Prior to our analysis we had expected that CMBs would associate with cavitating lacunes and WMHs. These lesions all represent different manifestations of SVD, and we presumed an (at least partially) common underlying pathophysiology. Moreover, the primary purpose of this study was to identify a possible association between lipid levels (and other potentially modifiable clinical parameters) and CMB risk, and we did not want the potentially strong influences of WMHs and cavitating lacunes to mask them. For this reason we performed a separate logistical regression analysis in which WMHs and cavitating lacunes were not included.

4.4. Secondary findings: non-associations and non-significant trends

4.4.1. Cerebral microbleeds and hypertension

In the univariate analysis previously diagnosed and currently medicated hypertension was not a significant predictor of CMBs, but diastolic blood pressure was. Hypertension has been associated with both WMHs [26] and cavitating lacunes [36,39]. A number of studies have also reported an association between hypertension and CMBs, both in healthy cohorts and in patients with known cerebrovascular diseases [9,10,16,29,31,76,87,90–92,102].

In our multivariate analysis, however, hypertension did not emerge as one of the strongest predictors for CMBs. This may be due to the confounders of kidney function and WMHs, which were stronger. Indeed, other studies of CMBs and hypertension also found that after controlling for confounders hypertension was no longer a clear and significant predictor [10,65].

Hypertension is strongly associated with kidney disease (as well as subclinical reductions in kidney function), and although it is not the only cause of kidney damage it is a major one in the industrialized world [161]. While hypertension may represent a risk factor, kidney function provides a more concrete measure of damage that has occurred, and even very mild kidney impairment is associated with an increased risk of cardiovascular disease [162].

Another possible explanation for the ambivalent findings on hypertension is that many studies (including our own) have relied on a single blood pressure measurement. Isolated blood pressure measurements (especially in the clinical setting) are notoriously poor at estimating long-term blood pressure effects [163]. Average daily blood pressure measurements, collected over weeks, have proven to be better predictors of cerebrovascular disease [164]. Furthermore, blood pressure is often elevated in the first days following a stroke [165], and the hypertensive values reflected in our in-hospital measurements may not always be representative. As regards CMBs, blood pressure variation over time may be more significant than absolute blood pressure values [166]. This is not surprising, considering that it is the cumulative and long-term effects of blood pressure that ultimately result in damage. As discussed above, this damage is often well reflected in the kidney function, which we found to be a stronger predictor than history of hypertension or current blood pressure readings.

4.4.2. Stroke severity and CRP levels

Our univariate analysis suggested the possibility of an association between CMBs and stroke severity, based on NIHSS (p=0.05). CMBs and higher NIHSS [167] are both associated with poorer functional outcome after stroke. After controlling for confounding factors in the multivariate analysis, however, NIHSS was not significant in our study. Kim et al. found that CMBs were associated with poorer NIHSS at time of hospital discharge, but they did not consider the role of kidney function in their analysis [70] – which may indeed have been the decisive confounder in our study. Cho and Oh, on the other hand, who both considered kidney function, both determined that NIHSS was not significantly associated with CMBs [91,92].

Our univariate analysis suggested a possible association between CMBs and CRP levels (p=0.06), but after adjusting for confounders this association was not significant. Elevated CRP levels have been linked to WMH severity and other markers of SVD, and inflammation does appear to play a role in the pathogenesis of SVD [100]. Moreover, CRP levels are known to associate with stroke severity and poorer functional outcome [168]. In the context of our study, however, the recent ischemic stroke makes for a significant confounder.

4.4.3. Cerebral microbleeds and TSH

The univariate analysis suggested a possible association between lower TSH and CMBs (p=0.07). Thyroid dysfunction and depressed levels of TSH in particular are common in acute critical illness, including the immediate period following ischemic stroke [169], and subclinical hyperthyroidism (reflected in depressed TSH levels) may be associated with poorer functional outcome after stroke [170]. Additionally, another substudy of the Berlin Cream&Sugar study had found that deranged TSH levels were independently associated with WMH severity[171].

In our multivariate analysis, however, TSH did not emerge as one of the strongest predictors of CMBs. Much of the existing literature on CMBs draws from community based cohorts, and thyroid function has not been extensively investigated in this context. Thyroid function was not the focus of our inquiry either, and we have no data regarding triiodothyronine (T3) or thyroxine (T4) or TSH levels before stroke, making it impossible to determine whether the lower levels of TSH in patients with CMBs were related to the stroke or whether they represented preexisting thyroid dysfunction. Although the relationship between (dys)function of the hypothalamic-pituitary-thyroid axis and cerebral SVD represents an interesting field of inquiry, we had too little information to discuss the meaning of this finding at any great length. Studies in independent cohorts will be necessary to investigate a possible relationship here.

4.4.4. Smoking

Our study found no association between CMBs and smoking. In some ways this is surprising, considering that smoking has long been implicated as a serious cause of vascular injury [172,173] and has also been identified as a risk factor for WMHs [174]. Some studies of CMBs identified smoking as a risk factor [9,29,30], while others reported a non-association [10,11,31,65]. In existing studies of ischemic stroke patients smoking did not associate with CMBs [10,91,92]. One systematic review with a pooled cohort of 1107 patients with known cerebrovascular disease even found that smoking was negatively associated with CMBs [72].

Our finding of non-significance may be due to being underpowered (the studies with stroke patients, including ours, had significantly fewer patients than the population based studies). Additionally, our patients were simply grouped as former, current, or never-smokers. We did not consider total pack years, years since quitting, or other significant variables for evaluating overall nicotine burden. It may also be that smoking

simply plays a smaller role in the pathogenesis of SVD, compared to large vessel diseases.

4.4.5. Diabetes mellitus and associated risk factors

Additionally, we found no association between CMBs and diabetes mellitus, BMI, WHR, fasting glucose, fasting insulin, postchallenge glucose or insulin levels, or HOMA. Several existing studies are in concordance with this lack of association for diabetes mellitus [9,29,65,91,92]. A systematic review determined that diabetes only associated with CMBs in otherwise healthy patients, but not in patients with cerebrovascular diseases like stroke [10]. While some studies reported an association between WMHs and diabetes mellitus [39,65,175] others reported a non-association [26,176]. Romero et al. also excluded metabolic syndrome, HOMA, and obesity as risk factors for CMBs [31]. One (smaller) study of community-based elderly, however, reported that obesity did associate with CMBs [177]. Another study reported an association between increasing BMI and lacunes [94].

To our knowledge, no other CMB studies have included postchallenge glucose or insulin measurements. Once again, this lack of association may be due to the particular pathogenesis of SVD. Although diabetes is a known risk factor for large vessel diseases [178], it is possible that it does not affect the small cerebral vessels in the same way. Additionally, glucose levels can be altered and glucose tolerance can be impaired in the days and weeks following stroke, even in patients with no previous history of dysfunctional glucose metabolism [179].

4.5. Study strengths

The strengths of this study include use of an at risk population, prospective collection of extensive clinical and demographic information, and standardized means of evaluating TG and glucose metabolism.

While much of the existing data on CMBs is drawn from population based studies, we investigated CMBs in first-time ischemic stroke patients. This is significant as CMBs are a known risk factor for stroke, recurrent stroke, and poor functional outcome after stroke [6,70–72]. While CMBs in the context of hemorrhagic stroke have been more thoroughly investigated [180,181], there are fewer studies that have investigated CMBs in ischemic stroke patients specifically. Recent studies of CMBs in ischemic stroke patients draw from exclusively Asian cohorts [70,90–92], where CMB prevalence is known to be higher than in Western cohorts and where the associated risks differ [71].

Additionally, we prospectively collected a broad range of data on pre-existing conditions, current medications, physical characteristics, stroke severity, other radiological manifestations of cerebral SVD, and multiple serum laboratory parameters. This permitted us to investigate a broad spectrum of potentially modifiable risk factors and to adjust accordingly for potential confounders.

Although several larger population based studies have investigated cholesterol in a more differentiated manner (LDL-C, HDL-C, in some cases stratified), no study that we are aware of has taken such a differentiated approach to investigating TGs and TG metabolism as they relate to CMBs – either in a community based population or in ischemic stroke patients. The same is true for our standardized investigation of glucose and insulin metabolism. Although we ulitimately found no association between CMBs and any aspect of TG or glucose metabolism our standardized study design offered a number of advantages. In addition to the superiority of oral tolerance tests compared to fasting values (as described in previous sections), the clinical setting made it possible to monitor study participants and ensure (1) that they began the oral glucose and TG tolerance tests in a fasting state and (2) that the cream and glucose drinks were properly consumed. We also followed a very strict protocol for the timing of postchallenge blood draws, alowing for very specific measurements of TG, glucose, and insulin metabolism.

4.6. Study limitations

The main limitation of this study is the somewhat small sample size. Although our total sample size (n=291) is respectable for a patient based (as opposed to a population based) study, the CMB prevalence in our cohort was also low (9.6%). Related to this limitation, we had too few patients with CMBs to permit a meaningful analysis of CMB number as a continuous variable or to stratify based on number of CMBs. We therefore classified patients on the basis of CMB presence (yes/no). The same is true for cavitating lacunes. A large community-based study with 3,660 participants, however, found that comparison on the basis of single vs. multiple lacunes revealed no significant difference [33].

The small number of patients with CMBs also prevented a meaningful differentiation on the basis of anatomical location. As discussed previously, CMBs found exclusively in the lobar cortices are associated with Alzheimer's dementia and are thought to arise from CAA rather than SVD [13]. In our study, however, dementia was a clear exclusion criterion. Furthermore, even in cases of pathologically verified CAA there is some

evidence that underlying SVD-related damage to the blood brain barrier lays the groundwork for subsequent amyloid related damage in predisposed individuals [34]. At the same time, however, it is not possible to completely exclude the possibility that very early stages of CAA (before clinically manifest dementia) may have played a role in some of the CMBs in our study. Finally, although many (but not all) of the larger population based CMB studies differentiated on the basis of anatomical location, few studies provided precise definitions of the terms employed, and in those that did there is little consistency [72]. In short, although the lack of anatomical stratification in our study is a clear limitation, we do not consider it a serious limitation or one that is peculiar to our study.

In terms of generalizability of our results, it is important to emphasize that the strokes in our cohort were relatively mild. In patients with more severe strokes the findings may have been different. The relationship between kidney function and CMBs is an interesting finding, but since manifest kidney failure was an exclusion criterion, it was not possible to assess this relationship in patients with more severe kidney dysfunction in the Cream&Sugar study. We still consider this finding meaningful, however, as even only mildly impaired kidney function has been associated with significant risks for other serious morbidities [161].

Finally, we had too little follow-up data available to perform a meaningful analysis of CMBs and functional or imaging outcome[76]. In addition to providing information on new CMBs follow-up MRI studies would allow us to see how they develop in relation to other radiological findings like WMHs and lacunes, as well as in relation to clinical risk factors and functional outcome.

4.7. Recommendations for future research

Based on our findings we cannot recommend further investigation of a potential relationship between CMBs and TG metabolism. Although a much larger study could potentially discover a relationship that our study did not, we employed a broad and exhaustive means of investigating TG metabolism as it relates to CMBs and none of our employed measures even approached the level of significance. In light of the costliness and the discomfort for patients that the OTTT entails we believe there are better avenues to pursue in understanding CMBs in stroke patients.

Our observed association between lower TC levels and CMBs, for example, should be further explored. Although other studies have demonstrated this association they were

not in ischemic stroke patients, for whom the question of cholesterol modulation is of particular importance. A longitudinal investigation of progression or regression of CMBs after ischemic stroke would also be of interest. An examination of the changes in CMB status over time as they relate to lipid levels and lipid metabolism could be useful as well.

Although our data suggested that diabetes mellitus was not a risk factor for CMBs, in light of the well-established link between diabetes and renal vasculopathies [182] a further investigation of CMBs in a specifically diabetic population or in a population of patients with diabetic nephropathy could be of interest. Further investigation of the relationship between kidney function and CMBs can be recommended as well. Since kidney function was not the primary focus of this study, our investigation was restricted to calculating GFR. There is some evidence, however, that Cystein C, microalbuminuria, or albumin-to-creatinine ratio may be better predictors [63,92,145]

5. Conclusion

In conclusion, the purpose of this retrospective substudy was to determine whether plasma lipids were associated with CBMs in first time ischemic stroke patients. The analysis included all patients who had been enrolled in the Berlin Cream&Sugar study between January 2009 and October 2015 and who had received a T2*-weighted MRI to determine whether CMBs were present. Our analysis revealed that although CMBs were not associated with fasting TGs or any measure of TG metabolism based on the OTTT they were associated with low TC. Additionally, we determined that CMBs were associated with WMH severity and reduced kidney function.

Regarding kidney function, renal damage that has already occurred cannot be undone, but further damage can be prevented by stricter control of hypertension and particular caution in the use of nephrotoxic drugs. Patients presenting with CMBs and reduced renal function, may benefit in particular from modulators of the renin-angiotensin system, which have been shown to promote cerebral perfusion [152,153] as well as to reduce systemic blood pressure. Further studies, employing other measurements of renal function, may investigate this relationship further.

The observed association between TC and CMBs is a contribution to the existing literature on CMBs, lipids, and stroke risk, but the association is still too tenuous to suggest any modification to the current recommendations on cholesterol reduction following ischemic stroke. Despite the established role of pharmacological cholesterol reduction in the management of ischemic stroke patients, for patients with concurrent CMBs, less aggressive reduction may eventually prove to be appropriate, but only after confirmation of this finding in larger studies.

The OGTT is a costly and time-consuming means of evaluating TG metabolism, and in light of our failure to find significant associations between CMBs and any of our many measures of TG metabolism we cannot recommend this approach for future CMB research.

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7. Abbreviations

ALAT – alanine transaminase

ASAT – aspartate transaminase

AUC – area under the curve

sBP – systolic blood pressure

dBP – diastolic blood pressure

CAA – cerebral amyloid angiopathy

CI – confidence interval

CMB – Cerebral microbleed

CRP C-reactive protein

CT – computer tomography

DWI – diffusion weighted imaging

FLAIR – fluid-attenuated inversion recovery sequence

fTG – fasting triglycerides

GFR – glomerular filtration rate

HbA1c – glycolysated Hemoglobin

HDL-C – high-density lipoprotein cholesterol

HOMA-IR homeostasis model assessment insulin resistance index

ICH – intracerebal hemorrhage

IQR – interquartile range

LDL-C – low-density lipoprotein cholesterol

MRI – magnetic resonance imaging

NIHSS – National Insitutes of Health Stroke Severity

MDRD – modified diet and renal diseases (formula)

OGTT – oral glucose tolerance test

OTTT - oral triglyceride tolerance test

OR – odds ratio

RR – relative risk

SD – standard deviation

SE – standard error

SVD – small vessel disease

SWI – susceptibility weighted imaging

TC – total cholesterol

TG – TGs

TG AUC – triglyceride area under the curve

TG max – peak triglyceride level

fTG – fasting triglycerides

pTG – peak triglycerides

TG var – triglyceride variance

TIA – transient ischemic attack

TSH – thyroid stimulating hormone

VLDL-C – very low-density lipoprotein cholesterol

WHO – World Health Organisation

WMH – white matter hyperintensity

8. Tables

9. Figures

10. Appendix

10.1. Case reporting form for Cream&Sugar study

CSB Trial Team

Cream & Sugar II - CRF Source Data

Worksheet CreamSugar

Datum: **Unterschrift:**

Patientendaten:

Klebchen

getestet mit: **Landliebe-Sahne** ☐
Bärenmarke-Sahne ☐
Weihenstephan ☐

Hausarzt:

ScreeningNr. :
Einwilligung unterschrieben (Datum):
Adresse:

Wann letzte Mahlzeit:

Telefon Patient:
Telefon Angehörige:

Pseudonym (nur in Datenbank eingeben)

Angaben zum Ereignis:
erstmaliger ischämischer Schlaganfall, Symptombeginn am (Datum):
nachgewiesen CCT/MRT ☐ am:
Klinisch ☐
andere, Kommentar:

Diabetesanamnese (Arzt!):
Hat der Patient Diabetes? **J/N/U**nbekannt ☐ Einnahme von oralen Antidiabetika **J/N** ☐
Falls ja: Insulin J/N ☐ Seit wieviel Jahren Insulingabe?
oGTT Stunde 3 durchführen? J/N ☐

Datum u. Unterschrift Arzt: _____

Anamneseparameter:
Hat der Patient einen Hypertonus? **J/N/U**nbekannt ☐ Einnahme von Antihypertonika **J/N** ☐
Hat der Patient eine Hyperlipidämie? **J/N/U**nbekannt ☐ Einnahme von Lipidsenkern **J/N** ☐

Pos. Familienanamnese ☐ Männer vor 55 J. mit cerebrovask. Erkr. (HI,Stroke,TIA)
☐ Frauen vor 65 J. mit cerebrovask. Erkr. (HI, Stroke, TIA)
Rauchen ☐ Ja ☐ Nein ☐ Ex Rauchen bis vor weniger als 12 Monaten ist nicht Ex
Anzahl der Tage pro Woche mit frischem Obst oder Gemüse? ☐ 0-7 ☐ Regelmäßig Sterole J/N
Alkohol: Nie ☐ <3x/Woche ☐ >3x/Woche ☐ Täglich ☐
Gewichtsveränderung letzte 12 Monate ☐ ☐ Zunahme oder ☐ Abnahme in Kg

Immobilisierung ? J/N ☐ vorbestehend ? J/N ☐ seit wann?
Fieberhafter Infekt ☐ (wenn ja, seit vieviel Tagen?) ☐
Ursache: Harnwegsinf. ☐ unbekannt ☐
Pneumonie ☐ ander (Freitext):

CSB Trial Team Worksheet CreamSugar

Befragung nach Herzerkrankungen: **J= ja, N=nein, U=unbekannt**

Hatten Sie eine Herzkatheteruntersuchung? ☐ Falls Ja, wurde dabei eine Ballondilatation durchgeführt? ☐
Hatten Sie schon eine Herzechountersuchung? ☐
Hatten Sie eine Bypass- Op am Herzen? ☐
Haben Sie gefäßverkalkte Beine ☐
Leiden Sie unter einer KHK(koronare Herzkrankheit),falls ja welcher Grad der Erkrankung 1-3 ? ☐
Hatten Sie schon einen Herzinfarkt? ☐ Falls ja, wann: ☐
Wurde jemals eine Koronarangiografie durchgeführt oder ist eine geplant? ☐
Wurde bei Ihnen ein Stent implantiert? ☐

Untersuchungsparameter:

Größe ☐ cm BMI (FORMEL)
Gewicht ☐ kg
Taillenumfang: ☐ cm Waist-to-Hip-Ratio (FORMEL)
Hüftumfang: ☐ cm
Blutdruck ☐ ☐ mmHg sitzend gemessen ☐ liegend gemessen ☐
Puls ☐ bpm sitzend gemessen ☐ liegend gemessen ☐

Vorhofflimmern ☐ J/N (EKG) Intima Media Dicke (mm) ☐ re ☐ li

Medikamente am Testtag:

(Freitext)

Labor

Baseline: Datum: ☐ Uhrzeit: ☐

Hb	☐	g/dl	Triglyzeride	☐	mg/dl
Insulin	☐	mU/l	Kreatinin	☐	mg/dl
Glukose	☐	mg/dl	GOT (ASAT)	☐	U/l
HbA1c	☐	%	GPT (ALAT)	☐	U/l
Gesamt-Cholesterin	☐	mg/dl	CRP	☐	mg/dl
LDL-Cholesterin	☐	mg/dl	TSH	☐	µIU/ml
HDL- Cholesterin	☐	mg/dl			

oTTT (Stunde 0):

250 ml Sahne Datum: ☐ Uhrzeit: ☐

Labor

Stunde 3: Datum: ☐ Uhrzeit: ☐

Glukose, Sticks	☐		Insulin	☐	mU/l
Glukose, venös	☐	mg/dl	Triglyzeride	☐	mg/dl

oGTT (Stunde3):

75g Dextrose in 300 ml Wasser Datum: ☐ Uhrzeit: ☐

Labor

Stunde 4: Datum: ☐ Uhrzeit: ☐

Glukose, Sticks	☐		Insulin	☐	mU/l
Glukose, venös	☐	mg/dl	Triglyzeride	☐	mg/dl

Labor

Stunde 5: Datum: Uhrzeit:

Glukose, Sticks
Glukose, venös mg/dl
Insulin mU/l
Triglyzeride mg/dl

Adverse Events: **Datum mit Uhrzeit:**

Meteorismus nach oTTT	von/ bis	CTCAE Grad
Diarrhoen nach oTTT	von/ bis:	CTCAE Grad
Übelkeit nach oTTT	von/ bis:	CTCAE Grad
Völlegefühl nach oTTT	von/ bis:	CTCAE Grad
andere	von/ bis:	CTCAE Grad

keine AE´s

Datum u. Unterschrift Arzt: _____

Datum u. Unterschrift Arzt: _____

NIHSS (aktuell am Testtag)

1a Bewusstseinszustand
- 0 Wach unmittelbar antwortend
- 1 benommen aber durch geringe Simulation zum Befolgen von Aufforderungen, Antworten oder Reaktionen zu bewegen
- 2 Stuporös, bedarf wiederholter Stimulation um aufmerksam zu sein, oder ist somnolent und bedarf starker oder schmerzhafter Stimuli zum Erzielen von Bewegungen (keine Stereotypien)
- 3 Koma, antwortet nur mit motorischen oder vegetativen Reflexen oder reagiert gar nicht, ist schlaff und ohne Reflexe

1b Fragen zum Bewusstseinszustand (Monat, Alter des Patienten)
- 0 Beantwortet beide Fragen richtig
- 1 Beantwortet eine Frage richtig
- 2 Beantwortet keine Frage richtig

1c Aufforderungen zur Ermittlung des Bewusstseinszustandes (Augen öffnen und schließen, Faust machen und öffnen (nicht paretische Hand), ggf. Pantomime)
- 0 Führt beide Aufgaben richtig aus
- 1 Führt eine Aufgabe richtig aus
- 2 Führt keine Aufgabe richtig aus

2 Blickbewegungen (Blick folgt dem Finger des Untersuchers)
- 0 Normal
- 1 Partielle Blickparese
- 2 Forcierte Blickdeviation oder komplette Blickparese

3 Gesichtsfelder (Visuelle Gesten oder Finger zählen)
- 0 Keine Gesichtsfeldeinschränkung
- 1 Partielle Hemianopsie
- 2 Komplette Hemianopsie
- 3 Bilaterale Hemianopsie (Blindheit inkl. Kortikaler Blindheit)

4 Facialisparese (Zähne zeigen, Stirn runzeln, Augen schließen)
- 0 Normale symmetrische Bewegungen
- 1 Geringe Parese (abgeflachte Nasolabialfalte, Asymmetrie beim Lächeln)
- 2 Partielle Parese (vollständige oder fast vollständige Parese des unteres Gesichts)
- 3 Vollständige Parese von ein oder zwei Seiten (Fehlen von Bewegungen im oberen und unteren Teil des Gesichts)

5 Motorik Arme (Arme in 90° Position bringen)

0 links	0 rechts	Kein Absinken, die Extremität wird über 10 Sekunden in der 90° (oder45°) Position gehalten
1 links	1 rechts	Absinken, Extremität sinkt vor Ablauf von 10Sek ab
2 links	2 rechts	Anheben gegen Schwerkraft möglich
3 links	3 rechts	Kein (aktives) Anheben gegen Schwerkraft, Extremität fällt
4 links	4 rechts	Keine Bewegung
9 links	9 rechts	Amputation, Gelenkversteifung

Bewertung mit 9="Amputation" bitte als 0 zählen

6 Motorik Beine (Beine in 45° Position bringen)

0 links	0 rechts	Kein Absinken, Bein bleibt über 5 Sekunden in der 30° Position
1 links	1 rechts	Absinken, Bein sinkt am Ende der 5 Sekundenperiode, berührt das Bett jedoch nicht
2 links	2 rechts	Aktive Bewegung gegen Schwerkraft, das Bein sinkt binnen 5 Sekunden auf das Bett ab
3 links	3 rechts	Kein Anheben gegen Schwerkraft, Bein fällt sofort auf das Bett
4 links	4 rechts	Keine Bewegung
9 links	9 rechts	Amputation, Gelenkversteifung

Bewertung mit 9="Amputation" bitte als 0 zählen

7 Extremitäten Ataxie (Finger-Nase-Finger bzw. Ferse-Hacke-Versuch)

0	Fehlend
1	In einer Extremität vorhanden
2	In zwei Extremitäten vorhanden

Nur das erste Item "Extremitätenataxie" zählen, die Unterpunkte re/li Arm bzw. re/li Bein dienen nur der Information.

Ataxie besteht in:	Rechtem Arm	Nein	1
		Ja	2
		Amputation, Gelenkversteifung	9
	Linkem Arm	Nein	1
		Ja	2
		Amputation, Gelenkversteifung	9
	Rechtem Bein	Nein	1
		Ja	2
		Amputation, Gelenkversteifung	9
	Linkem Bein	Nein	1
		Ja	2
		Amputation, Gelenkversteifung	9

8 Sensibilität (Nadel-, Schmerzreize bei Armen, Beinen, Körper, Gesicht

0	Normal; kein Sensibilitätsverlust
1	leichter bis mittelschwerer Sensibilitätsverlust
2	Schwerer bis vollständiger Sensibilitätsverlust

9 Sprache (Benennen von Gegenständen, Beschreiben des Bildes, Lesen der Satzliste

0	Keine Aphasie; normal
1	Leichte bis mittelschwere Aphasie
2	Schwere Aphasie
3	Stumm, globale Aphasie, keine verwendbare Sprachproduktion oder kein Sprachverständnis

10 Dysarthrie (Vorlesen der Wortliste)

0	Normal
1	Leicht bis mittelschwer; der Patient spricht zumindest einige Wörter verwaschen
2	Schwer, die verwaschene Sprache des Patienten ist unverständlich
9	Intubation oder andere mechanische Behinderungen

Bewertung mit 9="Intubation oder andere mechanische Behinderunge" bitte als 0 zählen

11 Auslöschung und Nichtbeachtung, früher Neglect (Verwendung der vorangegangenen Untersuchungen)

- [ ] 0 Keine Abnormalität
- [ ] 1 Visuelle, taktile, auditive, räumliche oder personenbezogene Unaufmerksamkeit oder Auslöschung bei der Überprüfung von gleichzeitiger bilateraler Stimulation in einer der sensiblen Qualitäten
- [ ] 2 Schwere halbseitige Unaufmerksamkeit oder halbseitige Unaufmerksamkeit in mehr als einer Qualität. Kein Erkennen der eigenen Hand oder orientierung nur zu einer Seite des Raumes

Gesamtpunkte 1-11: [ ]

Barthel Index (BI) (am Testtag)

Essen
- [ ] 10 Unabhängig
- [ ] 5 Braucht etwas Hilfe,z.B. Fleisch oder Brot schneiden
- [ ] 0 Nicht selbständig, auch wenn o.g. Hilfe gewährt wird

Bett/(Roll-)Stuhltransfer
- [ ] 15 Unabhängig
- [ ] 10 Geringe Hilfe oder Beaufsichtigung notwendig
- [ ] 5 Erhebliche Hilfe beim Transfer, Lagewechsel, Liegen/Sitz selständig
- [ ] 0 Nicht selbständig, auch wenn o.g. Hilfe gewährt wird

Waschen
- [ ] 5 Unabhängig
- [ ] 0 Nicht selbständig

Toilettenbenutzung
- [ ] 10 Unabhängig
- [ ] 5 benötigt Hilfe,z.B. wg. Unzureich. Gleichgewichtes od. bei Kleidung/Reinigung
- [ ] 0 Nicht selbständig, auch wenn o.g. Hilfe gewährt wird

Baden
- [ ] 5 Unabhängig
- [ ] 0 Nicht selbständig

Gehen auf Flurebene bzw. Rollstuhlfahren
- [ ] 15 Unabhängig beim Gehen über 50m, Hilfsmittel erlaubt, nicht Gehwagen
- [ ] 10 Geringe Hilfe oder Überwachung erforderlich, kann m. Hilfsm. 50m gehen
- [ ] 5 Nicht selbständig beim Gehen aber Rollstuhl selbständig, auch um Ecken und an Tisch, Strecke min 50m
- [ ] 0 Nicht selbständig

Treppensteigen
- [ ] 10 Unabhängig
- [ ] 5 benötigt Hilfe oder Überwachung beim Treppensteigen
- [ ] 0 Nicht selbständig

An- und Auskleiden
- [ ] 10 Unabhängig
- [ ] 5 Benötigt Hilfe, kann aber 50% der Tätigkeit selbständig durchführen
- [ ] 0 Nicht selbständig, auch wenn o.g. Hilfe gewährt wird

Stuhlkontrolle
- [ ] 10 Ständig kontinet
- [ ] 5 Gelegentlich inkontinent, maximal einmal/Woche
- [ ] 0 Häufiger/ständig inkontinent

Urinkontrolle
- [ ] 10 Ständig kontinet, ggf.unabhängig bei Versorgung eines DK/Cystofix
- [ ] 5 Gelegentlich inkontinent, max. einmal/Tag, Hilfe bei externer Harnableitung
- [ ] 0 Häufiger/ständig inkontinent

Gesamtpunkte: [ ]

CSB Trial Team Worksheet CreamSugar

Modified Ranking Scale (am Testtag)

- ☐ 0 Keine Symptome
- ☐ 1 Keine wesentliche Funktionseinschränkung trotz Symptomen; kann allegewohnten Aufgaben und Aktivitäten verrichten
- ☐ 2 Geringgradige Funktionseinschränkung; unfähig alle früheren Aktivitäten zu verrichten, ist aber in der Lage, die eigenen Angelegenheiten ohne Hilfe zu erledigen
- ☐ 3 Mäßiggradige Funktionseinschränkung; bedarf einiger Unterstützung, ist aber in der Lage, ohne Hilfe zu gehen
- ☐ 4 Mittelschwere Funktionseinschränkung; unfähig ohne Hilfe zu gehen und unfähig, ohne Hilfe für die eigenen körperlichen Bedürfnisse zu sorgen
- ☐ 5 schwere Funktionseinschränkung; bettlägerig, inkontinent, bedarf ständiger Pflege und Aufmerksamkeit

Gesamtpunkte: ☐

TOAST Kriteria Datum vom Arztbrief: ☐

Ätiologischer Subtyp:

- ☐ Makroangiopathie/Atherosklerose
- ☐ Kardioembolisch
- ☐ Mikroangiopathie
- ☐ Andere Ursachen
- ☐ Undefinierter Typ (keine Ursache eruierbar)

Weitere Fragen durch Informationen aus Quelldaten, wie z.Bsp. Arztbrief:
(Nicht durch Befragung der Patienten)

LVEF(Bestimmung durch Herzechountersuchung) :

pe (postextrasystolisch- Bestimmung durch Koronarangiogrphie) :

Stentart: ☐ BMS(nicht beschichteter Metalstent)
☐ DES(Drug -Elutig- Stent) Medikamentenbeschichteter Stent

10.2. National Institutes of Health Stroke Score

1a. Bewusstseinszustand
- Wach unmittelbar antwortend ◯ 0
- Benommen, aber durch geringe Stimulation zum Befolgen von Aufforderungen, Antworten oder Reaktionen zu bewegen ◯ 1
- Stuporös, bedarf wiederholter Stimulation um aufmerksam zu sein, oder ist somnolent und bedarf starker oder schmerzhafter Stimuli zum Erzielen von Bewegungen (keine Stereotypien) ◯ 2
- Koma, antwortet nur mit motorischen oder vegetativen Reflexen oder reagiert gar nicht, ist schlaff und ohne Reflexe ◯ 3

1b. Fragen zum Bewusstseinszustand
Monat, Alter des Patienten
- Beantwortet beide Fragen richtig ◯ 0
- Beantwortet eine Frage richtig ◯ 1
- Beantwortet keine Frage richtig ◯ 2

1c. Aufforderungen zur Ermittlung des Bewusstseinszustandes
Augen öffnen und schließen, Faust machen und öffnen (nicht paretische Hand), ggf. Pantomime
- Führt beide Aufgaben richtig aus ◯ 0
- Führt eine Aufgabe richtig aus ◯ 1
- Führt keine Aufgabe richtig aus ◯ 2

2. Blickbewegungen
Blick folgt dem Finger des Untersuchers
- Normal ◯ 0
- Partielle Blickparese. Dieser Punktwert wird vergeben, wenn die Blickrichtung von einem oder beiden Augen abnormal ist, jedoch keine forcierte Blickdeviation oder komplette Blickparese besteht ◯ 1
- Forcierte Blickdeviation oder komplette Blickparese, die durch Ausführen des okulocephalen Reflexes nicht überwunden werden kann ◯ 2

3. Gesichtsfelder
Visuelle Gesten oder Finger zählen
- Keine Gesichtsfeldeinschränkung ◯ 0
- Partielle Hemianopsie ◯ 1
- Komplette Hemianopsie ◯ 2
- Bilaterale Hemianopsie (Blindheit inkl. kortikaler Blindheit) ◯ 3

4. Facialisparese
Zähne zeigen, Stirn runzeln, Augen schließen
- Normale symmetrische Bewegungen ◯ 0
- Geringe Parese (abgeflachte Nasolabialfalte, Asymmetrie beim Lächeln) ◯ 1
- Partielle Parese (vollständige oder fast vollständige Parese des unteren Gesichts) ◯ 2
- Vollständige Parese von ein oder zwei Seiten (Fehlen von Bewegungen im oberen und unteren Teil des Gesichts) ◯ 3

5. / 6. Motorik von Armen und Beinen

5. Arme links rechts
Arme in 90° Position bringen
- Kein Absinken, die Extremität wird über 10 Sekunden in der 90° (oder 45°) Position gehalten ◯ 0 ◯ 0
- Absinken, Extremität wird zunächst bei 90° (oder 45°) gehalten, sinkt aber vor Ablauf von 10 Sekunden ab; das Bett oder eine andere Unterlage wird nicht berührt ◯ 1 ◯ 1
- Anheben gegen Schwerkraft möglich; Extremität kann die 90° (oder 45°) Position nicht erreichen oder halten, sinkt auf das Bett ab, kann aber gegen Schwerkraft angehoben werden ◯ 2 ◯ 2
- Kein (aktives) Anheben gegen Schwerkraft, Extremität fällt ◯ 3 ◯ 3
- Keine Bewegung ◯ 4 ◯ 4
- Amputation, Gelenkversteifung ◯ 9 ◯ 9

Bewertung mit 9 = „Amputation" bitte als 0 zählen.
bitte erklären:

6. Beine links rechts
Beine in 45° Position bringen
- Kein Absinken, Bein bleibt über 5 Sekunden in der 30° Position ◯ 0 ◯ 0
- Absinken, Bein sinkt am Ende der 5 Sekundenperiode, berührt das Bett jedoch nicht ◯ 1 ◯ 1
- Aktive Bewegung gegen Schwerkraft, das Bein sinkt binnen 5 Sekunden auf das Bett ab, kann aber gegen die Schwerkraft gehoben werden ◯ 2 ◯ 2
- Kein Anheben gegen die Schwerkraft, Bein fällt sofort auf das Bett ◯ 3 ◯ 3
- Keine Bewegung ◯ 4 ◯ 4
- Amputation, Gelenkversteifung ◯ 9 ◯ 9

Bewertung mit 9 = „Amputation" bitte als 0 zählen.
bitte erklären:

7. Extremitäten Ataxie

Finger-Nase-Finger bzw. Ferse-Hacke-Versuch

- Fehlend ◯ 0
- In einer Extremität vorhanden ◯ 1
- In zwei Extremitäten vorhanden ◯ 2

Nur das erste Item „Extremitätenataxie" zählen, die Unterpunkte rechter/linker Arm bzw. rechtes/linkes Bein dienen nur der Information.

Falls vorhanden besteht die Ataxie in

Rechtem Arm

- Nein ◯ 1
- Ja ◯ 2
- Amputation, Gelenkversteifung ◯ 9
  bitte erklären:

.....

Linkem Arm

- Nein ◯ 1
- Ja ◯ 2
- Amputation, Gelenkversteifung ◯ 9
  bitte erklären:

.....

Rechtem Bein

- Nein ◯ 1
- Ja ◯ 2
- Amputation, Gelenkversteifung ◯ 9
  bitte erklären:

.....

Linkem Bein

- Nein ◯ 1
- Ja ◯ 2
- Amputation, Gelenkversteifung ◯ 9
  bitte erklären:

.....

8. Sensibilität

Nadel-, Schmerzreize bei Armen, Beinen, Körper, Gesicht

- Normal; kein Sensibilitätsverlust ◯ 0
- Leichter bis mittelschwerer Sensibilitätsverlust, Patient empfindet Nadelstiche auf der betroffenen Seite als weniger scharf oder stumpf oder es besteht ein Verlust des Oberflächenschmerzes für Nadelstiche, doch nimmt der Patient die Berührung wahr ◯ 1
- Schwerer bis vollständiger Sensibilitätsverlust, Patient nimmt die Berührung von Gesicht, Arm und Bein nicht wahr ◯ 2

9. Sprache

Benennung von Gegenständen, Beschreibung des Bildes, Lesen der Satzliste

- Keine Aphasie; normal ◯ 0
- Leichte bis mittelschwere Aphasie; deutliche Einschränkung der Wortflüssigkeit oder des Sprachverständnisses, keine relevante Einschränkung von Umfang und Art des Ausdruckes. Die Einschränkung des Sprachvermögens und/oder des Sprachverständnisses macht die Unterhaltung über die vorgelegten Untersuchungsmaterialien jedoch schwierig bis unmöglich. Beispielsweise kann der Untersucher in einer Unterhaltung über die vorgelegten Materialien anhand der Antwort des Patienten ein Bild oder eine Wortkarte zuordnen ◯ 1
- Schwere Aphasie; die gesamte Kommunikation findet über fragmentierte Ausdrucksformen statt. Der Zuhörer muss das Gesagte in großem Umfang interpretieren, nachfragen oder erraten. Der Umfang an Informationen, der ausgetauscht werden kann, ist begrenzt, der Zuhörer trägt im wesentlichen die Kommunikation. Der Untersucher kann die vorgelegten Materialien anhand der Antworten des Patienten nicht zuordnen ◯ 2
- Stumm, globale Aphasie; keine verwendbare Sprachproduktion oder kein Sprachverständnis ◯ 3

10. Dysarthrie

Vorlesen der Wortliste

- Normal ◯ 0
- Leicht bis mittelschwer; der Patient spricht zumindest einige Wörter verwaschen und kann, schlimmstenfalls nur mit Schwierigkeiten verstanden werden ◯ 1
- Schwer; die verwaschene Sprache des Patienten ist unverständlich und beruht nicht auf einer Aphasie oder übersteigt das auf eine Aphasie zurückführende Maß oder Patient ist stumm/anarthrisch ◯ 2
- Intubation oder andere mechanische Behinderungen ◯ 9

Bewertung mit 9 = „Intubation oder andere mechanische Behinderungen" bitte als 0 zählen.
bitte erklären:

.....

11. Auslöschung und Nichtbeachtung (früher: Neglect)

Verwendung der vorangegangenen Untersuchungen

- Keine Abnormalität ◯ 0
- Visuelle, taktile, auditive, räumliche oder personenbezogene Unaufmerksamkeit oder Auslöschung bei der Überprüfung von gleichzeitiger bilateraler Stimulation in einer der sensiblen Qualitäten ◯ 1
- Schwere halbseitige Unaufmerksamkeit oder halbseitige Unaufmerksamkeit in mehr als einer Qualität. Kein Erkennen der eigenen Hand oder Orientierung nur zu einer Seite des Raums ◯ 2

Punkte 7. + 8. ☐ Punkte 9. – 11. ☐

Gesamtpunkte 1. – 11. ☐

11. Affidavit

I, Samantha Taber, certify under penalty of perjury by my own signature that I have submitted the thesis “Associations between cerebral microbleeds and lipids in first time ischemic stroke patients.” I wrote this thesis independently and without assistance from third parties, other than as mentioned in “Acknowledgements.” 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 section on methodology (in particular practical work, laboratory requirements, statistical processing) and results (in particular images, graphics and tables) corresponds to the URM and are answered by me.

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

12. Curriculum vitae

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

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

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