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The Association Between Whole Blood Viscosity and Cerebral Infarct Growth in Acute Ischemic Stroke: A Pilot Study

Degree Project in Medicine

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Abstract

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Background: Ischemic stroke is caused by focal hypoperfusion in the brain due to an obstructed cerebral vessel. Viscosity is the internal friction in a fluid and determinates its resistance to flow. Higher whole blood viscosity (WBV) has previously been associated with increased risk of ischemic stroke as well as reduced blood flow and tissue perfusion. However, the impact of elevated WBV on infarct growth remains unclear.

Aim: The aim of the study was to explore the association between WBV and infarct growth in patients with acute ischemic stroke.

Method: Forty-three patients with ischemic stroke were included in the study. The blood viscosity was measured in a whole blood sample, obtained on arrival to hospital, at a temperature of 37 °C and a shear rate of 20 s^{-1} after adjusting it to 40% hematocrit. The infarct growth was calculated as the difference in infarct volume between an initial brain computer tomography and a follow up magnetic resonance imaging examination. The relationship between WBV and infarct growth was analyzed using simple linear regression.

Findings: A non-significant linear regression equation was found with a constant of –22.53 (95% CI: –64.41-19.36, p=0.28) and a slope of 5.86 (95% CI: –2.38-14.10, p=0.16). The correlation coefficient (R) for the linear model was 0.22 (p=0.16) and the coefficient of determination (R^2) was 0.05. A post-hoc power analysis showed that the study was under dimensioned with a power of 0.30. To obtain a power of 0.80 in a sample with the same effect size as in this study an estimate of 158 patients would be needed to include.

Interpretation: A linear relationship between WBV and infarct growth cannot be proven in acute ischemic stroke because of the low statistical significance of the results in this study. However, due to the limited statistical power, nor can it be ruled out. Further studies with lager sample size will be needed for any definite conclusions to be made. The results in this paper can thus be used as a support while designing future studies in the field.

Keywords: ischemic stroke, cerebral infarction, whole blood viscosity, infarct growth

Table of content

Abbreviations

ADC	Apparent diffusion coefficient
AF	Atrial fibrillation
ATP	Adenosine triphosphate
CBF	Cerebral blood flow
CBV	Cerebral blood volume
CI	Confidence interval
CNS	Central nervous system
cP	Centipoise
CPP	Cerebral perfusion pressure
CT	Computer tomography
CTA	Computer tomography angiography
CTP	Computer tomography perfusion
CVA	Cerebrovascular accident
CVR	Cerebrovascular resistance
DALY	Disability adjusted life years
DT	Delay time
DWI	Diffusion weighted imaging
ECG	Electrocardiography
ICP	Intracranial pressure
LVO	Large vessel occlusion
MAP	Mean arterial pressure
MCA	Middle cerebral artery
mCT	Multimodal computer tomography
mL	Milliliter
MRA	Magnetic resonance angiography
MRI	Magnetic resonance imaging
mRS	Modified Rankin Scale
NCCT	Non-contrast computer tomography
NIHSS	National Institutes of Health Stroke Scale
NOAC	Non-vitamin K antagonist oral anticoagulants
P	Poise
PI	Prediction interval
PWI	Perfusion-weighted imaging
Q-Q plot	Quantile-quantile plot
R	Correlation coefficient
R^2	Coefficient of determination
rtPA	Recombinant tissue plasminogen activator
s^{-1}	Reciprocal seconds
SAH	Subarachnoid hemorrhage
SD	Standard deviation
SE	Standard error
SWI	Susceptibility weighted imaging
T2-FLAIR	T2-weighted fluid-attenuated inversion recovery
TIA	Transient ischemic attack
tPA	Tissue plasminogen activator
WBV	Whole blood viscosity
WHO	World Health Organization

Background

Definition of stroke

Stroke is defined by the World Health Organization (WHO) as a rapid development of focal or global neurological symptoms lasting for more than 24 hours, or leading to death before that, with no obvious origin other than vascular. This definition includes cerebral infarction, intracerebral hemorrhage and subarachnoid hemorrhage (SAH) but not transient ischemic attack (TIA) or traumatic intracranial bleedings.[1-3]

The American Heart Association and the American Stroke Association have in more recent years proposed a new tissue-based definition of stroke. They suggest to define stroke as an episode of neurological symptoms derived from the central nervous system (CNS) caused by focal ischemic infarction, non-traumatic intracerebral hemorrhage or non-traumatic SAH. Infarction or hemorrhage caused by cerebral sinus thrombosis also fall under this definition of stroke. Ischemic stroke is thus defined as a focal infarction in the CNS accompanied by overt neurological symptoms while hemorrhagic stroke is defined as rapidly developing neurological symptoms attributed to a focal collection of blood in the cerebral parenchyma or ventricle system. A CNS infarction is in turn defined as neural cell death attributable to focal ischemia within a vascular distribution or watershed territory in the brain, spinal cord or retina. The infarction diagnosis can be made based on objective findings (such as radiological or autopsy findings) or clinical evidence of permanent ischemic injury. For clinical diagnosis, without imaging or autopsy findings, neurological symptoms must persist for not less than 24 hours, or until death, without evidence of any other etiology than ischemia. Infarction and cerebral hemorrhage without clinical symptoms are not classified as stroke but as a silent infarction and silent cerebral hemorrhage, respectively. Global cerebral ischemic injury is however not included in the definition of stroke proposed by the American Heart Association and the

American Stroke Association since the cause and treatment normally differ from the focal cerebral ischemic injury.[4]

TIA is defined as focal neurological symptoms caused by cerebral ischemia but which resolves within 24 hours.[2, 3, 5] In most cases however the symptoms will resolve within 2 hours.[3] Modern brain imaging has nevertheless shown persistent ischemic injury in many patients with clinically transient symptoms.[4] The American Heart Association and the American Stroke Association have therefor suggested a tissue-based definition in order to make a clear distinction between TIA and ischemic stroke. They propose to define TIA as a transient episode of neurological symptoms caused by focal ischemia in the brain, spinal cord or retina but without persistent infarction.[5]

Stroke epidemiology

The risk of stroke is exponentially increasing with higher age, from about 30 per 100,000 citizens per year in the population aged 30-40 years to 2,000-3,000 per 100,000 citizens per year in the population older than 85 years. The incidence is 25-40% higher among men than women in the same age-group.[3] Of all stroke cases 87% is due to ischemic stroke and 13% to hemorrhagic stroke.[6] Between 25-30% of all preventable strokes are recurrent events.[7]

The worldwide incidence of stroke was in 2010 estimated to16.9 million. This resulted in 5.9 million deaths and the loss of over 102 million disability adjusted life years (DALYs). DALYs is defined as the sum of years of life lost because of death and years lived with disability.[8] The deaths due to stroke constitute for 11.1% of all deaths globally, making stroke the second leading cause of death after ischemic heart disease. Ischemic and hemorrhagic stroke account for about half of the stroke related deaths each.[9]

Between 1990 and 2010 the overall incidence of stroke, DALYs lost because of stroke, stroke related deaths and stroke survival rate all increased.[10] This might be related to an aging population and improved stroke treatment and care. If the trend continues it is estimated that by year 2030 there will be about 12 million stroke related deaths and 200 million DALYs lost globally.[10]

The incidence of TIA has been estimated to 50 per 100,000 citizens and year but this might be an underestimation since not everyone with transient symptoms will seek medical care.[3]

Clinical presentation of stroke

Stroke typically present as a sudden onset of focal neurological symptoms due to loss of function in specific areas of the brain, spinal cord or retina.[11] However, symptoms can also progress gradually.[2] Typical symptoms from unilateral brain or spinal cord engagement are ipsi- or contralateral weakness or sensory loss depending on the localization of the infarct.[3, 11, 12] Lesions in the brain can also cause hemianopsia, speech disturbances (dominate hemisphere) or visual-spatial-perception dysfunction (non-dominant hemisphere). Ataxia and vertigo can be symptoms of a cerebellar stroke but vertigo can also be caused by a lesion in the pathway between the vestibulum and cerebellum.[3, 11, 12] Neural damage in the oculomotor pathway can cause double vision and lesions in the retina or optic nerve can cause monocular blindness.[11] Headache can be a prominent symptom in hemorrhagic stroke but is often, if at all present, overshadowed by other neurological deficits in ischemic stroke.[13] Atypical symptoms of stroke include personality changes, abnormal movements (rather than paralysis) and seizures.[11] Small infarctions in the areas of connections between central and peripheral neurons can also generate an atypical clinical picture more consistent with peripheral neural damage.

However, there are also other possible causes of focal neurological deficits and headache than stroke. Traumatic brain injury, migraine, intracranial tumors and CNS-infections are some examples.[12, 13] It is thus important to always consider radiological examination to verify the diagnosis and simultaneously differentiate between hemorrhagic and ischemic stroke. A fast and accurate stroke diagnosis is key to early interventional treatment which may improve survival, functional recovery and minimize the risk of recurrent stroke.[11]

Cerebral perfusion

The brain is in continuous need of high perfusion in order for the neurons to receive oxygen and glucose, since these cells do not have the ability of anaerobic metabolism.[3] Cerebral perfusion refers to tissue level blood flow in the brain.[14] During normal circumstances the cerebral blood flow (CBF) is about 45-50 mL per 100 g brain tissue per minute. That is about 15-20% of the cardiac output at rest. Different functions in the brain cells are affected at different levels of CBF reduction. When CBF is reduced to 25-30 mL per 100 g brain tissue per minute some functions are impaired but the main functions and metabolism in the cells are maintained through compensatory mechanisms. At a CBF below 20 mL per 100 g brain tissue per minute the neurons can no longer transmit electrical impulses and the cells are at risk for irreversible damage. However, it is not until the CBF is reduced to about 10 mL per 100 g brain tissue per minute that the essential ion pumps in the cell membrane stop working, resulting in death of the cells.[3]

The CBF is determined by the cerebral perfusion pressure (CPP) and cerebrovascular resistance (CVR). The CPP is the difference between the mean arterial pressure (MAP) and the intracranial pressure (ICP) while the CVR according to Poiseuille's law is determined by the diameter and length of the intracranial arteries together with blood viscosity.[15, 16] When the

MAP is within the limits of about 60-150 mmHg the CBF is relatively constant in healthy brain tissue. This phenomenon is called cerebral autoregulation and is due to reciprocal changes in arterial tone thus regulating the CVR to meet the perfusion demands of the brain. If MAP is outside of this interval the cerebral autoregulation becomes compromised and the regulation of CBF becomes a more passive process.[17] In patients with chronic hypertension both the upper and lower MAP limits might be elevated.[18] Patients with acute ischemic stroke often present with an elevated blood pressure and it is believed that this might be a compensatory mechanism to increase the CBF in the ischemic tissue through collateral artery flow.[19]

There are three principal collateral systems in the human brain but their occurrence varies markedly between individuals. The primary collateral circulation is the circle of Willis which, if fully developed, connects the anterior and posterior intracranial circulation as well as the circulation of the left and right hemispheres. The major cerebral and cerebellar arteries are also connected by anastomoses in the pia mater, forming a leptomeningeal collateral circulation.[19, 20] This system is normally more prominent in individuals with a chronic stenosis in a more proximal artery.[19] There can also be anastomoses between the pial arteries and extracranial arteries (such as the facial, maxillary, middle meningeal and occipital arteries) thus connecting the extra- and intracranial circulation.[19, 20]

Pathophysiology of ischemic stroke

Ischemic stroke is caused by focal hypoperfusion in the brain due to an obstructed cerebral vessel.[2] When the CBF is reduced to about 10 mL per 100 g brain tissue per minute the restricted supply of oxygen and glucose causes insufficient production of intracellular adenosine triphosphate (ATP) and essential ion pumps in the cell membrane stop working. Sodium and calcium start to build up within the cell causing cytotoxic edema, free radical

formation, glutamate release and breakdown of the cell membrane. The cell death stimulates inflammation and vasogenic edema.[21, 22]

In the acute setting of stroke there are typically different levels of cellular impairment within different parts of the hypoperfused brain parenchyma, corresponding to the level of reduced CBF. Areas where the brain cell have died are referred to as the "ischemic core" while the hypoperfused area with cells at risk of irreversible damage is referred to as the "ischemic penumbra". The ischemic penumbra is thus tissue that have not yet infarcted and can be saved if adequate perfusion is restored in time.[21] This time frame is highly individual[2] and a well-developed collateral circulation plays a protective role by maintaining blood flow in the damaged tissue and slow the progression of penumbral tissue to irreversible infarction.[15]

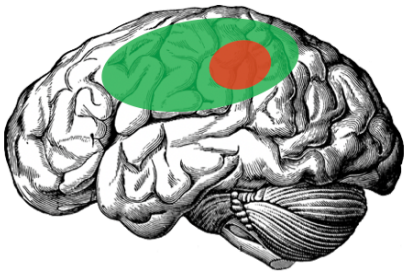

Figure 1: *A brain with a schematic visualization of the hypoperfused area (red and green) consisting of the irreversible damaged ischemic core (red) and the reversible damaged ischemic penumbra (green). If the cerebral blood flow remains compromised in the hypoperfused area the tissue in the penumbra will eventually infarct as well.*

Etiology of ischemic stroke

The etiology of ischemic stroke can be classified by the ASCOD classification system as atherosclerosis, small-vessel disease, cardiac pathology, other causes and dissection.[23] Stroke caused by atherosclerosis, small-vessel disease and cardiac pathology each represents about 25%, 20% and 25% of all symptomatic cerebral infarcts. The remaining 30% are mainly cryptogenic.[3]

Atherosclerosis most commonly causes stroke by artery to artery embolus but thrombosis and hemodynamic effects of a high-grade stenosis also occur.[3] Rupture of an arteriosclerotic plaque triggers aggregation of platelets and coagulation of fibrinogen which produces a clot. This can either thrombose the vessel at the location of formation (in situ thrombosis) or embolize to a more distal vessel.[2] Artery to artery embolus often occur from a more proximal cerebral artery, the carotids or the aortic arch.[23]

Atrial fibrillation (AF) is the most common cardiac pathology causing cardioembolic stroke[3] but other causes such as mitral stenosis, mechanical heart valve, recent myocardial infarction, mural thrombus in the left cavities, left ventricle ejection fraction less than 35%, endocarditis and a patent foramen ovale in combination with deep venous thrombosis or thrombus in situ also occur.[23] Embolic strokes tend to be multifocal since several embolies may be released and disseminate in the peripheral vessel tree.

Small-artery occlusion is caused by small vessel disease and causes lacunar infarcts, mainly in the basal ganglia, capsula interna and pons, where the arterioles terminates. The infarcts caused by small-artery occlusion are often silent and might thus only be an incidental finding on radiological examination.[3] Several pathogeneses are thought to cause the narrowing of the small cerebral vessels seen in small vessel disease but the underlying mechanisms are not yet fully understood.[24]

Dissection of the carotid or vertebral arteries is a relatively uncommon cause of ischemic stroke and mainly affects children, young adults, and trauma patients.[25] Vascular dissection can cause ischemia by two main mechanisms. The tear in the vessel wall enables formation of a false lumen causing an intramural hematoma that can obstruct the true lumen and thus reduces the CBF. The damaged vessel however also stimulates coagulation, causing clot formation that can thrombose the dissected vessel or embolize to a more distal vessel branch.[26]

Other potential causes of ischemic stroke are conditions with systemic, intracerebral or vascular inflammation, conditions with risk of excessive blood clotting and conditions with increased whole blood viscosity (WBV).[23]

Risk factors for ischemic stroke

Hypertension, high LDL cholesterol, diabetes mellitus, current smoking, excessive alcohol use, obesity, psychosocial factors, carotid stenosis and AF are all risk factors for ischemic stroke.[27-34] Regular physical activity and healthy diet have been shown to decrease the risk.[32] Primary prevention of stroke should thus aim to prevent and treat the risk factors and maintain a healthy lifestyle. The risk of future ischemic stroke is also increased after an index event of ischemic stroke or TIA with the risk being highest immediately after the index event.[7, 35-37] The incidence of recurrent stroke has however since the turn of the millennium decreased due to more effective secondary prevention.[36]

Hemorheology and whole blood viscosity

Rheology is the science of the deformation and flow of matter.[38] Hemorheology is thus the field describing the flow properties of blood and is mainly influenced by the WBV. Viscosity is the internal friction in a fluid.[39] All fluids are more or less viscous and it determinates their resistance to flow.[38] Friction forces between the fluid and a solid surface, as well as between adjacent layers of fluid, causes streamlining and laminar flow where each layer of flowing fluid is moving with its own velocity and keeping the same distance to the solid surface. Even when the fluid is in motion there is a thin boundary layer of the fluid which is nearly at rest with respect to the solid surface. The cells and particles in the second outermost layer roll over the outermost layer giving it a slightly higher velocity. The content in the third

outermost layer rolls over the second outermost, and so on. In a vessel the velocity of the fluid is thus gradually increasing from the vessel wall towards the center, **Figure 2**.[16, 39]

Viscosity is defined as the ratio between shear stress and shear rate and holds the unit of poise (P) which is equal to pressure (newtons per square meter or pascal) multiplied with time (seconds).[38] Shear stress is the shear force one layer of fluid exerts tangentially per area along another layer of fluid or the solid surface when the two layers are moving with different velocity and is measured in newtons per square meters or pascals.[40, 41] Shear rate is the gradient of change in velocity between layers of fluid and is measured in reciprocal seconds (s^{-1}).[38, 42] This is mathematically calculated as:

$$sheer\ rate = \frac{v_1 - v_2}{h}$$

where v_1 and v_2 are the velocity of the fluid in the innermost respectively the outermost layer in meters per second and h is the distance between the two layers in meters.[42]

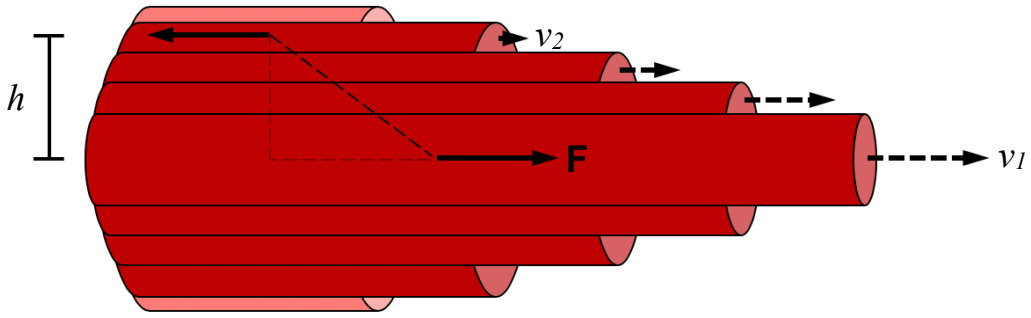

Figure 2: *Schematic image of the laminar flow in a blood vessel. The different layers of blood flows with different velocities (v_1 and v_2) which generates shear stress by frictional drag (F) between the laminar layers. The increasing velocity towards the center of the vessel also creates a velocity gradient within the fluid called shear rate. h is the distance between the innermost and the outermost laminar layers.*

Newtonian fluids have a linear relationship between shear rate and shear stress [38, 43] and as a consequence of this have a constant viscosity.[38] The characteristic of a non-Newtonian

fluid is thus a nonlinear relationship between shear rate and shear stress, **Figure 3**. Blood is a non-Newtonian fluid and its viscosity varies mainly in relation to shear rate, level of hematocrit and temperature.[38, 42] Lower shear rate, higher hematocrit and lower temperature increases blood viscosity.[42] Since WBV decreases with increased shear rate it is defined as a shear thinning fluid.[38]

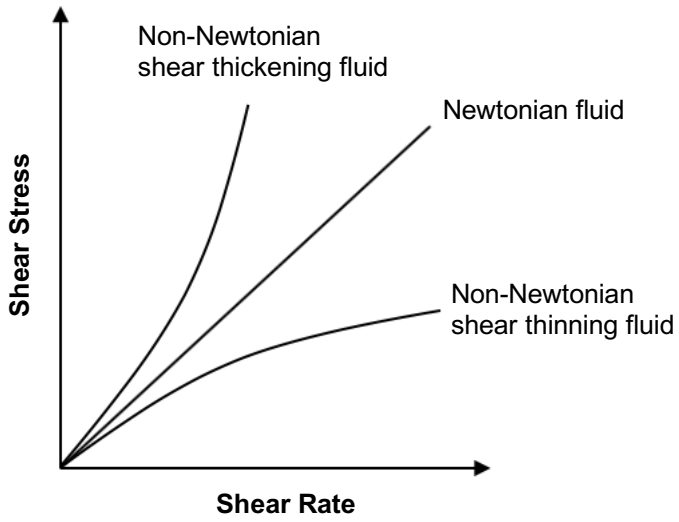

Figure 3: *The relationship between shear rate and shear stress in a Newtonian respectively a non-Newtonian shear thinning and a non-Newtonian shear thickening fluid. The viscosity in a fluid at a given shear rate is defined as the slope of the line corresponding to that shear rate value.*

Other factors that affect the WBV are the plasma content and erythrocyte deformability.[16, 38] Both the types of plasma proteins and the total amount will affect the viscosity but compared to the hematocrit the effect is minimal.[16] The viscosity of isolated plasma however also decreases with increased shear rate and temperature.[42] The deformability properties of erythrocytes will mainly affect the WBV during high shear rates and in the smallest of capillaries where flow is determined by the ability of the erythrocytes to change shape. The main factors that in turn determine erythrocyte deformability is the viscoelasticity of the cell membrane (and thus the molecular structure of the membrane), the cell geometry (the ratio of

surface area to cell volume) and the internal viscosity of the cell (determined by the physical state of the hemoglobin).[38]

Of all the factors determining WBV the hematocrit is the most influential.[16, 38] The erythrocytes increase the internal friction in blood by exerting frictional drag against other cells and the blood vessel wall. In healthy adult males the hematocrit averages about 42% and in healthy adult females about 38%. The specific level for a single individual can however vary a lot depending on factors such as whether the person has anemia, the degree of bodily activity and the altitude of residence.[16] Since hematocrit vary between individuals and is the most influential factor for the WBV it is customary to adjust it to a standardized level in most studies to not obscure the effects of other determinants of viscosity. An exception is of course if the study specifically aiming to investigate the effects of the hematocrit in terms of blood viscosity.[38]

Since hematocrit and temperature is relatively constant within the circulatory system it is mainly the shear rate that determines the viscosity of the blood in an individual.[42] The velocity of blood changes with the pressure gradient and vessel size and is thus highly different in arteries, veins and capillaries. In large vessels with high pressure the blood flow is markedly laminar.[16] In the small capillaries essentially all the blood is close to the vessel wall, not enabling laminar flow. The velocity of the centermost blood in capillaries is thus relatively low, causing decreased shear rate and resulting in a higher viscosity than in lager vessel with greater flow velocity.[16, 42] The relative low velocity also allow the blood components (mainly erythrocytes) to interact with one another and thus increasing the shear stress.[38] The Fåhræus-Lindqvist effect is a physiological phenomenon that lowers the shear stress in the smallest capillaries by lowering the hematocrit in these vessels. Since erythrocytes cannot pass the vessel endothelium their center is at least the half diameter length of an erythrocyte from the vessel

wall. The result of this is a partially lower concentration of erythrocytes in the outermost blood just by the vessel wall, **Figure 4**. This has no obvious effect in lager vessels but in the smallest capillaries this layer of blood with reduced hematocrit gets proportionally large, decreasing the overall hematocrit in the smaller vessels compared to the larger. Since lower hematocrit reduces shear stress the viscosity in the small capillaries gets lower which help to offset the increase in viscosity that occur because of reduced shear rate.[44-46] Because shear rate and shear stress vary the WBV also vary within the vessel tree.[42]

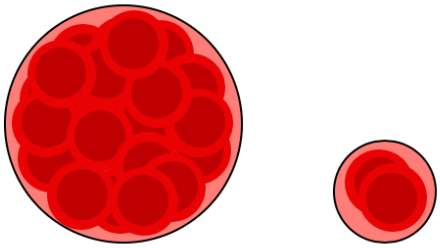

Figure 4: *Schematic cross section of two blood vessels containing erythrocytes (red circles) and plasma (light red marginal). The concentration of erythrocytes is lower in the outermost marginal of the vessel since their center is at least the half diameter length of an erythrocyte from the vessel wall. The volume of blood with a lower hematocrit becomes proportionally large in the smallest vessels compared to lager vessels thus reducing the total whole blood viscosity in the small vessels. This is called the Fåhræus-Lindqvist effect.*

Viscosity measured at a shear rate of 300 s^{-1} may be referred to as systolic WBV (and is often equated with the viscosity in large, high flow arteries) whereas viscosity measured at a shear rate of 1 s^{-1} may be referred to as diastolic WBV.[47] A shear rate of 20 s^{-1} could therefore be assumed to correspond with the conditions in the smaller arteries.

WBV is one of the factors determining the CVR and thus also the CBF.[14] Higher blood viscosity retards the blood flow in the vessels.[16] Measured at different shear rates, higher WBV has previously been demonstrated to be associated with[48-55] and increase the risk of[56] ischemic stroke and silent cerebral infarction. Higher plasma viscosity has also been associated with ischemic stroke[51-53, 57] and has been suggested to be an independent risk

factor for the condition.[56] How WBV affect the progress of cerebral infarctions in acute ischemic stroke has however, to the best of our knowledge, not yet been studied.

Assessment of ischemic stroke

Clinical examination

Clinical neurological examination is crucial in the initial assessment of stroke and radiological investigation, although informative, should always be considered a compliment to clinical examination. The physician should aim to conclude the symptoms, analyze potential differential diagnoses, conclude the localization and nature of the lesion and assess the need and possibility of thrombolytic or endovascular thrombectomy treatment.[3] To facilitate and equalize the reporting several scales can be used for different purposes. Two generally used are the National Institutes of Health Stroke Scale (NIHSS) and the modified Rankin Scale (mRS).

The NIHSS is an impairment scale used to measure stroke severity. It includes assessment of level of consciousness, eye movements, integrity of visual fields, facial movements, arm and leg muscle strength, sensation, coordination, language, speech and neglect. The scale ranges from 0 to 42 with a higher score indicating a more severe stroke.[58]

The mRS ranges from 0 to 5 and measures the functional outcome in terms of symptom burden and dependence in daily activities of a patient after stroke.[59] A score of six is often added to denote death of the patient.[60] The mRS is a valuable tool in the assessment of the patient. The scale can also be used to evaluate the patient's disability prior to stroke onset as part in evaluating the potential gain of interventional treatment.

Stroke imaging

All patients with suspected acute stroke should receive emergency brain imaging on arrival to a hospital before initiating any acute stroke treatment.[61]

Computer tomography

Multimodal computer tomography (mCT) of the brain is an imaging protocol consisting of non-contrast computer tomography (NCCT), computer tomography angiography (CTA) of both brain and neck vessels and computer tomography perfusion (CTP). This protocol is developed to answer four questions in the setting of an acute stroke: (1) Is there hemorrhage? (2) Is there intravascular thrombus that can be target for thrombolysis? (3) Is there a core of critically ischemic irreversibly infarcted tissue? (4) Is there a penumbra of severely ischemic but potentially salvageable tissue?[14] In recent years thrombectomy has gained a large role in the treatment arsenal and would today be as relevant as thrombolysis for some selected patients with visible occlusions. While NCCT and CTA answer the questions one and two respectively, CTP plays an important role in answering the questions tree and four.[62, 63]

The raw data from CTP may be postprocessed to generate a number of hemodynamic maps derived from the volume-time analysis of the contrast flowing through the brain tissue. The total hypoperfused lesion is typically defined as tissue with a delay time (DT) of more than 3 seconds (or Tmax more than 6 seconds) while the ischemic core typically is defined by a CBF of less than 30%, both qualities compared to the corresponding areas on the contralateral hemisphere. The penumbra is defined as the mismatch between these volumes.[64] CTP is thus of great importance in both visualization of ischemia as well as in determining the potential gain of interventional treatment.[63]

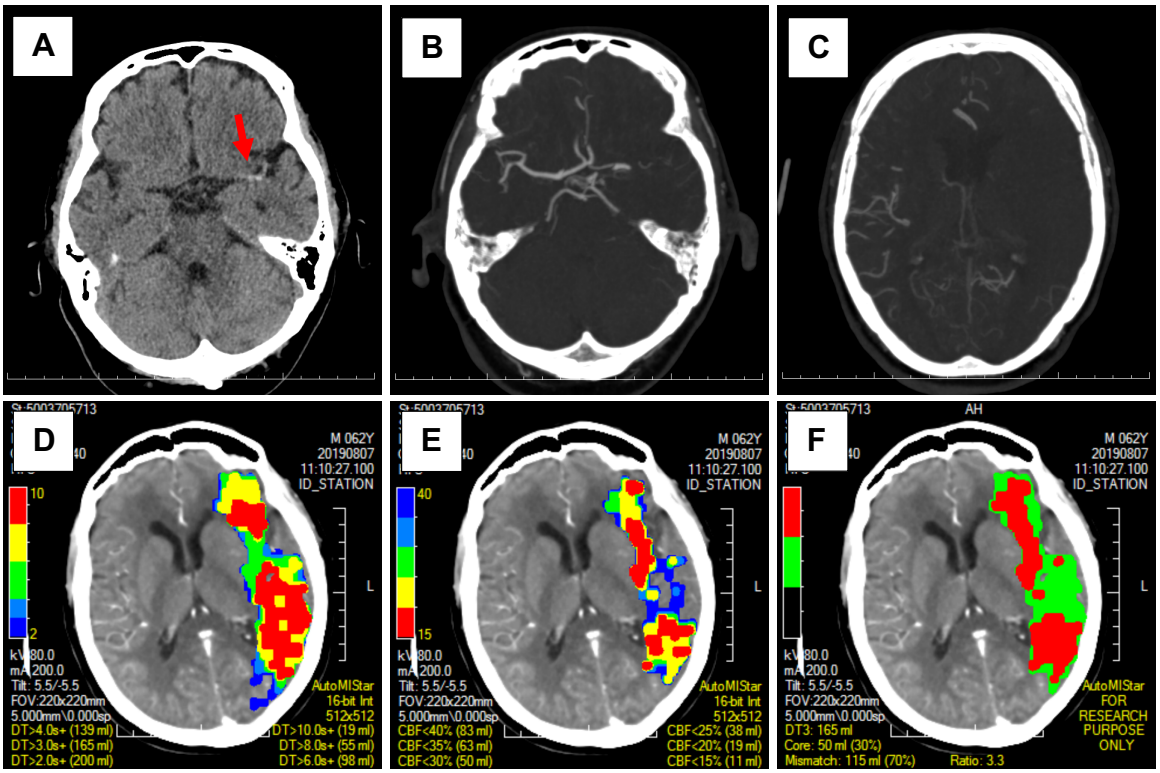

Figure 5: *Multimodal computer tomography images from a patient with acute ischemic stroke stemming from an occlusion in the M1 segment of the left middle cerebral artery (MCA).* ***A:*** *Non-contrast computer tomography with hyperdense sign (arrow) in the M1 segment of the left MCA.* ***B:*** *Computer tomography angiography (CTA) with abrupt interrupted blood flow in the M1 segment of the left MCA.* ***C:*** *CTA showing poor collateral flow in the left MCA territory.* ***D:*** *Delay time (DT) band map postprocessed from computer tomography perfusion (CTP) highlighting areas with different levels of prolonged DT.* ***E:*** *Cerebral blood flow (CBF) band map postprocessed from CTP highlighting areas with different levels of decreased CBF.* ***F:*** *Lesion map postprocessed from CTP showing the total hypoperfused area (green and red) defined as a DT of more than 3 seconds, the ischemic core (red) defined as CBF of less than 30% and the ischemic penumbra (green) defined as the mismatch between the total hypoperfused area and the ischemic core.*

Magnetic resonance imaging

Magnetic resonance imaging (MRI) is a technique based on electromagnetism and mainly measures the movements of protons (hydrogen nucleus) in different magnetic fields. This differentiates the elements of a tissue depending on their composition and can be used to process a number of different imaging sequences. An MRI protocol often used in acute stroke consists of T2-weighted fluid-attenuated inversion recovery (T2-FLAIR), magnetic resonance angiography (MRA), diffusion weighted imaging (DWI), apparent diffusion coefficient (ADC), perfusion-weighted imaging (PWI) and susceptibility weighted imaging (SWI).

The DWI and ADC is sensitive to the random translational motion of water molecules in tissue thus showing the degree of cellular swelling (cytotoxic edema) or concentration of cells within the tissue by which free water molecules diffuses in the area. Restricted diffusion shows as increased signal intensity on DWI, and decreased signal intensity on ADC maps. This area is generally considered to be irreversibly damaged in the setting of ischemic stroke.[65] T2-FLAIR imaging incorporates a T2-weighting with suppression of cerebrospinal fluid and is often used to visualize subacute to chronic ischemic brain lesions by vasogenic edema.[65, 66] MRA can, like CTA, be used to visualize vessel occlusion or stenosis.[65] The raw data from the PWI is like the CTP data used to construct hemodynamic maps and the application of the information generated is more or less the same from both investigations.[62, 65] SWI uses tissue magnetic susceptibility to generate a visual map. Because of the iron content within erythrocytes it is thus highly sensitive to differ fresh blood as well as old bleedings from other tissue.[67]

When MRI is used in the acute setting of stroke the ischemic core is typically defined by the restricted diffusion on DWI while the penumbra is defined from mismatch between this volume and the total hypoperfused volume which is visualized on the PWI.[65] MRI is however mainly used in follow up imaging of stroke. The DWI visualizes the expanse of the infarct and the SWI demonstrates any hemorrhagic transformation. A comparison with the ADC and T2-FLAIR is usually also performed to exclude artifacts (such as T2 shine-through and ADC pseudonormalization) and differ the acute infarction from any older lesions and other cerebral pathology.

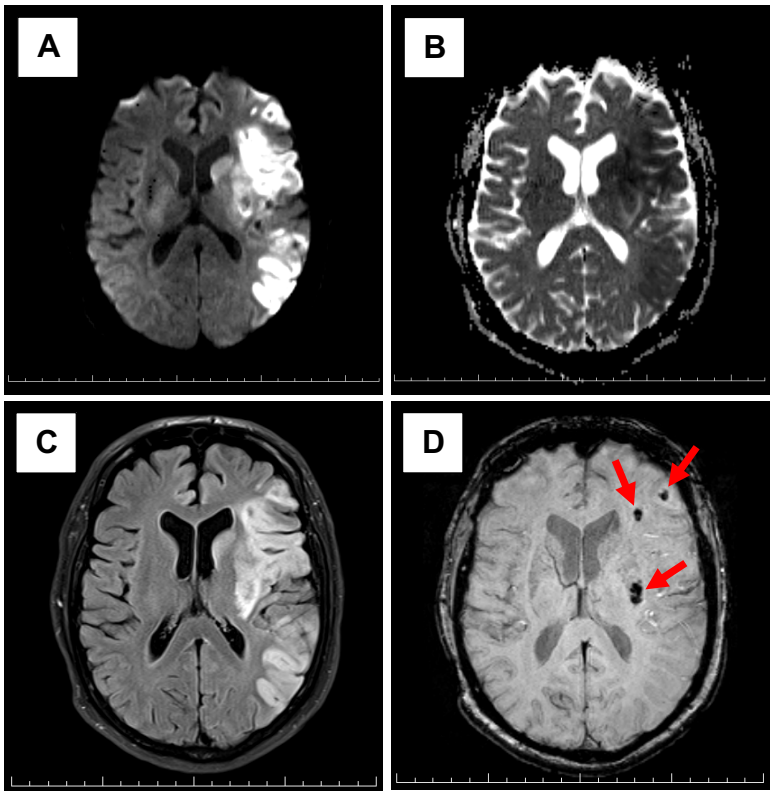

Figure 6: *Follow up magnetic resonance imaging sequences of the same patient as in Figure 5, exerted 27.5 hours after the initial multimodal computer tomography.* ***A:*** *Diffusion weighted imaging showing the infarcted area with restricted diffusion as high signal (white).* ***B:*** *Apparent diffusion coefficient showing the infarcted area with restricted diffusion as low signal (black).* ***C:*** *T2-weighted fluid-attenuated inversion recovery showing the lesion as hyperintense attenuation (white).* ***D:*** *Susceptibility weighted imaging showing hemorrhagic transformation as black areas (arrows) in three foci of the infarcted tissue.*

Choice of imaging technique

MRI with DWI are much more sensitive than NCCT in detecting acute ischemic stroke.[68] However, CTP in the acute setting have been shown to generate reliable predictions of the lesion core and penumbra when compared to MRI with DWI.[69, 70] MRI, particular with DWI, is however superior to mCT in detecting small infarcts and brainstem ischemia.[64] Computer tomography (CT) has practical advantages over MRI by generally being more available, accessible and rapid in execution.[63, 70] Because of this CT is normally the technic of choice in the acute investigation of stroke.[64]

Acute phase investigations

Acute phase investigations and examinations aims to find out the cause of the thromboembolism and to identify potential risk factors. Physiological cardiac examination and electrocardiography (ECG), often as Holter-ECG, should be performed to exclude a cardioembolic source and ultrasound of the carotid arteries to exclude symptomatic (causing stroke, TIA or retinal infarction) carotid stenosis. CTA often gives an indication of carotid stenosis but a complimentary investigation with ultrasound is normally desirable prior to surgical intervention.[3] Frequent general observations, normally including vital signs and neurological assessment, at the ward are important to early identify any complications or recurrence of stroke. In the aftermath other risk factors, such as blood pressure and blood lipid levels, should be investigated in order to adjust the dosages of secondary prevention medicines.

Treatment and management of ischemic stroke

In the initial setting of acute ischemic stroke the main goal is to prevent further progress of the lesion and minimize the risk of early recurrence of stroke. In the longer term risk factor management by medical and surgical interventions are important to minimize the risk of recurrence of stroke.[3]

Acute stroke treatment

Human tissue plasminogen activators (tPA) work by activating plasminogen to plasmin which in turn have fibrinolytic and thus thrombolytic effects.[71] Recombinant tPA (rtPA), such as alteplase or tenecteplase, can thus be used in thromboembolic diseases such as stroke. Intravenous treatment with rtPA increases the risk of intracranial hemorrhage compared to placebo but despite that improves the three-month clinical outcome if initiated within 4.5 hours

of stroke symptom onset.[72, 73] Earlier initiation of treatment is associated with greater benefit. The risk of intracranial hemorrhage is not significantly affected by the time from onset to treatment but increases in relation to the severity of the stroke (measured by the baseline NIHSS).[74] After thrombolytic treatment antithrombotic treatments should not be initiated within 24 hours to minimize the risk of intracranial hemorrhage.[75]

Endovascular thrombectomy is an interventional therapy where the occluding thrombus is mechanically extracted from the vessel via an arterial puncture in the groin or radial artery. Trials have shown that patients can benefit from this treatment up to 24 hours after stroke onset if the radiological imaging shows favorable patterns.[76] Endovascular thrombectomy both in combination with rtPA and as only acute intervention improves the functional outcome 90 days after stroke in patients with occlusion in proximal cerebral vessels.[77] Patients eligible for endovascular thrombectomy have an improved functional outcome and higher rate of revascularization than patients treated with rtPA alone.[78]

The benefit of both treatment with rtPA and endovascular thrombectomy is limited by the relatively narrow criteria for patient selection. In general terms patients with recent symptom onset and radiological signs of cerebral ischemia are considered candidates for these treatments. Patients with increased risk of major bleeding should however be elected with great caution for treatment with rtPA.[61]

Patients not suitable for interventional treatment with rtPA or endovascular thrombectomy are treated conservatively with early initiation of secondary prophylaxis and nursing by needs. Care in a dedicated stroke-care ward has been shown to increase the likelihood of discharge to home and reduce death and dependence compared with care in a general ward.[79] This is mainly attributed to increased knowledge and attention to any complications. The care is typically performed by a multidisciplinary team consisting of physicians, nurses,

physiotherapists, occupational therapists, speech-language pathologists, curators and psychologists. Early mobilization and rehabilitation are important for the patient to regain as much as possible of the disturbed functions.[3]

Secondary stroke prophylaxis

After the diagnosis is verified and initial treatment is given it is important to find the cause of the infarction since this in many cases will affect the choice of secondary prophylaxis. The aim of this treatment is to prevent a second stroke (or first stroke after a TIA). Early and effective secondary prevention can reduce the risk of early recurrent stroke by 80%.[80]

Patients with a stroke stemmed from arteriosclerotic disease will benefit from treatment with antiplatelet agents. Long term use of aspirin or clopidogrel has both been shown to reduce the risk of recurrent stroke, with a slight advantage for clopidogrel.[81] Monotherapy with dipyridamole is equivalent to aspirin or clopidogrel but dipyridamole in combination with aspirin significantly decreases the risk of recurrent stroke.[82] The benefit from a combination of aspirin and clopidogrel compared to the substances individually is ambiguous and results varies between different studies.[83, 84] Nevertheless, a short-term therapy (up to one month) with the combination of aspirin and clopidogrel, initiated adjacent to the ischemic stroke or TIA, seems to be associated with a reduced risk of recurrent stroke without any increase in risk of intracranial or major bleeding compared to monotherapy.[85]

Since the risk of stroke is higher after a previous event[35-37] the overall risk factor reduction is at least as important for prevention of recurrent stroke as it is to prevent the initial onset. Lowering of blood pressure and LDL cholesterol have both proven to lower the risk of recurrent stroke in patients with arteriosclerotic cause of stroke regardless of if the patient fulfills the criteria for hypertension or hyperlipidemia.[27, 28, 86, 87]

A patient with a symptomatic extracranial carotid stenosis of 70-99% will benefit from surgical endarterectomy.[88] Endovascular carotid stenting is an alternative treatment to endarterectomy but have a higher rate of recurrent and peri-procedural stroke in patients over 70 years old.[89, 90] However, long-term functional outcome and the risk of fatal stroke appears to be similar.[89, 91] Endarterectomy or stenting should optimally be done early after stroke or TIA since the risk of recurrent stroke is higher during the early period after the primary event.[92] Both procedures can be done within 15 days with a risk of peri-procedural stroke of less than 5%.[93]

Patients with cardioembolic stroke should be treated with anticoagulant agents as secondary prophylaxis. Depending on the patients overall risk of stroke (estimated from the CHA_2DS_2-VASc score) and the severity of the stroke (estimated from the NIHSS score) treatment is preferably initiated between four to fourteen days from stroke onset to minimize the risk of both recurrent stroke and hemorrhagic transformation.[94] The long term risk of significant bleeding should be assessed by comparing the CHA_2DS_2-VASc score to the ABC-bleeding score.[94, 95] If the biomarker analyzes needed to assess the patient by the ABC-bleeding score are not available the HAS-BLED score can serve as an alternative clinical tool in determining the risk of significant bleeding.[96] Warfarin in therapeutic interval lowers the risk of recurrent cardioembolic stroke in patients with AF with about two thirds but also significantly increases the risk of hemorrhage.[97] Non-vitamin K antagonist oral anticoagulants (NOAC) have shown similar effects in lowering the risk of recurrent cardioembolic stroke in patients with AF but have a lower risk of intracranial hemorrhagic than warfarin.[98] However, warfarin still shows a greater benefit than dabigatran in patients with mechanical heart valves.[99] Left atrial appendage closure is an option for patients with AF that do not tolerate anticoagulants.[100]

Aim

The aim of the study was to explore the association between WBV and infarct growth in patient with acute ischemic stroke. We hypothesized that higher WBV would be associated with increased infarct growth.

Material and methods

Five hundred and fourteen patients admitted to John Hunter Hospital in Newcastle, Australia, between September 2018 and February 2020 with symptoms of stroke were initially considered candidates for the study. A whole blood sample for viscosity analysis was obtained from 84 patients with suspected ischemic infarction. Of these, five patients were excluded because the specimen was too small in order to run the viscosity analysis. One patient had received treatment with rtPA before the blood sample for viscosity analysis was obtained and was therefore excluded. Of the remaining 78 patients 33 did not undergo a follow up MRI within seven days from the initial mCT examination and two had no evidence of ischemic infarction on the MRI. These patients were therefore also excluded. In result a sample of 43 patients were included in the study.

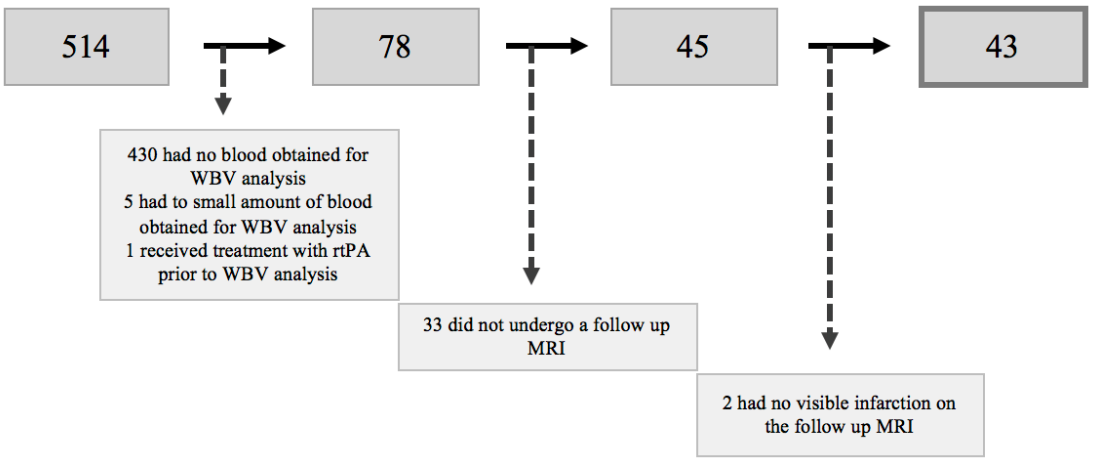

Figure 7: *Flowchart demonstrating the exclusion process of patients in the study. Of the 514 patients initially considered candidates for the study 43 were included in the analyses. WBV = whole blood viscosity; rtPA = recombinant tissue plasminogen activator; MRI = magnetic resonance imaging.*

At arrival to hospital the patients underwent a mCT examination (consisting of NCCT, CTA and CTP) and a whole blood sample was collected in a sample tube prefilled with ethylenediaminetetraacetate (EDTA-tube) before any treatment was given. The whole blood sample was brought to the lab and the viscosity was measured at a temperature of 37 °C and a

shear rate of 20 s^{-1}. To compensate for any eventual dehydration or dilutive effect of early intravenous fluid therapy the whole blood was adjusted to 40% hematocrit prior to the analysis. Whole blood samples with a hematocrit less than 40% had plasma removed in order to increase the proportion of erythrocytes to plasma in the specimen. Samples with hematocrit over 40% had autologous plasma from another sample tube added in order to reduce the proportion of erythrocytes to plasma in the specimen. The viscometer used was a Brookfield DV2T™ Viscometer.

The volume of the ischemic lesion at the time of arrival to hospital was automatically calculated from the CTP raw maps using MIStar software version 3.2 (release 3.2.62.03) (Apollo Medical Imaging Technology, Melbourne, Australia). The total hypoperfused tissue volume was defined as tissue with a DT of more than 3 seconds and the ischemic core as tissue with a CBF of less than 30%, both qualities compared to the corresponding areas on the contralateral side. The penumbra was defined as the difference between the total hypoperfused volume and the volume of the estimated ischemic core.

The included patients underwent a follow up MRI within seven days after admission to John Hunter Hospital on either a 1.5 Tesla Siemens Aera or 3 Tesla Siemens Verio MRI (Siemens AG, Healthcare Sector, Erlangen, Germany). The final lesion volume was manually calculated from the DWI images using the region of interest tool in MIStar software version 3.2 (release 3.2.62.03) (Apollo Medical Imaging Technology, Melbourne, Australia). All suspected lesions on DWI images were compared to the corresponding ADC images and in uncertain cases to the T2-FLAIR images in order to exclude artifacts such as T2 shine-through and ADC pseudonormalization.

Infarct core growth was calculated as the difference between the estimated lesion volume on mCT and final infarct volume on the DWI images of the follow up MRI.

Patient demographics and background were collected retrospectively from medical records. Hypertension and hyperlipidemia were defined as a diagnosis prior to the admission for stroke care or medication for the conditions on admission. Patients diagnosed with isolated hypercholesterolemia or isolated hypertriglyceridemia were included in the broader definition of hyperlipidemia. Diabetes mellitus, AF, ischemic heart disease, chronic cardiac failure and previous TIA were defined as diagnosis prior to admission. Previous stroke was defined as diagnosis prior to admission or radiological findings indicating old lesions during admission. Radiological findings of small vessel disease were not recorded as previous stroke.

Statistical analyses

Descriptive statistics were calculated for sample characteristics in terms of demography, past medical history, stroke severity and choice of acute stroke treatment. Proportions of the study sample are presented as frequency and percentage while central tendencies are presented as mean and standard deviation (SD) for normally distributed data and as median and range for data with skewed distributions. The relationship between the 40% hematocrit adjusted WBV measured at a temperature of 37 °C and a shear rate of 20 s^{-1} (independent variable) and infarct growth (dependent variable) were analyzed using a simple linear regression model in IBM SPSS Statistics version 26. Outcomes with a $p \leq 0.05$ were considered significant.

Ethical considerations

The study was approved by Hunter New England Human Research Ethics Committee (HNE HREC) (Reference No: 14/10/15/4.02). All participants, or if they themselves were incapable their next of kin, gave written consent to the additional sampling and all sensitive and personal information were handled with strict secrecy.

Results

Descriptive sample statistics

Forty-three patients were included in the analyses. A summary of the sample characteristics is shown in **Table 1**. The patients included were overall considered representative for the population. As expected, more males than females were included. Characteristics of the stroke severity and the proportion of patients receiving the different treatments can be observed in **Table 2** and **Table 3**, respectively.

Table 1: *Descriptive characteristics of the included patients prior to the onset of stroke. Pre-stroke disability was defined as a modified Rankin Scale (mRS) score of 2 or more prior to the onset of stroke.*

	Frequency (percentage)	Median (range)
Male	28 (65.1)	
Female	15 (34.9)	
Age (years)		72 (33-94)
Pre-stroke disability	6 (14.0)	
Pre-stroke mRS		0 (0-3)
Hypertension	32 (74.4)	
Hyperlipidemia	18 (42.9)*	
Diabetes Mellitus	8 (18.6)	
Type 1	0 (0)	
Type 2	8 (18.6)	
Previous CVA	7 (16.3)	
TIA	2 (4.7)	
Ischemic infarct	2 (4.7)	
Hemorrhagic infarct	0 (0)	
Multiple infarcts	0 (0)	
Unspecified type	3 (7.0)	
AF	6 (14.0)	
Ischemic heart disease	10 (23.3)	
Chronic cardiac failure	1 (2.3)	
Smoking		
Never smoked	9 (20.9)	
Ex-smoker	11 (25.6)	
Current smoker	7 (16.3)	
Not specified	16 (37.2)	
Antiplatelets on admission	10 (23.3)	
Anticoagulants on admission	1 (2.3)	

mRS = modified Rankin Scale; CVA = cerebrovascular accident; TIA = transient ischemic attack; AF = atrial fibrillation.
**Data were missing from one patient and the statistics are based on the 42 patients remaining in the sample.*

Tabell 2: *Descriptive characteristics of the stroke severity. Large vessel occlusion (LVO) was defined as a visible occlusion in the proximal M2 branch of the middle cerebral artery or a more proximal vessel.*

	Frequency (percentage)	Median (range)
NIHSS		8 (0-23)
LVO	21 (48.8)	
mCT DT >3 s (mL)		47 (0-354)
mCT CBF <30% (mL)		6 (0-96)
mCT mismatch (mL)		35 (0-258)
mCT to MRI time (hours)		27.75 (8.62-157.47)
DWI lesion (mL)		11 (0-156)

NIHSS = National Institutes of Health Stroke Scale; LVO = large vessel occlusion; mCT = multimodal computer tomography; DT = delay time; CBF = cerebral blood flow; MRI = magnetic resonance imaging; DWI = diffusion weighted imaging.

Tabell 3: *Descriptive characteristics of the stroke treatment. The recombinant tissue plasminogen activator (rtPA) used was either alteplase or tenecteplase.*

	Frequency (percentage)
Conservative treatment	18 (41.9)
rtPA	6 (14.0)
Thrombectomy	16 (37.2)
rtPA + Thrombectomy	3 (7.0)

rtPA = recombinant tissue plasminogen activator.

Variable characteristics

Both the viscosity data and the infarct growth data were continuous variables. The viscosity data obtained from the 43 included patients were approximately normally distributed with a skewness of 0.222 (SE=0.361), a kurtosis of 0.391 (SE=0.709) and a non-significant Shapiro-Wilk test with a p=0.742. The approximately normal distribution can also be visualized in the Normal quantile-quantile plot (Q-Q plot) in **Figure 8c**. The 40% hematocrit adjusted WBV measured at a temperature of 37 °C and a shear rate of 20 s^{-1} had a mean of 4.97 cP (SD=1.09).

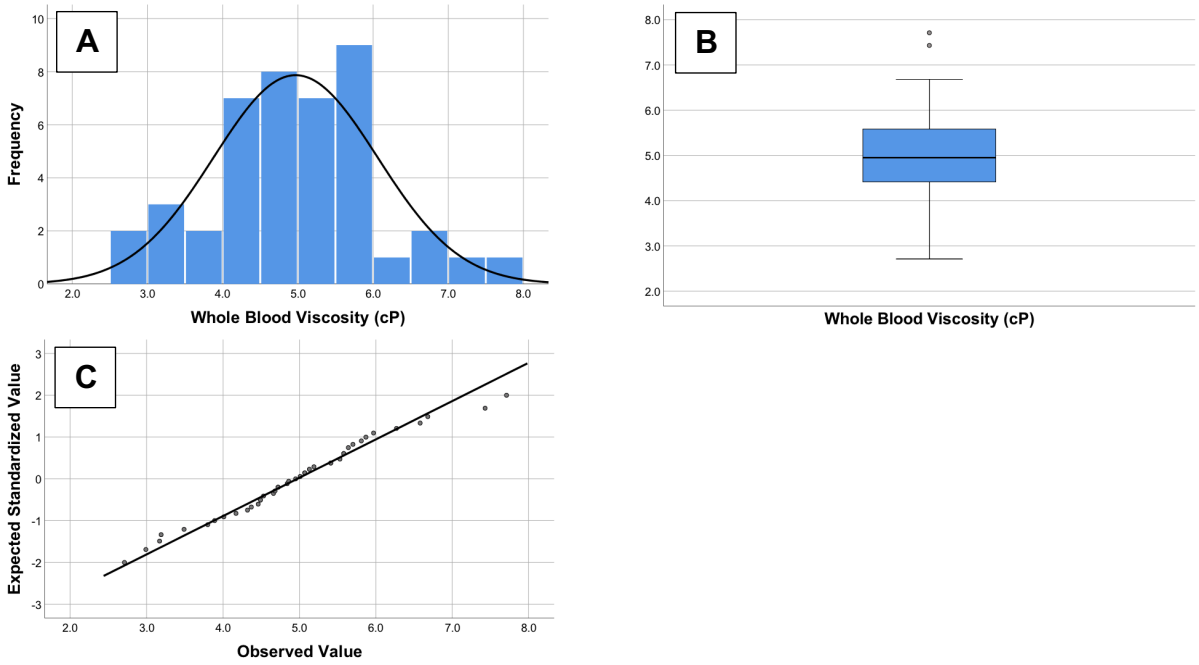

Figure 8: A: *Histogram with a normal distribution curve showing the frequency of whole blood viscosity (WBV) measurements in intervals of 0.5 cP.* ***B:*** *Box plot showing the distribution of WBV measurements in quartiles. There are two outliners (dots) with values exceeding the third quartile with >1.5 times the interquartile range.* ***C:*** *Normal quantile-quantile plot comparing the observed quantile values of the WBV with the expected standardized quantile values in a normal distribution. As can be observed, the dots follow the line marking the values of a perfect normal distribution, indicating an approximately normal distribution in the sample.*

The infarct growth data obtained from the 43 included patients were not normally distributed. The skewness was 0.998 (SE=0.361), the kurtosis was 9.543 (SE=0.709) and the Shapiro-Wilk test was significant with a $p<0.001$. The deviation from normal can also be visualized in the Normal Q-Q plot in **Figure 9c**. The data were not substantially more normally distributed after different transformations and were therefore kept in its original form in the regression analysis. The median value for the infarct growth was 3 mL (range –90-130).

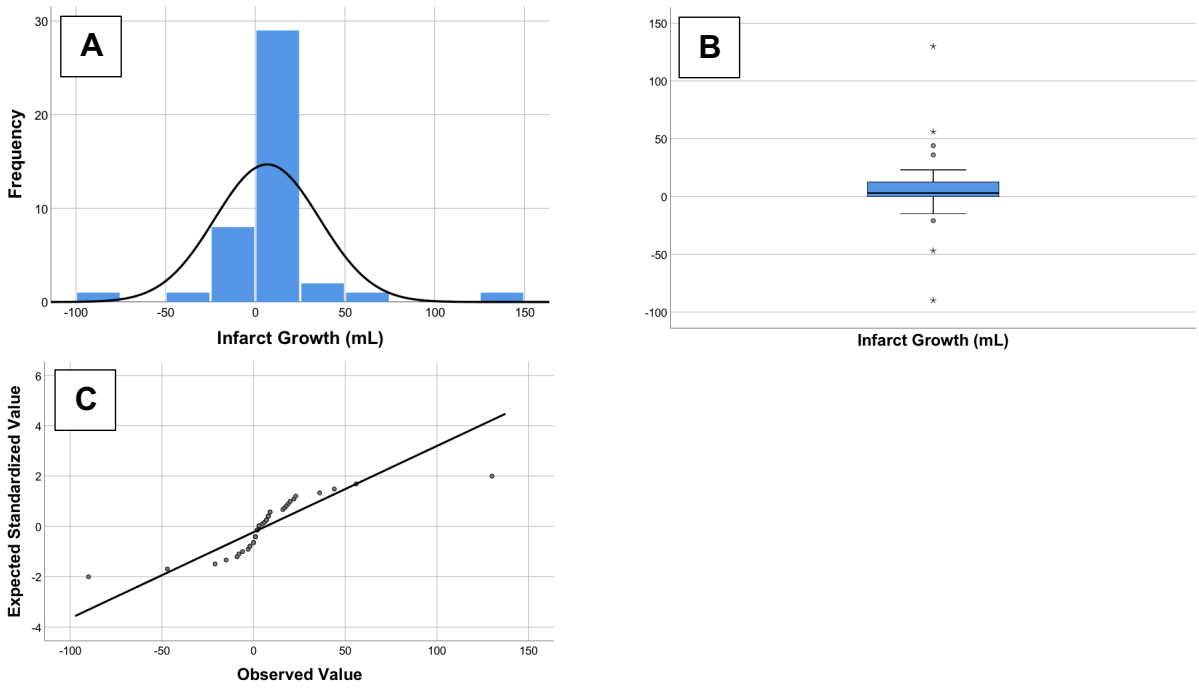

Figure 9: A: *Histogram with a normal distribution curve showing the frequency of infarct growth measurements in intervals of 25 mL.* ***B:*** *Box plot showing the distribution of the infarct growth measurements in quartiles. There are three outliners (dots) with values exceeding the first respectively the third quartile with >1.5 times the interquartile range. There are also four extreme values (asterisks) with values exceeding the first respectively the third quartile with >3 times the interquartile range.* ***C:*** *Normal quantile-quantile plot comparing the observed quantile values of the infarct growth with the expected standardized quantile values in a normal distribution. As can be observed, the dots do not follow the line marking the values of a perfect normal distribution. They are rather forming a S-shape, indicating a clustered center and a proportional large amount of extreme values in both ends of the sample.*

Simple linear regression analysis

A simple linear regression analysis was performed aiming to predict infarct core growth from blood viscosity. A non-significant regression equation was found with a constant of –22.53 (95% CI: –64.41-19.36, p=0.28) and a slope of 5.86 (95% CI: –2.38-14.10, p=0.16). The correlation coefficient (R) for the linear model was 0.22 (p=0.16) and the coefficient of determination (R^2) was 0.05. The equation for the regression line can be expressed as:

$$Y = -22.53 + 5.86X$$

where Y is the infarct growth in mL and X is the 40% hematocrit adjusted WBV at a temperature of 37 °C and a shear rate of 20 s^{-1} in cP.

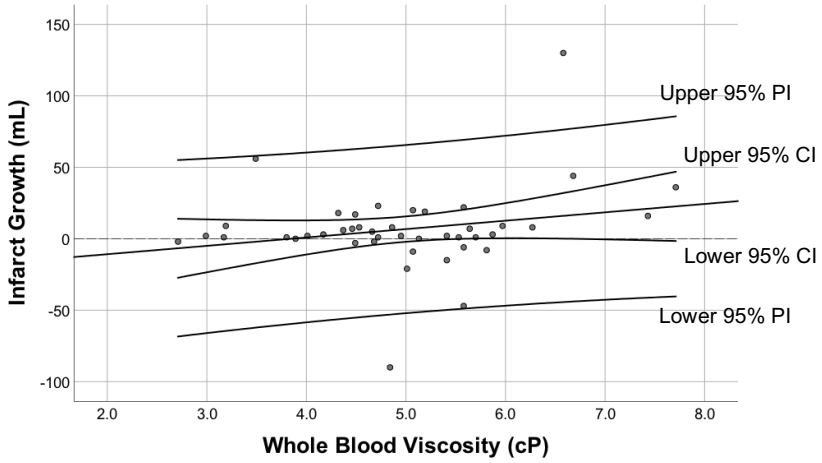

Figure 10: *Scatter plot of the 40% hematocrit adjusted whole blood viscosity measured at a temperature of 37 °C and a shear rate of 20 s^{-1} in relation to the infarct growth. The lines plotted are, from the center and outwards, the regression line of best fit (Y = –22.53 + 5.86X), the 95% confidence interval for the regression line and the 95% prediction interval for new observations.*

Because of the relatively small sample size the linear regression analysis was not adjusted for the choice of acute stroke treatment. However, no obvious trends can be observed in any of the respective treatment groups, **Figure 11**.

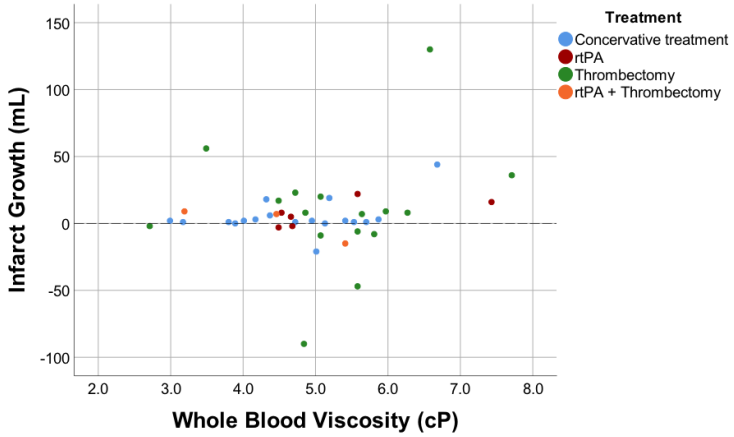

Figure 11: *Scatter plot of the 40% hematocrit adjusted whole blood viscosity (WBV) measured at a temperature of 37 °C and a shear rate of 20 s^{-1} in relation to the infarct growth with patients marked by color depending on which acute stroke treatment they received. No obvious relationship between WBV and infarct growth can be observed in any of the respective treatment groups. rtPA=recombinant tissue plasminogen activator.*

The residuals in the simple linear regression analysis had a mean of 0.00 (SD=28.47), a skewness of 0.664 (SE=0.361), a kurtosis of 8.363 (SE=0.709) and a significant Shapiro-Wilk test with a $p<0.001$. The deviation from normal can also be visualized in the Normal Q-Q plot in **Figure 12c**.

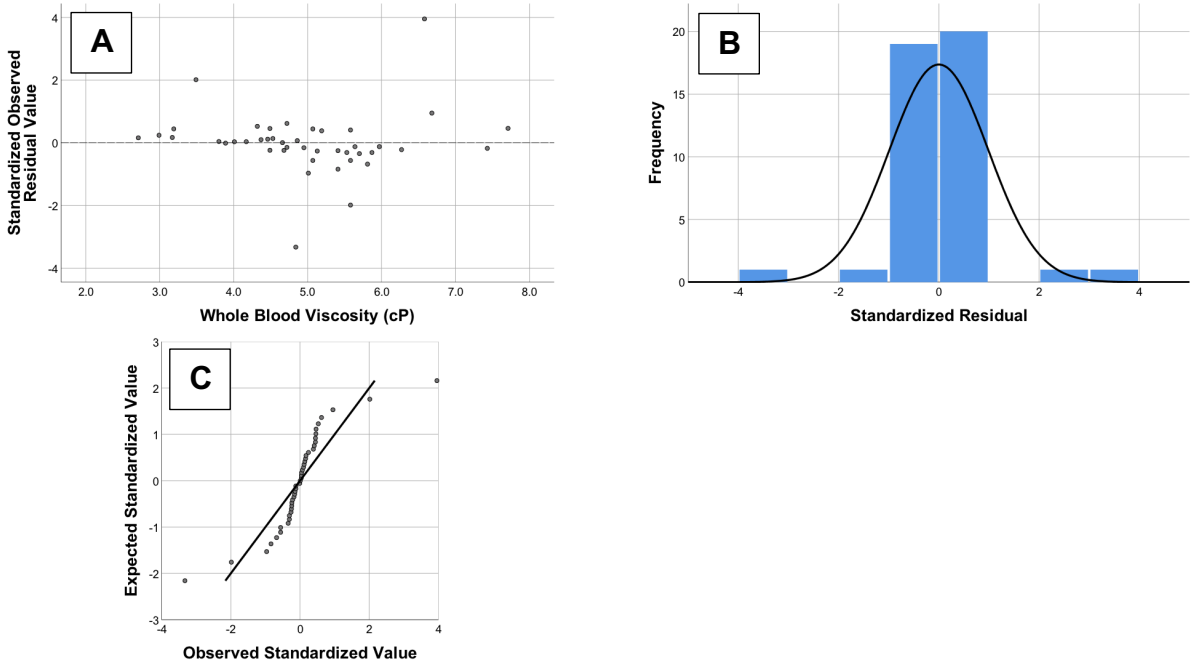

Figure 12: A: *Scatter plot of the standardized observed residual values in relation to the observed values of the independent variable (whole blood viscosity).* ***B:*** *Histogram with a normal distribution curve showing the frequency of standardized residual values in intervals of 1.* ***C:*** *Normal quantile-quantile plot comparing the observed standardized quantile values of the residuals with the expected standardized quantile values in a normal distribution. As can be observed, the dots do not follow the line marking the values of a perfect normal distribution. They are rather forming a S-shape, indicating a clustered center and a proportional large amount of extreme values in both ends of the sample.*

Discussion

Variable characteristics

The independent variable (40% hematocrit adjusted WBV measured at a temperature of 37 °C and a shear rate of 20 s^{-1}) was approximately normally distributed. The significant Shapiro-Wilk test of the dependent variable (infarct growth) indicates a non-normal distribution of the data in the sample. The test for skewness in the dependent variable was positive and more than 1.96 times the standard error, indicating a right sided skewness of the sample data. The kurtosis was also positive and more than 1.96 times the standard error, indicating a proportional large amount of extreme values in the sample. This is visualized by the S-shape of the Normal Q-Q plot for the infarct growth data in **Figure 9c** where it also can be seen that the extreme values occur in both ends of the distribution. If the proportion of extreme values is the same in the population or if the obtained distribution occurred by chance remains unclear because of the relatively small sample size. However, since infarct size can increase but not decrease (infarcted brain tissue is permanently damaged) it would be logical to expect a skewed distribution with a right tail in the population. The fact that we observed negative infarct growth between the initial mCT and follow up MRI might thus seem confusing. However, this is probably due to an incorrect overestimation of the true infarct core on the initial radiological examination. The traditional threshold used to determinate the infarct core on CTP is a CBF of less than 30% compared to the contralateral hemisphere.[64] With early and effective treatment, such as thrombolysis or thrombectomy, this threshold might overestimate the infarct core since the blood flow can be heavily impaired but the brain tissue in the area might not yet have died and thus is salvaged with early intervention. Future studies should therefore aim to investigate whether a lower set threshold, or any other factors, would increase the precision in estimating the truly infarcted tissue. This would be valuable both for future research and clinical considerations.

The infarcted brain tissue swells during the first three to five days and thereafter starts to shrink.[3] This might affect the accuracy of the measurement of the final infarct volume but since most follow up scans were performed within two days, median 27.75 hours (range 8.62-157.47), this error can be considered negligible.

In this study the relationship between WBV and the absolute value of infarct growth have been targeted. However, it would also be of interest to investigate the relationship between WBV and the shrinking of the total hypoperfused volume. Or put in other words, to investigate whether WBV is able to affect how much of the initial penumbra that converts to irreversible ischemic infarct. To adjust for the fact that a lesion with larger penumbra has a greater ability to increase the infarct volume than a lesion that by the time the patient arrives to hospital already is completely infarcted future studies could analyze the relationship between WBV and the ratio of infarct growth (or hypoperfused volume shrinking) and the initial penumbra volume. This would thus investigate how large proportion of the initial penumbra that infarcts (respectively that is salvaged) in relation to the level of WBV.

Simple linear regression analysis

In the simple linear regression analysis the unstandardized regression coefficient for the slope was 5.86, indicating that for every 1.00 cP increase in adjusted viscosity the infarct growth increased with 5.86 mL. However, the 95% CI as well as the p-value indicated a non-significant result and no conclusions about a true relationship can thus be made.

The equation constant was –22.53 and helps to orientate the regression equation line by the Y-axis and is the predicted infarct growth if the WBV was zero. Since the blood cannot have a viscosity level of zero (or below) the specific number of the constant does however not tell us

anything by its own. As per the above stated assumption, that infarct growth cannot have a negative value, the regression model would only be valid for viscosity values generating infarct growth values of zero or more. However, the 95% CI and the p-value were non-significant also for the constant in the equation so no certain conclusions about the true value can be made.

The correlation coefficient is a value from zero to one telling us how well the linear model fits the sample data. In the analysis the correlation coefficient was non-significant and had a value of 0.22. Even if the correlation coefficient would have been significant this would only indicate a weak fit of the linear model. However, it only tells us about the linear relationship between the variables. There might still be a non-linear correlation even if the linear correlation coefficient is close to zero.

The coefficient of determination was 0.05 meaning that if the correlation coefficient would have been significant still only 5% of the variance in infarct growth in the sample could have been explained by the level of WBV. This is obviously a very small proportion telling us that there are one or more other factors responsible for the vast majority of the variance in infarct growth. It is easy to assume that the choice of treatment, penumbra size at arrival to hospital, location of the clot and many other factors play a major role in determining how much the infarct can grow. WBV might thus only have a small, but maybe still clinically relevant, role. Future studies with larger sample size should aim to explore the effects of WBV within these subgroups.

Apart from the statistical significance three assumptions need to be fulfilled in order to draw any conclusions from a regression analysis. These are: (1) the values of the dependent variable (infarct growth) should have a normal distribution for each value of the independent variable (WBV), meaning that the residuals to the regression line of best fit should be normally distributed; (2) the variability of the dependent variable (infarct growth), as assessed by the

variance or standard deviation, should be the same for each value of the independent variable (WBV), meaning that it should be no heteroscedasticity of the data; and (3) the relationship between the two variables should be linear.[101]

When examining the kurtosis, Shapiro Wilk test and Normal Q-Q plot of the standardized residuals in **Figure 12c** normal distribution of the residuals cannot be concluded. As can be observed in the Normal Q-Q plot, the dots do not follow the line marking the values of a perfect normal distribution but rather forming a S-shape. This indicates a clustered center and a proportional large amount of extreme values in both ends of the sample. If this is because of no true linear association or because of chance due to the small sample size is however hard to tell. However, there is no noteworthy skewness of the residuals. The scatter plot in **Figure 12a**, showing the standardized observed residual values in relation to the observed values of the independent variable (WBV), shows no obvious pattern, systematic curvature or heteroscedasticity of the residuals. A constant variability of the dependent variable (infarct growth) for all values of the independent variable (WBV) can thus be assumed. However, two data points were obvious outliers with an observed value exceeding 3 SD from the mean. No reason was found to exclude these patients from the original analysis but a sensitivity analysis with exclusion of them was performed. All results remained non-significant and are presented in the Appendix. By observing the scatterplot of the two analyzed variables in **Figure 10** no obvious pattern, systematic curvature or trend can be seen. A linear relationship between the two variables in the population can thus be assumed even if the linear correlation coefficient tells us the fit is poor in this sample. In conclusion, the regression line of best fit is not statistically significant and do not make a perfect fit in the sample. Whether this would stay true in a larger sample can however not be concluded.

The power for the simple linear regression analysis was calculated post-hoc using the software G*Power version 3.1.9.6. The effect size (f^2) was calculated as:

$$f^2 = \frac{R^2}{1 - R^2} = \frac{0.22^2}{1 - 0.22^2} \approx 0.05$$

with R being the correlation coefficient obtained in the simple linear regression analysis. With a sample size of 43, an effect size of 0.05 and a significance threshold of 0.05 the power of the simple linear regression analysis was only 0.30. It is therefore not possible to state whether the non-significant result indicates no true linear regression in the population or if a true linear regression just could not be statistically proven because of the low power. Further studies with lager material will be needed for certain conclusions.

G*Power was also used to calculate the sample size needed to obtain a statistical power of 0.80 in a simple linear regression analysis. The significance threshold was set to 0.05 and the effect size obtained in this study was used as the estimated effect sizes. In order to obtain a power of 0.80 an estimate of 158 patients would be needed to include in the simple linear regression analysis. It is in other words clear that the study was under dimensioned in proportion to the effect size.

Spearman's rank correlation test

Spearman's rank correlation test was performed in IBM SPSS Statistics version 26 to visualize a possible monotonous correlation other than the linear assumed in the regression analysis. A non-significant correlation coefficient of 0.15 (p=0.34) was found in the analysis. The coefficient of determination was 0.02 meaning that if Spearman's rank correlation would have been significant still only 2% of the variability in infarct growth could have been explained

by a monotonous correlation with WBV. The power for the results in the correlation analysis was calculated post-hoc, once again using G*Power version 3.1.9.6. With a sample size of 43 patients and a correlation coefficient of 0.15 a power of 0.16 was obtain. To show a correlation of 0.15 and with a statistical power of 0.80 a total of 346 patients would be needed to include in the analysis. All calculations were done as two-tailed tests. Once again the low statistical power makes it not possible to state whether the non-significant result indicates no true correlation in the population or if a true correlation just could not be statistically proven. Further studies with larger material will be needed for certain conclusions.

Clinical relevance

Several previous studies have shown an association between higher WBV[48-56] respectively higher plasma viscosity[51-53, 56, 57] and the incidence of ischemic stroke but, to the best of our knowledge, no previous study have aimed to investigate the relationship between WBV and the characteristics of the infarct. A relevant question regarding the results in this study is thus whether they would be clinically relevant even if statistically significant. Formulated in another way, firstly, one would wonder if abnormal levels of WBV causes increased infarct growth, if increased infarct growth causes abnormal levels of WBV or if the two factors just covaries. Secondarily, if the level of WBV affects the infarct growth, one would wonder how large effect the WBV would need to have on the infarct growth in order to be a relevant target for further research and interventional therapy. If we assume the results in this study to be true for the population in general a decrease in WBV by 1.00 cP would be associated with a decrease of the final infarct volume by 5.86 mL. This is equivalent to about 0.5% of the total brain volume in healthy adults.[102] The reference interval for WBV differs depending on the conditions under which the viscosity is measured. To the best of our knowledge, no

reference interval has been established for 40% hematocrit adjusted WBV at a temperature of 37 °C and a shear rate of 20 s^{-1} but in our sample the measurements were normally distributed with a range of 2.71-7.71 cP. If a patient thus presents at hospital with a large penumbra and a WBV in the upper part of the interval a relatively large volume of brain tissue might be possible to save by lowering the WBV to the lower part of the interval.

The methodological approach in this study however only lets us investigate the potential relationship between the WBV at presentation to hospital and the infarct growth. Even if the results would have been significant we would not be able to conclude whether viscosity lowering treatment in the acute setting would reduce the infarct growth. Nor would we be able to conclude whether WBV directly affects the infarct growth or if the viscosity only is a surrogate marker for some other factor. To the best of our knowledge, no previous study has aimed to investigate these questions. Since decreased WBV entail an increased blood flow[16] it would however be logical to assume that early treatment affecting the WBV would increase the CBF and in the presence of collateral vessels benefit the patient if there still is penumbra to save. Randomized clinical trials with acute viscosity altering treatment will be needed in order to conclude whether the infarct growth can be minimized by lowering the WBV. This kind of study must also be done to confirm any possible hazards with viscosity lowering treatment.

Limitations

The study has several limitations. The first and most obvious one being the small sample size. As have been shown above a much larger sample is necessary to obtain the statistical power needed to draw any conclusions from the results. Another limitation related to the small sample size is that the analyzes are not adjusted for the choice of acute stroke treatment. It is easy to conclude that active treatment with thrombectomy or thrombolysis have a large impact

on the infarct growth. As can be observed in **Figure 11,** no obvious indication of distinct relationship between WBV and infarct growth could however be visualized in any of the respective treatment groups in this study. Future studies with larger sample size should nonetheless aim to analyze the effect of WBV in the different treatment groups separately to reach more certain conclusions.

As can be observed in **Table 1** the sample seems to be relatively representative for the population of patients with acute ischemic stroke. However, five hundred fourteen patients were considered candidates for the study but most of them were excluded because they missed the viscosity data or had not underwent a follow up MRI. It is uncertain how patients were selected for these investigations and it is thus hard to evaluate the risk of bias in the selection of the sample. For example, patients who were suspected to have very large or very small infarcts might not have been offered to undergo a follow up MRI since there might not have been a clinical indication for this. Future studies in the field will need to have more strict study protocols in order to prevent this uncertainty.

In this study we calculated the infarct growth as the difference in lesion volume on CTP and the DWI of MRI. It has previously been stated that the methods are comparable[69, 70] but as have been argued above CTP sometimes overestimates the initial infarct volume which thus might result in a negative infarct growth. Since the DWI sequence shows restricted diffusion in an infarcted area within a couple of minutes MRI is not inferior to CT in detection of acute brain ischemia.[65] To elude the methodically uncertainty descended from the comparison of two different radiological techniques it would thus have been preferable to do the initial scan, as well as the follow up, in an MRI scanner. However, as have been stated before, CT examination has practical advantages over MRI by generally being more available, accessible

and rapid in execution[63, 70] and is thus normally the technique of choice in the acute investigation of stroke.[64]

The determination of the final infarct volume on the DWI sequences of the MRI is subjective since it is based on visual examination rather than a computed calculation or a fix threshold. This has been shown to imply that different examiners might evaluate the final infarct volume differently.[103] The same examiner might also evaluate the lesion differently in the same patient on different times. In this study three medical students did the evaluation individually. This implies three limitations. Firstly, the three examiners had no noteworthy experience in neuroradiologically evaluation prior to the study. Secondly, since three different examiners independently evaluated the images there might be an interobserver bias in how the infarcts were determined. Thirdly, there might be an intraobserver bias in how each one of the examiners evaluated the images from day to day. The optimal way to handle these limitations would be to let two experienced neuroradiologist examine the images separately. If there were only small difference between their conclusions a mean value from the two observations could be used. In cases with significant differences between their conclusions either conclusion by consensus or consultation of a third party with experience in the field could be applied.

Lastly the patient history in this study was collected retrospectively from medical records. This might affect the accuracy of the data and future studies should aim to obtain all information needed for the study during the time the patients are hospitalized.

Interpretation

A linear relationship between WBV and infarct growth cannot be proven in acute ischemic stroke because of the low significance of the results in this study. However, due to the limited

statistical power, nor can it be ruled out. Further studies with lager sample size will be needed for any definite conclusions to be made. The results in this paper can thus be used as a support while designing future studies in the field. The information from these might be important to improve stroke care and motivate future trials targeting specific drugs affecting blood viscosity.

Populärvetenskaplig sammanfattning

Sambandet mellan blodets viskositet och infarktens tillväxt vid ischemisk stroke - En pilotstudie

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När en blodpropp täpper till ett av hjärnans blodkärl får inte hjärnan längre något syre eller någon näring. Detta gör att nervcellerna inte kan fungera som de skall och om inte blodflödet inom några minuter återställs i det tilltäppta kärlet kommer hjärncellerna att dö. Då uppstår irreversibel hjärnskada. Detta kallas för ischemisk stroke eller slaganfall. Patienten får då vanligen olika grad av bestående symptom från nervsystemet så som exempelvis förlamning, känselstörning och svårt att hitta ord. Ett specifikt område i hjärnan kan dock försörjas med blod från flera blodkärl. Det extra blodflödet räcker då sällan för att förhindra hjärnskada men kan göra att hjärncellerna överlever under en lägre tid (minuter till timmar) och om blodproppen släpper innan hjärncellerna dör kan de i viss mån återhämta sig.

Viskositet är ett mått på hur trögflytande en vätska är. Högre viskositet i blodet leder till sämre blodflöde. Syftet med den här studien var att undersöka om högre blodviskositet är associerat med en större tillväxt av det skadade området i hjärnan vid ischemisk stroke. Vi hade som hypotes att högre blodviskositet skulle leda till ett sämre blodflöde via de extra blodkärlen och därmed vara associerat med en större infarkttillväxt i akutskedet.

Patientens blodviskositet mättes genom ett blodprov som togs när patienten kom till sjukhuset. Hjärnskadans tillväxt kartlades genom att jämföra röntgenbilder på patientens hjärna vid ankomsten till sjukhuset och cirka ett dygn senare. Hjärnskadans tillväxtvolym jämfördes sedan med blodviskositeten för att se om det fanns ett samband mellan hur hög blodviskositeten var och hur mycket hjärnskadan tillväxte.

Resultaten kan tolkas som en indikation på att det finns en association mellan högre blodviskositet och större tillväxt av det skadade området i hjärnan vid ischemisk stroke men för få patienter ingick i studien för att man skall kunna dra några säkra slutsatser. Fortsatta studier med fler patienter behöver därför genomföras inom området. Om blodviskositeten visar sig ha en inverkan på hur mycket det skadade området i hjärnan tillväxer skulle framtida studier kunna undersöka om man kan minska tillväxten av skadan, och därmed minska den totala hjärnskadan, genom att med läkemedel påverka blodviskositeten hos en patient som insjuknat i ischemisk stroke.

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Appendix

Sensitivity analysis

A sensitivity analysis was performed by excluding the two patients which in the simple linear regression analysis gave rise to residuals exceeding values of +3 SD respectively –3 SD from the mean. A non-significant regression equation was found with a constant of –2.45 (95% CI: –27.45-22.56, p=0.84) and a slope of 1.70 (95% CI: –3.25-6.65, p=0.49). The correlation coefficient (R) for the linear model was 0.11 (p=0.49) and the coefficient of determination (R^2) was 0.01. The equation for the regression line can be expressed as:

$$Y = -2.45 + 1.70X$$

where Y is the infarct growth in mL and X is the 40% hematocrit adjusted whole blood viscosity at a temperature of 37 °C and a shear rate of 20 s^{-1} in cP. As can be observed, all results thus remained non-significant after exclusion of the most extreme outliers.

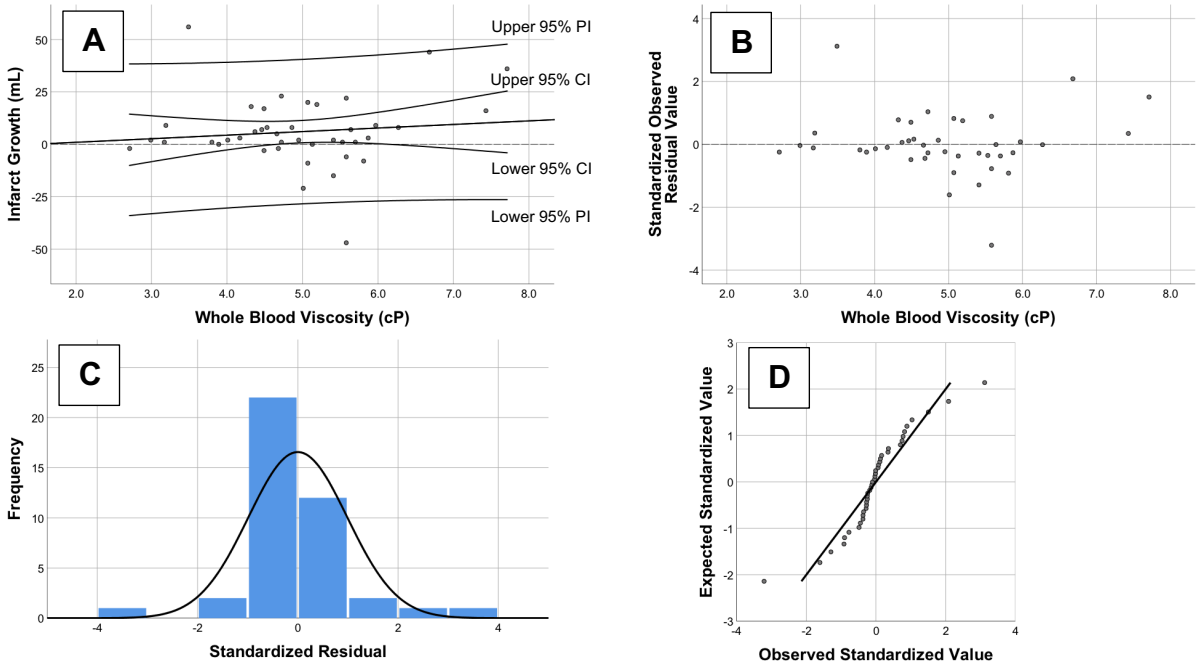

Figure 13: A: *Scatter plot of the 40% hematocrit adjusted whole blood viscosity measured at a temperature of 37 °C and a shear rate of 20 s^{-1} in relation to the infarct growth. The lines plotted are, from the center and outwards, the regression line of best fit (Y = –2.45 + 1.70X), the 95% confidence interval for the regression line and the 95% prediction interval for new observations.* ***B:*** *Scatter plot of the standardized observed residual values in relation to the observed values of the independent variable (whole blood viscosity).* ***C:*** *Histogram of the standardized residuals in intervals of 1.* ***D:*** *Normal quantile-quantile plot comparing the observed standardized quantile values of the residuals with the expected standardized quantile values in a normal distribution. As can be observed, the dots do not follow the line marking the values of a perfect normal distribution. They are rather forming a S-shape, indicating a clustered center and a proportional large amount of extreme values in both ends of the sample. However, the sample is closer to a normal distribution than the original sample, visualized in Figure 12c.*