Stroke, atrial fibrillation, and the management of oral anticoagulation

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2. List of publications in the context of the PhD with first authorship

2.1. Original research articles constituting the main body of the PhD thesis

1. **Polymeris AA**, Macha K, Paciaroni M, Wilson D, Koga M, Cappellari M, Schaedelin S, Zietz A, Peters N, Seiffge DJ, Haupenthal D, Gassmann L, De Marchis GM, Wang R, Gensicke H, Stoll S, Thilemann S, Avramiotis NS, Bonetti B, Tsivgoulis G, Ambler G, Alberti A, Yoshimura S, Brown MM, Shiozawa M, Lip GYH, Venti M, Acciarresi M, Tanaka K, Mosconi MG, Takagi M, Jäger RH, Muir K, Inoue M, Schwab S, Bonati LH, Lyrer PA, Toyoda K, Caso V, Werring DJ, Kallmünzer B, and Engelter ST for the NOACISP-LONGTERM, Erlangen Registry, CROMIS-2, RAF, RAF-DOAC, SAMURAI-NVAF and Verona Registry collaborators. Oral anticoagulants in the oldest old with recent stroke and atrial fibrillation. Published in Annals of Neurology; 2022 Jan; 91(1):78-88

2. **Polymeris AA***, Meinel TR*, Oehler H, Hoelscher K, Zietz A, Scheitz JF, Nolte CH, Stretz C, Yaghi S, Stoll S, Wang R, Haeusler KG, Hellwig S, Klammer MG, Litmeier S, Guerrero CRL, Moeini-Naghani I, Michel P, Strambo D, Salerno A, Bianco G, Cereda CW, Uphaus T, Gröschel K, Katan M, Wegener S, Peters N, Engelter ST, Lyrer P, Bonati LH, Grunder L, Ringleb P, Fischer U, Kallmünzer B, Purrucker JC*, and Seiffge DJ*. Etiology, secondary prevention strategies and outcomes of ischemic stroke despite oral anticoagulant therapy in patients with atrial fibrillation. Published in Journal of Neurology, Neurosurgery, and Psychiatry; 2022 Apr 8; jnnp-2021-328391

3. **Polymeris AA**, Coslovksy M, Aeschbacher S, Sinnecker T, Benkert P, Kobza R, Beer J, Rodondi N, Fischer U, Moschovitis G, Monsch AU, Springer A, Schwenkglenks M, Wuerfel J, De Marchis GM, Lyrer PA, Kühne M, Osswald S, Conen D, Kuhle J, and Bonati LH for the Swiss-AF Investigators. Serum neurofilament light in atrial fibrillation: clinical, neuroimaging and cognitive correlates. Published in Brain Communications; 2020 Oct 6; 2(2):fcaa166

4. **Polymeris AA**, Helfenstein F, Benkert P, Aeschbacher S, Leppert D, Coslovksy M, Willemse E, Schaedelin S, Blum MR, Rodondi N, Reichlin T, Moschovitis G, Wuerfel J, De Marchis GM, Engelter ST, Lyrer PA, Conen D, Kühne M, Osswald S, Bonati LH*, and Kuhle J* for the Swiss-AF Investigators. Renal function and body mass index contribute to serum neurofilament light chain levels in elderly patients with atrial fibrillation. Published in Frontiers in Neuroscience; 2022 Apr 14; 16:819010

2.2. Additional publications in the context of the PhD thesis with shared first authorship

1. Seiffge DJ*, **Polymeris AA***, Fladt J, Lyrer PA, Engelter ST, and De Marchis GM. Management of patients with stroke treated with direct oral anticoagulants. Published in Journal of Neurology; 2018 Dec; 265(12):3022-3033

2. Hert L*, **Polymeris AA***, Schaedelin S, Lieb J, Seiffge DJ, Traenka C, Fladt J, Thilemann S, Gensicke H, De Marchis GM, Bonati L, Lyrer P, Engelter ST, and Peters N. Small vessel disease is associated with an unfavourable outcome in stroke patients on oral anticoagulation. Published in European Stroke Journal; 2020 Mar; 5(1):63-72

*shared first / last authorship

3. Meya L*, **Polymeris AA***, Schaedelin S, Schaub F, Altersberger VL, Traenka C, Thilemann S, Wagner B, Fladt J, Hert L, Yoshimura S, Koga M, Zietz A, Dittrich T, Fisch U, Toyoda K, Seiffge DJ, Peters N, De Marchis GM, Gensicke H, Bonati LH, Lyrer PA, and Engelter ST. Oral Anticoagulants in Atrial Fibrillation Patients With Recent Stroke Who Are Dependent on the Daily Help of Others. Published in Stroke; 2021 Jul 27; STROKEAHA120033862

4. **Polymeris AA***, Albert V*, Hersberger KE, Engelter ST, Schaedelin S, Arnet I*, and Lyrer PA*. Protocol for MAAESTRO: Electronic Monitoring and Improvement of Adherence to Direct Oral Anticoagulant Treatment-A Randomized Crossover Study of an Educational and Reminder-Based Intervention in Ischemic STROke Patients Under Polypharmacy. Published in Frontiers in Neurology; 2018 Dec 21; 9:1134

5. Albert V*, **Polymeris AA***, Dietrich F, Engelter ST, Hersberger KE, Schaedelin S, Lyrer PA*, and Arnet I*. Insights Into Direct Oral Anticoagulant Therapy Implementation of Stroke Survivors with Atrial Fibrillation in an Ambulatory Setting. Published in Journal of Stroke and Cerebrovascular Diseases; 2021 Feb; 30(2):105530

6. Dietrich F*, **Polymeris AA***, Verbeek M, Engelter ST, Hersberger KE, Schaedelin S, Arnet I*, and Lyrer PA*. Impact of the COVID-19 lockdown on the adherence of stroke patients to direct oral anticoagulants: a secondary analysis from the MAAESTRO study. Published in Journal of Neurology; 2021 Jun 3; 1-7

*shared first / last authorship

3. Overview of the PhD thesis

Atrial fibrillation (AF) is the most common arrhythmia, becomes more prevalent with increasing age and is linked to neurological complications, including most notably ischemic stroke, but also cognitive dysfunction. The recent introduction of direct oral anticoagulants (DOAC) significantly advanced the management of patients with AF. Large-scale randomized controlled trials showed that – for most patients – DOAC are at least as effective as vitamin K antagonists (VKA) in preventing ischemic stroke but have the advantage of a lower risk for intracranial hemorrhage (ICH). However, clinically important patient populations were underrepresented or excluded in these trials, and more refined aspects which are important in clinical practice, including concomitant stroke etiologies or brain pathologies, medication adherence, neuroimaging characteristics or biomarker signatures were not addressed. Therefore, several research gaps and challenges remained for neurologists treating patients with AF, of which we selected 4 aspects to focus on in the following topics that comprise this PhD thesis.

The **first topic** focused on 3 high-risk subgroups of patients with AF treated with oral anticoagulants who were underrepresented or excluded from the large randomized trials. These were (i) stroke patients aged 85 years and older ("the oldest-old"), (ii) severely affected stroke patients dependent on the daily help of others, and (iii) stroke patients with concomitant cerebral small vessel disease. In the first main project of this PhD thesis we examined the performance of DOAC versus VKA in the oldest-old patients with recent stroke and AF in a large pooled analysis across 7 cohort studies. Facing the paucity of randomized evidence, many physicians have been reluctant to use DOAC in these patients. With this project, we provided new evidence that the benefits of DOAC over VKA are preserved in the oldest old with recent stroke, without any signal of a safety concern regarding risk of ICH. In an additional project from our local cohort, we showed that the favourable profile of DOAC over VKA was preserved also among patients with AF and recent stroke who were dependent on the daily help of others, a patient subgroup for which no data existed previously. Finally, we showed in the same cohort that concomitant cerebral small vessel disease in anticoagulated patients with AF and recent stroke was associated with an unfavorable clinical course, but the risk for ischemic stroke remained higher than the risk for ICH, even in the presence of small vessel disease. The latter two projects fall within the scope of this PhD thesis, but do not constitute its main body. Although both manuscripts were published in peer-reviewed journals, for the purpose of this thesis their presentation is restricted to abstracts. Our findings in the first topic of this PhD thesis advance the evidence for the use of anticoagulants in high-risk patient subgroups with AF and recent stroke.

The **second topic** examined ischemic stroke occurring despite anticoagulant therapy in AF patients. With the increasing use of oral anticoagulants, this scenario represents a growing challenge in everyday clinical practice, indicating the need to elucidate the underlying causes and – based on these – the optimal subsequent management strategies. We addressed this issue in the second main project of this PhD thesis in a large retrospective analysis pooling data of prospectively collected patients from 11 stroke centers. We found that the causes of stroke despite anticoagulation in AF patients were heterogeneous, but form three main clusters, all of which were comparably important. These included (i) competing stroke mechanisms other than AF-related cardioembolism, and (ii) insufficient anticoagulation due to prescription errors and nonadherence, suggesting that individualized treatment approaches to address these causes are necessary. Importantly, the third and most common cause was AF-related cardioembolism despite sufficient anticoagulation, indicating the need to develop novel preventive strategies beyond the currently available anticoagulants.

Furthermore, in this project we were able to demonstrate that AF patients with stroke despite anticoagulation represent a high-risk patient population, with higher than expected rates of stroke recurrence and other unfavorable outcomes. Finally, we showed also in this population that subsequent treatment with DOAC was associated with better outcomes than VKA treatment. Interestingly, neither any specific switch between DOAC nor antiplatelets as add-on treatment to anticoagulation seemed to confer any benefit, although both approaches are often employed in clinical practice. This study advanced the evidence for the preferential use of DOAC over VKA in AF patients with stroke despite anticoagulation, for whom no data existed so far, while demonstrating the need for more individualized and novel treatment approaches in these high-risk patients.

The **third topic** of this PhD thesis was the adherence of stroke patients to DOAC. Unlike VKA, DOAC require no coagulation monitoring and have short half-lives, which has raised concerns about nonadherence in AF patients treated with DOAC. This is particularly pertinent to patients with stroke, as shown in the second topic of this PhD thesis. In order to examine the medication-taking behaviour and the effect of an adherence-enhancing intervention in patients with recent stroke, we designed, initiated, and undertook the MAAESTRO study. MAAESTRO has been a joint venture with the Pharmaceutical Care Research Group of the University of Basel and has used electronic monitoring as the main method to assess adherence, data on which have been scarce so far. MAAESTRO comprises an initial observational phase and a subsequent randomized controlled interventional phase. MAAESTRO successfully concluded recruitment in July 2021 with reaching the predefined goal of n=130 participants. The observational study phase has now been completed, but as follow-up in the interventional phase is ongoing, the main study results are not part of this PhD thesis. Still, we present the published study protocol and the first results from the observational phase on the patterns of DOAC-taking behaviour, as well as an exploratory analysis on how adherence was impacted by the COVID-19 lockdown as abstracts, as these publications fall within the scope of this PhD thesis, but do not formally constitute its main body.

The **fourth and final topic** of this PhD thesis focused on cognitive dysfunction as a neurological complication of AF. While stroke is a well-known consequence of AF, there is increasing evidence that AF is also linked to cognitive dysfunction independent of ischemic stroke, but the mechanisms underlying this association are unclear. To preserve cognitive function in the growing population of elderly AF patients, a better understanding of these mechanisms is needed. Using data from the multicenter Swiss-AF Cohort Study, in the third main project of this PhD thesis we investigated serum neurofilament light chain (sNfL), a novel blood-based biomarker of neuronal damage, as a tool to explore the mechanisms through which neurological disease occurs in AF. In a cross-sectional analysis, we showed that sNfL is inversely associated with brain volume and cognitive function, thereby demonstrating that it represents a relevant biomarker of brain health in AF patients. Furthermore, we showed that neuronal loss measured by sNfL is associated with age, diabetes mellitus, heart failure, blood pressure and vascular brain lesions, observations which provide mechanistic insights into the occurrence of neurological disease in AF. Finally, in the fourth main project of this PhD thesis, we additionally investigated in this elderly cardiovascular cohort how renal function and body mass index contribute to sNfL levels in order to gain insights into the homeostasis (i.e., clearance and distribution) of this biomarker in the blood compartment. A better understanding of this is necessary towards further establishing this neurological biomarker in cardiovascular and dementia research. We showed that both renal function and body mass index were strongly, inversely associated with sNfL, but only renal function explained a relevant proportion of its variance. With this project we provided evidence

for the importance of accounting for renal function in future sNfL-based investigations in elderly cardiovascular populations, in whom chronic kidney disease is highly prevalent.

4. Introduction

4.1. Atrial fibrillation

Atrial fibrillation (AF) is the most common sustained arrhythmia in the adult population, and it becomes more prevalent with increasing age.[1, 2] Among individuals aged 85 years or older, the prevalence of AF is estimated at approximately 20%.[3] Besides age, risk factors for AF include hypertension, obesity, smoking, diabetes mellitus, coronary and valvular heart disease, as well as obstructive sleep apnoea.[4]

AF is characterized by high-frequency excitation of the atrium that results in disorganized atrial contraction and irregularity of ventricular response. On electrocardiography, this is evident by QRS complexes that occur at irregular intervals, with variable oscillation of the baseline between beats and no discrete P waves.[1, 4] Based on episode duration and spontaneous termination, AF is traditionally classified as paroxysmal, persistent, or permanent.[5]

The pathophysiological basis of AF is complex, resulting from an interplay between triggers, perpetuators and the development of an abnormal electrical and structural substrate, with alterations of atrial myocytes and the extracellular matrix including atrial fibrosis.[1, 4, 5] AF is characterized by a hypercoagulable state, in which various mechanisms including blood stasis due to hypocontractility, endothelial dysfunction and inflammation are implicated.[4, 5] This abnormal thrombogenic atrial substrate, termed atrial cardiopathy, is responsible for thrombus formation in the heart − particularly in the left atrial appendage − and consequent thromboembolism.[4, 6]

The clinical manifestations of AF are variable, ranging from asymptomatic to minimal or even incapacitating symptoms including palpitations, chest pain, dizziness, dyspnea, decreased exercise tolerance, and generalized fatigue.[1, 5] Furthermore, AF is linked to a multitude of severe clinical complications, the most catastrophic of which is ischemic stroke. Throughout all ages, AF independently confers an approximately 5-fold increased risk of stroke,[7-9] which can be stratified based on clinical risk factors using the CHA_2DS_2-VASc score.[10] Accumulating evidence indicates that AF is also linked to cognitive impairment and dementia, even in the absence of stroke.[11, 12] Finally, AF markedly increases the risk of heart failure and death, and AF patients have high rates of hospitalizations and often impaired quality of life.[5, 8] With the progressive ageing of the population, the socioeconomic burden of AF is increasing, and AF is considered a global public health concern.[3, 13]

4.2. Stroke

Stroke is the second most common cause of death and a leading cause of disability worldwide.[14] The incidence of stroke increases with age,[2] leading to a dramatic increase of the global burden of stroke as the population ages.[14, 15] Further risk factors for stroke include hypertension, diabetes mellitus, obesity, AF, smoking, chronic kidney disease, and obstructive sleep apnoea.[2] These are largely shared between ischemic and hemorrhagic stroke, which constitute two distinct types of stroke.

Ischemic stroke is the most common type, accounting for 87% of all strokes.[2] Ischemic stroke results from a decrease or interruption of focal cerebral blood flow leading to ischemic infarction.[16] The ischemic cascade involves the accumulation of sodium, calcium, and water in the injured brain cells, which leads to the release of excitatory neurotransmitters causing further cell injury.[17] Within the core

of the ischemic territory, where blood flow is most severely diminished, necrotic and excitotoxic cell death occurs within minutes. Thanks to collateral blood blow, cell death occurs less rapidly in the periphery of the ischemic area, which is termed ischemic penumbra.[17, 18] Hemorrhagic stroke comprises intracerebral and subarachnoid hemorrhage, which account for 10% and 3% of all strokes, respectively, and are caused by vessel rupture leading to bleeding into the brain or subarachnoid space.[2, 16] The pathophysiology of brain injury in hemorrhagic stroke includes tissue compression through the hematoma's mass effect and direct toxicity of blood on brain tissue.[16]

Clinically, stroke manifests as an acute or rapidly evolving episode of focal neurological dysfunction, and further delineation between ischemia and hemorrhage can be achieved with neuroimaging (i.e., computed tomography or magnetic resonance imaging [MRI]).[19] Brief transient episodes of neurological dysfunction attributable to focal ischemia but without neuroimaging evidence for infarction are termed transient ischemic attacks.[19, 20] Conversely, covert brain infarcts may frequently be detected on neuroimaging in patients without clinically manifest stroke as incidental findings, but their clinical importance is increasingly recognized.[21] The clinical severity of stroke is commonly estimated on the National Institutes of Health Stroke Scale, with increasing scores indicating higher severity.[22] The modified Rankin Scale, a measure of global disability or dependence in daily activities, is commonly used to quantify the functional outcome of stroke.[23]

The past few decades have witnessed major advances in stroke medicine. The introduction of intravenous thrombolysis in 1995 enabled the acute treatment of ischemic stroke,[24] which was revolutionized in 2015 with the advent of mechanical thrombectomy.[25] Advancements in neuroimaging included the widespread implementation of advanced imaging to guide acute reperfusion treatments and the introduction of several MRI modalities, expanding diagnostic capabilities.[26] Stroke unit care and stroke rehabilitation reduce mortality and disability,[27, 28] and have been established as standard components of comprehensive stroke pathways in healthcare systems.[29] Primary and secondary stroke prevention has continuously improved thanks to rigorous randomized clinical trials providing evidence for a vast array of effective interventions.[30, 31] Despite these developments, little progress has been made in other areas, such as the acute management of intracerebral hemorrhage and the development of neuroprotectants, but research is ongoing.[32, 33]

4.2.1. Etiology of ischemic stroke – cardioembolism and atrial fibrillation

The etiology of ischemic stroke is heterogeneous. Determining the underlying etiology is important in clinical practice to guide secondary prevention, and a number of classification systems have been developed.[34-36] Major ischemic stroke etiologies include cardiac embolism, occlusion of small cerebral arteries (i.e., microangiopathy, or cerebral small vessel disease, which is also a leading cause of intracerebral hemorrhage), and atherosclerosis of the large extracranial or intracranial arteries of the cerebral circulation (i.e., macroangiopathy). These account for 30%, 25% and 15% of ischemic stroke, respectively, while the etiology remains unclear in a significant number of patients.[34, 37]

Cardioembolic stroke typically manifests with acute neurological deficits that are maximal at onset, in contrast to stroke due to microangiopathy or macroangiopathy, which may have a more stuttering course. Moreover, cardioembolic stroke often includes neurological deficits indicative of cortical involvement, such as aphasia, neglect or hemianopia. On neuroimaging, cardioembolic stroke has a distinct profile which includes cortical lesions, acute lesions in multiple arterial territories, and occlusion of large intracranial arteries.[6, 38] In contrast, stroke due to small vessel disease typically

shows an acute small subcortical lesion and additional markers of microangiopathy, such as white matter hyperintensities and microbleeds.[39]

While a multitude of cardiac sources of embolism may lead to stroke, including recent myocardial infarction, aortic arch atheroma, prosthetic heart valves, endocarditis, and other rare structural heart abnormalities such as atrial myxoma, the most common cause of cardioembolic stroke is AF. [6, 40] The percentage of strokes attributable to AF increases steeply with age, from 1.5% at 50 - 59 years to over 20% at 80 - 89 years.[9] As the population ages and the treatment of cardiovascular risk factors improves, the incidence of stroke attributable to other etiologies declines, and AF-related cardiac embolism accounts for an increasing proportion of stroke.[41] The number of AF-related ischemic strokes at age over 80 years tripled during the past few decades and is projected to triple again by 2050.[42] This is important, because AF-related stroke is more often fatal, more severe and recurs more frequently than stroke due to other etiologies.[43] Electrocardiography to detect AF is an essential part of stroke work-up,[5] and accumulating evidence suggests that prolonged cardiac rhythm monitoring raises the detection rate of covert AF after ischemic stroke.[44]

4.2.2. Oral anticoagulants for stroke prevention in patients with atrial fibrillation

The cornerstone of stroke prevention in patients with AF is oral anticoagulation, which is superior to antiplatelet treatment and was exclusively based on vitamin K antagonists (VKA) for decades.[45-47] VKA exert their anticoagulant effect by impairing the synthesis of several vitamin K-dependent coagulation factors in the liver.[48] These drugs have a slow onset and offset of action, a narrow therapeutic window, and multiple drug and food interactions, which necessitate regular coagulation monitoring and dose adjustment to maintain optimal anticoagulation intensity.[48] As a result, a significant number of patients in clinical practice have suboptimal anticoagulation control with VKA,[49] exposing them to an increased risk of stroke, bleeding and death.[50] Additionally, the underuse of anticoagulation for stroke prevention in eligible AF patients has been a widespread concern in the VKA era.[51, 52]

To overcome the limitations of VKA, new non-vitamin K antagonist oral anticoagulants were developed, which directly inhibit specific factors of the coagulation cascade. These direct oral anticoagulants (DOAC) comprise the thrombin inhibitor dabigatran and the factor Xa inhibitors rivaroxaban, apixaban, and edoxaban. DOAC have predictable pharmacokinetics and pharmacodynamics with a rapid onset and offset of action and few drug and food interactions. They are therefore taken at fixed dosing schedules without the need for dietary restrictions or routine coagulation monitoring, making them a more convenient option for anticoagulation over VKA. Unlike VKA, all DOAC are eliminated renally at various rates.[48, 53] The DOAC dosing frequency differs between once-daily (rivaroxaban and edoxaban) and twice-daily (dabigatran and apixaban), and either a standard or reduced DOAC dose is used based on renal function, age and body weight.[5]

Four large randomized controlled clinical trials investigated DOAC against VKA in patients with AF.[54-57] The trials consistently showed comparable efficacy in terms of ischemic stroke prevention and superior safety with regards to intracranial hemorrhage (ICH), which is the most feared complication of anticoagulation, as well as lower mortality with DOAC.[58] Since the introduction of DOAC in clinical practice in the early 2010s, the overall use of oral anticoagulants for stroke prevention in patients with AF has increased steadily, owing mostly to the increasing uptake of DOAC.[59-61] The latest guidelines recommend the use of DOAC in preference over VKA for stroke prevention in AF patients.[31]

4.2.3. Remaining challenges and research gaps in the field of oral anticoagulation in stroke patients with atrial fibrillation

4.2.3.1. Oral anticoagulants in high-risk patient populations with atrial fibrillation and recent ischemic stroke

Although the benefits of DOAC over VKA were convincingly shown in the randomized trials,[58] concerns persist about the applicability of these findings to high-risk patient populations that are common in everyday neurological practice, but were underrepresented in the trials. These have been reviewed previously by us[62] and others,[63, 64] and encompass patients with recent stroke within 1 – 4 weeks or disabling stroke within 3 – 6 months who had been explicitly excluded from trial participation,[65] as well as very elderly patients aged over 85 years (the 'oldest old') who constituted less than 5% of the trial population.[66-68] In the absence of randomized data for these patient populations, high-quality observational data may bridge the evidence gap.[63, 69]

In a large pooled analysis of observational cohorts, we previously showed that DOAC maintain their favorable profile compared to VKA among AF patients with a recent ischemic stroke.[70] However, it remains unclear whether this also applies to the oldest-old stroke patients and those with disabling stroke who are dependent on the daily help of others. In these patients, who are common in clinical practice,[71, 72] DOAC pharmacokinetics may be altered in the presence of renal impairment, polypharmacy, or reduced body weight,[64, 73] and stroke-induced motor and cognitive deficits may interfere with drug adherence and the risk of falls.[62, 74] For these reasons, many physicians are reluctant to prescribe DOAC to these patients due to assumed safety concerns, particularly for fear of ICH.[64, 75] Another population of particular interest in this context are patients with an AF-related stroke and concomitant small vessel disease, especially those with cerebral microbleeds on MRI. In such patients, concerns have been raised that the ICH risk might outweigh the benefits of oral anticoagulant therapy.[76]

4.2.3.2. Ischemic stroke despite oral anticoagulant therapy in patients with atrial fibrillation

Anticoagulation with VKA or DOAC reduces but does not eliminate the risk of ischemic stroke. In fact, a substantial residual stroke risk persists despite anticoagulation in patients with AF, which ranges from 0.7% to 2.3% annually in primary and secondary prevention, respectively.[77-80] With the increasing use of oral anticoagulants,[59-61] the number of patients with AF suffering a stroke despite anticoagulation is steadily growing, too.[81, 82] In Switzerland, 1 in 3 patients with AF and an acute ischemic stroke were previously anticoagulated.[81] AF patients with breakthrough ischemic stroke despite oral anticoagulant therapy represent another common challenge in everyday neurological practice, with regards both to their acute and long-term management.

Previous work from our group focused on aspects of the acute management of these patients, including the use of DOAC plasma levels in the acute phase to guide reperfusion treatments.[83-85] Accumulating observational data have been reassuring about the safety of endovascular treatment and – in selected patients – intravenous thrombolysis.[86, 87] However, data on the long-term management of AF patients with stroke despite anticoagulation have been scarce.[87] We and others previously showed that these patients are at an increased risk of recurrence compared to patients who were naïve to anticoagulation before stroke.[88-90] However, the optimal subsequent treatment to prevent recurrence in patients with stroke despite anticoagulation is unclear, with the latest guidelines offering no recommendations about this,[31] and limited observational data showing no apparent

benefit from switching the anticoagulant type.[88, 90] To optimize subsequent management, a better understanding of the etiology of stroke despite anticoagulation is needed.[87] Several potential etiologies of stroke despite anticoagulation have been discussed,[87] including competing stroke mechanisms such as small vessel disease or large artery atherosclerosis, inappropriately low DOAC dosing and nonadherence, but data on their relative frequency have been scarce.[82]

4.2.3.3. Adherence of patients with recent stroke to direct oral anticoagulants

Nonadherence to anticoagulants is of special interest as a cause of stroke despite anticoagulation, because it may be amenable to interventions that could prevent breakthrough strokes. For DOAC in particular, nonadherence has been a matter of concern ever since they were introduced in clinical practice.[91, 92] While the absence of regular coagulation monitoring with DOAC is more convenient and eliminates costs incurred by laboratory testing, nurse visits, and physician consultations,[53] the reduced contact of patients to the healthcare system may render them more prone to nonadherence and hinder its early detection.[91] Importantly, the rapid offset of action and shorter half-lives of DOAC compared to VKA might translate to a more severe impact of nonadherence, as even few missed doses lead to a dramatic decrease of their anticoagulant effect.[93]

Within randomized trials, the adherence of patients to DOAC – as measured by pill counts – has been reported to be high, but the well-controlled trial environment does not necessarily reflect patients' adherence in 'real-world' conditions.[92] AF patients treated with DOAC after a recent stroke are of particular interest in this context, as they are at risk for both recurrence and nonadherence due to neurological and cognitive deficits.[94] We previously investigated the adherence of patients with AF and a recent stroke to oral anticoagulants – both VKA and DOAC – in a single-center study using self-reporting, and found generally high rates of adherence to all anticoagulants, with almost 80% of patients reportedly missing no DOAC doses.[95] Using prescription claims data, large observational studies in the broader population of AF patients yielded varying results regarding the extent of nonadherence to DOAC, with metanalyses indicating that it is present in up to one third of AF patients and is associated with unfavorable clinical outcomes including stroke and death.[96, 97]

However, pill counts, self-reporting and prescription claims, all have considerable limitations as methods to evaluate adherence. These methods are susceptible to various biases, may over- or underestimate adherence, and provide only crude information about treatment implementation.[98] Electronic monitoring is considered the most accurate method to assess adherence, and is the only source of detailed information on the patterns of medication-taking behavior.[93, 98] To develop tailored adherence-improving interventions for DOAC-treated patients, a deeper understanding of the patterns of nonadherence at the individual patient level is needed, but electronic data to that end have been scarce. Only few studies recently used electronic monitoring in DOAC-treated patients, with some additionally assessing the adherence-improving effect of educational and/or reminder-based interventions, but none examined patients with recent stroke. Rates of electronically monitored adherence to DOAC were consistently high in these studies,[99-103] and the adherence-enhancing interventions showed mixed results.[101-103]

4.3. Atrial fibrillation and cognitive dysfunction

While the most widely recognized consequence of AF is ischemic stroke, there is increasing evidence that AF is also linked to cognitive impairment and dementia, even in the absence of clinically manifest

stroke.[104, 105] In metanalyses of observational studies, AF increased the hazard for dementia by 40% in mixed populations with or without stroke,[106] and by 30% independently from prevalent or incident stroke.[107] Several potential mechanisms have been postulated to explain this association, including covert cerebral infarcts, cerebral small vessel disease (through shared risk factors such as diabetes and hypertension), rhythm-related cerebral hypoperfusion, and AF-induced systemic inflammation, but tangible evidence is lacking.[104, 105] With the progressive ageing of the population, both AF and dementia are growing public health concerns, and a deeper understanding of the pathophysiological pathways underlying their association will be crucial in developing strategies to preserve cognitive function in the elderly.[108]

4.3.1. Gaps in research and the value of serum neurofilament light

Recently, a cross-sectional analysis from the Swiss-AF study showed that cortical and large noncortical infarcts were common in AF patients and were independently associated with a lower score on the Montreal Cognitive Assessment,[109] supporting the hypothesis that covert cerebral embolic infarcts are implicated in the occurrence of cognitive dysfunction in AF.[110] In line with this, several randomized trials are underway to investigate the effect of DOAC on cognitive function among AF patients,[111, 112] supported by encouraging observational data that showed a lower risk for dementia with oral anticoagulation, particularly DOAC.[113-115]

Regarding the hypoperfusion hypothesis, observational data showed that AF is linked to decreased brain blood flow,[116] and that the restoration of sinus rhythm was associated with improved brain perfusion among AF patients.[117] An association between cerebral hypoperfusion and worse cognitive outcomes has been demonstrated in the general population,[118] and observational studies suggested that ablation of AF may improve cognitive performance and reduce dementia risk.[119, 120] However, a recent randomized trial showed no apparent benefit from rhythm control on cognitive function among AF patients.[121]

To elucidate the mechanisms of incident dementia in AF, further prospective studies with large numbers of AF patients and extensive observation are needed.[122, 123] Complementary to such data and until they become available, surrogate markers of brain health such as blood-based biomarkers of neuronal damage may be of value.[123, 124] Neurofilaments are neuron-exclusive cytoskeletal proteins that are released in the extracellular space, cerebrospinal fluid and eventually peripheral blood after neuroaxonal damage.[125] Recently, sNfL emerged as a biomarker of neuroaxonal injury in various nervous system diseases, including inflammatory and vascular neurological disorders, as well dementia and neurodegenerative diseases.[125, 126] However, it has not been investigated as a marker of neurological disease in AF so far. As the importance of heart-brain interactions is increasingly recognised,[127] the application of novel neurological biomarkers like sNfL in the emerging field of neurocardiology seems promising.

5. Aims of the PhD thesis

To address the aforementioned challenges and research gaps in the field of atrial fibrillation, stroke and oral anticoagulation, this PhD thesis had the following aims:

1. In the first topic, we used 'real-world' observational data to investigate the safety and effectiveness of direct oral anticoagulants compared to vitamin K antagonists in high-risk patient populations with AF and recent ischemic stroke, for whom randomized evidence has been limited. This includes the oldest old, who represent a growing and increasingly important patient group in the ageing population that we addressed in the first main project of this thesis in a pooled analyses of 7 prospective cohorts (section 6.1). In an additional single-center project on this topic, we focused on patients with disabling stroke and dependency on the daily help of others, who represent another common clinical challenge where randomized data are lacking (section 6.2). Finally, we investigated in an additional single-center project on this topic the prognostic importance of neuroimaging markers of small vessel disease in anticoagulated AF-stroke patients, in whom small vessel disease may contribute not only to the risk of recurrent ischemic stroke, but also ICH, raising concerns about the safety of anticoagulation (section 6.3).

2. The second topic addressed the issue of breakthrough ischemic stroke despite oral anticoagulant therapy in patients with AF. With the increasing use of oral anticoagulants, the number of patients with AF who suffer ischemic stroke despite therapy is similarly increasing. However, knowledge about the etiology of breakthrough stroke has been lacking so far, and the optimal subsequent management of these patients is unclear, with the latest guidelines offering no recommendations on this. We therefore pooled observational data from 11 experienced stroke centers in the second main project of this thesis in order to investigate not only the etiology, but also the subsequent management and outcomes of these patients (section 7.1).

3. The third topic focused on the adherence of stroke patients to direct oral anticoagulants. Nonadherence has been implicated as a potential cause of stroke despite anticoagulation, and has been a matter of concern since the introduction of the new class of direct oral anticoagulants. A nuanced appraisal of the medication-taking behaviour of stroke patients requires detailed electronic adherence data, but these have been scarce, as have been data on effective adherence-improving interventions. With these considerations, we developed the single-center MAAESTRO study with an observational and a randomized controlled interventional phase, in order to investigate the adherence of stroke patients to direct oral anticoagulants using electronic monitoring, and to evaluate the adherence-improving effect of an educational and reminder-based intervention. MAAESTRO has successfully concluded recruitment of its target sample size and completed the observational phase, while patient follow-up in the interventional phase is ongoing and final analyses are therefore not yet available. Still, we present the study design (section 8.1) and the first results from the observational study phase (sections 8.2, 8.3).

4. Independent of stroke, AF has also been linked to cognitive dysfunction. As the fourth and final topic of this PhD thesis, we focused on sNfL as a tool to explore the mechanisms that underly neuronal damage and cognitive dysfunction in AF. A deeper understanding of these – so far poorly understood – mechanisms is crucial to develop strategies to preserve cognitive function in AF patients. sNfL is a novel blood-based biomarker of neuronal damage with successful research

applications in multiple neurological diseases, which has not been utilized in AF research so far. Therefore, in the third main project of this thesis, we aimed to investigate the association of sNfL with cognitive function, clinical and neuroimaging characteristics in a large cross-sectional study of well-characterised AF patients from the multicenter Swiss-AF study (section 9.1). Finally, in the fourth main project of this thesis we aimed to gain insights into the homeostasis of sNfL (i.e., clearance and distribution in the blood compartment), which is an important step towards further establishing this neurological biomarker in cardiovascular research. In a cross-sectional analysis we therefore investigated how renal function and body mass index contribute to sNfL levels in the elderly cardiovascular population of Swiss-AF (section 9.2).

6. First topic: Oral anticoagulants in the oldest old and other high-risk patient populations with atrial fibrillation and recent ischemic stroke

This topic includes the first main project of the PhD thesis, which examined the performance of DOAC versus VKA in patients with AF and recent stroke aged over 85 years. These patients are increasingly common in everyday neurological practice, but were severely underrepresented in the randomized DOAC trials. Owing to the limited randomized evidence, many physicians are reluctant to prescribe DOAC in this age group due to safety concerns. Therefore, this project aimed to advance the evidence using observational data in a large pooled analysis from prospective cohort studies.

In two additional projects using data from our single-center cohort we examined (i) the performance of DOAC versus VKA in patients with AF and recent disabling stroke who are dependent on the daily help of others and (ii) the impact of concomitant small vessel disease on the clinical outcome of orally anticoagulated patients with AF and recent stroke. These projects are presented here briefly as abstracts.

6.1. Oral anticoagulants in the oldest old with recent stroke and atrial fibrillation

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Abstract

Objective

To investigate the safety and effectiveness of direct oral anticoagulants (DOAC) versus vitamin-K-antagonists (VKA) after recent stroke in patients with atrial fibrillation (AF) aged ≥85 years.

Methods

Individual patient data analysis from 7 prospective stroke cohorts. We compared DOAC versus VKA treatment among patients with AF and recent stroke (<3 months) aged ≥85 versus <85 years. Primary outcome was the composite of recurrent stroke, intracranial hemorrhage (ICH) and all-cause death. We used simple, adjusted and weighted Cox regression to account for confounders. We calculated the net benefit of DOAC versus VKA by balancing stroke reduction against the weighted ICH risk.

Results

In total, 5,984 of 6,267 (95.5%) patients were eligible for analysis. Of those, 1,380 (23%) were aged ≥85 years and 3,688 (62%) received a DOAC. During 6,874 patient-years follow-up, the impact of anticoagulant type (DOAC versus VKA) on the hazard for the composite outcome did not differ between patients aged ≥85 ($HR_{\geq 85y}$=0.65, 95%-CI [0.52, 0.81]) and <85 years ($HR_{<85y}$=0.79, 95%-CI [0.66, 0.95]) in simple ($p_{interaction}$=0.129), adjusted ($p_{interaction}$=0.094) or weighted ($p_{interaction}$=0.512) models. Analyses on recurrent stroke, ICH and death separately were consistent with the primary analysis, as were sensitivity analyses using age dichotomized at 90 years and as a continuous variable. DOAC had a similar net clinical benefit in patients aged ≥85 (+1.73 to +2.66) and <85 years (+1.90 to +3.36 events/100 patient-years for ICH-weights 1.5 to 3.1).

Interpretation

The favorable profile of DOAC over VKA in patients with AF and recent stroke was maintained in the oldest old.

Introduction

Atrial fibrillation (AF) becomes more prevalent with increasing age, and both are independent risk factors for ischemic stroke.[128] As the population ages, the number of patients aged 85 years and older – often termed the *oldest old* – suffering AF-related ischemic stroke is growing.[71]

In the current guidelines,[31] direct oral anticoagulants (DOAC) are recommended in patients with AF for recurrent stroke prevention in preference to vitamin K antagonists (VKA) based on the results of the pivotal DOAC randomized controlled trials (RCTs).[58] However, it is less clear whether this preference can be generalized to include patients: (i) aged ≥85 years, who made up less than 5% of the RCTs population;[66-68] or, (ii) with recent ischemic stroke, who had been excluded from the RCTs for at least some weeks after stroke.[62, 63]

Facing the paucity of randomized evidence, many physicians are reluctant to prescribe DOAC to the oldest old due to assumed safety concerns due to clinical situations particularly prevalent in the oldest old (e.g., altered DOAC pharmacokinetics in the presence of unstable or declining renal function, polypharmacy, frailty, malnutrition or reduced body weight),[64, 73] especially for fear of intracranial hemorrhage (ICH).[75, 129] Instead, they may favor VKA[64] or withhold oral anticoagulant (OAC) treatment, even in patients who had had an ischemic stroke.[129] To bridge this evidence gap, systematically ascertained, standardized observational data – known as 'real-world' data – may be useful.[63, 69]

With these considerations in mind, we investigated the safety and effectiveness of DOAC compared to VKA in the oldest old with AF and a recent ischemic stroke. In the absence of randomized data, we used prospectively collected, individual patient data pooled within an international collaboration of cohort studies on the use of OAC following ischemic stroke in patients with AF.

Methods

Study design, patient population and data collection

We used prospectively collected, individual patient data pooled from an established international collaboration of investigator-initiated cohort studies of patients with AF, recent ischemic stroke or transient ischemic attack (TIA) and OAC treatment, as described previously[70]. This included 3 single-center (Basel, Switzerland[130] [NOACISP-LONGTERM; NCT03826927]; Erlangen, Germany[131]; Verona, Italy[132]) and 4 multicenter cohorts (CROMIS-2 [NCT02513316][133]; RAF[134]; RAF-DOAC[135]; SAMURAI-NVAF [NCT01581502][72, 136]). The number of patients contributed by each cohort, as well as the recruitment period and follow-up duration are summarized in Supplementary Table 6.1.1.

In this study, we included consecutive patients with (i) an index recent (i.e., <3 months) ischemic stroke or TIA (as defined previously[70]); (ii) nonvalvular AF (either known before index event or first diagnosed thereafter); (iii) treatment with DOAC [i.e., apixaban, dabigatran, edoxaban, rivaroxaban] or VKA [i.e., phenprocoumon, warfarin], initiated within 3 months after the index event; and (iv) prospectively ascertained follow-up data for at least 3 months after the index event for the outcomes recurrent ischemic stroke, ICH and all-cause death, defined as reported previously[70, 130]. We excluded patients with missing follow-up or information on age, those with OAC initiation > 3 months or unknown, and those with outcome events occurring before OAC initiation.

Data were collected as described in prior research[70] using standardized forms with predefined variables and pooled in the coordinating center in Basel, Switzerland, where the analysis was

performed. We used the following baseline variables: age; sex; National Institutes of Health Stroke Scale (NIHSS) score at baseline; dichotomized type of OAC after index event (DOAC or VKA); time to OAC initiation; concomitant antiplatelet use; history of ischemic stroke or TIA before the index event; history of ICH; diabetes mellitus; hypertension or dyslipidemia; the CHA_2DS_2-VASc score (congestive heart failure, hypertension, age 65-74 or ≥ 75 years, diabetes mellitus, IS or TIA, vascular disease, sex)[10]; estimated glomerular filtration rate (eGFR) using the Chronic Kidney Disease Epidemiology Collaboration [CKD-EPI] equation[137] and current smoking, as described previously[70].

Follow-up data included length of follow-up and absence or occurrence and timing of any of the following outcome events, which were defined in line with prior research[70, 130]: (i) recurrent ischemic stroke (defined as new neurological deficits with a corresponding finding on neuroimaging); (ii) ICH (defined as new neurological deficits with detection of intracranial bleeding on neuroimaging); and (iii) all-cause death, defined as every death irrespectively of the cause and regardless of whether the cause was known or not.

Outcomes

The primary outcome was the time to occurrence of the composite of recurrent ischemic stroke, ICH and all-cause death, in accordance with prior research[70, 130]. Secondary outcomes were the time to occurrence of each of these outcomes separately.

Statistical analysis

We stratified patients' characteristics by dichotomized age (≥85 vs. <85 years) and type of OAC (VKA vs. DOAC). We presented categorical data using frequencies and percentages and continuous data using the median and interquartile range (IQR) or mean and standard deviation (SD) as appropriate. We compared categorical variables using the Chi^2-test and continuous variables using the Mann-Whitney U test or t-test as appropriate. We calculated the annualized rate of outcome events as the total of observed events divided by patient-years of follow-up for each outcome.

As for the main analysis, we modelled time to primary outcome using Cox proportional hazards regression. For this, we analyzed time to first event after OAC initiation, without considering further events. To assess the effect of age on the performance of OACs, we included type of OAC (DOAC vs. VKA), dichotomized age (≥85 vs. <85 years) and an interaction term between these variables as fixed effects in the model. A significant interaction would indicate that the association between type of OAC and the composite outcome is modified by age and therefore differs in the oldest old compared to their younger counterparts. The model included the participating cohort study as a stratum.

We fitted the model three times according to the predefined analysis plan: (i) simple model including type of OAC and dichotomized age, with and without interaction term; (ii) adjusted model taking into account the known prognostic importance of sex, NIHSS at baseline and CHA_2DS_2-VASc score[10] (without the age and sex components, modified as in prior research[130]); (iii) weighted model, using the stabilized inverse probability of treatment weights (SIPTW)[138]. We constructed comparable treatment groups (DOAC vs. VKA) with regard to the following potentially outcome-modifying variables, as in previous research[70, 130]: sex, NIHSS at baseline, diabetes mellitus, hypertension, dyslipidemia, eGFR, history of prior stroke or TIA, history of ICH, current smoking, concomitant antiplatelet use and cohort study. We calculated the SIPTW using logistic regression and used robust standard errors for the 95% confidence intervals (CI) and p-values of the weighted analysis. We imputed missing values in the covariables used in the adjusted and weighted models with simple imputation rules (i.e., using the median / mean for continuous variables and the mode [most frequent

category] for categorical variables), and report the rate of missing values for all variables. For all models we report the model-based hazard ratio (HR) estimates along with the 95%-CI and p-values. We present the composite outcome data stratified to type of OAC and age group in weighted Kaplan-Meier curves using SIPTW (i.e., by weighting each observation by its stabilized inverse probability of treatment with DOAC vs. VKA),[139] for which we show both the crude and weighted numbers at risk.

We performed the following secondary analyses:

(a) We fitted the Cox models (i) – (iii) described above separately for the individual outcomes recurrent ischemic stroke, ICH and death. To account for competing risks, for these analyses we fitted Cox proportional cause-specific hazards models treating competing outcomes as censored observations.[140] With this approach, the competing outcomes influence the measure of association for the outcome of interest by removing at risk patient-years from the risk set over time.

(b) We analyzed the net clinical benefit (NCB) of DOAC over VKA in patients aged ≥85 and <85 years. We calculated the NCB by subtracting the weighted rate of excess ICH attributable to DOAC from the rate of excess ischemic stroke prevented by DOAC according to the following formula, as in prior research[141-144]:

NCB = (rate of recurrent ischemic stroke [VKA group] - rate of recurrent ischemic stroke [DOAC group]) – ICH weight x (rate of ICH [DOAC group] - rate of ICH [VKA group])

The ICH weight reflects the more severe clinical impact in terms of death and disability of ICH relative to ischemic stroke, with values ranging from 1.5 to 3.1 according to previously published weights[141-143]. We performed the NCB analyses for the entire range of weights according to previously used methodology[144]. We corrected the rate of ischemic stroke and ICH for baseline imbalances between DOAC- and VKA-treated patients using SIPTW as described above and report the NCB in events per 100 patient-years along with 95%-CI, calculated based on 1000 bootstrap replications. For the NCB analyses we considered all patients but those with death as first outcome.

As sensitivity analyses, we repeated the main analysis for the primary (composite) outcome using age as

(a) a categorical variable, dichotomized to ≥90 vs. <90 years. For this, we refitted all Cox models (i) – (iii) as described above.

(b) a continuous variable, using cubic B-splines to model the nonlinear association between age and log-hazard for the composite outcome. For this, we fitted the weighted model (iii) described above twice, with and without the interaction OAC type by age, and compared the two models using a likelihood ratio test. We graphically present the predicted rate of the composite outcome by age stratified to OAC type.

Statistical analyses were performed using R version 3.6.2 (2019-12-20) (R Core Team, 2019).

We conducted this study in accordance with the STROBE Statement for observational studies[145].

Ethics

The NOACISP-LONGTERM registry and the current analysis of pooled individual patient data were approved by the ethics committee in Basel, Switzerland (EKNZ 2014-027; PB_2016_00662). Patients provided written informed consent for participation in NOACISP-LONGTERM. The requirement for additional local ethical approval and patient informed consent differed among participating studies

and was acquired by the local investigators as necessary. CROMIS-2 was approved by the National Research Ethics Committee, London Queen Square and patients with capacity gave informed written consent. When patients could not consent, written consent from a proxy was obtained as defined by relevant local legislation. The SAMURAI-NVAF registry and the current collaboration were approved by the ethics committee in the National Cerebral and Cardiovascular Center (M23-18-3 and M29-077).

Results

In total, 5,984 of 6,267 (95.5%) patients were eligible for analysis. Information on OAC type was complete. Seven patients were excluded for missing age and 125 patients for missing follow-up data (study flowchart in Figure 6.1.1).

Baseline characteristics

The index event was ischemic stroke in 5,593 patients (93.5%) and TIA in 391 (6.5%); 2,858 patients (47.8%) were female. The median age was 78 years (IQR 71 – 84, range 24 – 102); 1,380 (23.1%) patients were aged ≥85 years and 4,604 (76.9%) were aged <85 years. OAC was initiated at a median (IQR) of 5 (2 – 11) days after the index event with DOAC in 3,688 patients (61.6%) and VKA in 2,296 patients (38.4%).

Patients aged ≥85 vs. <85 years

The baseline characteristics of the oldest old compared to their younger counterparts are displayed in Table 6.1.1. Patients aged ≥85 years were more commonly female and had higher NIHSS scores and lower eGFR, more often hypertension and previous ischemic stroke or TIA, as well as higher CHA_2DS_2-VASc scores compared to younger patients. Diabetes mellitus was more common among younger patients, as was dyslipidemia and current smoking. Time to OAC initiation after index event was shorter in the oldest old.

Patients with DOAC vs. VKA

There were no substantial differences between DOAC- and VKA-treated patients regarding age (median [IQR] 78 [71 – 84] years in both groups, p=0.179), sex (48.8% vs. 46.2% female, p=0.055) and time to OAC initiation (median [IQR] 5 [2 – 10] vs. 5 [2 – 14] days, p=0.075). Compared to patients with VKA treatment, DOAC-treated patients had less often ischemic stroke as index event (92.0% vs. 95.8%, p<0.001), reflected in their lower NIHSS scores (median [IQR] 5 [2 – 10] vs. 6 [2 – 13], p<0.001). They had less often concomitant antiplatelets (26.0% vs. 36.5%, p<0.001) and diabetes (23.7% vs. 27.6%, p=0.001), but more commonly hypertension (79.1% vs. 74.8%, p<0.001) and dyslipidemia (53.8% vs. 39.4%, p<0.001). DOAC-treated patients had higher CHA_2DS_2-VASc scores (mean [SD] 5.2 [1.5] vs. 5.0 [1.6], p<0.001). This was largely consistent in the subgroups of patients aged ≥85 vs. <85 years (Table 6.1.1).

Main analysis – primary composite outcome

During a total follow-up of 6,874 patient-years we observed a total of 279 recurrent ischemic strokes, 69 ICH and 737 deaths. This amounted to 994 primary (composite) outcome events, a primary outcome event rate of 14.5%/year. The follow-up time, number and crude rate of events for the primary outcome stratified to age group and OAC type are given in Table 6.1.2.

In the simple Cox model, age <85 years was associated with a significantly lower hazard for the primary outcome compared to age ≥85 years, as indicated by a HR of 0.46 (95%-CI [0.40, 0.52]). Likewise, DOAC-treated patients had a lower hazard than VKA-treated patients with a HR of 0.74 (95%-CI [0.63,

0.86]). There was no evidence for an interaction between age group and OAC type on their impact on the composite outcome (HR DOAC vs. VKA among patients aged ≥85 years 0.65, 95% CI [0.52, 0.81]; HR DOAC vs. VKA among patients aged <85 years 0.79, 95% CI [0.66, 0.95]; $p_{interaction}$ =0.129). Consistent findings resulted from repeated analyses refined by adjustment for potential confounders ($HR_{\geq 85y}$ 0.70, 95%-CI [0.56, 0.88] and $HR_{<85y}$ 0.87, 95%-CI [0.73, 1.05]; $p_{interaction}$=0.094) and weighting ($HR_{\geq 85y}$ 0.79, 95%-CI [0.61, 1.01] and $HR_{<85y}$ 0.88, 95%-CI [0.71, 1.09]; $p_{interaction}$=0.512). Thus, the better performance of DOAC over VKA with regard to the composite outcome was not dependent on age and was maintained in the oldest old. The detailed results of all Cox models for the composite outcome are presented in Table 6.1.3. The weighted Kaplan-Meier estimates for the composite outcome stratified to type of OAC and age group are presented in Figure 6.1.2.

Secondary analysis – individual outcomes recurrent IS, ICH and death

Table 6.1.2 shows the follow-up time, number and crude rate of events for the secondary (individual) outcomes in the entire study population and stratified to age group and OAC type. In line with the main analysis of the composite outcome, there was no evidence for an interaction between age and OAC type on the recurrence of ischemic stroke nor the occurrence of ICH in the simple, adjusted and weighted Cox proportional cause-specific hazards models accounting for competing risks (all $p_{interaction}$>0.05). For the outcome death, there was evidence for a weak interaction only in the simple ($p_{interaction}$= 0.054) and adjusted models ($p_{interaction}$=0.029), indicating that the lower hazard for death among patients treated with DOAC as compared to VKA-treated patients was even more pronounced in the oldest old than in their younger counterparts (simple model: $HR_{\geq 85y}$ 0.61, 95%-CI [0.47, 0.79] and $HR_{<85y}$ 0.83, 95%-CI [0.65, 1.05]; adjusted model: $HR_{\geq 85y}$ 0.66, 95%-CI [0.51, 0.86] and $HR_{<85y}$ 0.94, 95%-CI [0.74, 1.19]). The detailed results of all Cox cause-specific hazards models for the individual outcomes are presented in Supplementary Table 6.1.2 and Figure 6.1.3.

Secondary analysis – net clinical benefit

The point estimates for the NCB of DOAC over VKA were similar in patients aged ≥85 (+1.73 to +2.66) and <85 years (+1.90 to +3.36 events per 100 patient-years) and remained positive over the entire range of ICH weights used (1.5 to 3.1), with wide confidence intervals crossing zero in the smaller group of patients aged ≥85 years. Figure 6.1.4 depicts the NCB for 3 previously published ICH weights[141-143]. The detailed results of the NCB analysis are presented in Supplementary Table 6.1.3.

Sensitivity analyses – age dichotomized at 90 years and as a continuous variable

The sensitivity analyses using age dichotomized to ≥90 (N=451) vs. <90 years (N=5,533) showed no evidence for interaction between age and OAC type on the hazard for the composite outcome in the simple ($p_{interaction}$=0.283), adjusted ($p_{interaction}$=0.514) and weighted ($p_{interaction}$=0.433) models, consistent with the main analysis (Supplementary Table 6.1.4).

Using age as a continuous variable, the favorable profile of DOAC over VKA regarding the composite outcome was maintained across the entire age spectrum in the weighted model (Figure 6.1.5). There was no evidence that the association between OAC type and composite outcome was modified by age upon comparison of the weighted model with vs. without interaction term ($p_{likelyhood\ ratio\ test}$ = 0.623). The hazard for the composite outcome continuously increased with increasing age in a nonlinear fashion.

Discussion

This study focused on the safety and effectiveness of DOAC versus VKA in the oldest-old patients with AF and recent stroke or TIA in a real-world setting. The key finding was that the benefits of DOAC over VKA were consistently maintained in the oldest old, even when potential confounders were accounted for.

In our study, DOAC were associated with a lower hazard than VKA for the composite outcome of recurrent ischemic stroke, ICH and all-cause death in patients with AF and recent ischemic stroke, independent of age. The favorable profile of DOAC was maintained in the oldest old, whether defined as aged 85 or 90 years or older. This observation is highly relevant for clinical practice as it contradicts the assumptions of many clinicians who are reluctant to use DOAC in this age group, particularly in multimorbid patients[64, 73]. In this context, it is clinically important that the beneficial effect of DOAC over VKA persisted after taking into account the high-risk profile of the oldest old. Reassuringly, simple, adjusted, as well as weighted models which controlled for the nonrandomized treatment assignment, all yielded consistent results.

Notably, there was no signal of a safety concern regarding ICH risk among the oldest-old DOAC-treated patients with recent ischemic stroke, which is a widespread concern[75, 129, 146]. In NCB analyses balancing the benefit in stroke reduction against the weighted risk of ICH, the net benefit of DOAC over VKA in these patients was preserved, as indicated by NCB point estimates that were similar in the oldest old as in their younger counterparts, and remained consistently positive across a broad spectrum of ICH weights. Taken together, these findings provide new evidence that the overall beneficial effect of DOAC treatment following recent ischemic stroke is maintained in the oldest old.

These results are clinically important because limited randomized data exist for such patients, as patients with recent stroke within 7 days,[56] 14 days,[54, 55] or 30 days,[68] respectively, were excluded from the pivotal DOAC RCTs and the oldest old were severely underrepresented, constituting less than 5% of the RCTs population.[62, 63] While several large observational studies later confirmed the benefits of DOAC in elderly patients with AF,[147-152] they did not examine patients with a recent ischemic stroke. The fact that the elderly in our study had a recent stroke matters, as such patients – compared to those without recent stroke – have a higher risk for hemorrhagic complications, including ICH[153] and hemorrhagic transformation of the ischemic infarct[154], concomitant active small vessel disease[155, 156] and stroke-induced motor and cognitive deficits with an increased risk of falls.[62, 74]

For patients with recent stroke, subgroup analyses in observational studies suggested the safety of DOAC versus VKA for the age groups of ≥75[72] and >80 years[70, 157]. As we are not aware of any studies investigating AF patients with both (i) age over 85 years and (ii) a recent ischemic stroke, our data address an important evidence gap, mitigating concerns about the applicability of the RCT findings in everyday clinical stroke practice and supporting the current guidelines for prevention of stroke recurrence.[31]

Strengths and Limitations

Our study has the following strengths: (i) we used individual patient data pooled within an established collaboration of prospective observational studies from Europe and Asia; (ii) the high data completeness limits the risk of spurious findings; and (iii) the consistency of results both in unadjusted and in adjusted and – most importantly – weighted analyses accounting for potential confounders, as

well as in net benefit analyses and in sensitivity analyses focusing on patients ≥ 90 years or using age as a continuous measure, underlines the robustness of our key finding.

We are aware of the following limitations: (i) as our data are observational rather than randomized, baseline imbalances in the allocation to the type of OAC that were unaccounted for might have introduced bias or confounding; (ii) our study included exclusively OAC-treated patients, so age-matched stroke patients without OAC treatment were not available for comparison. Of note, the placebo-controlled ELDERCARE-AF trial suggested the benefit of anticoagulation even in very elderly patients with AF who were not appropriate candidates for standard anticoagulant treatment;[73] (iii) we did not consider extracranial bleeding or myocardial infarction in our analyses, as these outcomes were not available in all participating cohorts; (iv) the follow-up time in the participating cohorts differed, with some reporting over 2 years of follow-up data, while others were limited to 3 months; (v) our study did not include information on adherence to oral anticoagulants, which was not systematically assessed in most cohorts, although our previous work from the single-center NOACISP-LONGTERM cohort indicated high rates of self-reported adherence both in VKA- and DOAC-treated patients also among the oldest old;[95] (vi) Dementia was not an explicit exclusion criterion in any of the contributing cohorts. However, as our study lacked information on the frequency of dementia, it remains unclear whether our key findings are applicable to demented patients, too.

In conclusion, our study provides new and compelling evidence indicating that the benefits of DOAC over VKA in patients with AF and recent stroke are maintained among the oldest old.

Tables

Table 6.1.1. Patient characteristics stratified to age group and OAC type

	Age ≥85 years	Age <85 years		Missing values rate	Age ≥85 years			Age <85 years		
					DOAC	VKA		DOAC	VKA	
Patients, n (%)	1,380 (23.1)	4,604 (76.9)	p-value		865 (62.7)	515 (37.3)	p-value	2,823 (61.3)	1,781 (38.7)	p-value
Demographics										
Age, years, median (IQR)	88.0 (86.0-90.0)	75.0 (69.0-80.0)	<0.001	0%	88.0 (86.0-90.0)	88.2 (86.0-91.0)	0.001	75.1 (69.0-80.0)	75.0 (69.0-80.0)	0.119
Female sex, n (%)	881 (63.8)	1,977 (42.9)	<0.001	0%	549 (63.5)	332 (64.5)	0.753	1,249 (44.2)	728 (40.9)	0.027
Stroke characteristics										
Ischemic stroke as index event, n (%)	1,292 (93.6)	4,301 (93.4)	0.836	0%	794 (91.8)	498 (96.7)	<0.001	2,599 (92.1)	1,702 (95.6)	<0.001
NIHSS at baseline, median (IQR)	6 (3-12.5)	5 (2-11)	<0.001	7.8%	5 (2-11)	8 (3-16)	<0.001	4 (2-10)	6 (2-12)	<0.001
Medication details										
Time to OAC initiation, days, median (IQR)	4 (2-10)	5 (2-11)	0.001	5.1%*	4 (2-9)	4 (2-12)	0.620	5 (2-10)	5 (2-14)	0.081
Concomitant antiplatelet use, n (%)	370 (30.9)	1,255 (30.1)	0.622	10.4%	196 (27.7)	174 (35.6)	0.005	620 (25.4)	635 (36.8)	<0.001
Risk factors										
Previous stroke or TIA, n (%)	389 (28.2)	1,110 (24.1)	0.002	0.03%	250 (28.9)	139 (27.0)	0.497	686 (24.3)	424 (23.8)	0.724
Previous ICH, n (%)	17 (1.5)	37 (1.1)	0.322	24.0%	12 (1.7)	5 (1.1)	0.577	17 (0.8)	20 (1.5)	0.078
Diabetes mellitus, n (%)	309 (22.4)	1,200 (26.1)	0.006	0.07%	181 (20.9)	128 (24.9)	0.104	694 (24.6)	506 (28.4)	0.004
Hypertension, n (%)	1,146 (83.3)	3,474 (75.7)	<0.001	0.3%	742 (85.9)	404 (79.1)	0.001	2,169 (77.0)	1,305 (73.6)	0.011
Dyslipidemia, n (%)	521 (43.4)	1,908 (48.7)	0.001	14.5%	365 (51.8)	156 (31.4)	<0.001	1,177 (54.5)	731 (41.7)	<0.001
CHA_2DS_2VASc-Score, mean (SD)	6.0 (1.1)	4.9 (1.6)	<0.001	1.1%	6.0 (1.2)	5.9 (1.1)	0.038	5.0 (1.6)	4.8 (1.6)	<0.001
Modified CHA_2DS_2VASc-Score (without age and sex), mean (SD)	3.3 (1.1)	3.1 (1.2)	<0.001	1.1%	3.4 (1.1)	3.2 (1.0)	0.018	3.2 (1.2)	3.0 (1.3)	<0.001
eGFR, ml/min, mean (SD)	51.0 (24.4)	62.3 (29.3)	<0.001	14.0%	50.4 (25.6)	51.9 (21.9)	0.303	64 (48 – 83)	66 (51 – 81)	0.039
Current smoking, n (%)	70 (5.3)	803 (18.0)	<0.001	3.4%	49 (6.0)	21 (4.2)	0.205	501 (18.5)	302 (17.3)	0.331

*exact time missing, but all <30 days

OAC, oral anticoagulant; DOAC, direct oral anticoagulant; VKA, Vitamin-K-antagonist; NIHSS, National Institutes of Health Stroke Scale; eGFR, estimated glomerular filtration rate; ICH, intracranial hemorrhage

Table 6.1.2. Follow-up time, number and crude rate of events for the primary and secondary outcomes

		Patient-years of follow-up	Number of events (annualized rate)			
			Composite outcome	Recurrent ischemic stroke	Intracranial hemorrhage	All-cause death
All		6,874	994 (14.5%)	279 (4.1%)	69 (1.0%)	737 (10.7%)
Stratified to age						
≥85 years		1,502	387 (25.8%)	72 (4.8%)	18 (1.2%)	337 (22.4%)
<85 years		5,372	607 (11.3%)	207 (3.9%)	51 (0.9%)	400 (7.4%)
Stratified to OAC type						
DOAC		3,559	491 (13.8%)	150 (4.2%)	26 (0.7%)	351 (9.9%)
VKA		3,316	503 (15.2%)	129 (3.9%)	43 (1.3%)	386 (11.6%)
Stratified to age and OAC type						
≥85 years	DOAC	779	181 (23.2%)	40 (5.1%)	8 (1.0%)	152 (19.5%)
	VKA	723	206 (28.5%)	32 (4.4%)	10 (1.4%)	185 (25.6%)
<85 years	DOAC	2,780	310 (11.2%)	110 (4.0%)	18 (0.6%)	199 (7.2%)
	VKA	2,593	297 (11.5%)	97 (3.7%)	33 (1.3%)	201 (7.8%)

OAC, oral anticoagulant; DOAC, direct oral anticoagulant; VKA, Vitamin-K-antagonist

Table 6.1.3. Cox models for time to composite outcome

Model (N = 5,984)	**Variable**	**Hazard ratio**	**95% confidence interval**	**p-value**
simple model	DOAC (vs. VKA)	0.74	[0.63, 0.86]	<0.001
	Age <85 years (vs. ≥85 years)	0.46	[0.40, 0.52]	<0.001
simple model with interaction term	DOAC (vs. VKA)	0.65	[0.52, 0.81]	<0.001
	Age <85 years (vs. ≥85 years)	0.41	[0.34, 0.49]	<0.001
	Interaction OAC by age	1.22	[0.94, 1.58]	0.129
adjusted model* with interaction term	DOAC (vs. VKA)	0.70	[0.56, 0.88]	0.002
	Age <85 years (vs. ≥85 years)	0.42	[0.35, 0.50]	<0.001
	Interaction OAC by age	1.25	[0.96, 1.61]	0.094
weighted model† with interaction term	DOAC (vs. VKA)	0.79	[0.61, 1.01]	0.060
	Age <85 years (vs. ≥85 years)	0.48	[0.37, 0.61]	<0.001
	Interaction OAC by age	1.12	[0.81, 1.55]	0.512

*adjustment for sex, National Institutes of Health Stroke Scale score at baseline, modified CHA_2DS_2-VASc score (without the age and sex components)

†weighting for sex, National Institutes of Health Stroke Scale score at baseline, history of prior stroke or transient ischemic attack, history of intracranial hemorrhage, diabetes mellitus, hypertension, dyslipidemia, estimated glomerular filtration rate, current smoking, concomitant antiplatelet use, cohort study

OAC, oral anticoagulant; DOAC, direct oral anticoagulant; VKA, Vitamin K antagonist

Figures

Figure 6.1.1. Study Flowchart

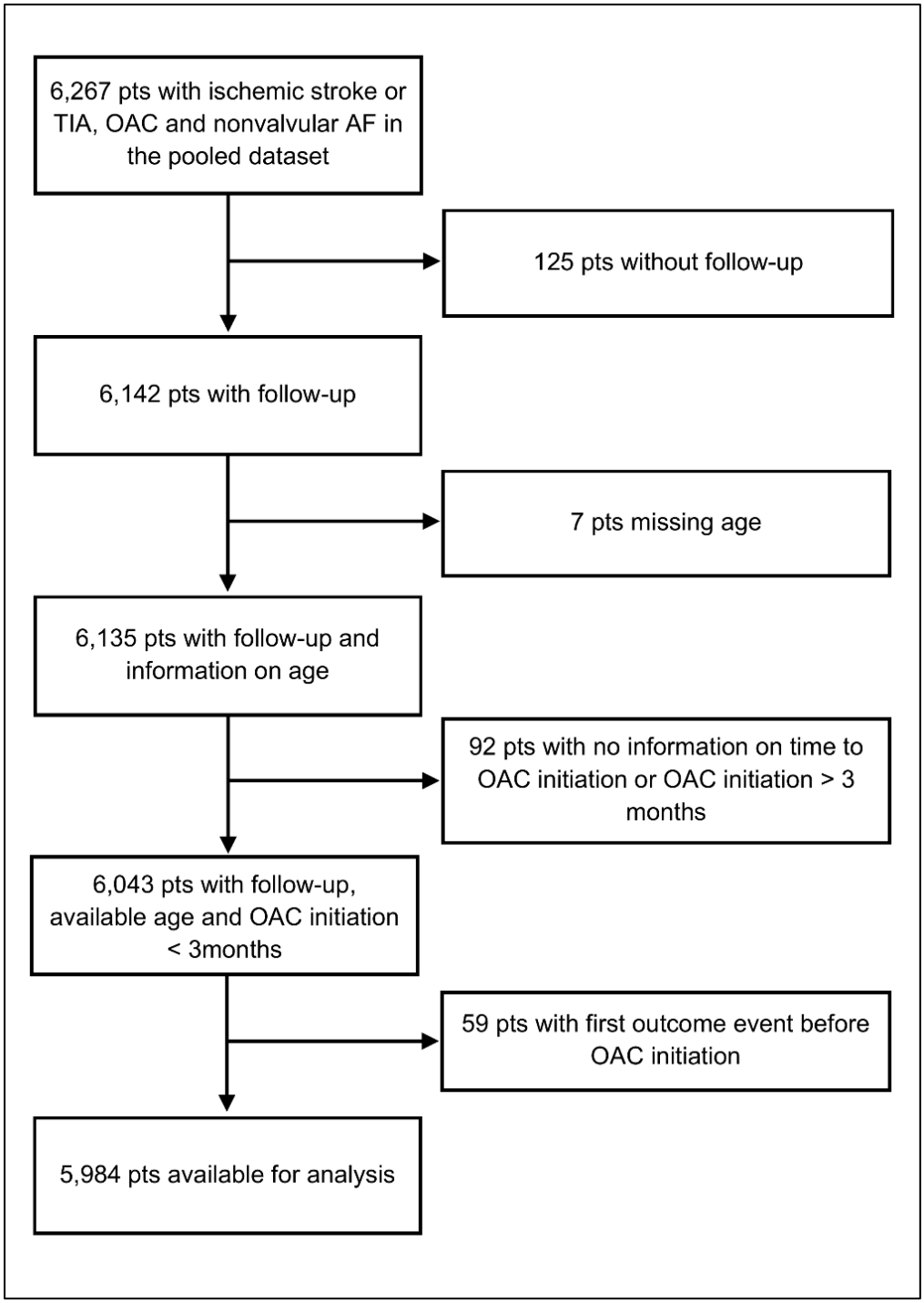


Figure 6.1.2. Weighted Kaplan-Meier curves for the composite outcome stratified to anticoagulant type (DOAC / VKA) and age group (≥85 / <85 years)

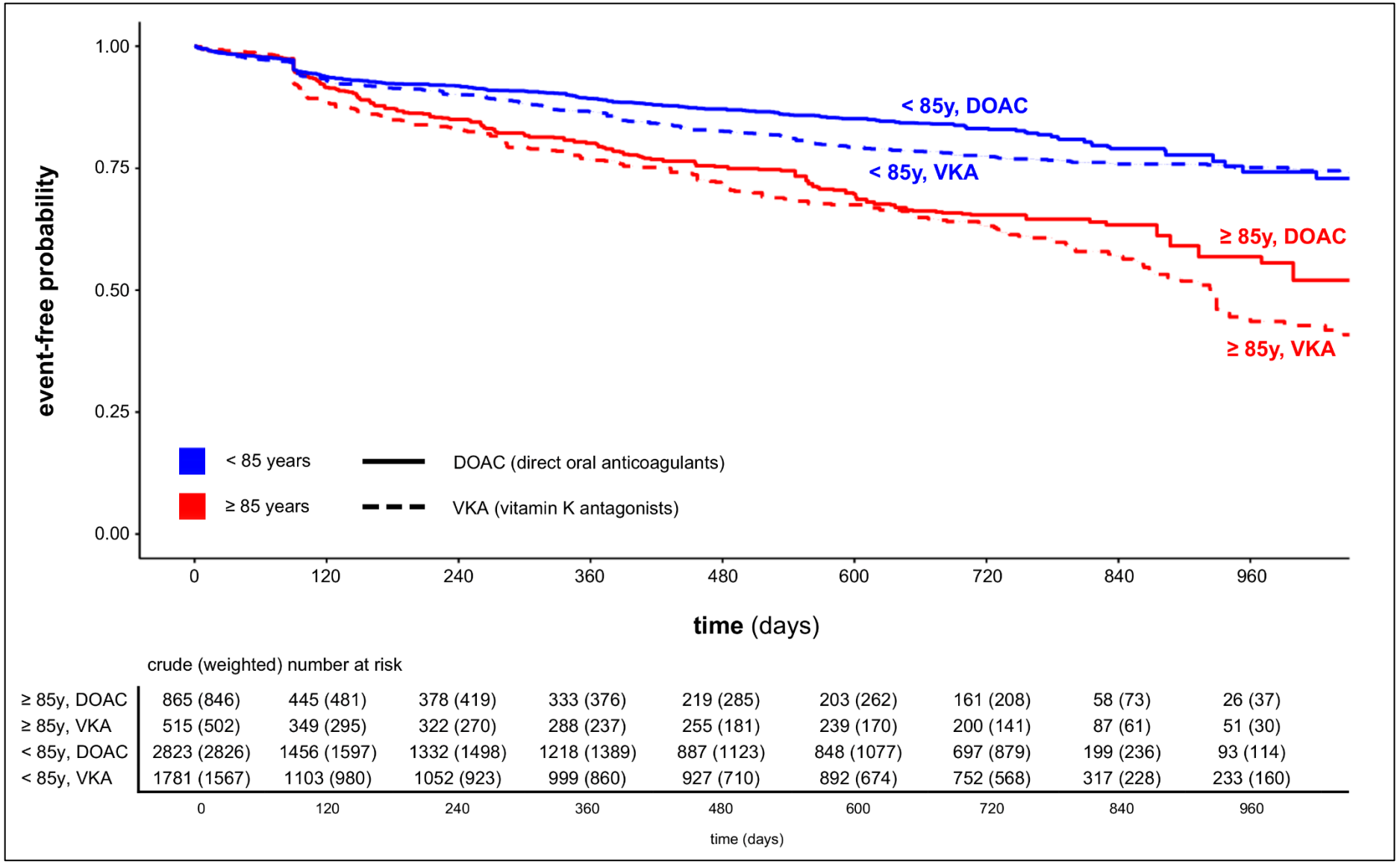


Figure 6.1.3. Hazard ratio estimates for the effect of DOAC vs. VKA on the primary composite outcome and all its individual components (accounting for competing risks) stratified to patients aged ≥85 versus <85 years based on the weighted model

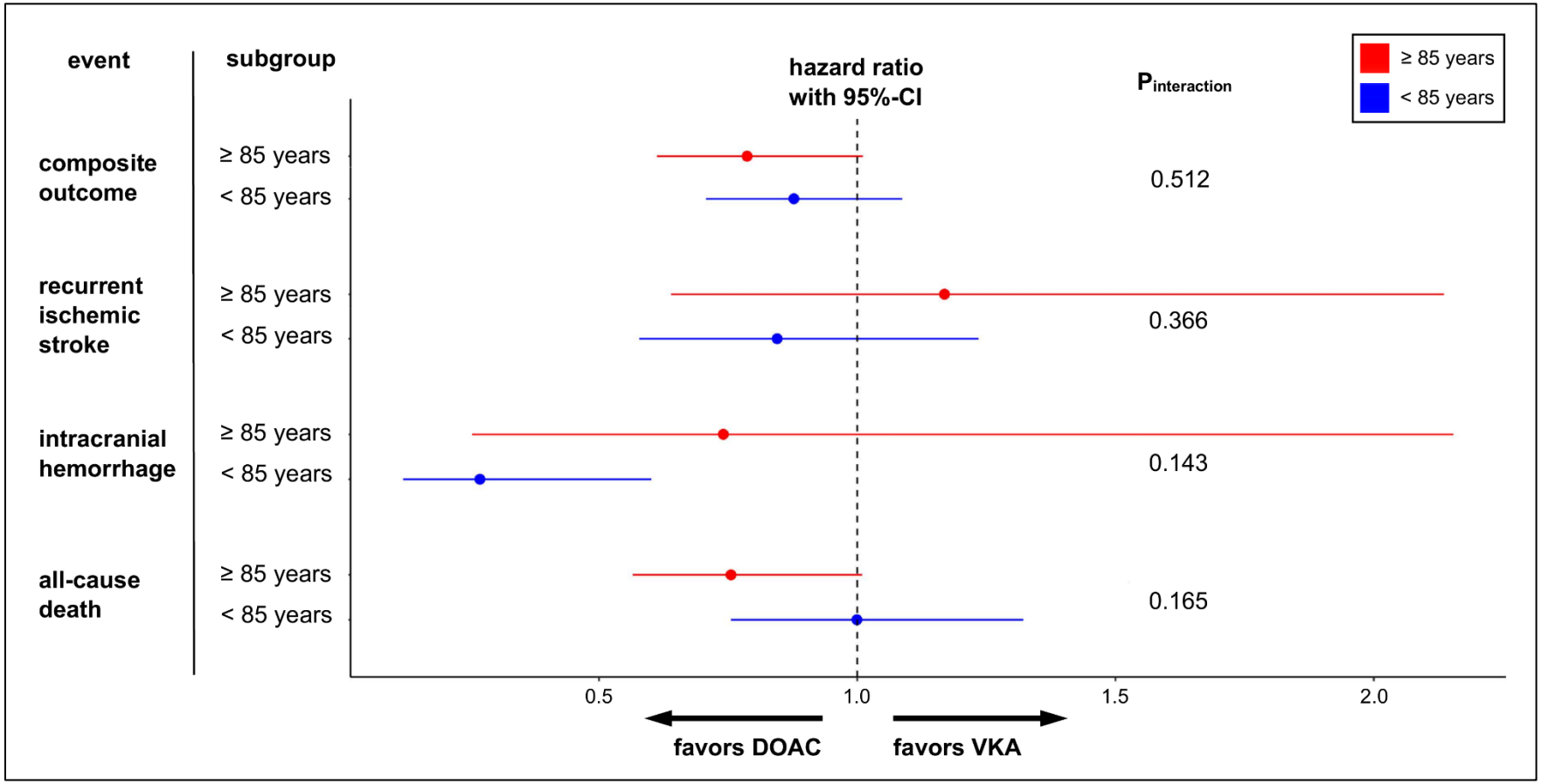


Figure 6.1.4. Net clinical benefit of DOAC over VKA with 95% confidence intervals stratified to age group (≥85 / <85 years), using 3 previously published ICH weights

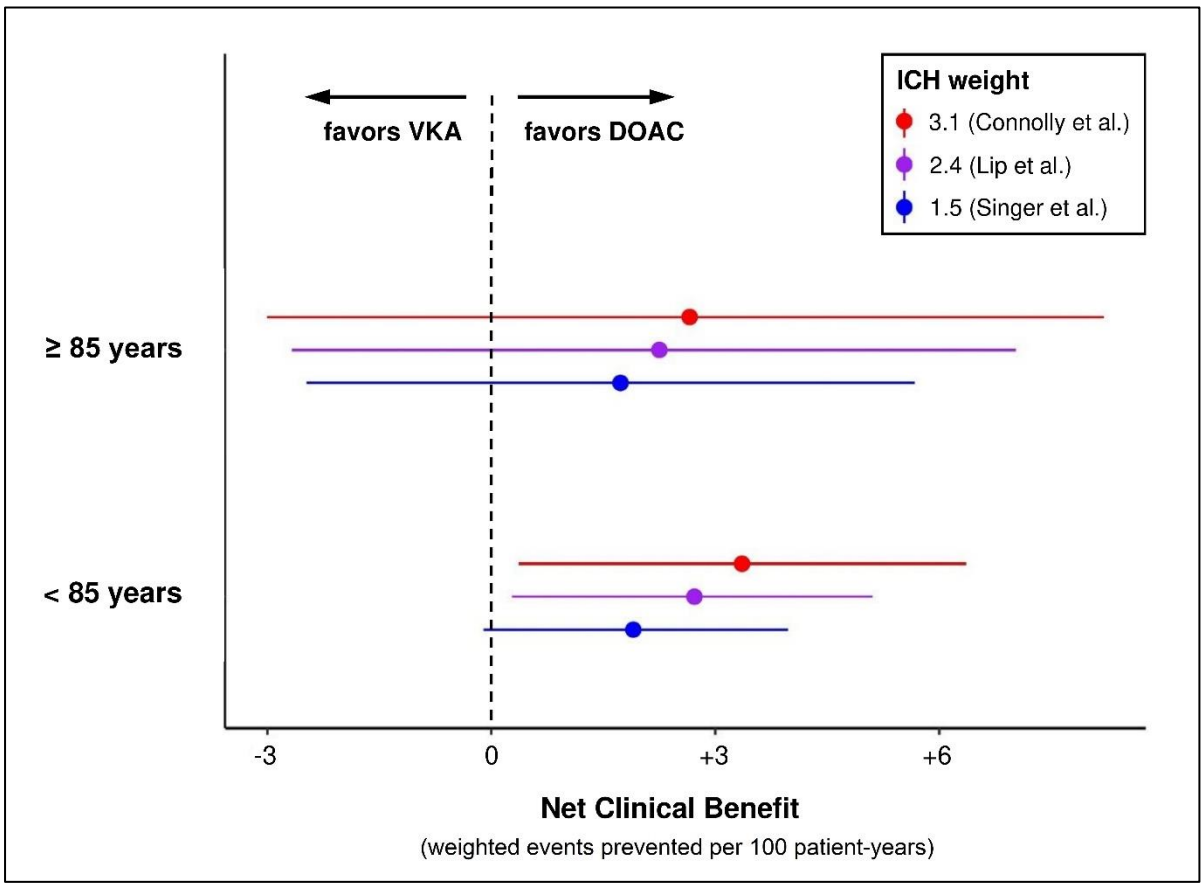
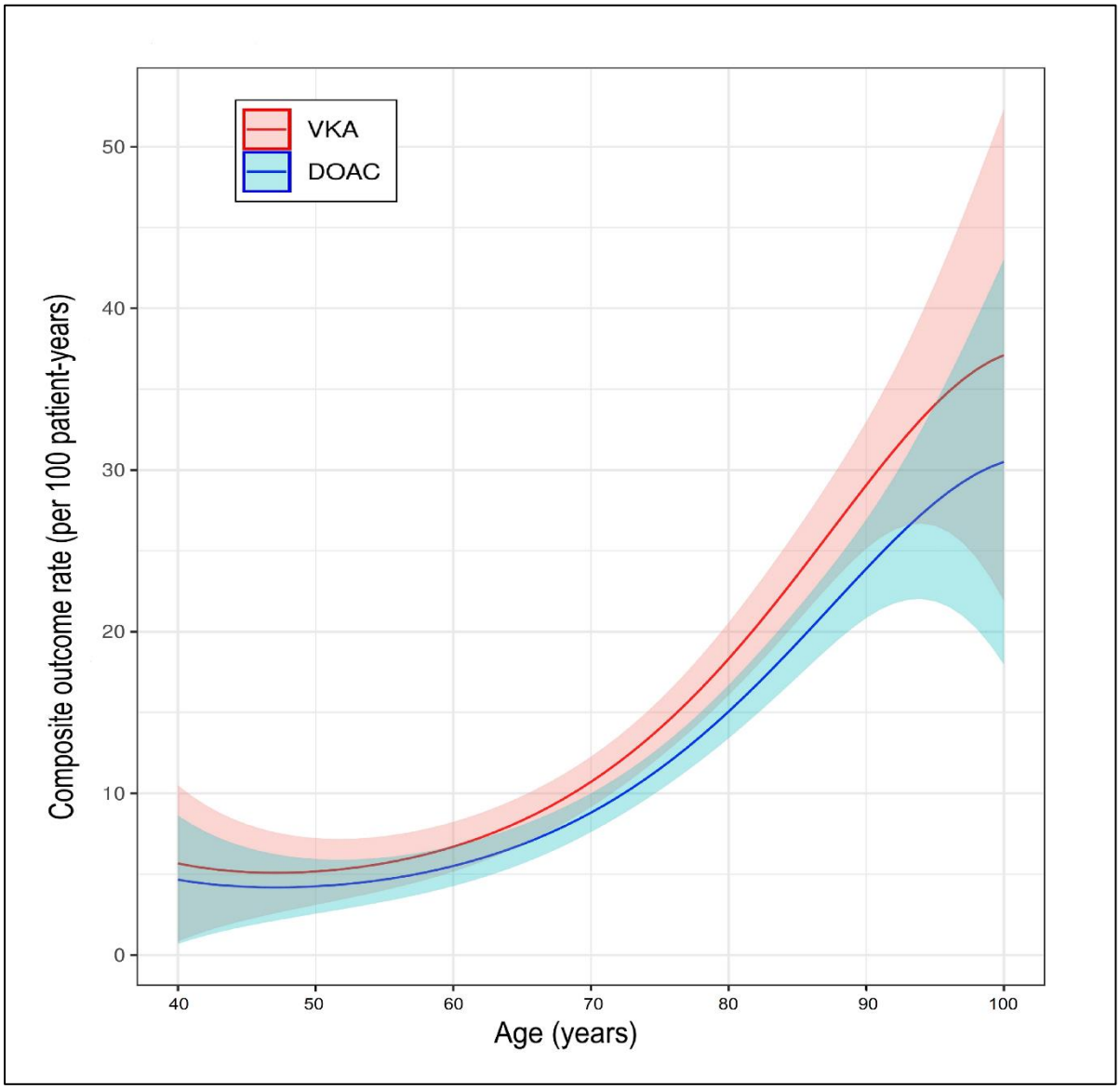


Figure 6.1.5. Rate of the composite outcome by age as a continuous variable stratified to type of anticoagulant (DOAC / VKA). The solid lines represent the estimates for each year of age from the weighted model without interaction term and the shaded areas the 95%-CI.



Supplementary Material

Supplementary Table 6.1.1. *Participating cohort studies*

Cohort study	**Study period**	**Number of patients contributed to the final pooled cohort in this study**	**Median follow-up** (days)
Single-center			
NOACISP-LONGTERM*	2012 – 2019	798	717
Erlangen Registry*	2011 – 2013 2016 – 2019	1,174	107
Verona Registry	2013 – 2015	222	90
Multicenter			
CROMIS-2	2011 – 2015	1,245	786
RAF	2012 – 2014	553	90
RAF-DOAC	2014 – 2016	868	90
SAMURAI-NVAF	2011 – 2014	1,124	717

* The datasets of these ongoing studies were updated since the initial data pooling (Seiffge et al. Ann Neurol 2019) to include additional patients with index event up to December 31st, 2019.

Supplementary Table 6.1.2. Cox proportional cause-specific hazards models for time to recurrent ischemic stroke, intracranial hemorrhage and death, accounting for competing risks

Model (N = 5,984)	Variable	Recurrent ischemic stroke			Intracranial hemorrhage			All-cause death		
		Hazard ratio	95 % confidence interval	p-value	Hazard ratio	95 % confidence interval	p-value	Hazard ratio	95 % confidence interval	p-value
simple model	DOAC (vs. VKA)	0.90	[0.67, 1.20]	0.463	0.38	[0.20, 0.72]	0.003	0.72	[0.60, 0.88]	<0.001
	Age <85 years (vs. ≥85 years)	0.83	[0.63, 1.10]	0.193	0.78	[0.45, 1.37]	0.391	0.34	[0.29, 0.39]	<0.001
simple model with interaction term	DOAC (vs. VKA)	0.87	[0.53, 1.44]	0.591	0.60	[0.22, 1.69]	0.334	0.61	[0.47, 0.79]	<0.001
	Age <85 years (vs. ≥85 years)	0.82	[0.54, 1.22]	0.323	1.01	[0.48, 2.12]	0.988	0.29	[0.23, 0.36]	<0.001
	Interaction OAC by age	1.04	[0.60, 1.81]	0.885	0.53	[0.17, 1.66]	0.277	1.36	[1.00, 1.86]	0.054
adjusted model* with interaction term	DOAC (vs. VKA)	0.90	[0.55, 1.50]	0.692	0.58	[0.21, 1.63]	0.303	0.66	[0.51, 0.86]	0.002
	Age <85 years (vs. ≥85 years)	0.85	[0.57, 1.29]	0.449	0.91	[0.43, 1.95]	0.814	0.29	[0.23, 0.36]	<0.001
	Interaction OAC by age	1.06	[0.61, 1.85]	0.825	0.55	[0.18, 1.71]	0.300	1.42	[1.04, 1.94]	0.029
weighted model† with interaction term	DOAC (vs. VKA)	1.17	[0.64, 2.14]	0.612	0.74	[0.26, 2.15]	0.581	0.76	[0.57, 1.01]	0.058
	Age <85 years (vs. ≥85 years)	1.06	[0.59, 1.91]	0.835	1.41	[0.53, 3.74]	0.494	0.32	[0.24, 0.43]	<0.001
	Interaction OAC by age	0.72	[0.36, 1.46]	0.366	0.36	[0.09, 1.41]	0.143	1.32	[0.89, 1.96]	0.165

*adjustment for sex, National Institutes of Health Stroke Scale score at baseline, modified CHA_2DS_2-VASc score (without the age and sex components)

†weighting for sex, National Institutes of Health Stroke Scale score at baseline, history of prior stroke or transient ischemic attack, history of intracranial hemorrhage, diabetes mellitus, hypertension, dyslipidemia, estimated glomerular filtration rate, current smoking, concomitant antiplatelet use, cohort study

OAC, oral anticoagulant; DOAC, direct oral anticoagulant; VKA, Vitamin K antagonist

Supplementary Table 6.1.3. *Net clinical benefit of DOAC over VKA*

ICH weight	Patients aged ≥85 years			Patients aged <85 years		
	weighted rate of events* in VKA-treated patients [95%-CI] (per 100 patient-years)	**weighted rate of events* in DOAC-treated patients** [95%-CI] (per 100 patient-years)	**NCB of DOAC over VKA** [95%-CI] (per 100 patient-years)	**weighted rate of events* in VKA-treated patients** [95%-CI] (per 100 patient-years)	**weighted rate of events* in DOAC-treated patients** [95%-CI] (per 100 patient-years)	**NCB of DOAC over VKA** [95%-CI] (per 100 patient-years)
1.5	8.17 [4.76, 11.51]	6.44 [4.40, 8.60]	+1.73 [-2.47, +5.68]	6.84 [5.13, 8.64]	4.94 [3.93, 5.94]	+1.90 [-0.10, +3.98]
1.6	8.33 [4.70, 11.85]	6.54 [4.35, 8.78]	+1.79 [-2.32, +5.80]	6.98 [5.13, 8.76]	4.99 [3.98, 6.00]	+1.99 [-0.05, +4.14]
1.7	8.49 [4.98, 11.99]	6.64 [4.29, 8.91]	+1.85 [-2.32, +6.29]	7.12 [5.23, 8.96]	5.04 [3.96, 6.03]	+2.08 [+0.01, +4.10]
1.8	8.65 [4.99, 12.21]	6.74 [4.45, 9.01]	+1.91 [-2.60, +6.40]	7.25 [5.26, 9.14]	5.08 [4.03, 6.17]	+2.17 [+0.00, +4.28]
1.9	8.80 [5.21, 12.55]	6.84 [4.40, 9.13]	+1.96 [-2.65, +6.26]	7.39 [5.58, 9.27]	5.12 [4.06, 6.20]	+2.27 [+0.11, +4.48]
2.0	8.95 [5.02, 12.67]	6.93 [4.47, 9.30]	+2.02 [-2.51, +6.27]	7.53 [5.45, 9.55]	5.17 [4.09, 6.31]	+2.36 [+0.21, +4.59]
2.1	9.11 [5.02, 12.95]	7.03 [4.50, 9.50]	+2.08 [-2.66, +6.77]	7.67 [5.59, 9.64]	5.22 [4.14, 6.29]	+2.45 [+0.19, +4.82]
2.2	9.27 [5.16, 13.18]	7.13 [4.58, 9.58]	+2.14 [-2.62, +6.69]	7.80 [5.67, 9.85]	5.26 [4.08, 6.40]	+2.54 [+0.13, +4.93]
2.3	9.42 [5.56, 13.27]	7.23 [4.75, 9.68]	+2.19 [-2.59, +6.92]	7.94 [5.82, 10.15]	5.31 [4.17, 6.44]	+2.63 [+0.34, +5.07]
2.4	9.58 [5.54, 13.55]	7.33 [4.66, 9.98]	+2.25 [-2.67, +7.03]	8.07 [5.86, 10.41]	5.35 [4.19, 6.50]	+2.72 [+0.28, +5.11]
2.5	9.73 [5.65, 13.73]	7.42 [4.81, 10.00]	+2.31 [-2.84, +7.28]	8.21 [6.01, 10.38]	5.40 [4.27, 6.52]	+2.81 [+0.43, +5.37]
2.6	9.89 [5.72, 14.19]	7.52 [4.81, 10.15]	+2.37 [-2.53, +7.47]	8.34 [6.06, 10.74]	5.44 [4.30, 6.54]	+2.90 [+0.38, +5.37]
2.7	10.05 [5.64, 14.44]	7.62 [4.83, 10.39]	+2.43 [-2.70, +7.55]	8.48 [6.15, 10.86]	5.49 [4.33, 6.69]	+2.99 [+0.32, +5.64]
2.8	10.20 [5.81, 14.43]	7.72 [4.58, 10.55]	+2.48 [-2.71, +7.58]	8.62 [6.17, 11.09]	5.53 [4.32, 6.69]	+3.09 [+0.51, +5.77]
2.9	10.36 [6.00, 14.75]	7.82 [4.89, 10.68]	+2.54 [-2.71, +7.68]	8.76 [6.34, 11.23]	5.58 [4.41, 6.77]	+3.18 [+0.52, +5.88]
3.0	10.51 [5.76, 15.39]	7.91 [4.84, 10.87]	+2.60 [-2.93, +8.00]	8.89 [6.36, 11.43]	5.62 [4.41, 6.85]	+3.27 [+0.37, +6.12]
3.1	10.67 [5.93, 15.36]	8.01 [5.01, 11.00]	+2.66 [-3.01, +8.21]	9.03 [6.39, 11.65]	5.67 [4.48, 6.94]	+3.36 [+0.37, +6.36]

*calculated as [rate of ischemic stroke + (ICH weight x rate of ICH)]

DOAC, direct oral anticoagulant; VKA, Vitamin K antagonist; ICH, intracranial hemorrhage

Supplementary Table 6.1.4. Cox models for time to composite outcome using age dichotomized at 90 years

Model (N = 5,984)	**Variable**	**Hazard ratio**	**95% confidence interval**	**p-value**
simple model	DOAC (vs. VKA)	0.74	[0.63, 0.86]	<0.001
	Age <90 years (vs. ≥90 years)	0.45	[0.38, 0.56]	<0.001
simple model with interaction term	DOAC (vs. VKA)	0.62	[0.44, 0.88]	0.006
	Age <90 years (vs. ≥90 years)	0.41	[0.33, 0.52]	<0.001
	Interaction OAC by age	1.21	[0.85, 1.73]	0.283
adjusted model* with interaction term	DOAC (vs. VKA)	0.73	[0.52, 1.03]	0.077
	Age <90 years (vs. ≥90 years)	0.47	[0.37, 0.59]	<0.001
	Interaction OAC by age	1.13	[0.79, 1.60]	0.514
weighted model† with interaction term	DOAC (vs. VKA)	0.74	[0.52, 1.07]	0.111
	Age <90 years (vs. ≥90 years)	0.44	[0.33, 0.58]	<0.001
	Interaction OAC by age	1.18	[0.79, 1.76]	0.433

*adjustment for sex, National Institutes of Health Stroke Scale score at baseline, modified CHA_2DS_2-VASc score (without the age and sex components)

†weighting for sex, National Institutes of Health Stroke Scale score at baseline, history of prior stroke or transient ischemic attack, history of intracranial hemorrhage, diabetes mellitus, hypertension, dyslipidemia, estimated glomerular filtration rate, current smoking, concomitant antiplatelet use, cohort study

OAC, oral anticoagulant; DOAC, direct oral anticoagulant; VKA, Vitamin K antagonist

6.2. Oral anticoagulants in atrial fibrillation patients with recent stroke who are dependent on the daily help of others

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Abstract

Background and purpose

Data on the effectiveness and safety of direct oral anticoagulants (DOAC) versus vitamin K antagonists (VKA) in patients with stroke attributable to atrial fibrillation (AF) who were dependent on the daily help of others at hospital discharge are scarce.

Methods

Based on prospectively obtained data from the observational Novel-Oral-Anticoagulants-in-Ischemic-Stroke-Patients-longterm registry (NOACISP-LONGTERM) from Basel, Switzerland, we compared the occurrence of the primary outcome – the composite of recurrent ischemic stroke, major bleeding and all-cause death – among consecutive AF-stroke patients treated with either VKA or DOAC between patients dependent (defined as modified Rankin Scale 3-5) and patients independent at discharge. We used simple, adjusted and weighted cox proportional hazards regression to account for potential confounders.

Results

We analyzed 801 patients (median age 80 years, 46% female), of whom 391 (49%) were dependent at discharge and 680 (85%) received DOAC. Over a total follow-up of 1,216 patient-years, DOAC- compared to VKA-treated patients had a lower hazard for the composite outcome (hazard ratio [HR] 0.58, 95% confidence interval [CI] [0.42, 0.81]), as did independent compared to dependent patients (HR 0.54, 95%-CI [0.40, 0.71]). There was no evidence that the effect of anticoagulant type (DOAC vs. VKA) on the hazard for the composite outcome differed between dependent ($HR_{dependent}$ 0.68, 95%-CI [0.45, 1.01]) and independent patients ($HR_{independent}$ 0.44, 95%-CI [0.26, 0.75]) in the simple model ($p_{interaction}$=0.212). Adjusted ($HR_{dependent}$ 0.74, 95%-CI [0.49, 1.11] and $HR_{independent}$ 0.51, 95%-CI [0.30, 0.87]; $p_{interaction}$=0.284) and weighted models ($HR_{dependent}$ 0.79, 95%-CI [0.48, 1.31]) and $HR_{independent}$ 0.46, 95%-CI [0.26, 0.81]; $p_{interaction}$=0.163) yielded concordant results. Secondary analyses focusing on the individual components of the composite outcome were consistent to the primary analyses.

Conclusion

The benefits of DOAC in atrial fibrillation patients with a recent stroke were maintained among patients that were dependent on the help of others at discharge.

Registration

URL: https://www.clinicaltrials.gov; Unique identifier: NCT03826927

6.3. Small vessel disease is associated with an unfavorable outcome in stroke patients on oral anticoagulation

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Abstract

Introduction

Cerebral small vessel disease is an important cause for both ischemic stroke and intracranial haemorrhage. To date, knowledge on the impact of small vessel disease on the clinical course in stroke patients treated with oral anticoagulation for atrial fibrillation is limited.

Patients and methods

Registry-based prospective observational study of 320 patients (aged 78.2 ± 9.2 years) treated with anticoagulation following atrial fibrillation stroke. Patients underwent standardised magnetic-resonance-imaging assessing measures of small vessel disease, including cerebral microbleeds and white matter hyperintensities. Median follow-up was 754 (interquartile range [708-828]) days. Using adjusted logistic and Cox regression, we assessed the association of imaging measures with clinical outcome including recurrent ischemic stroke, intracranial hemorrhage and death and assessed disability (modified Rankin Scale).

Results

Overall, recurrent ischemic stroke was more common than intracranial hemorrhage (22 versus 8, respectively). Cerebral microbleeds were related to an increased risk of the composite endpoint (ischemic stroke, intracranial hemorrhage, death: odds ratio (OR) 2.05, 95% confidence interval (CI) 1.27-3.31; $P = 0.003$), as were white matter hyperintensities (OR 2.00, 95%CI 1.23-3.27, $P = 0.005$). This was also true in time-to-event analysis (cerebral microbleeds: HR 2.31, 95%CI 1.39-3.52; $P < 0.001$; white matter hyperintensities: HR 1.99, 95%CI 1.20-3.17; $P = 0.007$). Both measures were associated with an increased risk for recurrent ischemic stroke (cerebral microbleeds: HR 4.42, 95%CI 1.07-18.20; $P = 0.04$; white matter hyperintensities: HR 5.27, 95%CI 1.08-25.79, $P = 0.04$) and intracranial hemorrhage (cerebral microbleeds: HR 2.43, 95%CI 1.04-5.69; $P = 0.04$; white matter hyperintensities: HR 2.57, 95%CI 1.11-5.98, $P = 0.03$). Furthermore, confluent white matter hyperintensities were associated with increased disability (OR 4.03; 95%CI 2.16-7.52; $P < 0.001$) and mortality (HR 1.81, 95%CI 1.04-3.14, $P = 0.04$).

Discussion and conclusion

In atrial fibrillation stroke patients treated with oral anticoagulation, small vessel disease is associated with an unfavourable outcome. The presence of microbleeds indicated a risk higher for recurrent ischemic stroke than for intracranial hemorrhage.

7. Second topic: Ischemic stroke despite oral anticoagulant therapy in patients with atrial fibrillation

This topic comprises the second main project of the PhD thesis. A substantial residual stroke risk persists in patients with AF despite treatment with oral anticoagulants. A rising number of patients suffer ischemic stroke despite anticoagulation as the use of anticoagulants increases. This represents a growing challenge in clinical practice, because the etiology of stroke despite anticoagulation is poorly understood and the optimal subsequent management of these patients is unclear. Therefore, in this project we investigated the etiology, secondary prevention strategies and outcomes of stroke despite anticoagulation in AF patients in a large multicenter analysis.

7.1. Etiology, secondary prevention strategies and outcomes of ischemic stroke despite oral anticoagulant therapy in patients with atrial fibrillation

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Abstract

Aims

To investigate the etiology, subsequent preventive strategies, and outcomes of stroke despite anticoagulation in atrial fibrillation (AF) patients.

Methods

We analyzed consecutive AF patients with an index imaging-proven ischemic stroke despite vitamin K-antagonist (VKA) or direct oral anticoagulant (DOAC) treatment across 11 stroke centers (ISRCTN48292829). We classified stroke etiology as: (i) competing stroke mechanism other than AF-related cardioembolism; (ii) insufficient anticoagulation (nonadherence or low anticoagulant activity measured with drug-specific assays); or, (iii) AF-related cardioembolism despite sufficient anticoagulation. We investigated subsequent preventive strategies with regard to the primary (composite of recurrent ischemic stroke, intracranial hemorrhage, death) and secondary endpoint (recurrent ischemic stroke) within 3 months after index stroke.

Results

Among 2,946 patients (median age 81 years; 48% female; 43% VKA, 57% DOAC), stroke etiology was competing mechanism in 713 patients (24%), insufficient anticoagulation in 934 (32%), and cardioembolism despite sufficient anticoagulation in 1,299 (44%). We found high rates of the primary (27% of patients; completeness 91.6%) and secondary endpoint (4.6%; completeness 88.5%). Only DOAC (versus VKA) treatment after index stroke showed lower odds for both endpoints (primary: aOR [95%-CI] 0.49 [0.32, 0.73]; secondary: 0.44 [0.24, 0.80]), but not switching between different DOAC types. Adding antiplatelets showed higher odds for both endpoints (primary: aOR [95%-CI] 1.99 [1.25, 3.15]; secondary: 2.66 [1.40, 5.04]). Only few patients (1%) received left atrial appendage occlusion as additional preventive strategy.

Interpretation

Stroke despite anticoagulation comprises heterogeneous etiologies and cardioembolism despite sufficient anticoagulation is most common. While DOAC were associated with better outcomes than VKA, adding antiplatelets was linked to worse outcomes in these high-risk patients. Our findings indicate that individualized and novel preventive strategies beyond the currently available anticoagulants are needed.

Introduction

Oral anticoagulation with either direct oral anticoagulants (DOAC) or vitamin K-antagonists (VKA) reduces the risk of ischemic stroke in patients with non-valvular atrial fibrillation (AF). However, there is a substantial residual stroke risk in patients with AF despite anticoagulation ranging from 0.7% to 2.3% annually in primary and secondary prevention, respectively.[77-80] Since the introduction of DOAC, the overall use of oral anticoagulants for stroke prevention in patients with AF has increased steadily, particularly in AF patients at the highest stroke risk.[60] Due to this development, the number of patients with AF suffering a stroke despite anticoagulation is expected to increase, too.[81, 82] Accumulating evidence suggests that patients with AF and stroke despite anticoagulation are at a higher risk for future recurrence than patients who were naive to anticoagulation treatment before stroke.[88-90]

For stroke physicians, ischemic stroke despite anticoagulation in patients with AF represents a challenge in everyday clinical practice, as its etiology is not well-understood.[87] Competing stroke mechanisms such as large artery and small vessel disease, as well as nonadherence or inappropriately dosed anticoagulation have been discussed as potential causes of stroke despite anticoagulation,[87-90] but few data on their relative frequency exist.[82] A better understanding of the etiology of stroke despite anticoagulation is needed to inform strategies to prevent recurrence after a stroke despite anticoagulation.[87] So far, limited data suggested no benefit from switching the anticoagulant type.[88, 90] Indeed, the optimal management of patients with stroke despite anticoagulation remains unclear, and the latest guidelines offer no recommendations on this.[31]

We therefore sought to (i) describe the etiology of stroke despite oral anticoagulation and (ii) investigate subsequent preventive strategies and outcomes in a large collaborative study of patients with AF and stroke despite anticoagulation from 11 experienced stroke centers.

Methods

Study design, patient population, and data collection

We pooled individual data of consecutive stroke patients in a collaborative effort across 11 experienced stroke centers from Switzerland, Germany and the United States with a special research interest in stroke despite anticoagulation. Stroke patients were identified using local prospective registries complemented by hospital admission records, as in prior research.[81, 82] Local investigators collected data that were not available in the prospective databases by retrospectively reviewing patient charts. All data were collected using predefined variables in a standardized manner. De-identified patient data were pooled and analyzed at the University Hospitals Basel and Bern.

We included patients with previously known AF and an imaging-proven acute ischemic stroke (hereafter referred to as index stroke) occurring while on oral anticoagulant therapy (i.e., prescribed anticoagulation for long-term stroke prevention, excluding nonpersistent patients and those with physician-initiated pauses for medical reasons at the time of the stroke). We excluded stroke patients with missing data on anticoagulant treatment, and those with mechanical heart valves. The reporting period was limited to patients presenting no earlier than January 2012 and no later than December 2020.

A detailed description of collected baseline clinical, neuroimaging and laboratory variables, as well as preventive treatments following the index stroke, is presented in the Supplement.

Etiology of stroke despite anticoagulation

The presumed most likely etiology of stroke was determined by local investigators according to the following predefined categories:
(a) competing stroke mechanism other than AF-related cardioembolism (such as small vessel disease, large artery atherosclerosis, or other established pathologies as the most likely stroke mechanism in line with the TOAST classification criteria, i.e., "two or more mechanisms"[34]);
(b) insufficient anticoagulation defined (adapting prior research[82]) as (i) self-reported nonadherence (i.e., history of missing intake of anticoagulants within the last 3 days before index stroke); (ii) low anticoagulant activity on admission (i.e., INR <2.0 in VKA-treated patients; plasma level <30 ng/ml in DOAC-treated patients[158]) or (iii) inappropriately low DOAC dose or dosing frequency (according to current product labeling by the Swiss Agency for Therapeutic Products, European Medicines Agency and the United States Food and Drug Administration, as applicable). Patients with evidence for both (a) and (b) were classified solely as (a), i.e., competing stroke mechanism;
(c) AF-related cardioembolism despite sufficient anticoagulation, defined as stroke without evidence for either (a) or (b).

To investigate the reproducibility of the classification, a random sample from the two largest datasets (Basel and Bern, 25 patients each) was reclassified by a different local investigator, showing high inter-rater agreement (82%, kappa 0.73). Additionally, a random sample of 25 patients from the entire dataset was reclassified based on available baseline variables by blinded raters from 3 of the largest centers (Basel, Bern, and Heidelberg), showing high inter-center agreement (87%, kappa 0.80).

3-month outcomes

Out of 11 centers, 8 routinely collected standardized information on the following 3-month outcomes: (i) recurrent ischemic stroke, (ii) intracranial hemorrhage (ICH), (iii) all-cause death, and (iv) functional outcome on the mRS. The primary endpoint was the composite of recurrent ischemic stroke, ICH and all-cause death within 3 months, defined as in previous research[70, 88]. Secondary endpoint was recurrent ischemic stroke within 3 months. An additional combined endpoint of recurrent ischemic stroke and ICH within 3 months was defined post-hoc.

Statistical Analyses

Main analysis

We presented data on the etiology of stroke despite anticoagulation using descriptive statistics. We stratified patient characteristics according to stroke etiology and type of anticoagulant (DOAC vs. VKA) at the time of the index stroke. Categorical data are presented using frequencies and percentages and continuous data using the median and interquartile range (IQR). We compared categorical variables using the Chi^2-test or Fisher's exact test, as appropriate, and continuous variables using the Mann-Whitney U test.

Secondary analyses

To investigate the prognostic significance of the etiology of stroke despite anticoagulation with regard to the primary and secondary endpoints, we used univariable and multivariable logistic models adjusted for preselected common risk factors (i.e., age, sex, hypertension, diabetes, ischemic heart

disease, dyslipidemia, renal impairment, prior ischemic stroke or ICH, current smoking, and active malignancy).

To explore the association of preventive strategies with the primary and secondary endpoints, we fitted univariable and multivariable logistic models adjusted for preselected common outcome predictors, as described in detail in the Supplement. As a post-hoc analysis we additionally examined the association of all preventive strategies with the combined endpoint of recurrent ischemic stroke and ICH.

For all models, we report the (adjusted) odds ratio ([a]OR) along with 95% confidence intervals (95%-CI) and two-sided p-values. Additionally, the number of missing values are indicated for all data. We fitted all models using only complete cases without data imputation and report the number of patients (and number of events) included in each model.

Statistical analyses were performed using Stata version 17.0 (StataCorp LLC, College Station, Texas 77845 USA). We conducted this study in accordance with the STROBE Statement for observational studies[145]. This study is registered with the International Standard Registered Clinical/Social Study Number Registry (ISRCTN48292829).

Ethics

The use and pooling of de-identified patient data from all Swiss centers participating in the study and the study itself were approved by the responsible ethics committee (Cantonal Ethics Committee Bern [Kantonale Ethikkommission (KEK) Bern] 2019-01010). The requirement for additional local ethical approval differed among non-Swiss participating centers and was acquired by the local principal investigators if necessary. This study complies with the Declaration of Helsinki.

Results

In total, 2,946 patients were eligible for analysis. Figure 7.1.1 shows the study flowchart and Supplementary Table 7.1.1 the center contributions. The median (IQR) age was 81 (76–86) years, 1,404 patients were female (47.7%), and stroke was moderate to severe (NIHSS 6 [2–14]). At the time of the index stroke, 1,674 patients (56.8%) were taking DOAC and 1,272 (43.2%) were taking VKA; their detailed characteristics are shown in Supplementary Table 7.1.2. Plasma level on admission was available for 913 patients on DOAC (54.5%), and INR on admission was available in 1,267 patients on VKA (99.6%).

Etiology of stroke despite anticoagulation

Information on the presumed most likely etiology of stroke was available for all patients and was classified as competing stroke mechanism in 713 (24.2%), insufficient anticoagulation in 934 (31.7%) and cardioembolism despite sufficient anticoagulation in 1,299 (44.1%) patients. The distribution of stroke etiologies in patients with stroke from January 2012 to June 2016 versus July 2016 to December 2020 did not differ substantially (Supplementary Figure 7.1.1). The detailed characteristics of all patients stratified to stroke etiology are presented in Table 7.1.1.

Among patients with competing mechanisms other than AF-related cardioembolism as stroke etiology, information on the exact competing mechanism was available for 685 of 713 patients (96.1%). Of those, 658 patients (96.1%) had one and 27 (3.9%) had more than one competing stroke

mechanism as the most likely stroke etiology. The most common competing mechanism was large artery atherosclerosis, which was present in 415 (60.6%) patients, followed by small vessel disease (present in 180 [26.3%] patients). Less common etiologies included coagulopathies (i.e., cancer-related coagulopathy, antiphospholipid syndrome and others; 5.3%), peri-interventional stroke (3.4%), endocarditis (3.2%) and other cardio-aortic pathologies (3.8%). There were no substantial differences in the distribution of competing mechanisms among patients with DOAC versus VKA therapy at the time of the index stroke. Details are given in Table 7.1.2.

Preventive treatments

Information on antithrombotic treatment after the index stroke was available for 2,875 of 2,946 patients (completeness 97.6%). At hospital discharge, 2,437 patients (84.8%) were treated with anticoagulants, 120 (4.2%) received antiplatelets alone, 286 (9.9%) received no antithrombotic treatment, and 32 (1.1%) received parenteral anticoagulation.

Of patients who received anticoagulants, 13.4% were prescribed VKA and 86.8% DOAC, whereby 80.5% received a twice-daily DOAC and 66.6% received DOAC at full dose. Antiplatelets as add-on therapy to anticoagulation were prescribed in 367 patients (12.8% of all patients; 15.1% of all patients with anticoagulation), which was more common among those with competing mechanism as stroke etiology. Most patients were prescribed statins and antihypertensives after the index stroke, and both drug types were more commonly prescribed after stroke due to competing mechanisms than due to other etiologies. The detailed preventive treatments stratified to stroke etiology are given in Supplementary Table 7.1.3. An overview of the changes in oral anticoagulant therapy before versus after stroke is presented in Figure 7.1.2.

Information on nonpharmacologic preventive treatments was available for revascularization treatments in 2,774 of 2,946 patients (94.2%) and for left atrial appendage occlusion in 1,762 of 2,496 patients (59.8%). Revascularization treatments including carotid endarterectomy or stenting were administered to 94 patients (3.4%) and left atrial appendage occlusion was performed in 17 patients (1.0%), both more commonly among patients with competing mechanisms as stroke etiology (Supplementary Table 7.1.3).

3-month outcomes and their association with stroke etiology

Information on 3-month outcomes was available in all but three centers for 2,082 patients in total. Here, information on the primary and secondary endpoint was complete in 1,906 (91.6%) and 1,842 (88.5%) patients, respectively. The primary endpoint (i.e., the composite of recurrent ischemic stroke, intracranial hemorrhage, or all-cause death) occurred in 516 patients (27.1%) and the secondary endpoint (i.e., recurrent ischemic stroke) in 84 patients (4.6%) within 3 months. Detailed information on 3-month outcomes is given in Table 7.1.3.

Compared to patients with cardioembolism despite sufficient anticoagulation, those with competing mechanisms had higher odds for recurrent ischemic stroke in unadjusted and adjusted analyses, but not for the composite outcome. The outcomes of patients with stroke due to insufficient anticoagulation did not differ from those with cardioembolism despite sufficient anticoagulation with regard to the primary and secondary endpoints (Table 7.1.3).

Association of preventive strategies with the primary and secondary endpoint

Figure 7.1.3 shows the adjusted estimates for the association of preventive strategies after the index stroke with the primary and secondary endpoints; the detailed unadjusted and adjusted models are given in Table 7.1.4. Among patients who received anticoagulant treatment at hospital discharge and for whom outcome data were available, 1,279 (85.4%) patients received DOAC and 219 (14.6%) patients received VKA. Treatment with DOAC versus VKA was associated with lower odds for the primary and secondary endpoints, both in unadjusted and adjusted analyses (Table 7.1.4). This remained true independent of the type of anticoagulant (DOAC vs. VKA) at the time of the index stroke and whether the drug was switched or not after the index stroke in additional models accounting for the anticoagulant type at the time of the index stroke and its interaction with the type of anticoagulant after stroke (composite outcome: $aOR_{DOACvs.VKA}$ [95%-CI] 0.50 [0.31, 0.81], $p_{interaction\ (before*after)}$ = 0.855; recurrent ischemic stroke: $aOR_{DOACvs.VKA}$ [95%-CI] 0.47 [0.21, 1.03], $p_{interaction\ (before*after)}$ = 0.429).

Any anticoagulant switch and switch from VKA to DOAC were associated with lower odds for the composite outcome, but not for recurrent stroke. Among patients with cardioembolism despite sufficient anticoagulation, the use of twice-daily DOAC was associated with lower odds for the composite outcome, but not for recurrent stroke. No other strategy was associated with lower odds for any of the endpoints, while the addition of antiplatelets to anticoagulants was even associated with higher odds for the primary and secondary endpoints.

A post-hoc analysis focusing on the combined endpoint of recurrent ischemic stroke and ICH revealed largely consistent results. Among patients treated with VKA at the time of the index stroke, switching to any DOAC was associated with lower odds for this combined endpoint (Supplementary Table 7.1.4).

Discussion

This study revealed the following key findings: (1) The etiology of stroke despite anticoagulation in patients with AF is heterogeneous, with about 1/4 of cases attributable to competing stroke mechanisms and about 1/3 to insufficient anticoagulation, while AF-related cardioembolism despite sufficient anticoagulation was the most common etiology. (2) Following stroke despite anticoagulation, unfavorable outcomes are common and recurrence rate is high. (3) Anticoagulation with DOAC was linked to better outcomes than VKA after stroke despite anticoagulation, while additional antiplatelet therapy was not associated with better outcomes.

Among patients with a competing mechanism other than AF-related cardioembolism as the most likely stroke etiology, large artery atherosclerosis and small vessel disease were the most frequent mechanisms. Previous case-control and cohort studies on anticoagulated patients with AF demonstrated that vascular risk factors such as diabetes[159] and dyslipidemia,[159, 160] but also large artery atherosclerosis[159, 161] and small vessel disease[155] *per se* were associated with higher stroke risk. In line with this, a previous single-center study on patients with AF and stroke despite anticoagulation using the ASCOD classification indicated that the coexistence of competing stroke mechanisms is common.[82] These findings stress the importance of a thorough work-up in patients with AF and stroke despite anticoagulation in order to uncover non-cardioembolic pathologies that might be less responsive to anticoagulation and warrant additional preventive therapies. Of note, our data indicated that underlying coagulopathies may – less commonly – also account for stroke despite anticoagulation in

AF patients. In these cases, abnormal blood count findings, elevated lactate dehydrogenase, C-reactive protein and particularly D-dimer levels should raise suspicion and prompt further testing including hematologic work-up and cancer-screening with imaging of the chest and abdomen to uncover potential relevant comorbidities such as myeloproliferative or other neoplasms.[162-164]

Our study further highlights the problem of insufficient anticoagulation as an important etiology of stroke despite anticoagulation in patients with AF. Prior reports either lacked this information[88-90] or were of small sample size.[160] Our definition of insufficient anticoagulation comprised not only self-reported nonadherence and inappropriately low DOAC dosing, combining findings from previous studies,[96, 160, 165] but also included the anticoagulant activity measured on admission. For this, DOAC level was available in over 50% of DOAC-treated patients and INR in almost all VKA-treated patients in our dataset. While low time in therapeutic range among VKA-treated patients has been previously reported as a contributor to stroke risk in AF patients,[50] only few data about DOAC levels existed in this context so far[82, 85]. Our finding that a relevant proportion of stroke despite anticoagulation is attributable to insufficient anticoagulation is important because these strokes may potentially be preventable. Such prevention strategies would entail interventions to increase physicians' awareness about the importance of per-label dosing, and also ways of identifying patients at high risk for nonadherence,[95] a more nuanced evaluation of drug intake behavior[166] and adherence-enhancing interventions.[167]

Another main finding of our study is that the largest proportion of stroke despite anticoagulation was attributable solely to AF-related cardioembolism without evidence for insufficient anticoagulation or competing mechanisms. The profile of these patients resembled more the profile of patients with insufficient anticoagulation in terms of traditional cardiovascular risk factors and neuroimaging characteristics than the profile of patients with competing stroke mechanisms. This suggests shared stroke mechanisms in these patients, in whom inadequate anticoagulant activity might be ultimately implicated. Besides nonadherence and inappropriate dosing leading to insufficient anticoagulation, emerging evidence suggests that a high inter-individual variation in DOAC pharmacokinetics and pharmacodynamics exists, which may be attributable to genetic factors.[168] More research is needed to evaluate whether tailored pharmacogenomics approaches might mitigate the risk of AF-related cardioembolism despite anticoagulation.

Furthermore, our data show that the burden of unfavorable outcomes within 3 months after stroke despite anticoagulation is high, expanding on findings from previous smaller studies that focused mostly on ischemic stroke recurrence.[88-90] In our study, over 1/5 of patients died and over 1/2 of patients had a mRS ≥3 at 3 months. While ICH occurred infrequently at <1%, 4.6% of patients suffered ischemic stroke recurrence, clearly identifying this patient group as high risk and stressing the need to define optimal treatment strategies.

Overcoming limitations of previous studies, the large sample size of this pooled analysis enabled us to comprehensively investigate a series of preventive strategies. Regardless of the type of anticoagulant at the time of the index stroke, treatment with DOAC after the index stroke was associated with lower odds for both the primary and secondary endpoints as opposed to treatment with VKA, even after adjustment for several outcome-modifying variables. Although residual confounding by indication – potentially left unaccounted for despite adjustment – may have influenced this finding by introducing bias against VKA, our data are reassuring for the use of DOAC and support the current guidelines for

recurrent stroke prevention which recommend DOAC in preference over VKA,[31] providing new evidence for patients with stroke despite anticoagulation.

In contrast to widespread practice, our data do not suggest that any specific switch between DOAC (including switching to different DOAC, or to DOAC with different dosing frequency or mechanism of action) may lead to better outcomes in patients with stroke while on DOAC therapy. Importantly, we found that adding antiplatelets to anticoagulants was not linked to better outcomes, but was instead associated with higher odds for both the primary and secondary endpoints. This finding expands on previous research showing no better or even worse cardiovascular outcomes in anticoagulated AF patients with add-on antiplatelets,[169, 170] indicating that this seems to apply also to AF patients with stroke despite anticoagulation. It is possible that residual confounding by indication that remained unaccounted for despite extensive adjustment for comorbidities may have influenced this finding, as discussed previously.[170]

The rates of the primary and secondary endpoints in our study were high, although most patients were treated with DOAC after stroke. This indicates that novel approaches to prevent stroke recurrence are needed in these patients. Besides strategies to optimize the currently available drug treatments discussed above, novel pharmacologic approaches, such as factor XIa inhibitors,[111] or nonpharmacologic interventional treatments, including the percutaneous occlusion of the left atrial appendage,[171] might advance stroke prevention in AF. Notably, surgical occlusion of the left atrial appendage was shown to confer additional protection against stroke when added to anticoagulation in a recent trial.[172]

Strengths and limitations

The strengths of this study include (i) its large sample size; (ii) the detailed patient characterization with high data completeness, allowing for a large number of analyses with extensive adjustment for confounders and limiting the risk of spurious findings; (iii) the standardized classification of the stroke etiology incorporating DOAC plasma levels, which were available in the majority of participating centers; and (iv) the homogeneity of the study population, which included only patients with imaging-confirmed stroke and previously known AF as the sole indication for anticoagulation.

We are aware of the following limitations: (i) Data were in part collected retrospectively rather than prospectively ascertained; (ii) Although experienced investigators determined the most likely stroke etiology, inherent limitations in the determination of competing stroke mechanisms may have led to misclassification of the stroke etiology, and heterogeneity among the participating centers may have introduced bias in the classification; (iii) Local investigators classified the stroke etiology as insufficient anticoagulation using a standardized definition, but availability of coagulation measurements (DOAC plasma levels vs. INR for VKA) differed. This is a potential source of bias, as it decreases the likelihood of patients on VKA (and increases the likelihood of patients on DOAC) to be classified as “AF-related cardioembolism despite sufficient anticoagulation” and may have caused more patients on VKA (and less patients on DOAC) to be classified as “insufficient anticoagulation”; (iv) The observational design of the study allowed only the assessment of association between treatment strategies and outcomes, but not causality thereof. Importantly, despite extensive adjustment for comorbidities, indication bias may still have confounded our findings, potentially contributing to the worse outcomes of patients treated with VKA or add-on antiplatelets after stroke despite anticoagulation. These results should therefore be interpreted cautiously, better serving as hypothesis-generating for potential future randomized trials that are necessary to provide robust evidence; (v) Despite the large sample size, the

short follow-up time of 3 months may have limited the number of outcomes, thus disallowing the detection of their association with treatment strategies; (vi) With only 17 patients undergoing left atrial appendage occlusion in our observational dataset, no meaningful statistical analyses for this preventive strategy were possible; (vii) The limited number of ICH events disallowed statistical analysis of ICH as a separate outcome. Finally, we did not consider extracranial bleeding in our analyses, as this outcome was not collected during follow-up.

In conclusion, this study on ischemic stroke despite anticoagulant therapy in patients with AF showed that the etiology of stroke is heterogeneous and unfavorable outcomes are common. While DOAC treatment after stroke despite anticoagulation was associated with better outcomes than VKA, add-on antiplatelets were linked to worse outcomes; further research into more personalized and novel preventive strategies is warranted.

Tables

Table 7.1.1. Patient characteristics stratified to stroke etiology

Characteristic	All (N=2,946)	N missing	Etiology of stroke despite anticoagulation			
			Competing mechanism (N=713)	Insufficient anticoagulation (N=934)	Cardioembolism despite sufficient anticoagulation (N=1,299)	p value
Demographics						
age, median (IQR), years	81 (76-86)	0	80 (74-85.1)	82.45 (77-86.9)	81 (75-86)	<0.001
female sex, N (%)	1,404 (47.7%)	0	254 (35.6%)	533 (57.1%)	617 (47.5%)	<0.001
Risk factors						
hypertension, N (%)	2,649 (89.9%)	0	632 (88.6%)	844 (90.4%)	1,173 (90.3%)	0.430
diabetes, N (%)	871 (29.6%)	0	239 (33.5%)	264 (28.3%)	368 (28.3%)	0.029
dyslipidemia, N (%)	1,768 (60.3%)	13	458 (64.6%)	569 (61.1%)	741 (57.3%)	0.005
renal impairment, N (%)	959 (33.2%)	58	229 (32.8%)	318 (34.7%)	412 (32.4%)	0.510
prior ischemic stroke, N (%)	984 (33.4%)	0	262 (36.7%)	290 (31.0%)	432 (33.3%)	0.052
history of ICH, N (%)	60 (2.0%)	0	13 (1.8%)	14 (1.5%)	33 (2.5%)	0.210
ischemic heart disease, N (%)	905 (30.7%)	0	232 (32.5%)	275 (29.4%)	398 (30.6%)	0.400
bioprosthetic heart valve, N (%)	151 (5.1%)	0	54 (7.6%)	34 (3.6%)	63 (4.8%)	0.001
current smoking, N (%)	249 (8.8%)	103	79 (11.8%)	69 (7.6%)	101 (8.0%)	0.006
active malignancy, N (%)	236 (8.1%)	15	79 (11.1%)	63 (6.8%)	94 (7.3%)	0.002
prestroke mRS ≥3, N (%)	567 (22.1%)	381*	118 (18.5%)	198 (24.6%)	251 (22.3%)	0.021
ipsilateral stenosis ≥50%, N (%)	452 (15.6%)	54	307 (43.4%)	65 (7.2%)	80 (6.3%)	<0.001
ipsilateral stenosis <50%, N (%)	496 (17.1%)	50	100 (14.1%)	200 (21.9%)	196 (15.3%)	<0.001
Medication at the time of stroke onset						
Oral anticoagulant		0				
VKA, N (%)	1,272 (43.2%)		249 (34.9%)	548 (58.7%)	475 (36.6%)	<0.001
DOAC, N (%)	1,674 (56.8%)		464 (65.1%)	386 (41.3%)	824 (63.4%)	
DOAC dose		32				
full, N (%)	925 (56.3%)		292 (63.8%)	121 (32.0%)	512 (63.5%)	<0.001
reduced, N (%)	717 (43.7%)		166 (36.2%)	257 (68.0%)	294 (36.5%)	
DOAC dosing frequency		215†				
once daily, N (%)	848 (58.1%)		247 (57.7%)	221 (66.4%)	380 (54.4%)	<0.001
twice daily, N (%)	611 (41.9%)		181 (42.3%)	112 (33.6%)	318 (45.6%)	
DOAC mechanism of action		0				
thrombin inhibitor, N (%)	152 (9.1%)		39 (8.4%)	29 (7.5%)	84 (10.2%)	0.270
factor Xa inhibitor, N (%)	1,522 (90.9%)		425 (91.6%)	357 (92.5%)	740 (89.8%)	
additional antiplatelet, N (%)	363 (12.3%)	4	119 (16.7%)	112 (12.0%)	132 (10.2%)	<0.001
statin, N (%)	1,354 (46.4%)	30	371 (52.3%)	387 (41.7%)	596 (46.6%)	<0.001
antihypertensive(s), N (%)	2,683 (91.9%)	27	652 (91.8%)	842 (90.7%)	1,189 (92.8%)	0.210
Stroke details						
NIHSS on admission, median (IQR)	6 (2-14)	33	4 (2-10)	8 (3-16)	6 (2-14)	<0.001
intravenous thrombolysis, N (%)	351 (11.9%)	2	46 (6.5%)	211 (22.6%)	94 (7.2%)	<0.001
endovascular treatment, N (%)	787 (26.8%)	6	110 (15.4%)	293 (31.4%)	384 (29.7%)	<0.001
embolic infarct pattern, N (%)	2,317 (81.7%)	111	468 (67.3%)	805 (89.6%)	1,044 (84.1%)	<0.001

large vessel occlusion, N (%)	1,345 (46.2%)	32	241 (34.2%)	513 (55.6%)	591 (46.0%)	<0.001
Laboratory parameters on admission						
INR, median (IQR)	1.4 (1.1-1.9)	100	1.4 (1.1-2.0)	1.3 (1.1-1.6)	1.4 (1.2-2.2)	<0.001
low anticoagulant activity, N (%)‡	957 (43.9%)	766§	128 (26.9%)	633 (82.1%)	196 (21.0%)	<0.001
low VKA activity, N (%)	737 (58.2%)		96 (39.3%)	528 (96.4%)	113 (23.8%)	<0.001
low DOAC activity, N(%)	220 (24.1%)		32 (13.8%)	105 (47.1%)	83 (18.1%)	<0.001
DOAC plasma level, ng/ml, median (IQR)	83.9 (30-164)	761§	110.1 (54.9-193.6)	34.6 (1.0-93.5)	100.9 (44.3-192.6)	<0.001
Outcome at discharge						
mRS ≥3, N (%)	1,543 (63.3%)	508¶	393 (62.8%)	516 (67.9%)	634 (60.3%)	0.004
in-hospital death, N(%)	204 (8.4%)		35 (5.6%)	78 (10.3%)	91 (8.7%)	0.007

* not collected in the center Berlin (reporting period 2013 – 2015)

† not collected in the center Erlangen

‡ defined in VKA-treated patients as INR <2.0 and in DOAC-treated patients as plasma level <30ng/ml

§ DOAC plasma level on admission not collected in the centers Berlin, Mainz and George Washington University

¶ not collected in the center Mainz

ICH, intracranial hemorrhage; VKA, vitamin K antagonist; DOAC, direct oral anticoagulant; NIHSS, National Institutes of Health Stroke Scale; INR, international normalized ratio; mRS, modified Rankin Scale

Table 7.1.2. Details of competing mechanisms

Competing mechanism	**All (N=685)***	**DOAC (N=441)**	**VKA (N=244)**
large artery atherosclerosis, N (%)	415 (60.6%)	255 (57.8%)	160 (65.6%)
small vessel disease, N (%)	180 (26.3%)	120 (27.2%)	60 (24.6%)
coagulopathy†, N (%)	36 (5.3%)	28 (6.3%)	8 (3.3%)
peri-interventional stroke‡, N (%)	23 (3.4%)	18 (4.1%)	5 (2.0%)
endocarditis, N (%)	22 (3.2%)	14 (3.2%)	8 (3.3%)
other cardio-aortic causes§, N (%)	26 (3.8%)	13 (2.9%)	13 (5.3%)
cervical artery dissection, N (%)	9 (1.3%)	6 (1.4%)	3 (1.2%)
vasculitis, N (%)	4 (0.6%)	2 (0.5%)	2 (0.8%)

*details were available for 685/713 patients (96.1%) who had competing mechanism as stroke etiology

†including suspected cancer-related coagulopathy, hereditary thrombophilia, myeloproliferative disorders, and antiphospholipid syndrome

‡including percutaneous transluminal coronary angioplasty, transcatheter aortic valve implantation, pulmonary vein isolation, cardioversion, and other cardiovascular procedures

§including intracardiac thrombus, aortic dissection, patent foramen ovale / atrial septal defect, heart valve fibroelastoma, and other structural heart abnormalities

DOAC, direct oral anticoagulant; VKA, vitamin K antagonist

Table 7.1.3. 3-month outcomes according to stroke etiology

A. All 3-month outcomes stratified to stroke etiology

3-month outcome	All (N=2,082)*	N missing	Stroke etiology		
			Competing mechanism (N=533)	Insufficient anticoagulation (N=729)	Cardioembolism despite sufficient anticoagulation (N=820)
composite outcome, N (%)	516 (27.1%)	176	125 (25.4%)	186 (27.8%)	205 (27.5%)
recurrent ischemic stroke, N (%)	84 (4.6%)	240	33 (6.8%)	23 (3.6%)	28 (3.9%)
intracranial hemorrhage, N (%)	15 (0.8%)	238	3 (0.6%)	6 (0.9%)	6 (0.8%)
all-cause death, N (%)	434 (22.8%)	177	93 (18.9%)	164 (24.5%)	177 (23.8%)
mRS ≥3, N (%)	1,081 (56.7%)	177	258 (52.5%)	421 (62.9%)	402 (54.0%)

B. Association of stroke etiology with the primary and secondary endpoint

Stroke etiology	Composite outcome						Recurrent ischemic stroke					
	unadjusted			adjusted†			unadjusted			adjusted†		
	OR [95%-CI]	p value	N in model	aOR [95%-CI]	p value	N in model	OR [95%-CI]	p value	N in model	aOR [95%-CI]	p value	N in model
competing stroke mechanism	0.90 [0.69, 1.16]	0.400	1,906	1.18 [0.83, 1.66]	0.363	1,773	1.80 [1.07, 3.02]	0.026	1,842	1.83 [1.05, 3.20]	0.034	1,697
insufficient anticoagulation	1.02 [0.81, 1.28]	0.891		0.93 [0.68, 1.27]	0.648		0.91 [0.52, 1.60]	0.751		0.99 [0.55, 1.79]	0.968	
cardioembolism despite sufficient anticoagulation	(reference)			(reference)			(reference)			(reference)		

* 3-month outcomes not collected in the centers Berlin, Heidelberg, and Mainz

† adjusted for age, sex, hypertension, diabetes, ischemic heart disease, dyslipidemia, renal impairment, prior ischemic stroke, ICH, current smoking, and active malignancy

mRS, modified Rankin Scale

Table 7.1.4. Association of preventive strategies after index stroke with the primary and secondary endpoint

Patients	Preventive strategy	Composite outcome						Recurrent ischemic stroke					
		Unadjusted			adjusted*			unadjusted			adjusted*		
		OR [95%-CI]	p value	N events / total N in model	aOR [95%-CI]	p value	N events / total N in model	OR [95%-CI]	p value	N events / total N in model	aOR [95%-CI]	p value	N events / total N in model
all patients	use of DOAC (vs. VKA) after stroke	0.49 [0.34, 0.71]	<0.001	194/1,498	0.49 [0.32, 0.73]	<0.001	179/1,394	0.51 [0.29, 0.90]	0.020	69/1,489	0.44 [0.24, 0.80]	0.007	62/1,368
	any anticoagulant switch	0.71 [0.52, 0.96]	0.024	194/1,498	0.69 [0.49, 0.96]	0.026	179/1,394	1.03 [0.62, 1.69]	0.916	69/1,489	1.03 [0.60, 1.77]	0.909	62/1,368
	addition of antiplatelets	1.34 [0.89, 2.03]	0.164	251/1,564	1.99 [1.25, 3.15]	0.004	225/1,448	2.38 [1.31, 4.32]	0.004	69/1,505	2.66 [1.40, 5.04]	0.003	62/1,382
patients with DOAC at the time of the stroke	switch to another DOAC	0.83 [0.54, 1.27]	0.380	94/829	0.81 [0.51, 1.29]	0.372	86/761	1.76 [0.89, 3.47]	0.105	39/826	1.87 [0.88, 3.99]	0.105	33/757
	switch to DOAC with different dosing frequency	0.65 [0.41, 1.03]	0.069	89/798	0.60 [0.36, 1.00]	0.051	81/730	1.31 [0.67, 2.58]	0.436	35/794	1.38 [0.64, 2.98]	0.410	29/725
	switch to DOAC with different mechanism of action	0.91 [0.55, 1.52]	0.722	89/799	1.00 [0.57, 1.76]	0.994	81/731	2.17 [1.09, 4.33]	0.027	35/795	2.12 [0.96, 4.69]	0.063	29/726
patients with VKA at the time of the stroke	switch to any DOAC	0.51 [0.33, 0.79]	0.002	100/669	0.55 [0.33, 0.91]	0.019	93/621	0.50 [0.24, 1.06]	0.070	30/663	0.56 [0.25, 1.29]	0.174	29/611
patients with competing stroke mechanism	addition of antiplatelets	1.02 [0.56, 1.87]	0.936	70/414	1.88 [0.93, 3.83]	0.080	63/361	1.83 [0.84, 3.99]	0.128	30/409	2.19 [0.92, 5.21]	0.075	27/359
patients with insufficient anticoagulation	switch to DOAC or correct DOAC dose	0.84 [0.50, 1.41]	0.501	69/498	1.05 [0.58, 1.90]	0.874	62/467	0.87 [0.33, 2.32]	0.778	17/480	1.05 [0.35, 3.12]	0.930	16/402
patients with cardioembolism despite sufficient anticoagulation	twice-daily DOAC (vs. any other anticoagulant)	0.29 [0.18, 0.45]	<0.001	96/617	0.55 [0.31, 0.97]	0.039	86/576	2.20 [0.64, 7.56]	0.212	21/592	2.02 [0.53, 7.69]	0.305	18/555

*adjusted for age, sex, hypertension, diabetes, ischemic heart disease, dyslipidemia, renal impairment, prior ischemic stroke, ICH, current smoking, active malignancy, use of statins, use of antihypertensives

VKA, Vitamin K antagonist; DOAC, direct oral anticoagulant

Figures

Figure 7.1.1. Study Flowchart

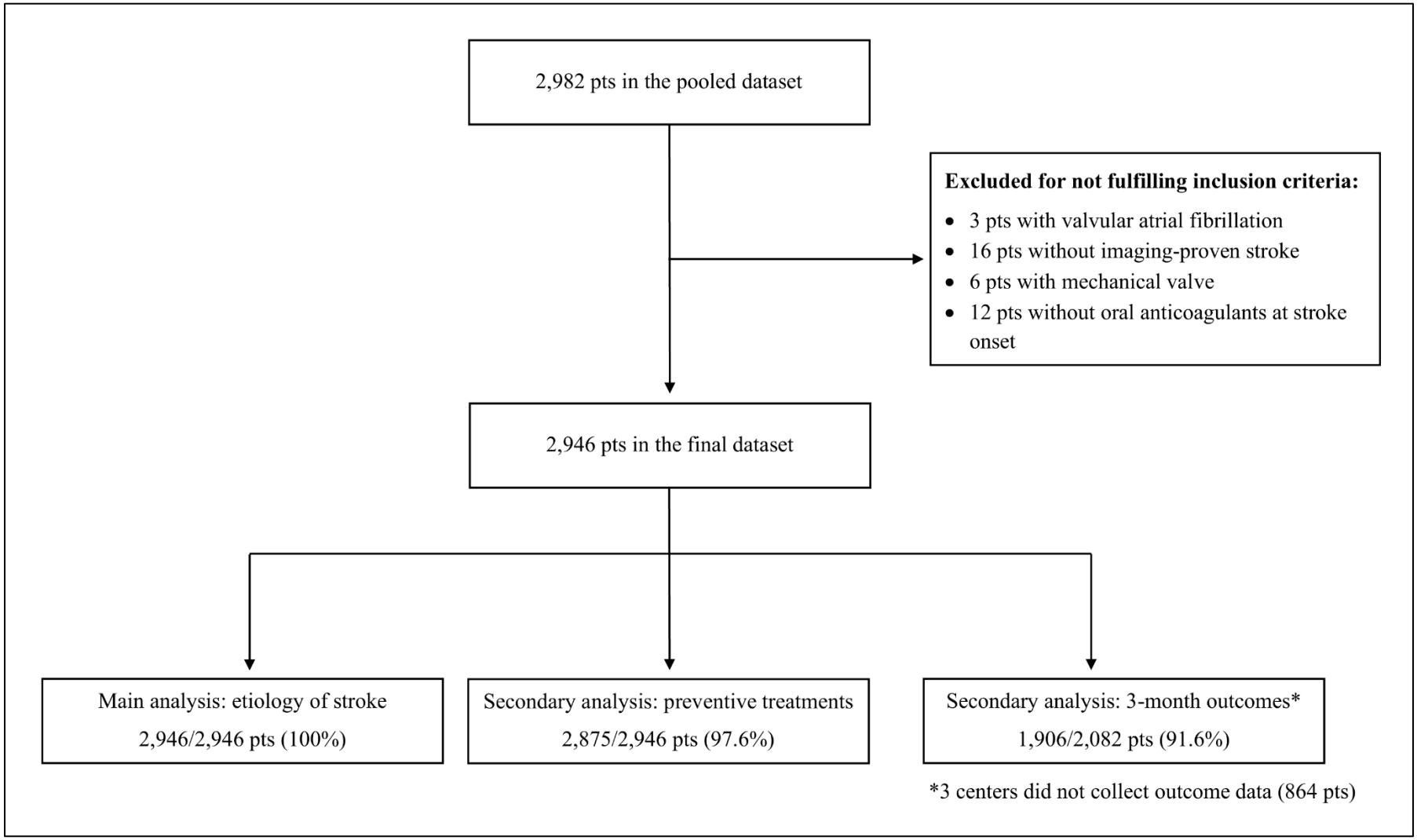


Figure 7.1.2. Changes in oral anticoagulant therapy at the time of the index stroke (before) versus at hospital discharge (after stroke). Patients not receiving oral anticoagulants after stroke and patients with missing type and dosing frequency of anticoagulants before or after stroke are not depicted. (VKA, vitamin K antagonist; DOAC, direct oral anticoagulant; QD, once daily; BID, twice daily; OAC, oral anticoagulant)

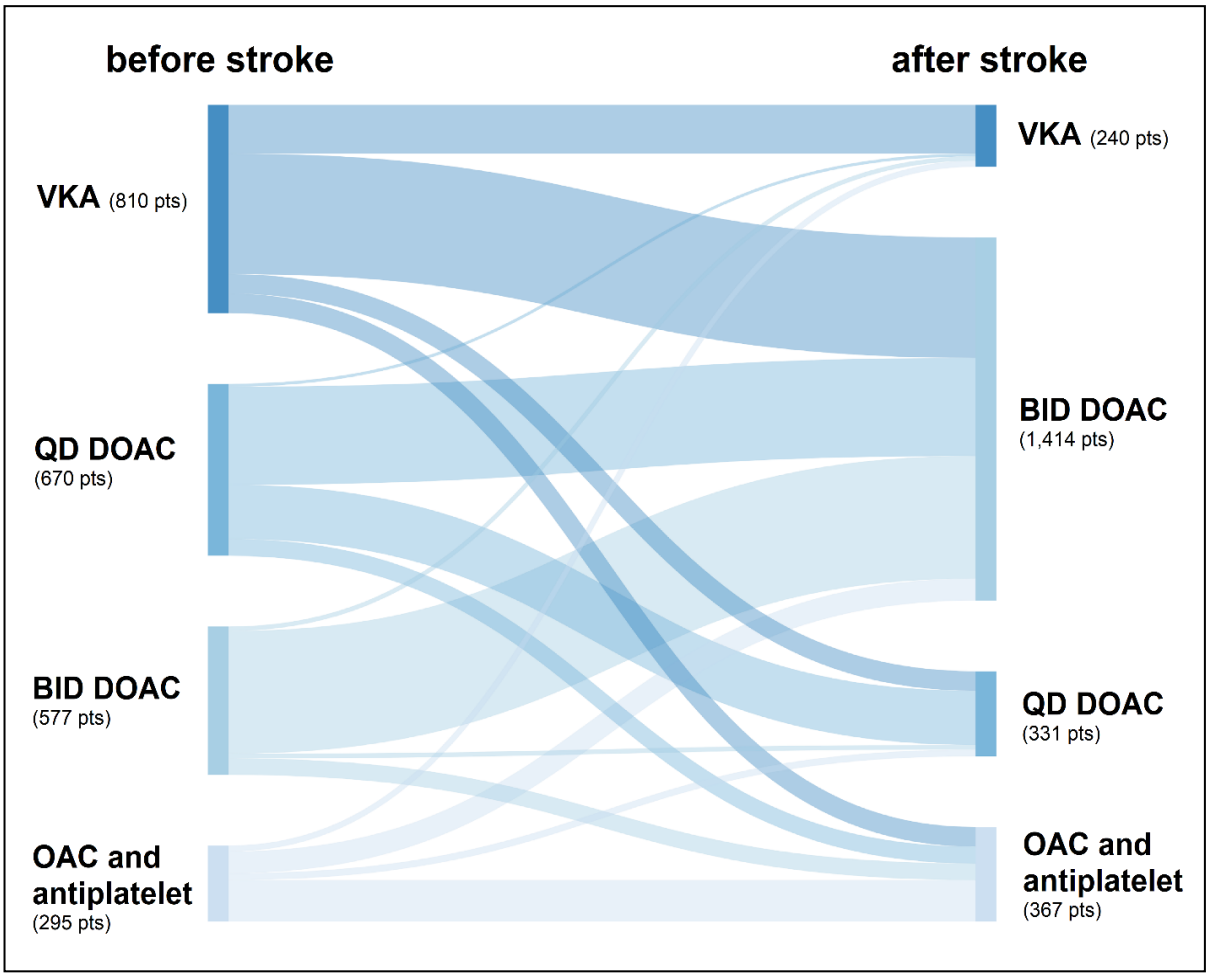
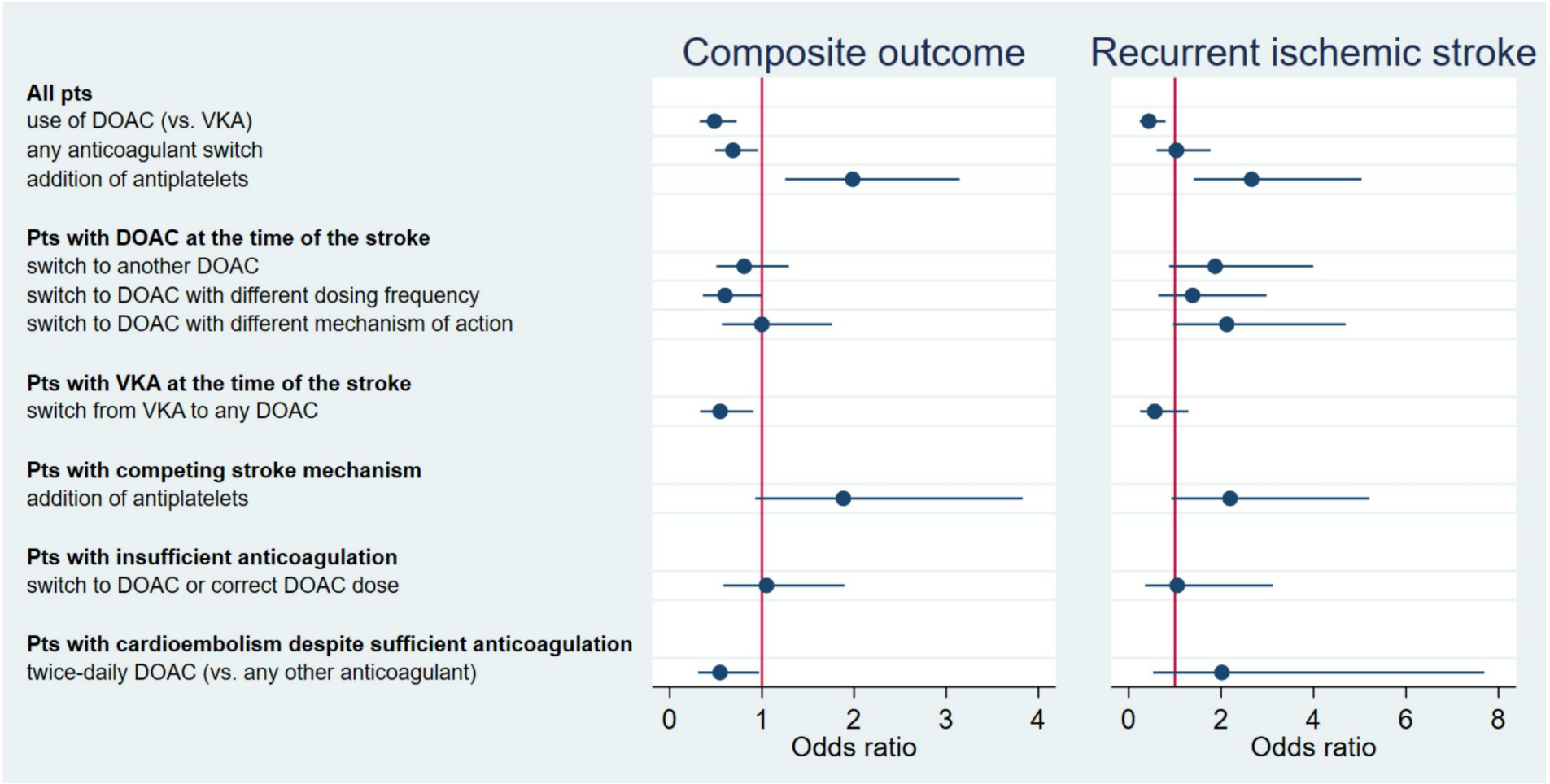


Figure 7.1.3. Association of preventive strategies after stroke despite anticoagulation with the primary and secondary endpoints from the adjusted models (pts, patients; VKA, vitamin K antagonist; DOAC, direct oral anticoagulant; estimates adjusted for age, sex, hypertension, diabetes, ischemic heart disease, dyslipidemia, renal impairment, prior ischemic stroke, history of intracranial hemorrhage, current smoking, active malignancy, use of statins and use of antihypertensives)



Supplementary Material

Supplementary methods

Study design, patient population, and data collection

Baseline clinical, neuroimaging and laboratory variables

We collected the following baseline variables: age; sex; risk factors defined in accordance with previous research,[70, 88] including history of prior ischemic stroke, intracranial hemorrhage, ischemic heart disease, hypertension, diabetes mellitus, dyslipidemia, current smoking, renal impairment (defined as glomerular filtration rate <50ml/min using the creatinine-based Chronic-Kidney-Disease-Epidemiology-Collaboration equation), as well as history of bioprosthetic heart valve replacement, stenosis ≥50% or <50% in arteries supplying the territory in which the index stroke occurred (hereafter referred to as ipsilateral stenosis), active malignancy, and prestroke modified Rankin Scale (mRS); medication details at the time of the index stroke, including the type of anticoagulant, i.e., DOAC (apixaban, dabigatran, edoxaban, or rivaroxaban with their respective dosing and frequency of daily intake) or VKA (acenocoumarol, fluindione, phenprocoumon, or warfarin), as well as use of antiplatelets, statins, or antihypertensives at stroke onset; stroke characteristics, including the National Institutes of Health Stroke Scale (NIHSS) on admission, presence of large vessel occlusion (LVO), acute treatment with intravenous thrombolysis or mechanical thrombectomy, infarct pattern classified as embolic (i.e., presence of LVO, cortical infarct, or other patterns[38]) vs. nonembolic (i.e., lacunar[39]) as in prior research,[82] as well as discharge outcome on the mRS; and laboratory data, including the international normalized ratio (INR) and DOAC-specific plasma level at the time of the stroke.

Preventive treatments

We collected the following information on preventive treatments after the index stroke: use of anticoagulants (including type and dosing), antiplatelets (either alone or as add-on therapy to anticoagulation), statins, and antihypertensives at hospital discharge. Furthermore, we collected information on revascularization treatments for symptomatic ipsilateral stenosis (such as carotid endarterectomy or stenting) and nonpharmacologic prevention strategies for AF (left atrial appendage occlusion) after the index stroke.

Statistical Analyses

Secondary analyses: Association of preventive strategies with the endpoints

To explore the association of preventive strategies with the primary and secondary endpoints, we fitted univariable and multivariable logistic models adjusted for preselected common outcome predictors (i.e., age, sex, hypertension, diabetes, ischemic heart disease, dyslipidemia, renal impairment, prior ischemic stroke or ICH, current smoking, active malignancy, and use of statins or antihypertensives after stroke) with the following preventive strategies after index stroke as an independent variable:

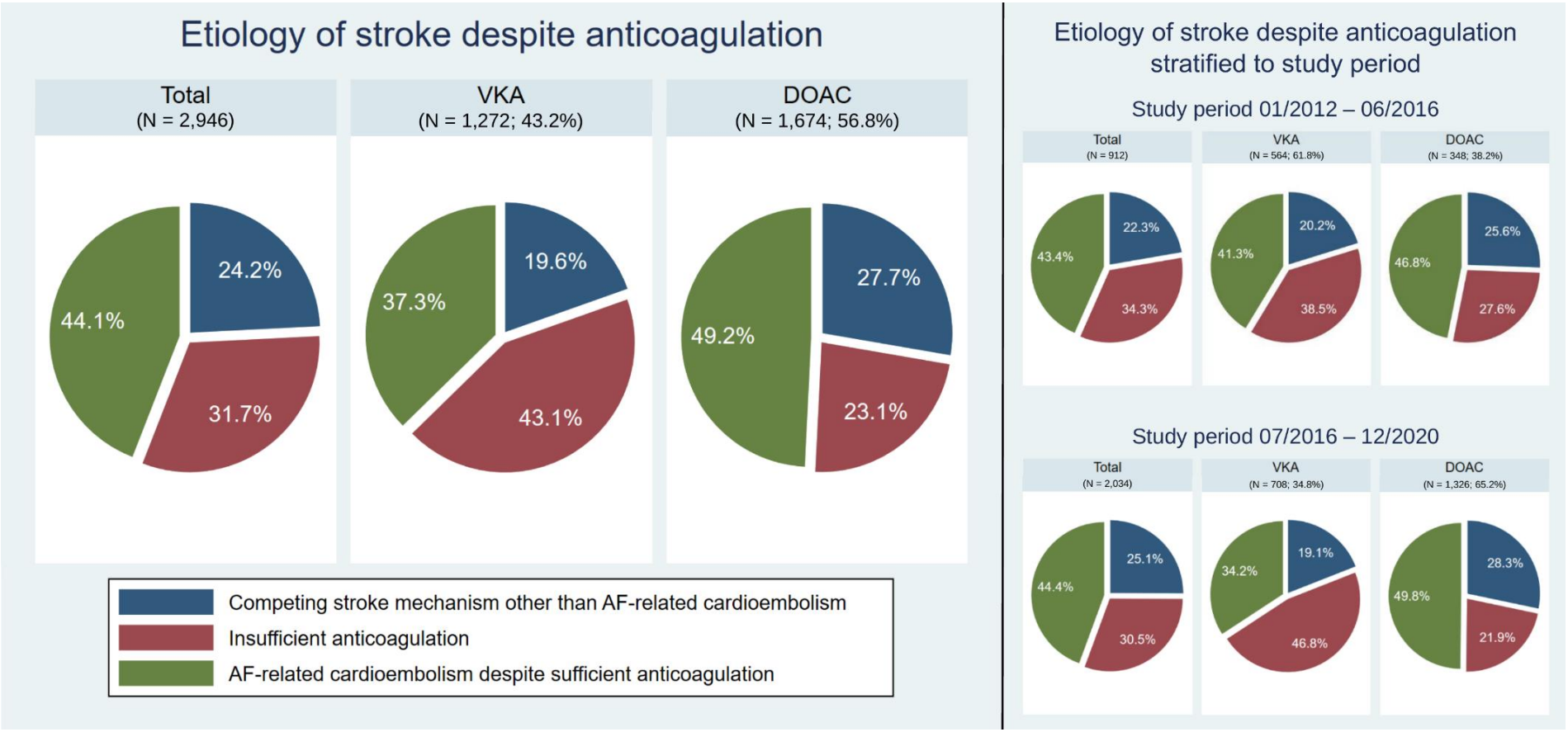
(i) for all patients:

- use of DOAC (versus VKA). For this, we fitted all models twice, with and without the type of anticoagulant (DOAC vs. VKA) at the time of the index stroke and an interaction term anticoagulant type before*after.
- any anticoagulant switch (including VKA to DOAC, DOAC to VKA, and DOAC to another DOAC)

- new addition of antiplatelets to anticoagulants after index stroke
(ii) for patients treated with DOAC at the time of the stroke:
- switch to another DOAC
- switch to DOAC with different dosing frequency (once to twice daily or vice versa)
- switch to DOAC with different mechanism of action (thrombin to factor Xa inhibitor or vice versa)
(iii) for patients treated with VKA at the time of the stroke:
- switch from VKA to any DOAC
(iv) for patients with competing mechanism as stroke etiology:
- new addition of antiplatelets to anticoagulants after stroke
(v) for patients with insufficient anticoagulation as stroke etiology:
- DOAC dose correction (i.e., switch from reduced to full dose) or switch from VKA to DOAC after stroke
(vi) for patients with cardioembolism despite sufficient anticoagulation as stroke etiology:
- use of twice-daily DOAC (vs. any other anticoagulant) after stroke

We limited these analyses to patients for whom follow-up data were available and to those receiving oral anticoagulant therapy after the index stroke (excluding patients with antiplatelet monotherapy, parenteral anticoagulation, or no antithrombotic treatment).

Supplementary Figure 7.1.1. *Distribution of stroke etiologies in the total study population and stratified to type of anticoagulant and study period at the time of the index stroke. While the distribution of DOAC vs. VKA differed between the two study periods January 2012 to June 2016 vs. July 2016 to December 2020 (p<0.001), the distribution of stroke etiologies did not differ substantially (p=0.08).*



VKA, vitamin K antagonist; DOAC, direct oral anticoagulant; AF, atrial fibrillation

Supplementary Table 7.1.1. Participating stroke centers, their contribution to the pooled dataset and complete list of collaborators

Stroke Center	Reporting period	Number of cases contributed to the analyzed pooled dataset	Complete list of collaborators
Basel, Switzerland	04/2012 – 12/2020	460	Stefan T Engelter, Philippe A Lyrer, Leo H Bonati, Christopher Traenka, Alexandros A Polymeris, Annaelle Zietz, Lilian Kriemler, Nils Peters, Gian Marco De Marchis, Sebastian Thilemann, Henrik Gensicke, Lisa Hert, Benjamin Wagner, Fabian Schaub, Louisa Meya, Nikolaos Symeon Avramiotis, Joachim Fladt, Tolga Dittrich, Urs Fisch
Berlin, Germany	01/2013 – 12/2015 01/2018 – 12/2019	438	Jan F Scheitz, Christian H Nolte, Karl Georg Haeusler, Simon Hellwig, Markus G Klammer, Simon Litmeier
Bern, Switzerland	02/2015 – 07/2020	456	Thomas R Meinel, Urs Fischer, David J Seiffge, Lorenz Grunder, Marcel Arnold, Simon Jung, Jan Gralla
Brown University, USA	01/2018 – 12/2019	50	Christoph Stretz, Shadi Yaghi, Xing (Cathy) Dai
Erlangen, Germany	04/2016 – 12/2018	334	Svenja Stoll, Ruihao Wang, Bernd Kallmünzer
George Washington University, USA	02/2016 – 12/2019	34	Christopher R. Leon Guerrero, Iman Moeini-Naghani
Heidelberg, Germany	01/2015 – 06/2018	339	Hannah Oehler, Kyra Hoelscher, Peter Ringleb, Jan C Purrucker
Lausanne, Switzerland	01/2012 – 06/2020	293	Patrik Michel, Davide Strambo, Alexander Salerno
Lugano, Switzerland	02/2014 – 08/2020	159	Giovanni Bianco, Carlo W Cereda
Mainz, Germany	09/2019 – 06/2020	87	Timo Uphaus, Klaus Gröschel
Zurich, Switzerland	01/2014 – 08/2020	296	Mira Katan, Susanne Wegener
Total	01/2012 – 12/2020	2,946	

Supplementary Table 7.1.2. Patient characteristics stratified to type of anticoagulant (DOAC vs. VKA) at the time of stroke

Characteristic	All (N=2,946)	N missing	VKA (N=1,272)	DOAC (N=1,674)	p value
Demographics					
age, median (IQR), years	81 (76-86)	0	82 (76.85-86)	80.75 (75-86)	<0.001
female sex, N (%)	1,404 (47.7%)	0	624 (49.1%)	780 (46.6%)	0.190
Risk factors					
hypertension, N (%)	2,649 (89.9%)	0	1,149 (90.3%)	1,500 (89.6%)	0.520
diabetes, N (%)	871 (29.6%)	0	362 (28.5%)	509 (30.4%)	0.250
dyslipidemia, N (%)	1,768 (60.3%)	13	791 (62.3%)	977 (58.7%)	0.047
renal impairment, N (%)	959 (33.2%)	58	457 (36.7%)	502 (30.5%)	<0.001
prior ischemic stroke, N (%)	984 (33.4%)	0	373 (29.3%)	611 (36.5%)	<0.001
history of ICH, N (%)	60 (2.0%)	0	17 (1.3%)	43 (2.6%)	0.019
ischemic heart disease, N (%)	905 (30.7%)	0	406 (31.9%)	499 (29.8%)	0.220
bioprosthetic heart valve, N (%)	151 (5.1%)	0	68 (5.3%)	83 (5.0%)	0.640
current smoking, N (%)	249 (8.8%)	103	97 (7.9%)	152 (9.4%)	0.170
active malignancy, N (%)	236 (8.1%)	15	79 (6.2%)	157 (9.4%)	0.002
prestroke mRS ≥3, N (%)	567 (22.1%)	381*	253 (23.4%)	314 (21.2%)	0.180
ipsilateral stenosis ≥50%, N (%)	452 (15.6%)	54	181 (14.6%)	271 (16.4%)	0.170
ipsilateral stenosis <50%, N (%)	496 (17.1%)	50	235 (18.8%)	261 (15.8%)	0.036
Medication details before stroke					
additional antiplatelet, N (%)	363 (12.3%)	4	162 (12.8%)	201 (12.0%)	0.540
statin, N (%)	1,354 (46.4%)	30	561 (44.8%)	793 (47.7%)	0.120
antihypertensive(s), N (%)	2,683 (91.9%)	27	1,160 (92.4%)	1,523 (91.5%)	0.380
Stroke details					
Etiology		0			
competing mechanism, N (%)	713 (24.2%)		249 (19.6%)	464 (27.7%)	<0.001
insufficient anticoagulation, N (%)	934 (31.7%)		548 (43.1%)	386 (23.1%)	
cardioembolism despite sufficient anticoagulation, N (%)	1,299 (44.1%)		475 (37.3%)	824 (49.2%)	
NIHSS on admission, median (IQR)	6 (2-14)	33	7 (3-15)	5 (2-13)	<0.001
intravenous thrombolysis, N (%)	351 (11.9%)	2	227 (17.8%)	124 (7.4%)	<0.001
endovascular treatment, N (%)	787 (26.8%)	6	340 (26.8%)	447 (26.8%)	1.000
embolic infarct pattern, N (%)	2,317 (81.7%)	111	1,036 (84.6%)	1,281 (79.6%)	<0.001
large vessel occlusion, N (%)	1,345 (46.2%)	32	628 (50.4%)	717 (43.0%)	<0.001
Laboratory parameters on admission					
INR, median (IQR)	1.4 (1.1-1.9)	100	1.8 (1.5-2.31)	1.2 (1.08-1.35)	<0.001
low anticoagulant activity, N (%)‡	957 (43.9%)	766‡	737 (58.2%)	220 (24.1%)	<0.001
Outcome at discharge					
mRS ≥3, N (%)	1,543 (63.3%)	508§	691 (64.5%)	852 (62.4%)	0.290
in-hospital death, N (%)	204 (8.4%)		104 (9.7%)	100 (7.3%)	0.035

* not collected in the center Berlin for the recruiting period 2013-2015
† not collected in the center Erlangen
‡ defined in VKA-treated patients as INR <2.0 and in DOAC-treated patients as plasma level <30ng/ml. DOAC plasma level not collected in the centers Berlin, Mainz, and George Washington University.
§ not collected in the center Mainz

VKA, Vitamin K antagonist; DOAC, direct oral anticoagulant; ICH, intracranial hemorrhage; NIHSS, National Institutes of Health Stroke Scale; INR, international normalized ratio; mRS, modified Rankin Scale

Supplementary Table 7.1.3. Preventive treatments after index stroke stratified to stroke etiology

	All (N=2,946)	N missing	Stroke etiology			
			Competing mechanism (N=713)	Insufficient anticoagulation (N=934)	Cardioembolism despite sufficient anticoagulation (N=1,299)	p value
Treatments						
Antithrombotic treatment		71				
oral anticoagulant monotherapy, N (%)	2,070 (72.0%)		404 (57.8%)	666 (73.1%)	1,000 (79.1%)	<0.001
oral anticoagulant and antiplatelets, N (%)	367 (12.8%)		198 (28.3%)	71 (7.8%)	98 (7.7%)	
antiplatelets monotherapy, N (%)	120 (4.2%)		28 (4.0%)	49 (5.4%)	43 (3.4%)	
parenteral anticoagulation, N (%)	32 (1.1%)		12 (1.7%)	10 (1.1%)	10 (0.8%)	
no treatment, N (%)	286 (9.9%)		57 (8.2%)	115 (12.6%)	114 (9.0%)	
Type of anticoagulant		80				
VKA, N (%)	315 (13.4%)		92 (15.7%)	95 (13.1%)	128 (12.2%)	0.140
DOAC, N (%)	2,042 (86.6%)		495 (84.3%)	630 (86.9%)	917 (87.8%)	
DOAC dose		199*				
full, N (%)	1,228 (66.6%)		289 (63.4%)	362 (66.2%)	577 (68.7%)	0.150
reduced, N (%)	615 (33.4%)		167 (36.6%)	185 (33.8%)	263 (31.3%)	
DOAC dosing frequency		23				
once daily, N (%)	393 (19.5%)		124 (25.3%)	145 (23.5%)	124 (13.6%)	<0.001
twice daily, N (%)	1,626 (80.5%)		367 (74.7%)	472 (76.5%)	787 (86.4%)	
DOAC mechanism of action		23				
thrombin inhibitor, N (%)	486 (24.1%)		108 (22.0%)	123 (19.9%)	255 (28.0%)	<0.001
factor Xa inhibitor, N (%)	1,533 (75.9%)		383 (78.0%)	494 (80.1%)	656 (72.0%)	
Other pharmacologic treatments						
statins, N (%)	2,126 (75.1%)	115	555 (81.3%)	643 (71.2%)	928 (74.5%)	<0.001
antihypertensives, N (%)	2,401 (84.8%)	116	592 (86.7%)	745 (82.5%)	1,064 (85.5%)	0.048
Nonpharmacologic treatments						
revascularisation treatments, N (%)	94 (3.4%)	172†	89 (13.2%)	1 (0.1%)	4 (0.3%)	<0.001
left atrial appendage occlusion, N (%)	17 (1.0%)	1,184‡	9 (1.9%)	2 (0.4%)	6 (0.8%)	0.045

* not collected in the center Lausanne
† not collected in the center Mainz
‡ not collected in the centers Berlin, Heidelberg, Lausanne and Mainz

VKA, Vitamin K antagonist; DOAC, direct oral anticoagulant

Supplementary Table 7.1.4. Association of preventive strategies after index stroke with the combined endpoint of recurrent ischemic stroke and intracranial hemorrhage

Patients	Preventive strategy	Combined endpoint of recurrent ischemic stroke and ICH					
		unadjusted			adjusted*		
		OR [95%-CI]	p value	N events / total N in model	aOR [95%-CI]	p value	N events / total N in model
all patients	use of DOAC (vs. VKA) after stroke	0.50 [0.29, 0.84]	0.009	80/1,489	0.41 [0.23, 0.72]	0.002	72/1,368
	any anticoagulant switch	0.83 [0.53, 1.31]	0.425	80/1,489	0.77 [0.47, 1.25]	0.286	72/1,368
	addition of antiplatelets	2.33 [1.33, 4.08]	0.003	80/1,505	2.61 [1.43, 4.76]	0.002	72/1,382
patients with DOAC at the time of the stroke	switch to another DOAC	1.30 [0.71, 2.40]	0.398	45/826	1.28 [0.65, 2.51]	0.472	39/757
	switch to DOAC with different dosing frequency	1.08 [0.57, 2.03]	0.820	41/794	1.05 [0.52, 2.14]	0.888	35/725
	switch to DOAC with different mechanism of action	1.65 [0.86, 3.19]	0.133	41/795	1.47 [0.70, 3.11]	0.307	35/726
patients with VKA at the time of the stroke	switch to any DOAC	0.45 [0.23, 0.90]	0.023	35/663	0.45 [0.21, 0.99]	0.046	33/611
patients with competing stroke mechanism	addition of antiplatelets	1.82 [0.86, 3.84]	0.118	33/409	2.22 [0.96, 5.12]	0.061	29/359
patients with insufficient anticoagulation	switch to DOAC or correct DOAC dose	0.81 [0.33, 1.95]	0.634	21/480	0.87 [0.33, 2.25]	0.770	20/402
patients with cardioembolism despite sufficient anticoagulation	twice-daily DOAC (vs. any other anticoagulant)	1.45 [0.54, 3.94]	0.464	25/592	1.36 [0.45, 4.08]	0.586	22/555

*adjusted for age, sex, hypertension, diabetes, ischemic heart disease, dyslipidemia, renal impairment, prior ischemic stroke, ICH, current smoking, active malignancy, use of statins, use of antihypertensives

VKA, Vitamin K antagonist; DOAC, direct oral anticoagulant; ICH, intracranial hemorrhage

8. Third topic: Adherence of patients with recent stroke to direct oral anticoagulants

In this topic we provide a brief overview of the MAAESTRO trial (electronic **M**onitoring and improvement of **A**dherence to direct oral **A**nticoagulant treatment – a randomized crossover study of an **E**ducational and reminder-based intervention in ischemic **STRO**ke Patients under polypharmacy). Nonadherence to DOAC has been a matter of concern since these drugs were introduced in clinical practice. We initiated MAAESTRO as a single-center interdisciplinary project in collaboration with pharmacists in order to investigate adherence to DOAC in patients with a recent stroke using electronic monitoring and evaluate the adherence-improving effect of an educational and reminder-based intervention. MAAESTRO had an observational phase and a randomized controlled interventional phase.

MAAESTRO successfully concluded recruitment of its target sample size in July 2021 and has now completed the observational phase. Patient follow-up in the interventional phase is ongoing and expected to conclude in April 2022. Final analyses are therefore not yet available. Still, we present here the study design and the first results from the observational study phase on (i) the patterns of DOAC-taking behaviour and appropriate metrics to describe adherence and (ii) the impact of the COVID-19 lockdown on the medication adherence of stroke patients.

8.1. Protocol for MAAESTRO: electronic Monitoring and improvement of Adherence to direct oral Anticoagulant treatment – a randomized crossover study of an Educational and reminder-based intervention in ischemic STROke patients under polypharmacy

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Abstract

Background

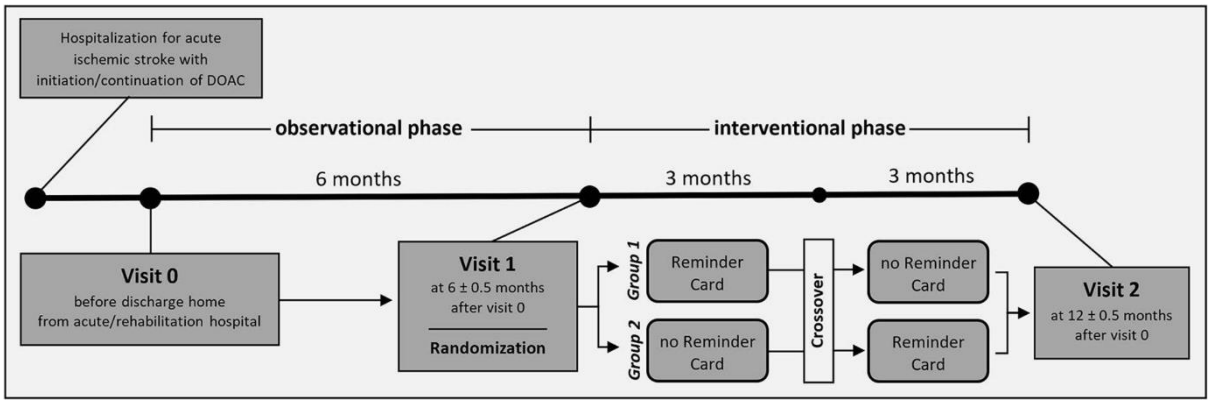
Nonadherence to direct oral anticoagulants (DOAC) remains a matter of concern, especially for patients with a recent stroke. However, data on electronically monitored adherence and adherence-improving interventions are scarce.

Aims

We aim to use electronic monitoring in DOAC-treated stroke patients to (i) evaluate the effect of an educational, reminder-based adherence-improving intervention, (ii) investigate predictors of nonadherence, (iii) identify reliable self-report measures of adherence, and (iv) explore the association of nonadherence with clinical outcomes.

Methods

Single-center, randomized, crossover, open-label study. Adherence to DOAC of polymedicated patients self-administering their medication will be monitored electronically throughout the 12-month-long study following hospitalization for ischemic stroke. After a 6-month observational phase, patients will receive pharmaceutical counselling with feedback on their intake history and be given a multi-compartment pillbox for the subsequent 6-month interventional phase. The pillbox will provide intake reminders either during the first or the last three interventional-phase months. Patients will be randomly allocated to reminders-first or reminders-last.



Study outcomes

Primary: nonoptimal timing adherence; Secondary: nonoptimal taking adherence; timing adherence; taking adherence; self-reported adherence; clinical outcomes including ischemic and hemorrhagic events; patient-reported device usability and satisfaction.

Sample size estimates

A sample of 130 patients provides 90% power to show a 20% improvement of the primary adherence outcome with intake reminders.

Discussion

MAAESTRO will investigate various aspects of nonadherence and evaluate the effect of an adherence-improving intervention in DOAC-treated patients with a recent stroke using electronic monitoring.

Clinical Trial Registration

ClinicalTrials.gov identifier: NCT03344146, Swiss National Clinical Trials Portal: SNCTP000002410

8.2. Insights into direct oral anticoagulant therapy implementation of stroke survivors with atrial fibrillation in an ambulatory setting

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Abstract

Objectives

To describe how stroke survivors with atrial fibrillation implement direct oral anticoagulant treatment and propose appropriate metrics to describe adherence.

Materials and methods

Stroke patients with atrial fibrillation electronically recorded their self-administered direct oral anticoagulants (apixaban, dabigatran, edoxaban, rivaroxaban) during a 6-month observation phase after hospitalisation for ischemic stroke. Taking and timing adherence, correct dosing days, drug holidays, time of the day and day of the week subsets, dose-to-dose intervals and longest intervals between two consecutive doses were calculated from electronic monitoring data to describe and discuss the implementation phase of adherence.

Results

Data from 41 patients were analysed. Median age was 77 (IQR 69-84), 63.4% were male and the majority suffered a mild stroke (median NIHSS 1). Mean taking and timing adherence exceeded 90%. Correct dosing occurred in 86.6% of the days. Seven patients (17.1%) had intake pauses of three or more consecutive days. Patients with twice-daily regimen (70.7%) had higher taking adherence in the morning than in the evening (94.4% versus 89.9%; p = 0.001). No therapy- or anamneses-related characteristic was associated with taking adherence.

Conclusions

Although adherence to direct oral anticoagulants of stroke patients with atrial fibrillation exceeded 90%, deviant intake patterns such as drug holidays and missed evening doses were common and raise concerns. Appropriate adherence metrics calculated from electronic monitoring data may guide healthcare professionals elucidating patient-tailored adherence-enhancing interventions.

ClinicalTrials.gov registration number

NCT03344146

8.3. Impact of the COVID-19 lockdown on the adherence of stroke patients to direct oral anticoagulants: a secondary analysis from the MAAESTRO study

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Abstract

Background

The negative impact of the COVID-19 outbreak on stroke care has been reported, but no data exist on the influence of the lockdown on medication adherence to antithrombotic treatment for stroke prevention. We present a comparison of electronic adherence data of stroke patients treated with direct oral anticoagulants (DOAC) prior to and during the COVID-19 lockdown in spring 2020 in Switzerland.

Methods

This is a secondary analysis using data from the ongoing MAAESTRO study, in which stroke patients with atrial fibrillation electronically monitor their adherence to DOAC treatment. Eligible patients for this analysis had at least four weeks of adherence data prior to and during the COVID-19 lockdown. Three adherence metrics (taking adherence, timing adherence, drug holidays) were calculated and compared descriptively.

Results

The analysis included eight patients (median age 81.5 years, IQR 74.8-84.5). Five patients had a pre-lockdown taking adherence over 90% (mean 96.8% ± 2.9), with no change during lockdown, high timing adherence in both periods and no drug holidays. The remaining three patients had pre-lockdown taking and timing adherence below 90%. Of those, two patients showed a moderate decline either in taking or timing adherence compared to pre-lockdown. One showed a substantial increase in taking and timing adherence during lockdown (both + 25.8%).

Conclusion

Our data suggest that a major disruption of social life (i.e., the imposed COVID-19 lockdown) is unlikely to relevantly affect the medication intake behaviour of patients with high pre-established adherence, but might have an impact in patients with previously suboptimal adherence.

Trial registration number

NCT03344146

9. Fourth topic: Serum neurofilament light chain as a tool to investigate cognitive dysfunction in atrial fibrillation

The final topic of this PhD thesis focused on sNfL – a novel biomarker of neuronal damage – as a tool to investigate the mechanisms of cognitive dysfunction in patients with AF. In the third main project of the PhD thesis, we investigated the association of sNfL with cognitive performance, as well as clinical and neuroimaging characteristics of AF patients in a large cross-sectional analysis from the multicenter Swiss-AF Cohort Study. In the fourth and last main project of this PhD thesis we additionally investigated in this elderly cardiovascular cohort how renal function and body mass index alter the levels of sNfL, in order to gain insights into its homeostasis (i.e., clearance and distribution) in the blood compartment – a necessary step towards further establishing this neurological biomarker in cardiovascular research.

9.1. Serum neurofilament light in atrial fibrillation: clinical, neuroimaging and cognitive correlates

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Abstract

Emerging evidence suggests that atrial fibrillation is associated with cognitive dysfunction independently of stroke, but the underlying mechanisms remain unclear. In this cross-sectional analysis from the Swiss-AF Study (NCT02105844), we investigated the association of serum neurofilament light protein, a neuronal injury biomarker, with (i) the CHA2DS2-VASc score (congestive heart failure, hypertension, age 65-74 or ≥ 75 years, diabetes mellitus, stroke or transient ischemic attack, vascular disease, sex), clinical and neuroimaging parameters and (ii) cognitive measures in atrial fibrillation patients.

We measured neurofilament light in serum using an ultrasensitive single-molecule array assay in a sample of 1,379 atrial fibrillation patients (mean age 72 years, 27% female). Ischemic infarcts, small vessel disease markers and normalized brain volume were assessed on brain MRI. Cognitive testing included the Montreal Cognitive Assessment, Trail Making Test, Semantic Verbal Fluency and Digit Symbol Substitution Test, which were summarized using principal component analysis. Results were analyzed using univariable and multivariable linear regression.

Neurofilament light was associated with the CHA2DS2-VASc score, with an average 19.2% (95% confidence interval [17.2%, 21.3%]) higher neurofilament per unit CHA2DS2-VASc increase. This association persisted after adjustment for age and MRI characteristics. In multivariable analyses, clinical parameters associated with neurofilament light were higher age (32.5% [27.2%, 38%] neurofilament increase per 10 years), diabetes mellitus, heart failure and peripheral artery disease (26.8% [16.8%, 37.6%], 15.7% [8.1%, 23.9%] and 19.5% [6.8%, 33.7%] higher neurofilament, respectively). Mean arterial pressure showed a curvilinear association with neurofilament, with evidence for both an inverse linear and a U-shaped association. MRI characteristics associated with neurofilament were white matter lesion volume and volume of large noncortical or cortical infarcts (4.3% [1.8%, 6.8%] and 5.5% [2.5%, 8.7%] neurofilament increase per unit increase in log-volume of the respective lesion), as well as normalized brain volume (4.9% [1.7%, 8.1%] higher neurofilament per 100 cm3 smaller brain volume). Neurofilament light was inversely associated with all cognitive measures in univariable analyses. The effect sizes diminished after adjusting for clinical and MRI variables, but the association with the first principal component was still evident.

Our results suggest that in atrial fibrillation patients, neuronal loss measured by serum neurofilament light is associated with age, diabetes mellitus, heart failure, blood pressure and vascular brain lesions, and inversely correlates with normalized brain volume and cognitive function.

Introduction

Atrial fibrillation (AF) and dementia are highly prevalent in the elderly. AF is linked to dementia through ischemic stroke, but evidence has emerged that even in the absence of clinically manifest stroke, the risk of cognitive impairment and dementia is increased in patients with AF.[11, 12, 104] Several potential mechanisms have been postulated to explain this association, including silent cerebral infarcts, cerebral small vessel disease (through shared risk factors such as diabetes and hypertension) and cerebral hypoperfusion,[104, 105] but tangible evidence is lacking. With the progressive ageing of the population, AF and dementia are a continuously growing public health concern, and a deeper understanding of the pathophysiological pathways underlying their association will be crucial in developing strategies to preserve cognitive function in the elderly.[108]

In a cross-sectional analysis from the Swiss-AF cohort study, we previously showed that cortical and large noncortical infarcts were common in AF patients and were independently associated with a lower score on the Montreal Cognitive Assessment (MoCA), lending support to the hypothesis that cognitive dysfunction in AF might – at least in part – be mediated through covert cerebral embolic infarcts.[110]

Here, we used serum neurofilament light protein (sNfL) to further explore the mechanisms that underly neuronal damage and cognitive dysfunction in AF. Neurofilaments are neuron-exclusive cytoskeletal proteins that are released in the extracellular space, cerebrospinal fluid and eventually peripheral blood following neuroaxonal damage. sNfL has emerged as a biomarker for neuronal injury in inflammatory, degenerative, traumatic and vascular neurological disorders,[173] but has not yet been investigated as a marker of neurological disease in AF. In this analysis from the Swiss-AF cohort study we investigated the association of sNfL with (i) clinical parameters and neuroimaging characteristics and (ii) measures of cognitive function.

Materials and methods

Study design, patient population and data collection

This was a cross-sectional analysis using baseline data from the ongoing prospective observational Swiss-AF cohort study (NCT02105844), that enrolled 2,415 AF patients between 2014 and 2017 across 14 centers in Switzerland. The detailed methodology of Swiss-AF has been described previously.[110, 123] In short, Swiss-AF included patients with documented AF aged 65 years or older, with an additional 15% of patients aged between 45 and 65 years. Patients with secondary forms of AF, those with a recent ischemic stroke, transient ischemic attack (TIA) or other acute illness (< 4 weeks) and those unable to provide informed consent (e.g., patients with dementia, psychosis or delirium) were excluded. Baseline information on sociodemographic parameters and comorbidities was collected based on patients' history and/or medical chart review as applicable, using standardized case report forms. Upon inclusion, weight, height and the mean of 3 consecutive blood pressure measurements were obtained, and patients underwent blood sampling, brain MRI and cognitive testing.

Baseline blood samples were collected following standard operating procedures.[123] After centrifugation, serum samples were aliquoted into cryotubes and stored at -80°C in a centralized biobank. sNfL concentrations were measured in duplicate using a previously described ultrasensitive single-molecule array assay.[174] Inter-assay coefficients of variation were 10% for low (mean, 6.9 pg/mL), 12% for medium (mean, 19.6 pg/mL) and 5% for high (mean, 84.5 pg/mL) concentration

quality control serum samples measured in duplicate in every run. The mean intra-assay coefficient of variation of duplicate determinations for concentration was 5%. Individuals performing sNfL measurements were blinded to clinical, MRI and cognitive patient data.

Baseline brain MRI was acquired on a 1.5 or 3.0 Tesla scanner using a standardized protocol including a 3D T1-weighted magnetization-prepared rapid gradient echo (MPRAGE), a 2D axial fluid-attenuated inversion recovery (FLAIR), a 2D axial diffusion-weighted imaging (DWI) and a 2D axial susceptibility-weighted imaging (SWI) or T2*-weighted sequence.[110, 123] All scans were analyzed centrally in a core lab (Medical Image Analysis Center AG, Basel, Switzerland) by expert raters blinded to clinical and cognitive patient data and sNfL measurements. We evaluated the following vascular brain lesions, which we defined adapting the STRIVE classification of small vessel disease,[39] as in previous research:[110] (i) Small noncortical infarcts (SNCIs), defined as hyperintense lesions on FLAIR, ≤20mm in diameter on axial sections and not involving the cortex, consistent with ischemic infarction in the territory of a perforating arteriole and located in the white matter, internal or external capsule, deep brain nuclei, thalamus or brainstem. (ii) Large noncortical infarcts were noncortical infarcts with a diameter >20mm. Cortical infarcts were defined as FLAIR hyperintense lesions involving the cortex irrespective of their size and whether they also involved subcortical areas. Large noncortical and cortical infarcts (LNCCIs) were grouped together in the analyses. All infarcts were characterized as recent (hyperintense) or chronic according to their appearance on DWI. (iii) FLAIR hyperintensities not meeting the aforementioned criteria for infarcts were identified as white matter lesions (WMLs). (iv) Microbleeds (MBs) were identified and counted as nodular, strongly hypointense lesions on either SWI or T2*-weighted sequences. T2-weighted volumes of SNCIs, LNCCIs and WMLs were segmented and quantified semi-automatically in mm^3 using Amira (Mercury Computer Systems Inc., Chelmsford, Massachusetts). Lesions with a central FLAIR hypointense core were segmented in total without differentiating between hyperintense and hypointense lesion areas. The normalized brain volume (nBV) was estimated in cm^3 on MPRAGE using SIENAX.[175]

Cognitive testing was performed by trained study personnel in a standardized manner and included:

(i) the MoCA, which assesses visuospatial and executive functions, confrontation naming, memory, attention, language and abstraction. Subjects could obtain a maximum of 30 points, with higher scores indicating better cognitive function. One point was added to the test score if the patient had ≤12 years of formal education.[109]

(ii) the Trail Making Test (TMT), which assesses visual attention, processing speed and executive functioning. It consists of two parts (A and B), in which the subject was instructed to connect a set of 25 points, either circled numbers in ascending order (TMT-A) or circled numbers and letters in alternating numeric and alphabetic ascending order (TMT-B), as quickly as possible while maintaining accuracy. The number of correct connections and the time to test completion in seconds were measured, with a maximum allowed time of 180 and 300 seconds for TMT-A and TMT-B, respectively. The test metric was the number of correct answers per second, with higher scores indicating better cognitive function.[176]

(iii) semantic verbal fluency (SVF), which assesses semantic memory and language production. Subjects were asked to name as many words as possible from the semantic category ‘animals’ within 60 seconds. The test metric was the number or correct responses, with higher scores indicating better cognitive function.[177]

(iv) the Digit Symbol Substitution Test (DSST) of the Wechsler Adult Intelligence Scale, which assesses processing speed, visuomotor coordination and attention. The subject received a key grid of numbers and matching symbols and a test section with numbers and empty boxes. The test

consisted of filling as many empty boxes as possible with the matching symbol from the key grid. The score was the number of correct number-symbol matches achieved within 120 seconds, with higher scores indicating better cognitive function.[178]

The detailed patient flowchart for the analyses of this study is given in Supplementary Figure 9.1.1. We included all Swiss-AF patients with quantifiable sNfL measurement and complete MRI data and excluded those with recent subclinical ischemic infarcts on DWI (which would expectedly raise sNfL concentrations disproportionately[179-181]). The Ethics Committee of Northwest and Central Switzerland approved Swiss-AF including the present study (PB_2016-00793). Written informed consent was obtained from all study participants according to the Declaration of Helsinki. This study was conducted in accordance with the STROBE Statement for cross-sectional studies.[145]

Statistical analyses

Analysis A: Association of the CHA_2DS_2-VASc score, clinical and MRI characteristics with sNfL

To investigate the association of patients' characteristics with sNfL, we fitted uni- and multivariable linear regression models with various sets of clinical and MRI variables as independent variables and the log-transformed sNfL concentration as dependent variable. Continuous independent variables were centered on their mean (or, in case of skewed data, median) values. We report the back-transformed model-based estimates, which represent multiplicative effects on the geometric mean of sNfL and are denoted by β_{mult} (so that a one-unit increase in the independent variable is associated with an average β_{mult}-fold change in sNfL), along with 95% confidence intervals (CI) and two-sided p-values. We interpret p-values as a continuous measure, with smaller values indicating stronger evidence for an association, but without specifying a threshold value. To compare between models, we used the Akaike's information criterion (AIC), which estimates the relative quality of different models fitted to a given dataset, while penalizing models for larger number of independent variables. Lower AIC values indicate a better fit. Additionally, we provide the coefficient of determination (R^2) of each model as a measure of the proportion of the observed sNfL variance explained by the model. Since R^2 tends to increase with the number of independent variables, we also provide the adjusted R^2 (R^2_{adj}), which penalizes R^2 for larger numbers of variables. We fitted the following predefined models with log-sNfL as the dependent variable:

(i) the CHA_2DS_2-VASc score (congestive heart failure, hypertension, age 65-74 or ≥ 75 years, diabetes mellitus, stroke or TIA, vascular disease, sex) models: CHA_2DS_2-VASc is a validated clinical score predicting stroke risk in AF patients.[10, 182] We opted to first investigate the association of sNfL with this risk score as a whole, independent of its individual components. Given the known association of sNfL with age,[173] we fitted univariable models for the association of sNfL with age, with the CHA_2DS_2-VASc score and with the CHA_2DS_2-VASc score after exclusion of its age component. Additionally, we fitted bivariable models for the age-adjusted association of sNfL with the CHA_2DS_2-VASc score and with the CHA_2DS_2-VASc score after exclusion of its age component. We selected the best fitting CHA_2DS_2-VASc score model based on AIC values, and proceeded to further adjust it for MRI markers of small vessel disease (SNCIs, MBs and WMLs),[39] as well as for all MRI variables, as detailed below, in two additional multivariable models.

(ii) the clinical model: We fitted a multivariable model for the association of sNfL with the following predefined clinical variables: age, sex, history of hypertension, diabetes mellitus, stroke or TIA, coronary heart disease, peripheral artery disease (PAD), heart failure, obstructive sleep apnea, AF type (paroxysmal, persistent, permanent), body mass index (BMI, calculated as weight in kg / height in m^2), smoking status (active, past, nonsmoker), alcohol consumption (number of standard drinks daily) and mean arterial pressure (MAP, calculated as 1/3 * systolic blood pressure + 2/3 *

diastolic blood pressure[183]). We opted to use the MAP instead of including both systolic and diastolic blood pressure in the models due to collinearity between those variables. There was no evidence of collinearity upon visual inspection of scatter plots for any of the other continuous clinical variables, but there was evidence for a curvilinear association between sNfL and MAP, which we modelled by introducing an additional quadratic term (MAP^2). We reduced the clinical model to a smaller set of variables via stepwise backward elimination based on AIC values. We imputed the few missing values in the clinical variables with simple single imputation, using the mode (i.e., the most common category) for categorical variables and the mean (or, in case of skewed data, the median) for continuous variables (Table 9.1.1; one missing value in smoking status and alcohol consumption, imputed with 'past' and 0.6 standard drinks daily, respectively; six missing values in systolic and diastolic blood pressure, imputed with 134.7mmHg and 78.4mmHg, respectively).

(iii) the MRI model: We fitted a multivariable model for the age-adjusted association of sNfL with the following predefined MRI variables: nBV, SNCIs' presence and log-transformed volume, LNCCIs' presence and log-volume, MBs' presence and count (truncated at 20 to reduce the influence of outliers) and WMLs' log-volume.

(iv) the combined clinical and MRI model: We fitted a final combined model for the association of sNfL with the chosen clinical and all MRI variables from the models (ii) and (iii).

Analysis B: Association of sNfL with measures of cognitive function

To investigate the association of sNfL with cognitive function we fitted linear regression models with the score of each of the cognitive tests (MoCA, TMT-A, TMT-B, SVF, DSST) as the dependent variable and the log-transformed sNfL concentration as independent variable. We report the model-based estimates, which represent additive effects on the mean of the test score and are denoted by β, along with the 95%CI and two-sided p-values. A one-unit increase in log-sNfL is associated with an average change in the test score of β units (or a 10% increase in sNfL is associated with a change of 0.095 * β units in the test score). For each cognitive test we fitted the following predefined models with test score as the dependent variable:

(i) univariable model (including only log-sNfL as independent variable)

(ii) age-adjusted model (including log-sNfL and age as independent variables)

(iii) clinical multivariable model, including log-sNfL, age, sex, education level (basic, middle, advanced), history of hypertension, diabetes mellitus, stroke or TIA, coronary heart disease, PAD, heart failure, obstructive sleep apnea, BMI, smoking status (active, past, nonsmoker) and alcohol consumption (number of standard drinks daily) as independent variables. We imputed the few missing values in the clinical variables with simple single imputation, as described above.

(iv) MRI multivariable model, including log-sNfL, age, nBV, SNCIs' presence and log-volume, LNCCIs' presence and log-volume, burden of MBs (categorized as 0, 1, 2 and ≥3) and WMLs' log-volume as independent variables

(v) combined clinical and MRI multivariable model, including all aforementioned variables

To summarize performance over all cognitive tests we used principal component analysis. The first principal component (PC1) explained 61.3% of the observed variance and the loading of each test on PC1 (representing the covariance between each test and PC1) was positive (MoCA: +0.40, TMT-A: +0.45, TMT-B: +0.50, SVF: +0.39, DSST: +0.49), thereby allowing for the use of PC1 as a single, summary measure of cognitive function, with higher values indicating better cognitive performance. We additionally fitted all models (i) – (v) described above with PC1 as the dependent variable.

As a sensitivity analysis, we repeated all models described under analyses A and B in the subset of patients without history of stroke or TIA.

All analyses were performed with R version 3.5.2 (2018-12-20) (R Core Team, 2019).

Data availability

The Swiss-AF consent forms, as approved by the ethics committee, do not allow for the data to be made publicly available. Researchers may contact the authors for the potential submission of research proposals for future analyses or independent verification of our results.

Results

A total of 1,379 patients (mean [SD] age 72.3 [8.6] years, 27.1% female) with quantifiable sNfL measurement and complete MRI data were available for analysis A (Supplementary Figure 9.1.1). The median (IQR) sNfL concentration was 38.2 (26.6 – 56.4) pg/ml. The detailed demographic, clinical and MRI characteristics of all patients are presented in Table 9.1.1.

Association of the CHA_2DS_2-VASc score with sNfL

The CHA_2DS_2-VASc score was associated with sNfL in univariable analysis, with an average 19.2% increase in sNfL concentration per point increase in the CHA_2DS_2-VASc score (β_{mult} = 1.192, 95%CI [1.172, 1.213], $p < 0.001$; Figure 9.1.1). Age was also strongly associated with sNfL in univariable analysis (β_{mult} = 1.489 per 10 years, 95%CI [1.440, 1.539], $p < 0.001$). The association between the CHA_2DS_2-VASc score and sNfL persisted after excluding the age component of the score, after adjusting for age and after both excluding the age component and adjusting for age. The model with the best fit was the one including the unmodified CHA_2DS_2-VASc score and adjusting for age, which was used in the rest of the analyses. The CHA_2DS_2-VASc score remained associated with sNfL after adjusting for MRI markers of small vessel disease and for all MRI variables combined (Supplementary Table 9.1.1).

Association of clinical and MRI characteristics with sNfL

The detailed results of the clinical, MRI and combined models are given in Table 9.1.2. The clinical model fitted the data better and explained a larger proportion of the observed sNfL variance compared to the MRI model. The combined clinical and MRI model fitted the data best. Figure 9.1.2 shows the effect size estimates for the association of all clinical and MRI variables with sNfL from the combined model: Parameters positively associated with sNfL were age (on average 32.5% higher sNfL per 10 years), history of diabetes mellitus (26.8% higher sNfL), PAD (19.5% higher sNfL) and heart failure (15.7% higher sNfL), as well as volume of LNCCIs and WMLs (5.5% and 4.3% higher sNfL per unit increase in log-volume of the respective lesion). MAP showed a curvilinear association with sNfL, with an inverse linear and U-shaped component (Figure 9.1.3A). Parameters inversely associated with sNfL were BMI (7.3% lower sNfL per 5kg/m^2 higher BMI), past smoker status (7% lower sNfL compared to nonsmoker), alcohol consumption (1.9% lower sNfL per 1 standard drink daily) and nBV (4.9% lower sNfL per 100 cm^3 larger nBV; Figure 9.1.3B).

Association of the sNfL with measures of cognitive function

Of the 1,379 patients with sNfL and MRI data, cognitive testing was incomplete in 16, leaving 1,363 patients available for analysis B. The median (IQR) MoCA score was 26 (24 – 28) points, TMT-A and TMT-B scores were 0.53 (0.39 – 0.68) and 0.21 (0.14 – 0.28) correct connections per second, respectively, SVF score was 19 (15 – 23) correct responses and DSST score was 45 (36 – 54) correct matches. The detailed results of all models for the association of sNfL with all cognitive measures are

presented in Table 9.1.3. In univariable analyses, log-sNfL was strongly associated with all cognitive tests and the PC1, with higher sNfL concentrations indicating worse cognitive performance. The effect sizes generally diminished for all cognitive measures after adjusting for age, clinical variables and MRI characteristics. In the combined models adjusting for all aforementioned parameters, the association of log-sNfL with TMT-A, TMT-B and PC1 persisted. Figure 9.1.4 shows the model-based estimates for the association of sNfL with PC1. The scatter plot of the association of sNfL with PC1 is presented in Figure 9.1.5.

Sensitivity analyses in patients without history of stroke or transient ischemic attack

After excluding those with history of stroke or TIA, 1,125 patients were available for sensitivity analysis A. Their median (IQR) sNfL concentration was 36.7 (25.5 – 53.2) pg/ml and their detailed demographic, clinical and MRI characteristics are presented in Table 9.1.1. Of those, 12 patients had incomplete cognitive testing, leaving 1,113 patients available for the sensitivity analysis B. Both sensitivity analyses in patients without history of stroke or TIA yielded consistent results with the main analysis (Supplementary Tables 9.1.2, 9.1.3 and 9.1.4).

Discussion

This cross-sectional study on the clinical, neuroimaging and cognitive correlates of sNfL in a large sample of AF patients showed the following key findings: (i) Higher CHA_2DS_2-VASc scores indicated increasing neuronal injury, independent of age and vascular brain lesions visible on MRI. (ii) Besides age, clinical factors associated with increased neuronal loss were diabetes mellitus, PAD, heart failure and lower MAP. (iii) MRI characteristics associated with higher sNfL were higher volume of WMLs and LNCCIs, as well as lower nBV. (iv) sNfL was associated with worse cognitive performance, an association which was largely but not exclusively explained by age, comorbidities and vascular brain lesions.

The CHA_2DS_2-VASc score, a validated clinical score predicting ischemic stroke risk in AF patients,[10, 182] was associated with sNfL. This was independent of age, history of stroke and ischemic infarcts visible on MRI, as well as MRI markers of small vessel disease, and might therefore reflect ongoing ischemic brain injury in AF that evades detection on conventional MRI.[184] Higher CHA_2DS_2-VASc scores have previously also been associated with an increasing risk for dementia in stroke-free AF patients.[11]

Our study in AF patients confirms the strong independent association of sNfL with age, which has been demonstrated across a wide variety of patient populations and healthy controls, probably reflecting neurodegenerative processes associated with normal ageing.[173, 185] Furthermore, we found that diabetes mellitus was associated with a sNfL increase by a similar magnitude as 10 years of age. In line with this, poor glycemic control was independently associated with sNfL in a previous study on sNfL among diabetics.[186] The association of diabetes mellitus with sNfL was independent of small vessel disease markers and potentially embolic infarcts on MRI. It remains therefore unknown whether this association reflects increasing ischemic neuronal injury in the presence of diabetes mellitus due to microinfarcts that go undetected on conventional MRI,[184] some other nonischemic, diabetes-induced mechanism of neuronal damage in the central nervous system[187] or the potential contribution of diabetic neuropathy in the peripheral nervous system.[188] Peripheral artery disease was another independent determinant of sNfL, even after adjustment for vascular MRI brain lesions, which might again reflect increasing ischemic brain injury that evades detection on conventional MRI[184] in the

presence of manifest atherosclerotic disease and is in line with cumulating evidence for the association of PAD with cognitive dysfunction independently of manifest cerebrovascular disease.[189]

Interestingly, we found a curvilinear association of MAP with sNfL, with evidence for both an inverse linear and a U-shaped relationship, indicating increasing neuronal loss with lower MAP. This novel finding is in contrast to a previous smaller study in diabetics, which found a positive linear association of systolic blood pressure and no association of diastolic blood pressure with sNfL.[186] Since MAP is a measure of the organ perfusion pressure,[183] our finding suggests that neuronal damage in AF may be partly attributable to cerebral hypoperfusion. This was independent of history of heart failure, which was another independent determinant of sNfL, suggesting that hemodynamic changes in AF might adversely affect brain health above and beyond clinically manifest heart failure. Taken together, these findings refine and further support the hypoperfusion hypothesis for the association of AF with cognitive dysfunction,[104, 105] which has been proposed based on the known associations of cerebral hypoperfusion with dementia,[118, 190, 191] AF with cerebral hypoperfusion,[116, 192] and heart failure with cerebral hypoperfusion[193] and cognitive dysfunction.[194] Of note, recent studies in healthy individuals provide evidence for a U-shaped association of blood pressure with cognitive dysfunction[195] and for an inverse association of diastolic blood pressure with white matter disease[196] and cognitive decline.[197] Putting our findings in this context, the curvilinear association of MAP with sNfL that we observed might not be specific to AF, but rather reflect a universal effect of blood pressure on brain health.

Of all vascular MRI brain lesions, the volume of WMLs and chronic LNCCIs were the strongest independent determinants of sNfL, also after excluding patients with stroke history. This indicates that both small vessel disease and infarcts of potentially embolic origin, even clinically silent ones, contribute to neuronal injury in AF. Our finding is in line with previous research on the association of sNfL with the burden of small vessel disease[180, 198, 199] and the size of acute ischemic infarcts,[180, 181] and might suggest a greater severity of ongoing neurodegenerative processes secondary to ischemia[181] or persistent, active microischemic phenomena in the brain of AF patients with a higher burden of established, embolic or microangiopathic, ischemic MRI lesions. These findings demonstrate the potential of sNfL as a blood biomarker to select AF patients that would benefit from further MRI investigations to uncover potential subclinical vascular brain disease, considering that mass screening with MRI is not feasible and that sNfL in our cohort appeared to be sensitive to potential mechanisms of brain injury independent of structural changes visualized on MRI. Of note, the presence of MBs was only marginally associated with sNfL after adjustment for other brain lesions, an association that was further weakened in the combined model. Thus, MBs might represent a proxy marker of vascular brain disease and contribute little if any to neuronal injury *per se*, in line with previous observations.[110, 200]

Another major finding was the inverse association of normalized brain volume with sNfL, which was independent of age, history of stroke and vascular MRI brain lesions. This association, which has been described in neurological diseases including multiple sclerosis and dementias,[173] might reflect an underlying ongoing neurodegenerative process in AF. Indeed, AF has been previously associated with reduced brain volume independent of ischemic infarcts, with putative explanations being cerebral microinfarcts or hypoperfusion leading to brain atrophy.[201, 202]

Despite the in-depth neuroimaging patient characterization, the clinical model still explained a larger proportion of the sNfL variance than the MRI model. We can only speculate on the reasons for this: It is possible that microischemic, hemodynamic, degenerative or other, yet unknown processes lead to neuronal damage in AF while remaining undetected on conventional MRI.

Finally, sNfL was inversely associated with cognitive performance in AF patients. This is in line with previous research on the association of sNfL with cognitive measures in patients with small vessel[198] and neurodegenerative diseases,[203-206] and suggests that sNfL is a nondisease-specific marker of neuronal damage resulting in cognitive dysfunction. The association of sNfL with cognitive measures grew markedly weaker after adjusting for age and was further attenuated in the multivariable clinical and MRI models, indicating that a multitude of factors including ageing, comorbidities and vascular brain lesions contribute to or mediate the association of neuronal damage with cognitive dysfunction in AF. Importantly, the association of sNfL with PC1, the summary cognitive measure, persisted after adjustment for all clinical and neuroimaging parameters, suggesting that additional unknown factors might contribute to this association. Among the separate cognitive measures, the strongest associations of sNfL were with TMT-A, TMT-B and – to a lesser extent – DSST, all measures of executive function, processing speed and attention. Preferential changes in these cognitive domains are known to reflect a vascular profile of cognitive dysfunction[207] and have been previously associated with AF.[208]

The strengths of our study include (i) its large sample size of AF patients with detailed clinical, neuroimaging and cognitive characterization, allowing for adjustment for several confounding factors and thus reducing the risk of spurious findings, (ii) the standardized manner of data acquisition, high rate of data completeness and blinded MRI assessment and sNfL measurement, reducing the risk of bias and (iii) its multicenter design, indicating a certain generalizability of our results, at least within the Caucasian population of central Europe. However, the following limitations must be acknowledged: (i) The study's cross-sectional design, which allows only for assessment of association but not causality thereof. (ii) As Swiss-AF included exclusively AF patients, we did not have a comparison group of patients with other heart diseases or healthy controls. It is therefore unknown whether our results are specific to AF. (iii) A large number of Swiss-AF patients did not undergo brain MRI due to contraindications or claustrophobia and were thus ineligible for this study. It is therefore unknown whether our results are generalizable to AF patients unsuited for brain MRI. (iv) We were not able to adjust our analyses for diseases of the peripheral nervous system, which were not systematically collected in Swiss-AF but might contribute to sNfL.[173] (v) Neuroimaging was performed on 1.5 or 3.0 Tesla scanners, which might miss a relevant proportion of microinfarcts compared to higher resolution MRI.[209]

In conclusion, our study demonstrates the potential of sNfL as a tool to explore the mechanisms that underly cognitive dysfunction in AF. It seems likely that neuronal damage in AF results from a complex interplay between subclinical brain ischemia, altered hemodynamics and neurodegeneration. sNfL holds promise not only as an instrument to investigate the intricate mechanisms underlying the heart-brain interactions, but also as a surrogate outcome parameter for brain health and cognitive function in cardiovascular research. In future Swiss-AF analyses we plan to investigate the prognostic significance of sNfL and other blood-based biomarkers of cardiovascular disease longitudinally with regards to the development of vascular brain lesions, brain atrophy and cognitive dysfunction over time.

Tables

Table 9.1.1. Patient demographic, clinical and MRI characteristics

	All patients (N = 1,379)		Patients without stroke/TIA (N = 1,125)
demographic and clinical data		missing values rate	
age, years, mean (SD)	72.3 (8.6)	0 %	71.7 (8.8)
sex, female, N (%)	374 (27.1)	0 %	297 (26.4)
AF-type, N (%)		0 %	
paroxysmal	636 (46.1)		510 (45.3)
persistent	408 (29.6)		349 (31.0)
permanent	335 (24.3)		266 (23.6)
history of			
hypertension, N (%)	930 (67.4)	0 %	741 (65.9)
diabetes mellitus, N (%)	190 (13.8)	0 %	146 (13.0)
stroke or transient ischemic attack, N (%)	254 (18.4)	0 %	0 (0)
coronary heart disease, N (%)	364 (26.4)	0 %	292 (26.0)
peripheral artery disease, N (%)	87 (6.3)	0 %	64 (5.7)
heart failure, N (%)	297 (21.5)	0 %	238 (21.2)
obstructive sleep apnea, N (%)	171 (12.4)	0 %	128 (11.4)
CHA_2DS_2-VASc score, median (IQR)	3 (2 – 4)	0 %	3 (2 – 4)
smoking status, N (%)		0.1 %	
nonsmoker	603 (43.8)		488 (43.4)
past smoker	671 (48.7)		553 (49.2)
active smoker	104 (7.5)		84 (7.5)
alcohol consumption, standard drinks / day, median (IQR)	0.6 (0.1 – 1.3)	0.1 %	0.6 (0.1 – 1.3)
education level, N (%)		0.1 %	
basic	157 (11.4)		128 (11.4)
middle	679 (49.3)		555 (49.3)
advanced	541 (39.3)		442 (39.3)
body mass index, kg/m^2, mean (SD)	27.5 (4.6)	0 %	27.6 (4.7)
systolic blood pressure, mmHg, mean (SD)	134.7 (18.7)	0.4 %	134.7 (18.6)
diastolic blood pressure, mmHg, mean (SD)	78.4 (11.9)	0.4 %	78.7 (11.9)
mean arterial pressure, mmHg, mean (SD)	97.2 (12.6)	0.4 %	97.3 (12.7)
oral anticoagulation, N (%)	1,240 (89.9)	0 %	1,004 (89.2)
MRI data			
small noncortical infarcts, N (%)	293 (21.2)	0 %	200 (17.8)
volume (if present), mm^3, median (IQR)	60 (30 – 150)		56 (30 – 123)
large noncortical and cortical infarcts, N (%)	288 (20.9)	0 %	153 (13.6)
volume (if present), mm^3, median (IQR)	1,374 (252 – 7,454)		585 (162 – 4,002)
white matter lesions, N (%)	1,368 (99.2)	0 %	1,116 (99.2)
volume (if present), mm^3, median (IQR)	3,662 (1,350 – 9,197)		3,335 (1,224 – 8,252)
microbleeds, N (%)	291 (21.1)	0 %	220 (19.6)
count (if present), median (IQR)	1 (1 – 2)		1 (1 – 2)
normalized brain volume, cm^3, median (IQR)	1,411 (1,354 – 1,478)	0 %	1,417 (1,358 – 1,487)

SD, standard deviation; IQR, interquartile range

Table 9.1.2. Association of patients' clinical and MRI characteristics with sNfL

Variables (N = 1,379)	**Clinical model*** AIC = 2079.05 R^2 = 0.36, R^2_{adj} = 0.36		**MRI model** AIC = 2148.29 R^2 = 0.33, R^2_{adj} = 0.32		**Combined model** AIC = 2040.56 R^2 = 0.39, R^2_{adj} = 0.38	
	β_{mult}† [95% CI]	**p-value**	**β_{mult}† [95% CI]**	**p-value**	**β_{mult}† [95% CI]**	**p-value**
age (per 10 years)	1.411 [1.365, 1.460]	< 0.001	1.367 [1.312, 1.424]	< 0.001	1.325 [1.272, 1.380]	< 0.001
BMI (per 5 kg/m^2)	0.925 [0.897, 0.955]	< 0.001			0.927 [0.899, 0.957]	< 0.001
MAP (per 10 mmHg)	0.961 [0.939, 0.983]	< 0.001			0.958 [0.937, 0.980]	< 0.001
MAP^2 (per 10 mmHg)	1.019 [1.008, 1.031]	0.001			1.019 [1.007, 1.030]	0.002
history of hypertension	1.068 [1.003, 1.138]	0.042			1.030 [0.967, 1.098]	0.351
history of diabetes mellitus	1.283 [1.181, 1.394]	< 0.001			1.268 [1.168, 1.376]	< 0.001
history of stroke or TIA	1.137 [1.059, 1.220]	< 0.001			1.056 [0.978, 1.141]	0.166
history of peripheral artery disease	1.231 [1.098, 1.380]	< 0.001			1.195 [1.068, 1.337]	0.002
history of heart failure	1.181 [1.102, 1.266]	< 0.001			1.157 [1.081, 1.239]	< 0.001
past smoker (ref: nonsmoker)	0.925 [0.873, 0.980]	0.008			0.930 [0.878, 0.984]	0.012
active smoker (ref: nonsmoker)	0.950 [0.850, 1.060]	0.358			0.944 [0.847, 1.053]	0.301
alcohol consumption (per 1 standard drink daily)	0.984 [0.965, 1.002]	0.086			0.981 [0.963, 1.000]	0.045
presence of LNCCIs			1.100 [1.025, 1.180]	0.008	1.049 [0.975, 1.128]	0.199
log-volume of LNCCIs			1.066 [1.035, 1.099]	< 0.001	1.055 [1.025, 1.087]	< 0.001
presence of SNCIs			1.055 [0.980, 1.136]	0.156	1.036 [0.965, 1.113]	0.330
log-volume of SNCIs			1.030 [0.978, 1.085]	0.262	1.020 [0.970, 1.072]	0.442
presence of MBs			1.104 [1.011, 1.207]	0.028	1.079 [0.991, 1.176]	0.079
count of MBs			1.017 [0.987, 1.047]	0.266	1.013 [0.985, 1.042]	0.372
log-volume of WMLs			1.045 [1.019, 1.071]	< 0.001	1.043 [1.018, 1.068]	<0.001
nBV (per 100cm^3)			0.945 [0.914, 0.978]	0.001	0.951 [0.919, 0.983]	0.003

* Sex, atrial fibrillation type, history of coronary heart disease and obstructive sleep apnea were eliminated from the final, reduced clinical model.

† The back-transformed model-based estimates β_{mult} represent multiplicative effects on sNfL (e.g., β_{mult} = 1.325 for age denotes an average 1.325-fold increase in sNfL concentration, that is an average 32.5% sNfL increase, per 10 years older age.)

AIC, Akaike's information criterion; BMI, body mass index; MAP, mean arterial pressure; TIA, transient ischemic attack; LNCCIs, large noncortical or cortical infarcts; SNCIs, small noncortical infarcts; MBs, microbleeds; WMLs, white matter lesions; nBV, normalized brain volume

Table 9.1.3. Association of log-sNfL with measures of cognitive function

Cognitive measures (N = 1,363)	univariable		age-adjusted		multivariable clinical model*		multivariable MRI model†		multivariable combined model‡	
	β§ [95% CI]	p-value	β§ [95% CI]	p-value	β§ [95% CI]	p-value	β§ [95% CI]	p-value	β§ [95% CI]	p-value
MoCA	-0.93 [-1.17, -0.69]	< 0.001	-0.37 [-0.65, -0.10]	0.008	-0.23 [-0.51, 0.05]	0.114	-0.22 [-0.51, 0.06]	0.123	-0.15 [-0.44, 0.14]	0.307
TMT-A	-0.11 [-0.13, -0.10]	< 0.001	-0.04 [-0.06, -0.02]	< 0.001	-0.04 [-0.06, -0.02]	< 0.001	-0.03 [-0.04, -0.01]	0.009	-0.03 [-0.04, -0.01]	0.012
TMT-B	-0.06 [-0.07, -0.05]	< 0.001	-0.02 [-0.03, -0.01]	< 0.001	-0.02 [-0.03, -0.01]	< 0.001	-0.01 [-0.02, -0.00]	0.015	-0.01 [-0.02, -0.00]	0.023
SVF	-1.56 [-2.00, -1.12]	< 0.001	-0.46 [-0.97, 0.05]	0.076	-0.29 [-0.81, 0.24]	0.283	-0.14 [-0.67, 0.38]	0.587	-0.09 [-0.62, 0.45]	0.752
DSST	-6.95 [-8.07, -5.83]	< 0.001	-2.40 [-3.64, -1.16]	< 0.001	-1.75 [-2.96, -0.54]	0.005	-0.98 [-2.23, 0.27]	0.124	-0.84 [-2.05, 0.37]	0.175
PC1	-0.98 [-1.11, -0.84]	< 0.001	-0.34 [-0.49, -0.20]	< 0.001	-0.27 [-0.41, -0.12]	< 0.001	-0.18 [-0.33, -0.03]	0.016	-0.16 [-0.31, -0.01]	0.032

* adjusted for age, sex, education level, history of hypertension, diabetes mellitus, stroke or TIA, coronary heart disease, peripheral artery disease, heart failure, obstructive sleep apnea, BMI, smoking status, alcohol consumption

† adjusted for age, normalized brain volume, presence and volume of small noncortical infarcts, presence and volume of large noncortical or cortical infarcts, burden of MBs, volume of white matter lesions

‡ adjusted for age, sex, education level, history of hypertension, diabetes mellitus, stroke or TIA, coronary heart disease, peripheral artery disease, heart failure, obstructive sleep apnea, BMI, smoking status, alcohol consumption, normalized brain volume, presence and volume of small noncortical infarcts, presence and volume of large noncortical or cortical infarcts, burden of MBs, volume of white matter lesions

§ The model-based estimates β represent additive effects on test score (e.g., β = -0.93 for the association of log-sNfL with MoCA denotes an average decrease of 0.93 points in the MoCA score per unit higher log-sNfL, or an average decrease of approx. 0.09 points in the MoCA score per 10% higher sNfL concentration)

MoCA, Montreal Cognitive Assessment; TMT, Trail Making Test; SVF, Semantic Verbal Fluency; DSST, Digit Symbol Substitution Test; PC1, first principal component

Figures

Figure 9.1.1. Boxplots of sNfL distribution stratified to CHA_2DS_2-VASc score

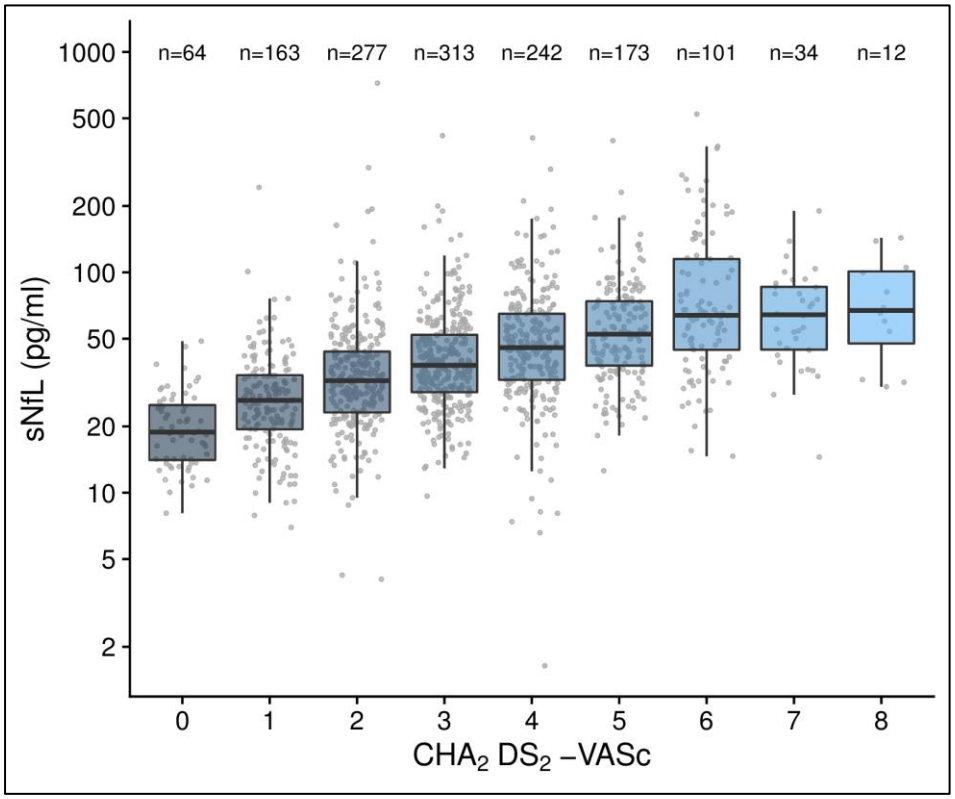


Figure 9.1.2. Multiplicative effect sizes of the association of clinical and MRI variables with sNfL from the combined model

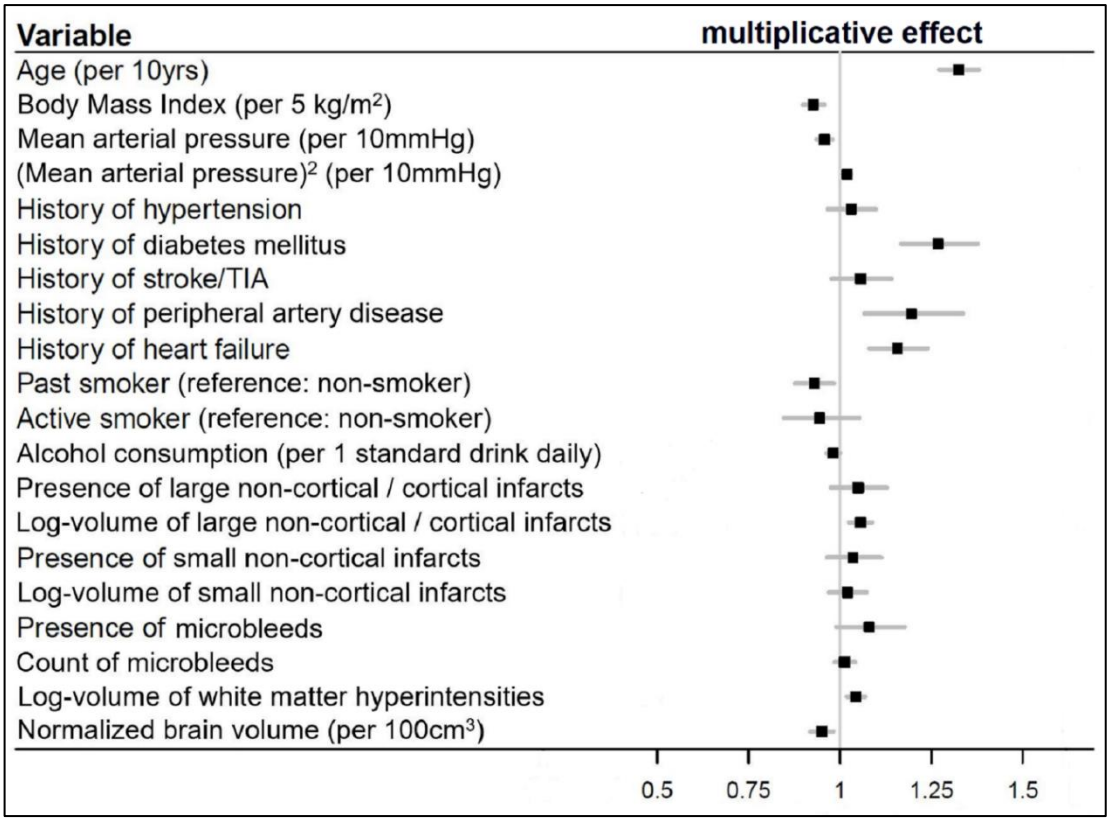


Figure 9.1.3. Scatter plot of the association of (A) mean arterial pressure and (B) normalized brain volume with sNfL. The solid line represents the predicted values from the combined clinical and MRI model and the dashed lines represent the 95% pointwise confidence intervals.

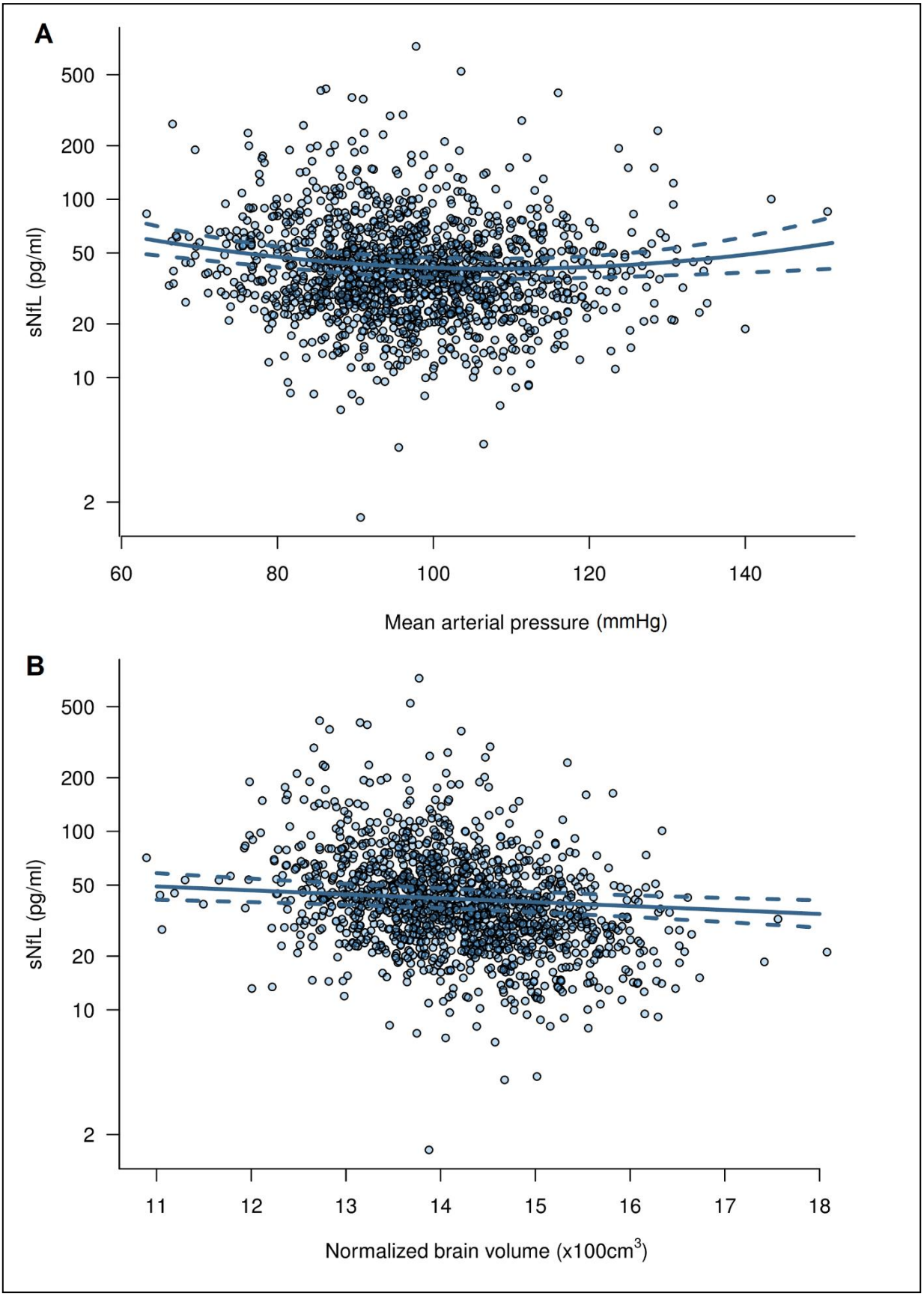


Figure 9.1.4. Model-based estimates for the association of log-sNfL with PC1

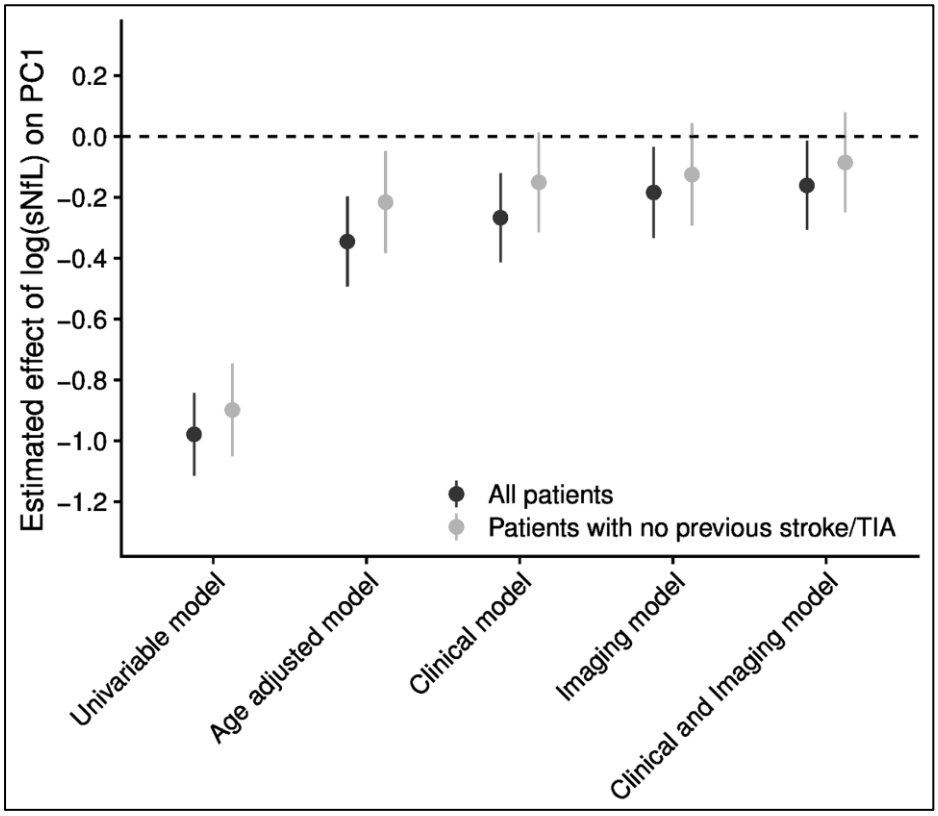
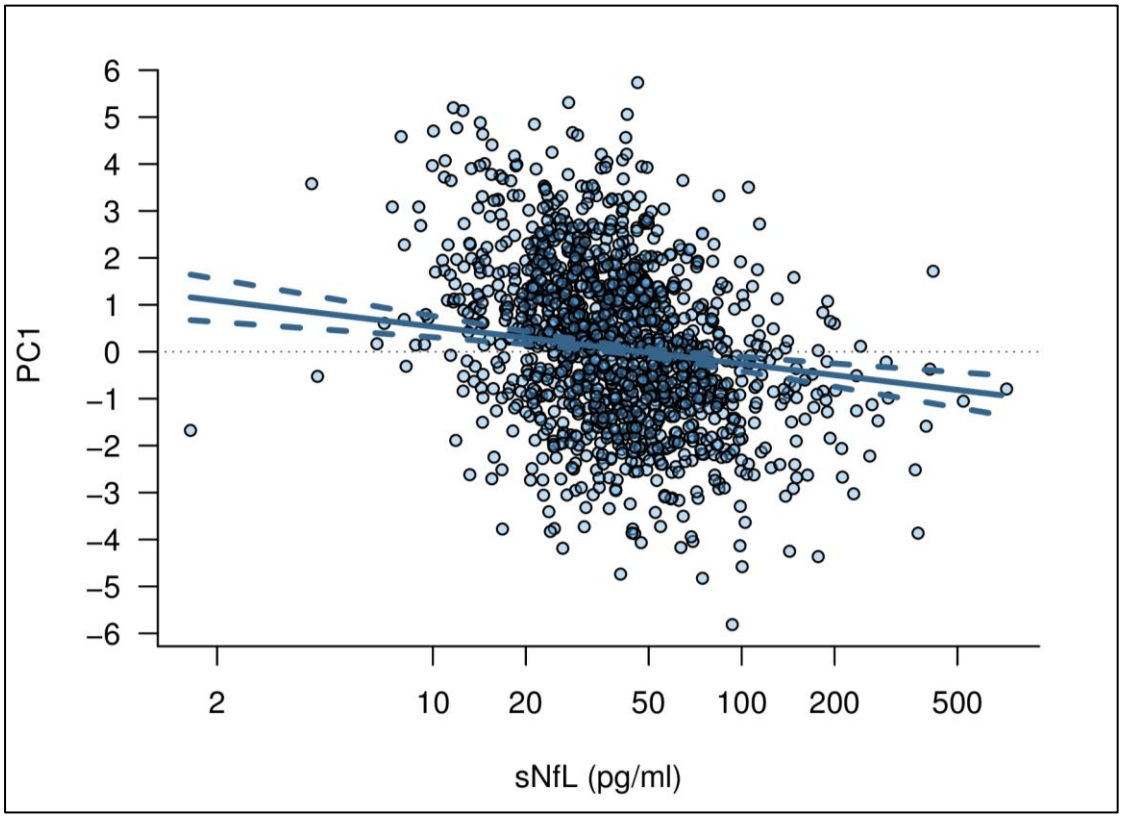
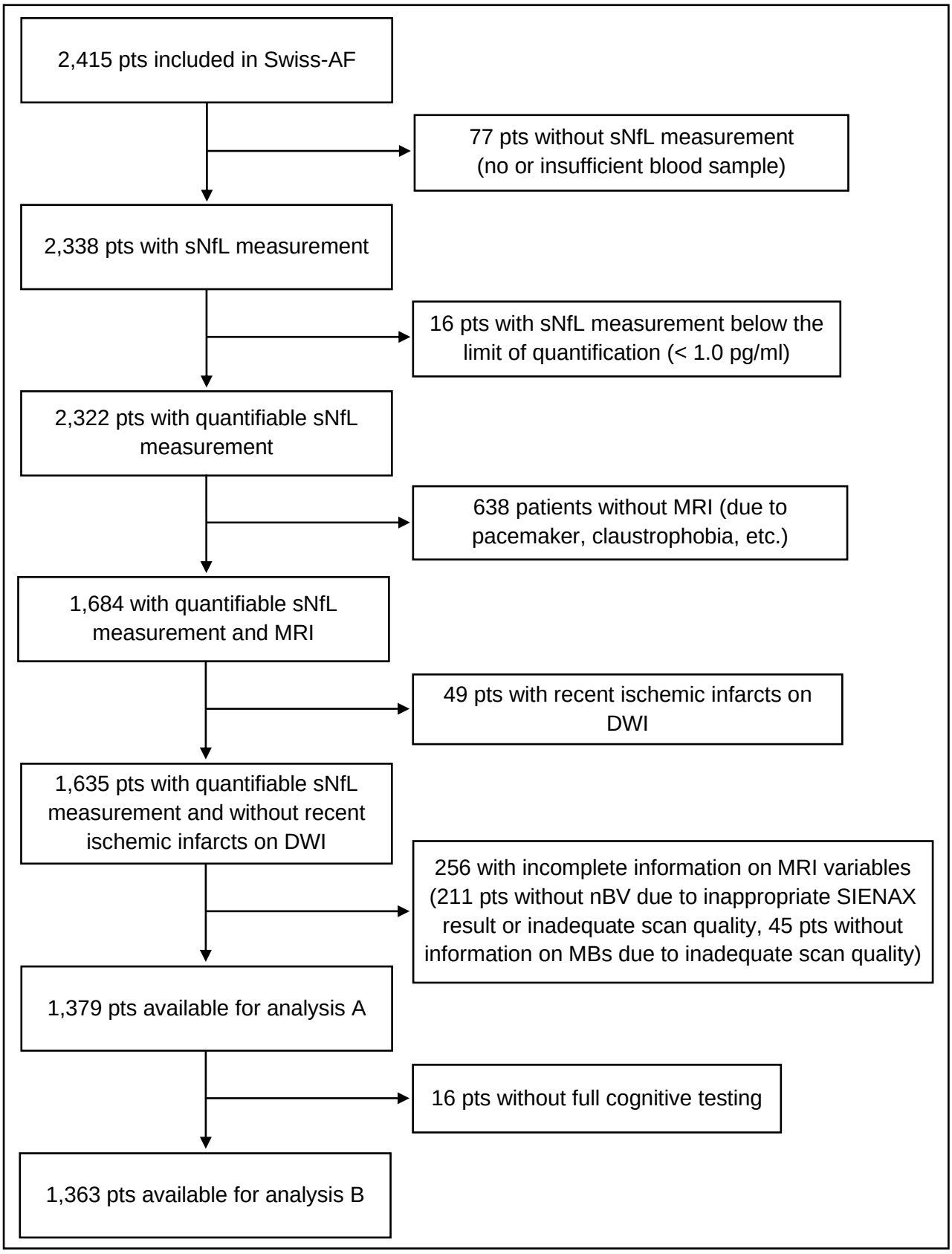


Figure 9.1.5. Scatter plot of the age-adjusted association of sNfL with PC1. The solid line represents the model-based predicted values and the dashed lines represent the pointwise 95% confidence intervals.



Supplementary Material

Supplementary Figure 9.1.1. Study flowchart



Supplementary Table 9.1.1. *Models for the association of the CHA_2DS_2-VASc score with sNfL*

Variables (N = 1,379)	**Univariable** AIC = 2326.82		**Univariable** AIC = 2513.85		**Bivariable (age-adjusted)** AIC = 2143.07		**Bivariable (age-adjusted)** AIC = 2149.04		**Multivariable (adjusted for age and MRI markers of small vessel disease)** AIC = 2123.22		**Multivariable (adjusted for age and all MRI variables)** AIC = 2109.12	
	β_{mult} [95% CI]	**p-value**	**β_{mult} [95% CI]**	**p-value**	**β_{mult} [95% CI]**	**p-value**	**β_{mult} [95% CI]**	**p-value**	**β_{mult} [95% CI]**	**p-value**	**β_{mult} [95% CI]**	**p-value**
CHA_2DS_2-VASc score	1.192 [1.172, 1.213]	< 0.001			1.094 [1.073, 1.116]	< 0.001			1.083 [1.061, 1.105]	< 0.001	1.071 [1.049, 1.094]	< 0.001
modified CHA_2DS_2-VASc score w/o age component			1.168 [1.141, 1.195]	< 0.001			1.094 [1.072, 1.118]	< 0.001				
age (per 10 years)					1.336 [1.283, 1.391]	< 0.001	1.425 [1.378, 1.475]	< 0.001	1.299 [1.245, 1.355]	< 0.001	1.282 [1.226, 1.341]	< 0.001
presence of SNCIs									1.042 [0.968, 1.121]	0.275	1.037 [0.964, 1.116]	0.330
log-volume of SNCIs									1.006 [0.956, 1.059]	0.816	1.016 [0.965, 1.069]	0.546
presence of MBs									1.075 [0.984, 1.174]	0.109	1.079 [0.988, 1.178]	0.089
count of MBs									1.020 [0.991, 1.050]	0.183	1.020 [0.991, 1.049]	0.184
log-volume of WMLs									1.039 [1.014, 1.065]	0.002	1.037 [1.012, 1.063]	0.004
presence of LNCCIs											1.045 [0.973, 1.122]	0.224
log-volume of LNCCIs											1.053 [1.022, 1.086]	< 0.001
nBV (per 100cm^3)											0.957 [0.926, 0.990]	0.010

AIC, Akaike's information criterion; SNCIs, small noncortical infarcts; MBs, microbleeds; WMLs, white matter lesions; LNCCIs, large noncortical or cortical infarcts; nBV, normalized brain volume

Supplementary Table 9.1.2. Models for the association of the CHA_2DS_2-VASc score with sNfL in patients without history of stroke or transient ischemic attack

Variables (N = 1,125)	**Univariable** AIC = 1829.11		**Univariable** AIC = 2022.98		**Bivariable (age-adjusted)** AIC = 1676.24		**Bivariable (age-adjusted)** AIC = 1680.38		**Multivariable (adjusted for age and MRI markers of small vessel disease)** AIC = 1667.01		**Multivariable (adjusted for age and all MRI variables)** AIC = 1659.79	
	β_{mult} [95% CI]	**p-value**	**β_{mult} [95% CI]**	**p-value**	**β_{mult} [95% CI]**	**p-value**	**β_{mult} [95% CI]**	**p-value**	**β_{mult} [95% CI]**	**p-value**	**β_{mult} [95% CI]**	**p-value**
CHA_2DS_2-VASc score	1.237 [1.210, 1.264]	< 0.001			1.103 [1.073, 1.133]	< 0.001			1.094 [1.064, 1.124]	< 0.001	1.089 [1.060, 1.119]	< 0.001
modified CHA_2DS_2-VASc score w/o age component			1.201 [1.163, 1.239]	< 0.001			1.104 [1.073, 1.136]	< 0.001				
age (per 10 years)					1.337 [1.279, 1.398]	< 0.001	1.433 [1.384, 1.485]	< 0.001	1.304 [1.245, 1.366]	< 0.001	1.288 [1.226, 1.352]	< 0.001
presence of SNCIs									1.031 [0.948, 1.121]	0.479	1.032 [0.949, 1.122]	0.462
log-volume of SNCIs									0.995 [0.934, 1.060]	0.878	0.996 [0.935, 1.061]	0.910
presence of MBs									1.070 [0.971, 1.179]	0.173	1.071 [0.972, 1.181]	0.164
count of MBs									1.016 [0.984, 1.048]	0.331	1.015 [0.984, 1.047]	0.353
log-volume of WMLs									1.035 [1.008, 1.063]	0.011	1.032 [1.006, 1.060]	0.018
presence of LNCCIs											1.076 [0.981, 1.179]	0.120
log-volume of LNCCIs											1.068 [1.023, 1.114]	0.003
nBV (per 100cm^3)											0.968 [0.933, 1.004]	0.079

AIC, Akaike's information criterion; SNCIs, small noncortical infarcts; MBs, microbleeds; WMLs, white matter lesions; LNCCIs, large noncortical or cortical infarcts; nBV, normalized brain volume

Supplementary Table 9.1.3. Results of the clinical, MRI and combined models for the association of clinical and neuroimaging parameters with sNfL in patients without history of stroke or transient ischemic attack

Variables (N = 1,125)	**Clinical model*** AIC = 1601.63 R^2 = 0.39, R^2_{adj} = 0.39		**MRI model** AIC = 1695.45 (R^2 = 0.34, R^2_{adj} = 0.33)		**Combined model** AIC = 1583.89 R^2 = 0.41, R^2_{adj} = 0.40	
	β_{mult} [95% CI]	**p-value**	**β_{mult} [95% CI]**	**p-value**	**β_{mult} [95% CI]**	**p-value**
age (per 10 years)	1.425 [1.377, 1.474]	< 0.001	1.389 [1.331, 1.451]	< 0.001	1.349 [1.293, 1.408]	< 0.001
BMI (per 5 kg/m^2)	0.937 [0.907, 0.968]	< 0.001			0.934 [0.904, 0.965]	< 0.001
MAP (per 10 mmHg)	0.967 [0.944, 0.991]	0.006			0.962 [0.939, 0.985]	0.001
MAP^2 (per 10 mmHg)	1.018 [1.005, 1.030]	0.005			1.017 [1.005, 1.030]	0.006
history of obstructive sleep apnea	0.931 [0.848, 1.021]	0.130			0.926 [0.845, 1.016]	0.104
history of diabetes mellitus	1.357 [1.241, 1.485]	< 0.001			1.338 [1.224, 1.463]	< 0.001
history of peripheral artery disease	1.251 [1.102, 1.420]	< 0.001			1.199 [1.056, 1.361]	0.005
history of heart failure	1.227 [1.140, 1.320]	< 0.001			1.201 [1.116, 1.291]	< 0.001
past smoker (ref: nonsmoker)	0.923 [0.869, 0.981]	0.010			0.928 [0.873, 0.985]	0.015
active smoker (ref: nonsmoker)	0.962 [0.856, 1.082]	0.521			0.959 [0.854, 1.077]	0.478
alcohol consumption (per 1 standard drink daily)	0.986 [0.967, 1.005]	0.156			0.984 [0.965, 1.003]	0.093
presence of LNCCIs			1.087 [0.990, 1.194]	0.079	1.046 [0.956, 1.143]	0.328
log-volume of LNCCIs			1.069 [1.024, 1.117]	0.002	1.060 [1.017, 1.105]	0.006
presence of SNCIs			1.034 [0.950, 1.126]	0.442	1.011 [0.933, 1.097]	0.786
log-volume of SNCIs			1.006 [0.943, 1.072]	0.864	0.991 [0.932, 1.053]	0.772
presence of MBs			1.101 [0.998, 1.215]	0.055	1.071 [0.975, 1.176]	0.152
count of MBs			1.012 [0.980, 1.045]	0.455	1.013 [0.983, 1.044]	0.404
log-volume of WMLs			1.040 [1.013, 1.069]	0.004	1.038 [1.012, 1.065]	0.004
nBV (per 100cm^3)			0.954 [0.920, 0.990]	0.013	0.959 [0.925, 0.994]	0.021

* Sex, atrial fibrillation type, history of hypertension and coronary heart disease were eliminated from the final, reduced clinical model.

AIC, Akaike's information criterion; BMI, body mass index; MAP, mean arterial pressure; TIA, transient ischemic attack; LNCCIs, large noncortical or cortical infarcts; SNCIs, small noncortical infarcts; MBs, microbleeds; WMLs, white matter lesions; nBV, normalized brain volume

Supplementary Table 9.1.4. Association of log-sNfL with measures of cognitive function in patients without history of stroke or transient ischemic attack

Cognitive measures (N = 1,113)	univariable		age-adjusted		multivariable clinical model*		multivariable MRI model†		multivariable combined model‡	
	β [95% CI]	p-value	β [95% CI]	p-value	β [95% CI]	p-value	β [95% CI]	p-value	β [95% CI]	p-value
MoCA	-0.89 [-1.15, -0.63]	< 0.001	-0.37 [-0.68, -0.06]	0.019	-0.22 [-0.53, 0.10]	0.174	-0.29 [-0.60, 0.02]	0.071	-0.17 [-0.49, 0.15]	0.287
TMT-A	-0.10 [-0.12, -0.08]	< 0.001	-0.03 [-0.05, -0.00]	0.024	-0.02 [-0.05, 0.00]	0.052	-0.02 [-0.04, 0.01]	0.158	-0.01 [-0.04, 0.01]	0.202
TMT-B	-0.05 [-0.06, -0.04]	< 0.001	-0.01 [-0.02, -0.00]	0.011	-0.01 [-0.02, -0.00]	0.048	-0.01 [-0.02, 0.00]	0.089	-0.01 [-0.02, 0.00]	0.162
SVF	-1.34 [-1.83, -0.84]	< 0.001	-0.16 [-0.75, 0.42]	0.589	0.02 [-0.59, 0.62]	0.955	0.02 [-0.57, 0.62]	0.940	0.15 [-0.46, 0.76]	0.630
DSST	-6.09 [-7.34, -4.85]	< 0.001	-1.07 [-2.47, 0.34]	0.136	-0.69 [-2.05, 0.67]	0.318	-0.30 [-1.71, 1.11]	0.674	-0.15 [-1.52, 1.21]	0.828
PC1	-0.90 [-1.05, -0.75]	< 0.001	-0.22 [-0.38, -0.05]	0.012	-0.15 [-0.31, 0.01]	0.072	-0.12 [-0.29, 0.04]	0.145	-0.08 [-0.25, 0.08]	0.309

* adjusted for age, sex, education level, history of hypertension, diabetes mellitus, stroke or TIA, coronary heart disease, peripheral artery disease, heart failure, obstructive sleep apnea, BMI, smoking status, alcohol consumption

† adjusted for age, normalized brain volume, presence and volume of small noncortical infarcts, presence and volume of large noncortical or cortical infarcts, burden of MBs, volume of white matter lesions

‡ adjusted for age, sex, education level, history of hypertension, diabetes mellitus, stroke or TIA, coronary heart disease, peripheral artery disease, heart failure, obstructive sleep apnea, BMI, smoking status, alcohol consumption, normalized brain volume, presence and volume of small noncortical infarcts, presence and volume of large noncortical or cortical infarcts, burden of MBs, volume of white matter lesions

MoCA, Montreal Cognitive Assessment; TMT, Trail Making Test; SVF, Semantic Verbal Fluency; DSST, Digit Symbol Substitution Test; PC1, first principal component

9.2. Renal function and body mass index contribute to serum neurofilament light levels in elderly patients with atrial fibrillation

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Abstract

Objective

Serum neurofilament light chain (sNfL) is increasingly used as a neuro-axonal injury biomarker in the elderly. Besides age, little is known about how other physiological factors like renal function and body mass index (BMI) alter its levels. Here, we investigated the association of estimated glomerular filtration rate (eGFR) and BMI with sNfL in a large sample of elderly atrial fibrillation (AF) patients.

Methods

This is a cross-sectional analysis from the Swiss-AF Cohort (NCT02105844). We measured sNfL using an ultrasensitive single-molecule array assay. We calculated eGFR using the Chronic-Kidney-Disease-Epidemiology-Collaboration creatinine ($eGFR_{crea}$) and creatinine-cystatin C ($eGFR_{crea\text{-}cys}$) formulas, and BMI from weight and height measurements. We evaluated the role of eGFR and BMI as determinants of sNfL levels using multivariable linear regression and the adjusted R^2 (R^2_{adj}).

Results

Among 2,277 Swiss-AF participants (mean age 73.3 years), $eGFR_{crea}$ showed an inverse curvilinear association with sNfL after adjustment for age and cardiovascular comorbidities. BMI also showed an independent, inverse linear association with sNfL. The R^2_{adj} of models with age, $eGFR_{crea}$ and BMI alone was 0.26, 0.35 and 0.02, respectively. A model with age and $eGFR_{crea}$ combined explained 45% of the sNfL variance. Sensitivity analyses (i)further adjusting for vascular brain lesions (N=1,402 participants with MRI) and (ii)using $eGFR_{crea\text{-}cys}$ yielded consistent results.

Interpretation

In an elderly AF cohort, both renal function and BMI were associated with sNfL, but only renal function explained a substantial proportion of the sNfL variance. This should be taken into account when using sNfL in elderly patients or patients with cardiovascular disease.

Introduction

Neurofilament light chain (NfL) is a cytoskeletal protein exclusive to neurons. Following neuro-axonal damage it is released into the extracellular space, cerebrospinal fluid and eventually peripheral blood. Over the past years, NfL has been established as the first blood-based biomarker reflecting disease activity and treatment response in traumatic brain injury and neurodegenerative diseases.[125, 210, 211] Considering the increasing use of blood NfL as a biomarker for neurological diseases in clinical research and the perspective of its diagnostic and prognostic applications in individual clinical practice, a deeper understanding of its homeostasis (including distribution and clearance) in the blood compartment is needed to elucidate physiological factors that might affect its association with disease processes.[210, 211] This is becoming increasingly important for NfL-based investigations of normal ageing,[212] as well as cerebrovascular disease,[181, 198, 213, 214] atrial fibrillation (AF)[215] and dementia[126], where accumulating age-related comorbidities might both interfere with the homeostasis of NfL and directly induce neuronal damage *per se*.[210, 211]

While the association of NfL blood levels with age has been consistently demonstrated across a variety of patient populations and healthy controls,[125, 212] their association with renal function and body mass index (BMI) was only recently reported in elderly diabetics and younger patients with multiple sclerosis, respectively.[186, 216, 217] However, little is known on how these factors impact on NfL concentrations relative to age, to one another, and to cardiovascular comorbidities and vascular brain lesions, which are increasingly prevalent in the elderly.[218, 219] Such data are necessary for a systematic appraisal of the importance of these factors as potential confounders and the need to account for them in future use of NfL as a laboratory measure in elderly individuals.

With this in mind, we investigated the association of (i) estimated glomerular filtration rate (eGFR) and (ii) BMI with serum NfL (sNfL) concentrations in a large, well-characterized cohort of elderly AF patients accounting for age, cardiovascular comorbidities, as well as vascular brain lesions and brain volume on neuroimaging.

Methods

Study design, patient population and data collection

This was a cross-sectional analysis using baseline data from the prospective observational Swiss-AF cohort study (NCT02105844), which was designed to investigate the relationship between AF, structural brain changes and cognition. We selected the Swiss-AF cohort for this analysis due to the large sample size, the detailed clinical and neuroimaging characterization with a relatively high prevalence of cardiovascular comorbidities, and the availability of blood biomarker measurements. Swiss-AF enrolled 2,415 patients with AF between 2014 and 2017 across 14 centres in Switzerland. Included were patients aged 65 years or older, with an additional 15% of patients aged < 65 years. Patients with a recent ischemic stroke, transient ischemic attack (TIA) or other acute illness (< 4 weeks) and those unable to provide consent (e.g., patients with dementia) were excluded. The detailed methodology of Swiss-AF has been described previously.[110, 123, 215] Baseline investigations included a standardized clinical assessment (sociodemographic parameters, comorbidities), weight and height measurements [from which BMI was calculated as weight in kg / (height in m)^2], blood sampling and brain MRI.

Baseline blood samples were collected following standard operating procedures. After centrifugation, serum samples were aliquoted into cryotubes and stored at -80°C in a centralized biobank. The concentration of sNfL was measured in duplicate using a previously described ultrasensitive single-molecule array assay (lower limit of quantification 1.0 pg/ml).[215, 220] Creatinine and cystatin C were measured using commercially available assays (cobas c 311 and Elecsys®; Roche Diagnostics, Mannheim, Germany). In order to calculate eGFR as a measure of renal function, we used the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) (i) creatinine equation ($eGFR_{crea}$) and (ii) the combined creatinine-cystatin C equation ($eGFR_{crea\text{-}cys}$).[221]

On baseline MRI we assessed the presence and volume of small noncortical infarcts (SNCIs), large noncortical infarcts or cortical infarcts (LNCCIs) and white matter lesions (WMLs), the presence and count of microbleeds (MBs), and estimated the normalized brain volume (nBV) using SIENAX,[222] as described previously in detail.[110, 215]

In this study we included all Swiss-AF patients with quantifiable sNfL measurement and available data on clinical variables, creatinine and cystatine C (Supplementary Figure 9.2.1). The Ethics Committee of Northwest and Central Switzerland approved Swiss-AF including this study (PB_2016-00793). Written informed consent was obtained from all study participants according to the Declaration of Helsinki. This study was conducted in accordance with the STROBE Statement for cross-sectional studies.[145]

Statistical analyses

Main analysis

As the first step to investigate the association of eGFR and BMI with sNfL, we fitted a multivariable linear regression model with log-transformed sNfL as the dependent variable and eGFR and BMI as independent variables. The model was adjusted for the following variables, known to be associated with sNfL from our previous work in this cohort[215]: age, history of hypertension, diabetes mellitus, stroke or TIA, peripheral artery disease, heart failure, as well as mean arterial pressure [calculated as (1/3 x systolic blood pressure) + (2/3 x diastolic blood pressure)], smoking status (non, past and current smoker) and alcohol consumption (in standard drinks per day). Continuous variables were centered on their mean (or, in case of skewed data, median) values, as appropriate. Visual inspection suggested curvilinear associations of age and eGFR with sNfL. We chose the best way to model these variables by fitting different univariable models (linear only; quadratic; cubic; cubic without quadratic term) and selecting the one with the best fit based on the Akaike's information criterion (AIC). Considering the known strong association of age and diabetes with sNfL,[215] we also included in the multivariable model the interactions GFR by age, BMI by age and GFR by diabetes. We report the back-transformed model-based estimates, which represent multiplicative effects on the geometric mean of sNfL and are denoted by β_{mult} (so that a one-unit increase in the independent variable is associated with an average β_{mult}-fold change in sNfL), along with 95% confidence intervals (95%-CI) and two-sided p-values.

In a second step, to investigate the relative contribution of eGFR, BMI and age to the variance of sNfL concentrations, we fitted linear models with log-sNfL as the dependent variable and these factors as independent variables, alone and in combination with one another and with their interactions with age. We report the coefficient of determination (R^2) and the adjusted R^2 (R^2_{adj}; penalized for larger numbers of independent variables) as a measure of the proportion of the observed sNfL variance explained by each model.

Sensitivity analyses

Considering patients who also had baseline brain MRI available (Supplementary Figure 9.2.1), we further adjusted the multivariable linear model from the main analysis for the following imaging measures: log-volume of WMLs, presence and log-volume of LNCCIs, presence and log-volume of SNCIs, presence and count of MBs, and nBV, which were previously reported to be associated with sNfL concentrations.[215] We reduced the model to a smaller set of variables via stepwise backward elimination based on AIC. As this is known to inflate the type I error, we refrained from providing p-values in this analysis and evaluated the explanatory importance of independent variables for sNfL concentrations based on whether they were selected or eliminated from the reduced model. Finally, we refitted the model without eGFR and report the R^2 and R^2_{adj} for both models. We repeated all models using $eGFR_{crea\text{-}cys}$ as a further sensitivity analysis.

All analyses were performed with R version 4.0.3 (2020-10-10).

Results

Main analysis

A total of 2,277 Swiss-AF patients were available for the main analysis, after exclusion of 77 patients without sNfL measurement (no or insufficient blood sample), 16 with sNfL measurement below the limit of quantification and 45 with other data missing (Supplementary Figure 9.2.1). The mean (standard deviation (SD)) age was 73.3 (8.5) years, mean (SD) $eGFR_{crea}$ / $eGFR_{crea\text{-}cys}$ was 59.1 (18.3) / 58.6 (20.0) ml/min/1.73m2, mean (SD) BMI was 27.6 (4.7) kg/m2 and the median (interquartile range) sNfL was 42.0 (29.0 – 65.1) pg/ml. All patients' characteristics are provided in Table 9.2.1.

In the multivariable model adjusted for all clinical variables (Table 9.2.2), $eGFR_{crea}$ showed a strong inverse curvilinear association with sNfL (Figure 9.2.1A). Modelling $eGFR_{crea}$ with a linear, quadratic and cubic component was chosen based on AIC (Supplementary Table 9.2.1). BMI also showed a strong, inverse linear association with sNfL in the multivariable model (Figure 9.2.2A).

Furthermore, age (modelled with a linear and cubic component based on AIC; Supplementary Table 9.2.1) was strongly, positively associated with sNfL in the multivariable model. There was evidence for an interaction between $eGFR_{crea}$ and age on their association with sNfL ($p_{interaction}<0.001$), indicating that, with older age, the negative association of $eGFR_{crea}$ with sNfL was steeper for lower values of $eGFR_{crea}$ (Figure 9.2.1B). There was also evidence for a weaker interaction between BMI and age on their association with sNfL ($p_{interaction}=0.013$), indicating a slightly stronger negative association of BMI with sNfL with increasing age (Figure 9.2.2B). Supplementary Table 9.2.2 presents the model-based estimates for the association of eGFRcrea and BMI with sNfL in four age-quartile subgroups.

Further variables with a strong association with sNfL in the multivariable model were diabetes mellitus and history of stroke or TIA. There was no evidence for an interaction between $eGFR_{crea}$ and diabetes mellitus and their association with sNfL.

Upon examination of the R^2_{adj} of different models fitted with sNfL as the dependent variable, models containing age, $eGFR_{crea}$ and BMI alone explained 26%, 35% and 2% of the variance in sNfL concentrations, respectively. Adding $eGFR_{crea}$ to the age model conferred a substantial increase in the sNfL variance explained by the model (R^2_{adj} 0.45 vs 0.26), while adding BMI to age increased the

model's explanatory power only marginally (R^2_{adj} 0.27 vs 0.26). The combined age, $eGFR_{crea}$ and BMI model explained 46% of the total sNfL variance. Adding the interaction terms $eGFR_{crea}$ by age, and BMI by age to the models conferred no substantial increase in R^2_{adj} (Table 9.2.3).

Sensitivity analysis adjusting for MRI variables

A total of 1,402 Swiss-AF patients were available for the MRI sensitivity analysis (Supplementary Figure 9.2.1). In the multivariable model including all variables from the main analysis, vascular brain lesions and nBV, both $eGFR_{crea}$ and BMI remained in the model after stepwise backward elimination, as did age, its interaction with $eGFR_{crea}$, diabetes mellitus and history of stroke or TIA (Supplementary Table 9.2.3). The R^2_{adj} of this model was 52%, and dropped to 36% after excluding $eGFR_{crea}$.

Sensitivity analysis using $eGFR_{crea\text{-}cys}$

As for the main analysis using $eGFR_{crea}$, a total of 2,277 Swiss-AF participants were available for sensitivity analysis using $GFR_{crea\text{-}cys}$. Consistent with the main analysis, in a multivariable model adjusting for all clinical variables (Table 9.2.2), $eGFR_{crea\text{-}cys}$ was strongly associated with sNfL, with a curvilinear relationship including a linear, quadratic and cubic component (modelled as such based on AIC, Supplementary Table 9.2.1). BMI was also strongly associated with sNfL, as was age, diabetes mellitus and history of stroke or TIA. For all associations, the coefficients were of similar magnitude as in the main analysis. Consistent with the main analysis, there was evidence for an interaction between $eGFR_{crea\text{-}cys}$ and age ($p_{interaction}<0.001$). The interaction BMI by age was even weaker than in the main analysis ($p_{interaction}=0.057$). Examination of the R^2_{adj} of different models containing age, $eGFR_{crea\text{-}cys}$ and BMI either alone or in combination with one another revealed similar results with the main analysis, with $eGFR_{crea\text{-}cys}$ explaining a substantial proportion of the sNfL variance beyond that explained by age (Table 9.2.3).

A total of 1,402 Swiss-AF patients were available for the sensitivity analysis including MRI data and using $eGFR_{crea\text{-}cys}$. As in the main analysis, in the multivariable model including all clinical variables, vascular brain lesions and nBV, both $eGFR_{crea\text{-}cys}$ and BMI remained in the model after backward variable elimination, as did age, its interaction with $eGFR_{crea\text{-}cys}$, diabetes mellitus and history of stroke or TIA (Supplementary Table 9.2.3). The R^2_{adj} of this model was 52% with $eGFR_{crea\text{-}cys}$, dropping to 36% after excluding $eGFR_{crea\text{-}cys}$.

Discussion

This cross-sectional study on the association of eGFR and BMI with sNfL concentrations in a large elderly cohort of AF patients showed that both eGFR (estimated using either creatinine or creatinine and cystatine C) and BMI were strongly associated with sNfL concentrations. This was true even after adjustment for other parameters known to contribute to sNfL concentrations, including age, clinical comorbidities and MRI characteristics. Furthermore, eGFR, but not BMI, conferred a substantial increase in the explanatory power of models predicting sNfL concentrations, which was additional and independent to the contribution of age.

Our finding of a strong negative association of eGFR with sNfL concentrations confirms and refines previous observations.[186, 216] An inverse association between eGFR and blood NfL concentrations was recently shown in smaller samples of elderly patients with diabetes and healthy controls, and the renal clearance of blood NfL was proposed as one potential explanation.[186, 216] Here, we show that this

association is independent of age, BMI, and pre-existing disease (diabetes, stroke history and other cardiovascular comorbidities). The association between eGFR and sNfL was maintained independent of the method used for calculating eGFR, i.e., based on creatinine alone or combined creatinine – cystatin C. Importantly, the association persisted even after adjustment for brain volume, as well as for the presence and burden of ischemic infarcts and small vessel disease markers on neuroimaging, which are known to be associated with sNfL concentrations,[181, 198, 213, 215] indicating that it is not mediated through structural brain pathology. These findings further support that, apart from NfL release from damaged neurons, renal clearance seems to be a predominant factor determining NfL levels. Combined investigations of NfL in cerebrospinal fluid (CSF), blood and urine to confirm this are now under way in our laboratory. Additionally, we show here that the association of GFR with sNfL is nonlinear, with a steeper slope in lower eGFR values. Taken together with our finding that the association between sNfL and eGFR depends on age (which indicates that the impact of renal impairment on sNfL levels is even more pronounced in older than in younger patients), these data stress the importance of accounting for renal function when evaluating blood NfL concentrations in elderly populations, in whom chronic kidney disease is highly prevalent.[223]

We also found a strong inverse association between BMI and sNfL. This is in line with a previous observation from a large cohort of young multiple sclerosis patients and healthy controls, where a larger distribution volume and specifically a larger total blood volume was postulated to be a modifier of blood NfL levels.[217] Here, we expand on these findings by demonstrating that the association holds true also among elderly individuals, and is independent of age, eGFR, cardiovascular comorbidities and the presence and burden of vascular brain lesions on neuroimaging. Furthermore, we confirmed the linearity of the association and found only a weak interaction with age. These findings strengthen the evidence for the validity of this relationship and for dilution as the underlying mechanism.[210] It seems therefore appropriate to account for BMI when examining blood NfL concentrations across the entire age spectrum.

Our study provides a comprehensive assessment of the relative contribution of eGFR and BMI in determining NfL serum levels as physiological factors important in its homeostasis in the elderly. After adjustment for age and comorbidities and regardless of the GFR estimation formula, both eGFR and BMI showed an independent, strong inverse association with sNfL levels, with effect sizes in a similar order of magnitude as age. However, only age and eGFR explained relevant proportions of the sNfL variance. Age alone explained about one fourth, GFR alone explained approximately one third, and their combination almost half of the variance of sNfL concentrations in this elderly cardiovascular cohort. Adding BMI did not substantially increase the explanatory power of the model. Taken together, these findings suggest that diagnostic and prognostic applications of sNfL in elderly populations should account not only for age, but also for renal function to increase their clinical meaningfulness, while the contribution of BMI seems to be less important.

Consistent with our findings, two very recent studies also demonstrated the importance of renal function as a contributor to sNfL levels.[224, 225] While these studies featured smaller samples from normal ageing cohorts, they further support the key conclusions of our study which examined a significantly larger sample of elderly patients with cardiovascular disease. Consequently, a large reference database for sNfL levels developed recently from data of younger individuals to optimize the use of sNfL for individual application in patients with multiple sclerosis excluded control persons with eGFR < 60 ml/min/1.73m^2.[226]

The strengths of this study include: (i) the large sample size of elderly patients with a detailed and standardized clinical and neuroimaging characterization, allowing for the exhaustive adjustment for multiple factors that are known to contribute to sNfL concentrations, indicating that the observed associations are not spurious but reflect true relationships; (ii) the estimation of GFR using two different approaches (the CKD-EPI formula using creatinine alone and the more accurate combined creatinine – cystatin C formula[221]) that yielded highly consistent results; (iii) comprehensive statistical modelling investigating not only the association of eGFR and BMI with sNfL concentrations, but also their relative contribution to the variance of sNfL concentrations.

We acknowledge the following limitations: (i) The study's cross-sectional design, which allows only for the assessment of association but not causality thereof. (ii) Although our results persisted after adjustment for brain MRI characteristics, we were not able to adjust our analyses for diseases of the peripheral nervous system, which were not systematically collected in Swiss-AF but might contribute to sNfL concentrations.[125] (iii) As Swiss-AF included exclusively AF patients, we did not have a comparison group of elderly individuals without this arrhythmia. However, in light of recent studies showing consistent results in other patient populations, this limitation is unlikely to have influenced our key findings. (iv) As the Swiss-AF biosampling protocol did not include the acquisition of CSF or urine, this study was not able to examine whether the observed associations are exclusive to blood concentrations of NfL, and we may only speculate on their underlying mechanisms.

In conclusion, this study represents a comprehensive appraisal of how physiological factors including renal function and BMI are associated with and contribute to blood NfL concentrations in the elderly, thereby providing important insights into the homeostasis of this increasingly used biomarker. The role of renal function and BMI in the prediction of neurological outcomes with sNfL needs to be evaluated in prospective studies.

Tables

Table 9.2.1. Patient characteristics

Clinical characteristics of 2,277 Swiss-AF patients (main analysis)	
Age, years, mean (SD)	73.3 (8.5)
Sex, female, N (%)	615 (27.0)
History of atrial fibrillation, N (%)	2,277 (100.0)
History of hypertension, N (%)	1,599 (70.2)
History of diabetes mellitus, N (%)	395 (17.3)
History of stroke or transient ischemic attack, N (%)	452 (19.9)
History of peripheral artery disease, N (%)	183 (8.0)
History of heart failure, N (%)	604 (26.5)
Smoking status, N (%) nonsmoker past smoker current smoker	 999 (43.9) 1,111 (48.8) 167 (7.3)
Alcohol consumption, std. drinks / day, median (IQR)	0.5 (0.1 – 1.3)
Mean arterial pressure, mmHg, mean (SD)	92.6 (12.6)
Body mass index, kg/m^2, mean (SD)	27.6 (4.7)
$eGFR_{crea}$, ml/min/1.73m^2, mean (SD)	59.1 (18.3)
$eGFR_{crea\text{-}cys}$, ml/min/1.73m^2, mean (SD)	58.6 (20.0)
Serum neurofilament light chain, pg/ml, mean (SD)	42.0 (29.0 – 65.1)
MRI characteristics of 1,402 Swiss-AF patients (sensitivity analysis)	
Small noncortical infarcts, N (%) Volume (if present), mm^3, median (IQR)	308 (22.0) 62 (30 – 150)
Large noncortical and cortical infarcts, N (%) Volume (if present), mm^3, median (IQR)	299 (21.3) 1,350 (252 – 7,086)
White matter lesions, N (%) Volume (if present), mm^3, median (IQR)	1,390 (99.1) 3,753 (1,368 – 9,353)
Microbleeds, N (%) Count (if present), median (IQR)	302 (21.5) 1 (1 – 2)
Normalized brain volume, cm^3, mean (SD)	1,416 (94)

SD, standard deviation; IQR; interquartile range; $eGFR_{crea}$/$eGFR_{crea\text{-}cys}$; estimated glomerular filtration rate based on creatinine / creatinine - cystatin C

Table 9.2.2. Multivariable models for the association of eGFR and BMI with sNfL

Variables (N = 2,277)	**using $eGFR_{crea}$**			**using $eGFR_{crea-cys}$**		
	β_{mult}	**95%-CI**	**p-value**	**β_{mult}**	**95%-CI**	**p-value**
Age* (per decade)	1.293	[1.244, 1.344]	<0.001	1.229	[1.182, 1.278]	<0.001
[Age* (per decade)]^3	0.988	[0.977, 0.999]	0.032	0.989	[0.978, 1.000]	0.046
eGFR* (per 10 ml/min/1.73m^2)	0.888	[0.869, 0.907]	<0.001	0.869	[0.854, 0.886]	<0.001
[eGFR* (per 10 ml/min/1.73m^2)]^2	1.030	[1.023, 1.036]	<0.001	1.029	[1.024, 1.033]	<0.001
[eGFR* (per 10 ml/min/1.73m^2)]^3	0.998	[0.996, 1.000]	0.017	0.998	[0.997, 1.000]	<0.001
BMI* (per 5 kg/m^2)	0.898	[0.878, 0.919]	<0.001	0.891	[0.871, 0.910]	<0.001
History of hypertension	1.043	[0.996, 1.094]	0.076	1.031	[0.985, 1.079]	0.195
History of diabetes mellitus	1.203	[1.137, 1.274]	<0.001	1.181	[1.117, 1.249]	<0.001
History of stroke or TIA	1.127	[1.072, 1.185]	<0.001	1.120	[1.067, 1.176]	<0.001
History of peripheral artery disease	1.071	[0.993, 1.154]	0.075	1.046	[0.972, 1.125]	0.229
History of heart failure	1.063	[1.014, 1.115]	0.012	1.023	[0.976, 1.071]	0.344
Mean arterial pressure (per 1mmHg)	0.998	[0.997, 1.000]	0.031	0.999	[0.998, 1.001]	0.277
Past smoker (ref: nonsmoker)	0.970	[0.930, 1.011]	0.151	0.967	[0.928, 1.007]	0.109
Current smoker (ref: nonsmoker)	0.963	[0.887, 1.044]	0.357	0.934	[0.863, 1.010]	0.088
Alcohol consumption (per 1 std.drink/d)	1.009	[0.988, 1.015]	0.795	1.004	[0.991, 1.017]	0.555
Interaction eGFR x age	1.032	[1.015, 1.050]	<0.001	1.035	[1.020, 1.049]	<0.001
Interaction BMI x age	0.971	[0.948, 0.994]	0.013	0.978	[0.956, 1.001]	0.057
Interaction eGFR x diabetes	0.993	[0.967, 1.020]	0.603	1.004	[0.980, 1.029]	0.738

eGFR, estimated glomerular filtration rate; BMI, body mass index; TIA, transient ischemic attack

*centered on its mean

Table 9.2.3. Performance of different models including age, eGFR and BMI to predict serum neurofilament light concentrations

	Model (N = 2,277)								
	age* alone	**eGFR† alone**	**BMI alone**	**age* and eGFR†**	**age* and eGFR† incl. interaction eGFR x age**	**age* and BMI**	**age* and BMI incl. interaction BMI x age**	**age*, eGFR† and BMI**	**age*, eGFR† and BMI incl. interactions eGFR x age, BMI x age**
using $eGFR_{crea}$									
R^2	0.26	0.35	0.02	0.45	0.45	0.27	0.27	0.46	0.47
R^2_{adj}	0.26	0.35	0.02	0.45	0.45	0.27	0.27	0.46	0.46
AIC	3927	3629	4568	3284	3269	3911	3910	3231	3208
using $eGFR_{crea-cys}$									
R^2	0.26	0.42	0.02	0.48	0.48	0.27	0.27	0.50	0.50
R^2_{adj}	0.26	0.42	0.02	0.48	0.48	0.27	0.27	0.49	0.50
AIC	3927	3386	4568	3148	3129	3911	3910	3073	3044

R^2, coefficient of determination; R^2_{adj}, adjusted R^2; AIC, Akaike's information criterion

*modelled with a linear and cubic component

†modelled with a linear, quadratic and cubic component

Figures

Figure 9.2.1. Scatter plot of the association of $eGFR_{crea}$ with sNfL (using the log scale) in the entire study population (A) and stratified to age quartiles (B). The solid line represents the predicted values from the main multivariable model and the grey shading represents the 95% confidence interval.

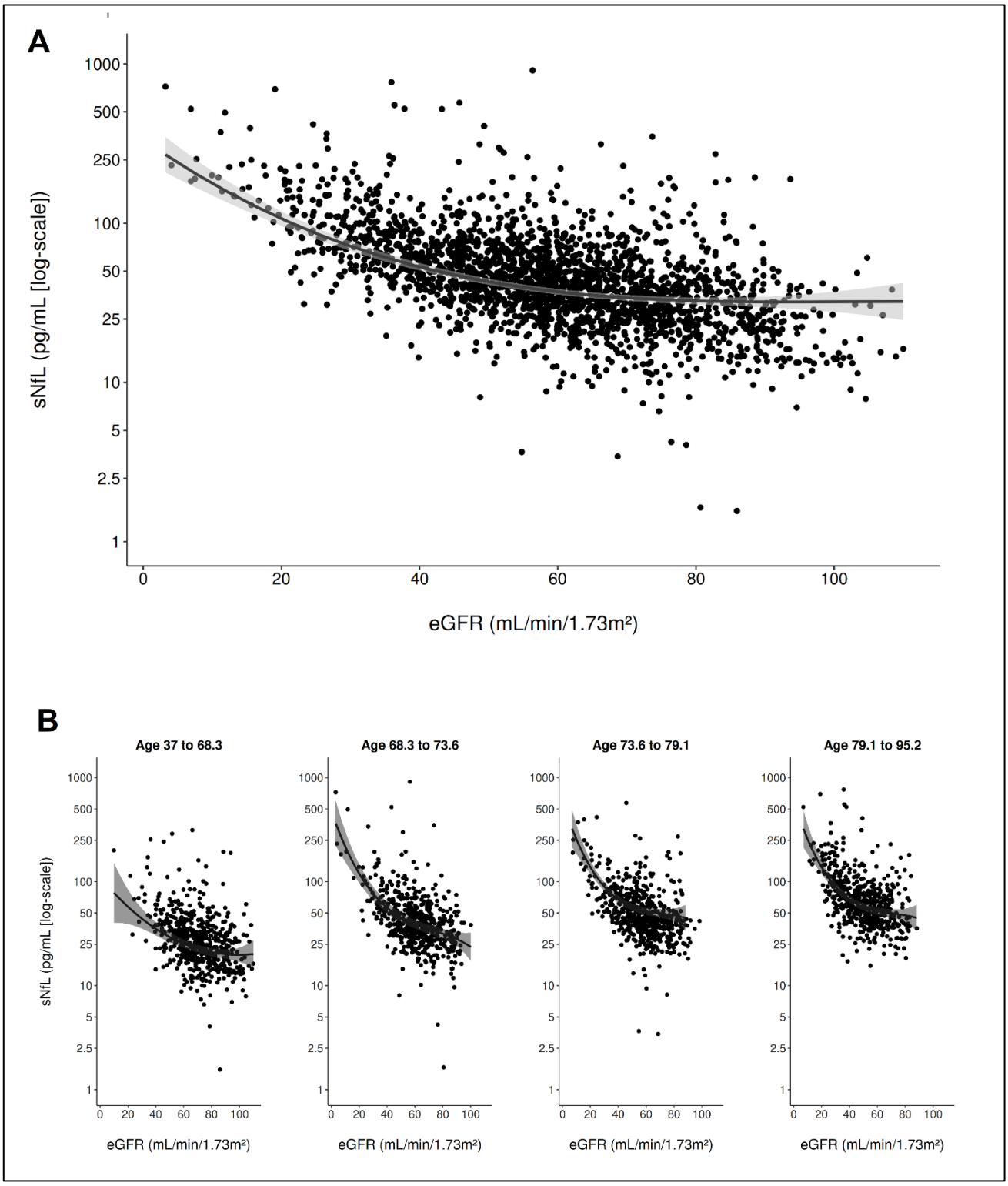
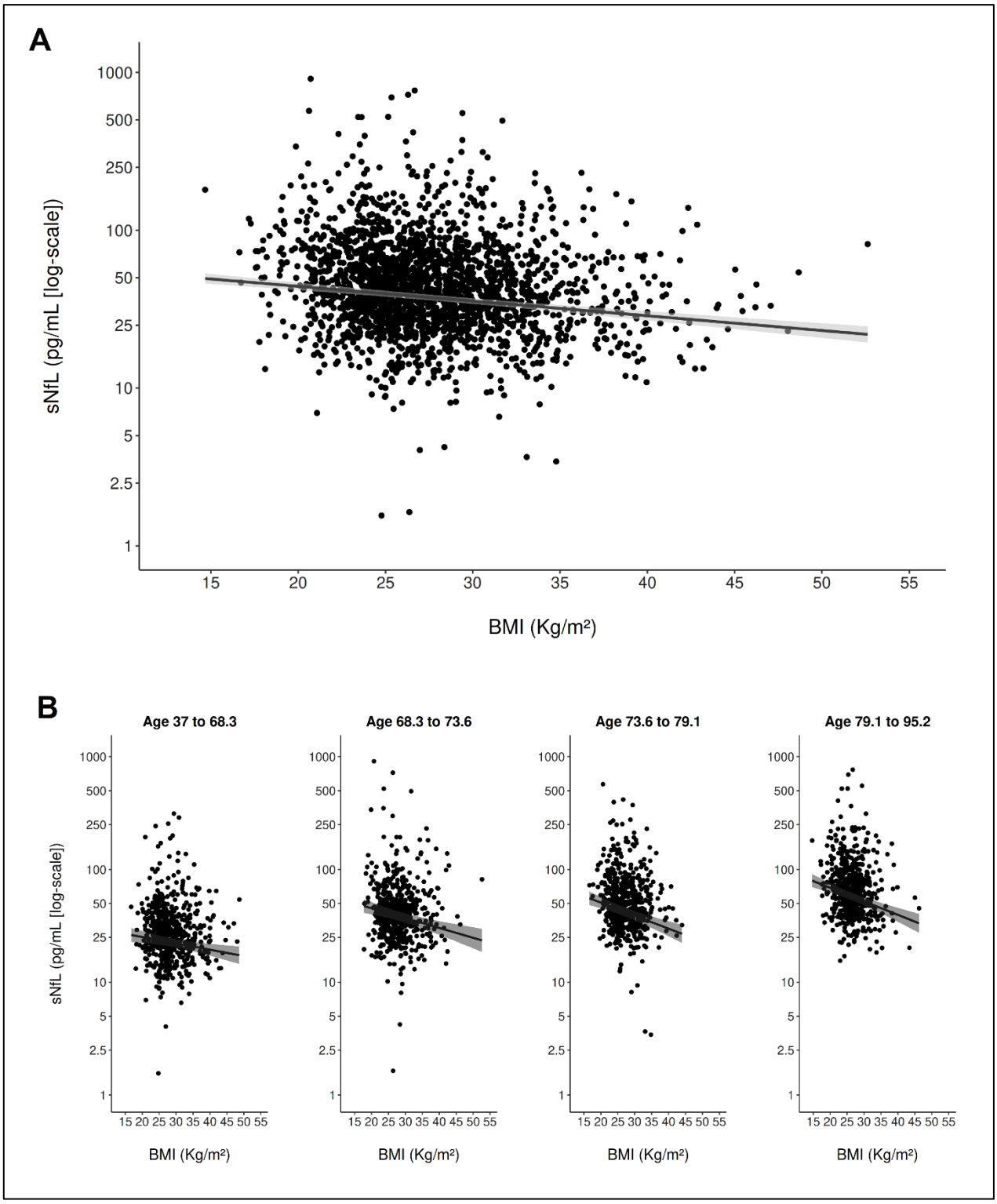
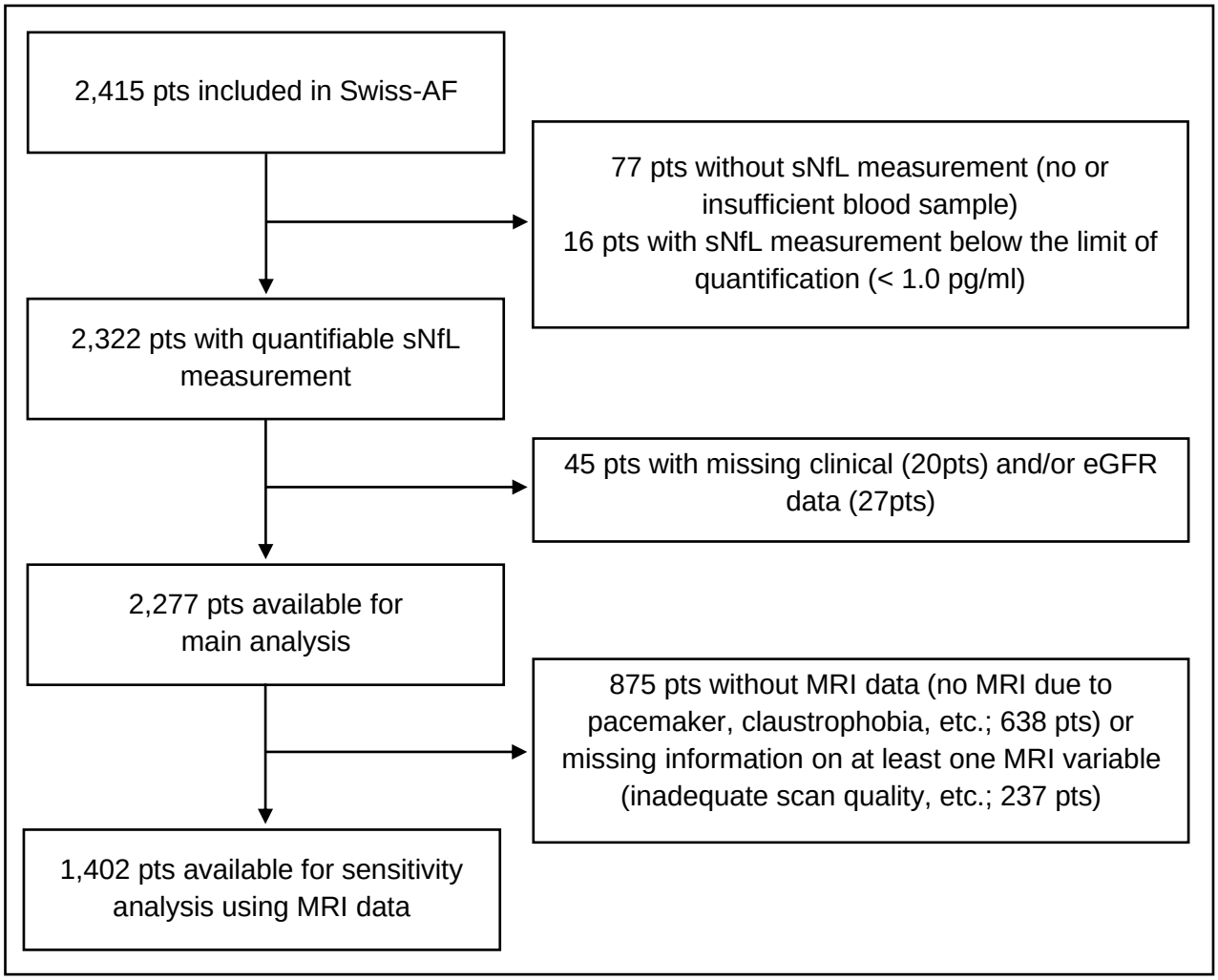


Figure 9.2.2. Scatter plot of the association of BMI with sNfL (using the log scale) in the entire study population (A) and stratified to age quartiles (B). The solid line represents the predicted values from the main multivariable model and the grey shading represents the 95% confidence interval.



Supplementary Material

Supplementary Figure 9.2.1. Study flowchart



Supplementary Table 9.2.1. *Linear, quadratic and cubic modelling of the association of eGFR and age with sNfL*

Model (N= 2,277)	**Component**	**$eGFR_{crea}$** (centered; per10 ml/min/1.73m^2)				**$eGFR_{crea-cys}$** (centered; per10 ml/min/1.73m^2)				**Age** (centered; per decade)			
		β_{mult}	**95%-CI**	**p-value**	**AIC**	**β_{mult}**	**95%-CI**	**p-value**	**AIC**	**β_{mult}**	**95%-CI**	**p-value**	**AIC**
Linear	linear	0.810	[0.800, 0.820]	<0.001	3702	0.811	[0.802, 0.819]	<0.001	3470	1.497	[1.456, 1.539]	<0.001	3930
Quadratic	linear	0.812	[0.802, 0.822]	<0.001	3644	0.807	[0.799, 0.816]	<0.001	3396	1.501	[1.457, 1.546]	<0.001	3932
	quadratic	1.020	[1.015, 1.025]	<0.001		1.018	[1.014, 1.021]	<0.001		1.005	[0.984, 1.027]	0.622	
Cubic	linear	0.842	[0.824, 0.860]	<0.001	**3629***	0.827	[0.813, 0.842]	<0.001	**3386***	1.571	[1.504, 1.641]	<0.001	3927
	quadratic	1.019	[1.014, 1.024]	<0.001		1.020	[1.016, 1.024]	<0.001		0.979	[0.952, 1.007]	0.147	
	cubic	0.996	[0.994, 0.998]	<0.001		0.998	[0.997, 0.999]	<0.001		0.978	[0.963, 0.994]	0.006	
Cubic without quadratic term	linear	0.848	[0.830, 0.866]	<0.001	3678	0.816	[0.802, 0.830]	<0.001	3471	1.555	[1.492, 1.621]	<0.001	**3927***
	cubic	0.995	[0.993, 0.997]	<0.001		0.999	[0.998, 1.001]	0.333		0.986	[0.974, 0.997]	0.016	

eGFR, estimated glomerular filtration rate; AIC, Akaike's information criterion

*best model, selected based on AIC values and degrees of freedom

Supplementary Table 9.2.2. *Model-based estimates for the association of eGFRcrea and BMI with sNfL in four age-quartile subgroups, describing the changes in curvature (eGFRcrea) or slope (BMI) in each age subgroup*

Variable	age 37.0 – 68.3 years (N = 570)			age 68.3 – 73.6 years (N = 569)			age 73.6 – 79.1 years (N = 569)			age 79.1 – 95.2 years (N = 569)		
	β_{mult}	95%-CI	p-value	β_{mult}	95%-CI	p-value	β_{mult}	95%-CI	p-value	β_{mult}	95%-CI	p-value
$eGFR_{crea}$* (per 10 ml/min/1.73m^2)	0.876	[0.836, 0.919]	<0.001	0.880	[0.839, 0.922]	<0.001	0.902	[0.864, 0.941]	<0.001	0.926	[0.890, 0.965]	<0.001
[$eGFR_{crea}$* (per 10 ml/min/1.73m^2)]^2	1.017	[1.003, 1.031]	0.017	1.021	[1.008, 1.034]	0.001	1.029	[1.015, 1.043]	<0.001	1.021	[0.999, 1.043]	0.067
[$eGFR_{crea}$* (per 10 ml/min/1.73m^2)]^3	0.998	[0.994, 1.002]	0.356	0.995	[0.991, 0.999]	0.022	0.997	[0.993, 1.002]	0.269	0.994	[0.988, 1.000]	0.051
BMI* (per 5 kg/m^2)	0.925	[0.886, 0.966]	<0.001	0.903	[0.863, 0.944]	<0.001	0.872	[0.831, 0.915]	<0.001	0.866	[0.827, 0.906]	<0.001

eGFR, estimated glomerular filtration rate; BMI, body mass index

*centered on its mean; estimates from multivariable models including history of hypertension, diabetes, stroke or transient ischemic attack, peripheral artery disease, heart failure, mean arterial pressure, past smoker, current smoker, alcohol consumption and the interaction $eGFR_{crea}$ x diabetes

Supplementary Table 9.2.3. Multivariable model for the association of eGFR and BMI with sNfL including adjustment for MRI variables

Variables (N=1,402)	**using $eGFR_{crea}$**		**using $eGFR_{crea-cys}$**	
	β_{mult}	**95%-CI**	**β_{mult}**	**95%-CI**
Age (centered; per decade)	1.249	[1.181, 1.320]	1.203	[1.140, 1.269]
[Age (centered; per decade)]^3	0.984	[0.969, 1.000]	0.984	[0.969, 0.999]
eGFR (centered; per 10 ml/min/1.73m^2)	0.889	[0.866, 0.912]	0.878	[0.859, 0.898]
[eGFR (centered; per 10 ml/min/1.73m^2)]^2	1.025	[1.017, 1.034]	1.023	[1.016, 1.029]
[eGFR (centered; per 10 ml/min/1.73m^2)]^3	0.998	[0.995, 1.000]	0.997	[0.996, 0.999]
BMI (centered; per 5 kg/m^2)	0.902	[0.877, 0.928]	0.893	[0.869, 0.917]
History of hypertension	eliminated*		eliminated*	
History of diabetes mellitus	1.172	[1.087, 1.263]	1.168	[1.087, 1.256]
History of stroke or transient ischemic attack	1.078	[1.005, 1.156]	1.073	[1.002, 1.148]
History of peripheral artery disease	eliminated*		eliminated*	
History of heart failure	1.064	[0.999, 1.133]	eliminated*	
Mean arterial pressure (per 1 mmHg)	0.998	[0.996, 1.000]	eliminated*	
Past smoker (ref: nonsmoker)	eliminated*		eliminated*	
Current smoker (ref: nonsmoker)	eliminated*		eliminated*	
Alcohol consumption (per 1 std.drink/d)	eliminated*		eliminated*	
Presence of SNCIs	eliminated*		eliminated*	
Volume of SNCIs (log-transformed, centered)	eliminated*		eliminated*	
Presence of LNCCIs	1.072	[1.003, 1.145]	1.064	[0.998, 1.134]
Volume of LNCCIs (log-transformed, centered)	1.047	[1.019, 1.076]	1.047	[1.020, 1.074]
Presence of microbleeds	1.076	[1.011, 1.145]	1.068	[1.006, 1.135]
Count of microbleeds	eliminated*		eliminated*	
Volume of WMLs (log-transformed, centered)	1.042	[1.021, 1.065]	1.037	[1.017, 1.059]
Normalized brain volume (per 1 cm^3)	0.9998	[0.9995, 1.0001]	eliminated†	
Interaction eGFR x age	1.021	[0.999, 1.044]	1.023	[1.004, 1.042]
Interaction BMI x age	eliminated*		eliminated*	
Interaction eGFR x diabetes	eliminated*		eliminated*	

BMI, body mass index; eGFR, estimated glomerular filtration rate; SNCIs, small noncortical infarcts; LNCCIs; large noncortical or cortical infarcts; WMLs, white matter lesions

*stepwise backward elimination based on Akaike's information criterion

10. Discussion and Outlook

This PhD thesis addressed several challenges and research gaps in the field of stroke, AF and oral anticoagulant treatment. The first topic focused on the use of oral anticoagulants in the oldest old and other challenging patient populations with AF and recent stroke, who are risk for both stroke recurrence and ICH, and are common in everyday neurological practice. The second topic examined the etiology, outcomes and subsequent preventive strategies of ischemic stroke despite anticoagulation in AF patients, which represents another growing challenge in clinical practice. As nonadherence to oral anticoagulants has been implicated as a potential etiology of breakthrough stroke, the third topic focused on the medication-taking behaviour of stroke patients treated with DOAC. Finally, AF is not only associated with ischemic stroke, but also cognitive dysfunction through mechanisms that are poorly understood; the fourth topic focused on sNfL, a novel biomarker of neuroaxonal injury, as a tool explore the mechanisms of neuronal damage and cognitive dysfunction in AF.

10.1. Oral anticoagulants in the oldest old and other high-risk patient populations with atrial fibrillation and recent ischemic stroke

The first main project of this thesis (section 6.1) was a large pooled analysis of individual patient data including 7 prospective AF cohorts with VKA or DOAC treatment following a recent stroke, which investigated the safety and effectiveness of DOAC versus VKA in the oldest old. The key finding was that the favorable profile of DOAC over VKA in terms of the primary composite outcome of recurrent ischemic stroke, ICH and death was preserved in the oldest old, whether defined as aged 85 or 90 years or older. This was consistent even after accounting for the high-risk profile of the oldest old in adjusted and weighted analyses. Additional analyses investigating the individual components of the composite outcome separately, as well as ancillary analyses of the net clinical benefit of DOAC versus VKA balancing the benefit in stroke reduction against the weighted risk of ICH, all yielded similar results, indicating the robustness of our findings. Importantly, there was no signal for safety issues regarding ICH risk, which has been a widespread concern.[75, 129, 146] Taken together, these findings provide new, 'real-world' evidence that the overall beneficial effect of DOAC treatment following recent ischemic stroke is maintained in the oldest old.

These findings are highly relevant for clinical practice, as they contradict the assumptions of many clinicians who are reluctant to use DOAC in the oldest old,[64, 73] and may favor VKA instead.[64] This has been mostly due to safety concerns, postulating that several clinical situations that are particularly prevalent in the oldest old, such as unstable or declining renal function, polypharmacy, frailty, malnutrition or reduced body weight, may interfere with DOAC pharmacokinetics and thereby heighten the risk of bleeding.[64, 73] The randomized evidence for these patients is limited, since patients aged over 85 years were severely underrepresented in the landmark DOAC trials, constituting less than 5% of the trial population.[66-68] In contrast, about 60% of all AF-related ischemic stroke cases seen in neurological practice occur in very elderly patients, a number projected to further increase in the coming decades.[42]

To bridge this evidence gap, systematically ascertained 'real-world' data are useful.[63, 69] While several large observational studies later confirmed the benefits of DOAC in elderly patients with AF,[147-152] they

did not examine patients with a recent ischemic stroke. Of note, patients with recent stroke within 1 – 4 weeks had also been excluded from the randomized DOAC trials.[65] The fact that the elderly in our analysis had a recent stroke matters, as such patients – compared to those without recent stroke – have a higher risk for hemorrhagic complications, including ICH[153] and hemorrhagic transformation of the ischemic infarct,[154] concomitant active small vessel disease[155, 156] and stroke-induced motor and cognitive deficits with an increased risk of falls.[62, 74]

For patients with recent stroke, subgroup analyses in observational studies suggested the safety of DOAC versus VKA for the age groups of ≥75[72] and >80 years.[70, 157] As no studies investigated the performance of DOAC versus VKA in AF patients with both age over 85 years and a recent ischemic stroke, our analysis addressed an important evidence gap, mitigating concerns about the applicability of the trials' findings in everyday clinical stroke practice and supporting the current guidelines for prevention of stroke recurrence, which recommend DOAC in preference over VKA.[31] Of note, the recent placebo-controlled ELDERCARE-AF trial suggested the benefit of anticoagulation even in very elderly patients with AF who were not appropriate candidates for standard anticoagulant treatment.[73]

As an additional project using data from our local single-center cohort of AF patients treated with oral anticoagulants following a recent stroke, we investigated the safety and effectiveness of DOAC versus VKA also in patients with dependency on the daily help of others at hospital discharge (section 6.2). Using a similar methodological approach as the previous project, our analyses demonstrated that the benefits of DOAC in patients with AF and a recent stroke are maintained among those that are dependent on the daily help of others. This is clinically important, because dependency after ischemic stroke is common in clinical practice, with approximately 40% of all patients with AF-related ischemic stroke being dependent at hospital discharge in previous studies.[72] However, no randomized evidence exists for these patients, as DOAC trials had explicitly excluded patients with disabling stroke within 3 – 6 months from participation.[65] Therefore, our findings in this additional project provided new, observational evidence that further supports the use of DOAC over VKA in AF patients with a recent ischemic stroke.

Finally, in an additional project from our single-center cohort, we investigated the prognostic importance of small vessel disease markers in anticoagulated patients with AF and a recent stroke (section 6.3). The first key finding was that the presence of small vessel disease, as indicated by MRI markers including white matter hyperintensities and microbleeds, was associated with an unfavorable clinical outcome, including a higher risk for recurrent ischemic stroke, ICH, death and disability. This indicates the need for thorough post-stroke medical guidance and cardiovascular risk factor control beyond oral anticoagulation, as well as effective rehabilitation measures in AF patients with a recent stroke who have concomitant small vessel disease. The second key finding was that the risk of ischemic stroke recurrence was higher than the risk of ICH, regardless of the presence or absence of small vessel disease markers and in particular microbleeds. This is important, because the presence of microbleeds has raised concerns that the ICH risk might outweigh the benefits of oral anticoagulant therapy in these patients.[76] Our findings indicate that withholding oral anticoagulants based solely on the presence of microbleeds does not seem justified. This is in line with a large pooled analysis of patients with or without AF and recent ischemic stroke across 38 cohorts including ours, which showed that the absolute risk of ischemic stroke was always higher than the risk of ICH, regardless of presence, burden, and anatomical distribution of microbleeds.[227]

In summary, our findings in these projects from the first topic of this PhD thesis advanced the evidence for the use of anticoagulants in high-risk patient populations with AF and a recent ischemic stroke.

10.2. Ischemic stroke despite oral anticoagulant therapy in patients with atrial fibrillation

The second main project of this thesis (section 7.1) was a large pooled analysis of individual patient data across 11 stroke centers, which investigated the etiology, outcomes and subsequent preventive strategies of ischemic stroke despite oral anticoagulant treatment in AF patients. With the increasing use of anticoagulants, particularly DOAC,[59-61] the number of AF patients suffering breakthrough stroke is growing, with about 1 in 3 AF-related strokes occurring in previously anticoagulated patients.[81, 82] This is a common challenge in everyday clinical practice, as the etiology of these strokes is poorly understood and the optimal subsequent management is unclear.[87]

The first key finding of this project was that the etiology of breakthrough stroke is heterogeneous, which has apparent implications for stroke prevention strategies, suggesting that more personalized treatment approaches may be needed. Competing stroke mechanisms besides AF-related cardioembolism were the main stroke etiology in about 1/4 of patients. Among those, large artery atherosclerosis and small vessel disease were the most common, stressing the importance of a thorough work-up in patients with AF and stroke despite anticoagulation in order to uncover competing noncardioembolic pathologies that might be less responsive to anticoagulation and warrant additional preventive therapies.[87] Insufficient anticoagulation, including inappropriately low DOAC dosing and nonadherence, was the main stroke etiology in about 1/3 of patients. This is important, because these strokes may be preventable through interventions to increase physicians' awareness about per-label dosing, and also through better identification strategies of nonadherent patients and effective adherence-enhancing interventions.[95, 167] Finally, the largest proportion of stroke despite anticoagulation was attributable solely to AF-related cardioembolism without evidence for insufficient anticoagulation or competing mechanisms. The profile of these patients resembled more the profile of patients with insufficient anticoagulation in terms of traditional cardiovascular risk factors and neuroimaging characteristics than the profile of patients with competing stroke mechanisms, suggesting shared stroke mechanisms, in which inadequate anticoagulant activity might be ultimately implicated. These data provided for the first time a comprehensive appraisal of the scope and relative frequency of the etiologies of stoke despite anticoagulation in a large multicenter sample.[82, 87]

The second main finding was that the burden of unfavorable outcomes within 3 months after stroke despite anticoagulation is high, expanding on findings from previous smaller studies.[88-90] We found that over 1/5 of patients died and over 1/2 of patients were dependent on the help of others at 3 months. While ICH occurred infrequently at <1%, 4.6% of patients suffered ischemic stroke recurrence, clearly identifying this patient group as high-risk, and stressing the need to define optimal treatment strategies.

The third key finding was that – regardless of the type of anticoagulant used before stroke – treatment with DOAC after breakthrough stroke was independently associated with better outcomes than VKA treatment. This provided new evidence for the preferential use of DOAC over VKA after stroke despite

anticoagulation, for whom no such data existed previously.[88, 90] Contradicting widespread practice,[87] our data showed that neither any specific switch between DOAC (i.e., switching to different DOAC, or to DOAC with different dosing frequency or mechanism of action), nor add-on treatment with antiplatelets conferred additional benefits after stroke despite anticoagulation. Taken together with our finding that the largest proportion of breakthrough stroke was attributable to cardioembolism despite sufficient anticoagulation and the high rate of unfavorable outcomes in these patients, our results indicate the need for novel preventive strategies beyond the currently available anticoagulants. These may include both pharmacologic (e.g., factor XIa inhibitors; currently under investigation in clinical trials)[111] and nonpharmacologic interventional approaches, such as carotid artery filters[228] and left atrial appendage occlusion[171], which may be worth exploring as add-on treatments to standard anticoagulation.[172]

10.3. Adherence of stroke patients to direct oral anticoagulants

Nonadherence to anticoagulation has been implicated as a cause of breakthrough stroke, as shown in our aforementioned project and elsewhere.[96, 97] This has been a matter of concern particularly for the increasingly used DOAC, as they do not require regular coagulation monitoring and have a rapid offset of action.[91, 92] During this PhD, we established the MAAESTRO study as a single-center interdisciplinary project in collaboration with pharmacists, in order to assess the medication-taking behaviour and the effect of an educational, reminder-based, adherence-enhancing intervention in patients with recent stroke treated with DOAC. To this end, we designed MAAESTRO with an initial observational phase, followed by an interventional phase with a randomized crossover design (section 8.1). MAAESTRO uses electronic monitoring as the primary method to assess adherence, which is the gold standard in adherence research.[98] So far, only few studies have used this method to assess adherence in DOAC-treated patients, with some also evaluating adherence-enhancing interventions, but none examined patients with recent stroke.[99-103] This is important, because stroke-induced neurological or cognitive deficits and polypharmacy, which is common in stroke patients, may interfere with adherence to medication.[94, 95] MAAESTRO recently concluded patient recruitment, and is now in the final stages of follow-up.

Using data from the observational phase of MAAESTRO (section 8.2), we demonstrated that electronic adherence monitoring after a recent stroke is feasible for the majority of patients who self-administer their medication. In line with previous work by us and others,[95, 99, 101-103] we found high rates of adherence to DOAC overall. However, the detailed electronic adherence data enabled us to identify several common patterns of suboptimal therapy implementation, such as drug holidays and missing evening doses of twice-daily DOAC. In this first MAAESTRO-based project, we showed that electronic monitoring enables the calculation of several adherence metrics and thereby provides a nuanced evaluation of DOAC-taking behavior, which may facilitate more targeted adherence-enhancing interventions.

In an additional exploratory analysis from the observational phase of MAAESTRO (section 8.3), which we conceived in the context of the COVID-19 pandemic, we investigated the impact of lockdown measures on the medication adherence of stroke patients by comparing several adherence metrics before and during lockdown in a small subset of MAAESTRO participants. We found that most patients had high adherence before lockdown, which remained unchanged during lockdown. In the few

patients with suboptimal pre-lockdown adherence, this either deteriorated further or improved during lockdown. Although limited by the small number of patients, this analysis suggested that a major disruption of social life (i.e., the imposed COVID-19 lockdown) seemed unlikely to relevantly affect the medication-taking behaviour of patients with high pre-established adherence, but might have an impact – either negative or positive – in patients with previously suboptimal adherence.

While the main results of MAAESTRO on the effect of our adherence-enhancing intervention are awaited, the data gained from our MAAESTRO-based projects so far provided important novel insights into the medication-taking behaviour of stroke patients treated with DOAC.

10.4. Serum neurofilament light chain and cognitive dysfunction in atrial fibrillation

Accumulating evidence links AF to cognitive dysfunction independent of ischemic stroke,[106, 107] but the mechanisms that drive this association are not well understood.[104, 105] In the third main project of this PhD thesis (section 9.1), we used sNfL as a tool to explore the mechanisms of neuronal damage in a large cross-sectional analysis on AF patients with a detailed clinical, neuroimaging, and cognitive phenotypization.

We found that higher a CHA_2DS_2-VASc score (i.e., a validated clinical score predicting ischemic stroke risk in AF patients),[10] as well as the presence of diabetes mellitus and peripheral artery disease indicated increasing neuronal injury measured by sNfL. This was independent of age, history of stroke and vascular brain lesions on MRI, suggesting that neuronal injury in AF goes beyond vascular brain lesions visible on MRI. Ongoing microembolism or concomitant small vessel dysfunction might be hypothesized as potential links. Indeed, at the level of vascular brain lesions, we showed that the burden of white matter hyperintensities and large noncortical or cortical chronic ischemic infarcts on MRI was strongly associated with sNfL levels, independent of history of clinically manifest stroke. This indicates that both small vessel disease and potentially embolic infarcts – even clinically covert ones – contribute to neuronal injury in AF. These findings complement recent evidence for the value of sNfL as a blood-based biomarker of cerebrovascular disease,[180, 181, 198, 199, 214] and demonstrate its potential as a screening tool to select AF patients who might benefit from MRI investigations to uncover subclinical vascular brain disease.

Another major finding was that blood pressure showed a complex curvilinear association with sNfL levels, with evidence for both an inverse linear and U-shaped relationship. Taken together with the strong independent association of heart failure with sNfL that we observed, these findings indicate the importance of hemodynamics and lend further support to the brain hypoperfusion hypothesis as a mechanism of neuronal damage in AF.[116-120] Additionally, we showed that age *per se* was one of the strongest contributors to sNfL, independent of comorbidities and vascular brain lesions, in line with other reports.[212] Considering also our observation of an inverse association between sNfL and brain volume on neuroimaging, these findings suggest that ongoing neurodegenerative processes may be at play and contribute to neuronal damage in AF.

Taken together, our results in the third main project of this PhD thesis showed the potential of sNfL as a tool to explore the mechanisms that underly neuronal damage in AF, which may result from a complex interplay between subclinical brain ischemia, altered hemodynamics and neurodegeneration. Importantly, in this project we also showed that sNfL was inversely associated

with cognitive performance across a battery of cognitive tests in AF patients. This is in line with previous research on the association of sNfL with measures of cognitive function in other patient populations with small vessel disease[198] and neurodegenerative disorders,[203-206] and demonstrates that sNfL may be useful as surrogate outcome parameter for brain health and cognitive function in cardiovascular research, in which it had not been utilized so far.

However, in order to further establish sNfL as a blood-based biomarker in cardiovascular research and enable potential applications in clinical practice, a better understanding of the homeostasis of this novel biomarker in the blood compartment, including its clearance and distribution, is necessary. Besides its use in cardiovascular disease, this is equally important for investigations of normal ageing, cerebrovascular and neurodegenerative disorders,[126, 181, 198, 212-214] where age-related physiological changes and comorbidities might affect the association of sNfL with disease processes.[210, 211] Therefore, in the fourth main project of this PhD thesis we used data from the aforementioned large sample of elderly AF patients (section 9.2) to investigate how renal function and BMI are associated with and contribute to sNfL levels in a cross-sectional analysis, in order to determine their importance as potential confounders in sNfL-based applications, and gain insights into the clearance and distribution of sNfL in the elderly.

The first key finding in this project was a strong inverse association between sNfL and both renal function and BMI, independently of each other. While the inverse association of sNfL with renal function and BMI had been reported previously in other patient populations, [186, 216, 217] it was unclear whether these observations were the result of confounding through comorbidities or vascular brain lesions. In our analysis, we showed that these associations persisted after extensive adjustment for other contributors to sNfL levels, including age, cardiovascular comorbidities and vascular brain lesions on neuroimaging, indicating that they reflect true relationships. These findings support the notion that sNfL is cleared by the kidney and may therefore be elevated when renal function is diminished,[216] as well as that a larger distribution volume, as indicated by higher BMI, may lead to lower sNfL levels through dilution.[217]

The second main finding was that besides age, only renal function explained a relevant proportion of the variance of sNfL levels, which was additional and independent to the contribution of age. This was not true for BMI, which did not meaningfully increase the explanatory power of models predicting sNfL levels. Having additionally shown that the inverse association of renal function with sNfL is nonlinear and is age-dependent, being even more pronounced with diminished renal function, and more so in older than in younger patients, our data provide evidence for the importance of accounting for renal function when evaluating sNfL concentrations in elderly populations, in whom chronic kidney disease is highly prevalent.[223] This finding is of particular relevance for the use of sNfL in patients with cardiovascular disease.

In summary, the fourth main project of this PhD thesis represents a comprehensive appraisal of the relative contribution of renal function and BMI in determining sNfL levels, thereby providing important insights into the homeostasis of this biomarker and informing ongoing efforts to establish normative values for sNfL in elderly populations.[211, 212]

10.5. Outlook and future perspectives

10.5.1. Oral anticoagulants in further high-risk patient populations with atrial fibrillation and recent stroke

In everyday clinical practice, stroke neurologists are confronted with a wide array of challenging scenarios regarding the management of oral anticoagulation in patients with AF and recent stroke, for which the randomized evidence from the large DOAC trials is insufficient.[62, 63]

While observational data about the 'real-world' performance of DOAC versus VKA, including the ones presented in this thesis, have been reassuring for some of these high-risk patient populations, several research gaps still remain. These include the use of DOAC in AF patients with:

- multimorbidity, in whom post-hoc analyses from the randomized showed that the favorable profile of DOAC was preserved,[229, 230] but more 'real-world' data are needed,[63] particularly on multimorbid patients with recent stroke.
- renal function impairment, particularly those with unstable or declining renal function, in whom altered DOAC pharmacokinetics may lead to bleeding complications and, conversely, the use of reduced DOAC doses may lower their effectiveness in thromboembolic protection.[231]
- combined antithrombotic therapy with add-on antiplatelets (e.g., due to concomitant coronary heart disease or stenting), in whom scarce observational data exist, showing unfavorable outcomes.[170]

We are currently investigating these topics both in our local single-center cohort in the context of Master theses of medical students, and within the international collaboration of cohorts on AF patients with recent stroke, on which the first main project of this PhD thesis was based.

10.5.2. Once- versus twice-daily direct oral anticoagulants after recent stroke in patients with atrial fibrillation

Since no direct comparisons between DOAC have been conducted, no evidence exists on which the choice of a specific DOAC over another can be based. Considering their pharmacokinetic properties, twice-daily DOAC are expected to maintain a better continuity of drug plasma levels with smaller peak-to-trough variability than once-daily DOAC, which might translate to a lower risk of bleeding and thrombotic events.[93] Indeed, in a metanalysis of the randomized DOAC trials, twice-daily DOAC appeared to offer a more balanced risk-benefit profile with respect to stroke prevention and ICH in indirect comparisons.[232] However, adherence to twice-daily DOAC has been shown to be lower than to once-daily DOAC in some 'real-world' studies, including large prescription claims studies[233] and studies using electronic monitoring including MAAESTRO,[100, 166] but not in others.[234] Although twice-daily dosing of DOAC has been hypothesized to be 'more forgiving' in the presence of suboptimal adherence,[93] this has not been proven.[233] Since several factors might apparently influence the performance of once- versus twice-daily DOAC, we intend to investigate their 'real-world' safety and effectiveness using data from our single-center cohort. Data collection for this project is completed and analyses are currently underway.

10.5.3. Timing of anticoagulation after recent ischemic stroke in patients with atrial fibrillation

Identifying the ideal timepoint to initiate oral anticoagulation after ischemic stroke in AF patients is a longstanding, common clinical challenge. While early anticoagulation should mitigate the risk of recurrence, it may put patients at risk of ICH.[154] Since the large DOAC trials excluded patients with recent stroke within 1 – 4 weeks,[65] no randomized data exist on this issue to guide clinical decision-making. Observational studies by us and others showed that DOAC initiation early after ischemic stroke was associated with a low frequency of ICH, whereas later initiation of DOAC was associated with an increased frequency of recurrent stroke.[154, 235] To definitively settle this, several multicenter randomized trials are currently underway,[154] including ELAN (Early versus Late initiation of DOAC in post-ischemic stroke patients with Atrial fibrillation; NCT03148457). During this PhD, 27 patients were enrolled in ELAN in Basel (9 of whom were also included in the newly initiated ELAN-MRI substudy), and recruitment is ongoing.

10.5.4. Main results of the MAAESTRO study

During this PhD, the single-center MAAESTRO study successfully recruited the target sample size of 130 patients in Basel. Recruitment concluded in July 2021, and follow-up is ongoing until April 2022. In close collaboration with the Pharmaceutical Care Research Group, we are planning to clean the data, close the database and perform the main analysis for the effect of our adherence-enhancing intervention in stroke patients treated with DOAC by the end of 2022.

10.5.5. Neurofilament light chain, cardiovascular biomarkers and brain atrophy in atrial fibrillation

Expanding on our sNfL-based projects presented in this PhD thesis (sections 9.1, 9.2), we are planning to investigate the association of sNfL and other neurological and cardiovascular serum biomarkers with the rate of brain atrophy over 2 years in the Swiss-AF Cohort study. This will provide further insights into the mechanisms that drive cognitive dysfunction in AF by leveraging longitudinal MRI data from this large multicenter study. A pilot analysis on the association of sNfL with 2-year brain atrophy from this dataset has been completed and was presented at the European Stroke Organisation Conference in 2019.[236] We have now finalized the estimation of brain atrophy rates in the entire Swiss-AF dataset in collaboration with the Medical Image Analysis Center in Basel and statistical analyses are currently underway.

10.5.6. Autoantibodies against autonomic G protein-coupled receptors in atrial fibrillation

G protein-coupled receptors (GPCR) are the largest superfamily of integral membrane proteins and play an essential role in human physiology, including the function of the nervous and cardiovascular systems. Accumulating evidence suggests that autoantibodies against GPCR may represent a physiological part of the immune system, which can become dysregulated and potentially causative in disease.[237-239] Autoantibodies against GPCR of the autonomic nervous system and vasculature are being increasingly recognized as putative novel players in the pathogenesis of several neurological and cardiovascular diseases, including Alzheimer's and vascular dementia,[240-242] heart failure,[243] and

particularly AF, where they seem to exert proarrhythmogenic effects, promote atrial fibrosis, and predict AF recurrence after ablation therapy.[244-246] However, no data exist about their association with neurological disease in the setting of AF.

We hypothesize that these autoantibodies may represent a novel link in the heart-brain connection, that could advance our understanding of how cognitive dysfunction occurs in AF. To pursue this hypothesis, we established an academic collaboration with Dr. Harald Heidecke, one of the leading experts in the field of GPCR-autoantibodies, in order to measure an antibody panel against 16 GPCR in a pilot study on 200 AF patients and 40 controls from Swiss-AF. In this project, we will explore the cross-sectional and longitudinal associations of the antibodies with clinical and neuroimaging characteristics, cognitive performance, sNfL and other blood-based neurological and cardiovascular biomarkers, as well as electrocardiographic markers of autonomic function and AF burden. This project constitutes my first independent postdoc research initiative, for which I applied for funding from the Swiss Heart Foundation with the support of my supervisors. Autoantibody measurement in Swiss-AF blood samples is currently underway, and results are expected in 2022.

11. Contributions by the PhD candidate

The projects from the first topic of this thesis were based on clinical registries that prospectively collected individual patient data. During my PhD, I recruited patients in our local single-center NOACISP-LONGTERM registry. NOACISP-LONGTERM concluded recruitment in December 2020, having reached 1,058 patients; over 500 patients were recruited during the PhD. Additionally, my tasks in NOACISP-LONGTERM included patient follow-up in the outpatient clinic, electronic data entry, management and updates of the electronic database in collaboration with the Clinical Trial Unit Basel, data cleaning, data pooling with other registries, as well as supervision of study nurses, master students and MD students in patient follow-up and data entry. Finally, I was involved either as a first author, shared first author or coauthor in the development of several NOACISP-LONGTERM-based research projects. This included the acquisition of funding from the University Hospital Basel and our Neurology Department, the drafting of analysis plans in collaboration with the statisticians of the Clinical Trial Unit, the interpretation of statistical results, the drafting or revising of manuscripts, and the presentation of our results in various academic events (European Stroke Organisation Conference, Neurology Research Retreat of the University Hospital Basel). Some of these projects, in which I contributed as shared first author or co-author, comprised the thesis of several MD students of our group, and are presented briefly as abstracts in this PhD thesis.

The project that constitutes the second topic of this PhD thesis was a joint venture with investigators from Basel, Bern and Heidelberg. I contributed to the conception, design and registration of this multicenter study and performed retrospective chart review of several hundred patients. Additionally, I undertook the pooling of data from all centers within this collaboration, as well as data cleaning of the pooled dataset. I rendered the first draft of the statistical analysis plan, performed statistical analyses in collaboration with another investigator from Bern, and drafted the manuscript. I share first authorship for this project with another investigator from our collaborating center Bern, who contributed significantly to data acquisition and statistical analysis.

The third topic of this thesis is based on the MAAESTRO study. We conceived and designed this study in collaboration with the Pharmaceutical Care Research Group of the University of Basel. With the support of my primary supervisor, I drafted the study protocol and subsequent amendments, secured ethical approval, registered the study, set up the electronic database in collaboration with the Clinical Trial Unit Basel, and successfully applied for financial support (ad personam grant from the Swiss Academy of Medical Sciences / Bangerter Foundation). During my PhD, I was primarily responsible for patient recruitment (which concluded in July 2021 having reached the target sample size of 130 patients), electronic data entry, as well as patient follow-up in the outpatient clinic, which I performed together with pharmacists - PhD students from the collaborating Pharmaceutical Care Group. I was involved as a shared first author in several projects from MAAESTRO, which included the presentation of results in multiple academic events (European Stroke Organisation Conference, European Academy of Neurology Congress, Basel/Aarau Stroke Summer School) and the drafting or revising of manuscripts. Some of these projects are part of the theses of PhD students from the Pharmaceutical Care Research Group, and are presented briefly as abstracts in this PhD thesis.

The projects from the fourth topic of this thesis were based on the Swiss-AF Cohort Study. For these projects, I was involved in the conception and design of analyses, drafted statistical analysis plans in collaboration with statisticians from the Clinical Trial Unit Basel, interpreted the statistical results,

presented our findings in several academic events (joint European-World Stroke Organisation Conference, Annual Meeting of the Swiss Stroke Society, Swiss Federation of Clinical Neuro-Societies Congress, Clinical Research Day University Hospital Basel) and drafted manuscripts.

Besides my work on the aforementioned projects, during my PhD I was involved as subinvestigator in several multicenter studies, both within the field of my PhD (ELAN trial [NCT03148457] on the timepoint of initiation of direct oral anticoagulants after stroke in atrial fibrillation patiens; GLORIA-AF registry [NCT01468701] of patients with atrial fibrillation and oral anticoagulant treatment; MICON collaboration [CRD42016036602] on the prognostic importance of cerebral microbleeds after stroke), but also within the wider field of vascular neurology (TRISP registry of acute stroke patients with intravenous thrombolysis; TICH-NOAC trial [NCT02866838] on the use of tranexamic acid in patients with acute anticoagulant-associated intracerebral hemorrhage; ESTREL trial [NCT03735901] on the pharmacological enhancement of stroke rehabilitation with levodopa, for which I served as the medical network advisor during 2021). Thanks to my wide involvement in the clinical and research activities of our Stroke Group at the University Hospital of Basel, during my PhD I had the opportunity to contribute as first author or coauthor in several publications from our group and provided independent reviews for several papers in peer-reviewed Journals (PLOS one, Frontiers in Pharmacology, Frontiers in Neurology, Stroke & Vascular Neurology, Patient preference and adherence), but was also able to keep in touch with clinical work in the outpatient neurological and cerebrovascular clinic. Additionally, I contributed to the revision of the standard operating procedures of our Stroke Center for the acute management of ischemic stroke despite anticoagulation and anticoagulant-associated intracerebral hemorrhage. Finally, I was involved in several teaching activities and delivered courses to medical and nursing professionals on neurology, neurosonology and stroke medicine.

12. Conclusion

In conclusion, the most important findings of this PhD thesis, which addressed a wide array of challenging topics in the field of AF, stroke and oral anticoagulation, are the following:

(1) In the first topic we provided 'real-world' evidence that the benefits of DOAC are preserved in high-risk patient populations with AF and recent stroke, including (i) the oldest old and (ii) patients who are dependent on the daily help of others. These findings support the current guidelines for recurrent stroke prevention, which recommend the use of DOAC in preference over VKA, by providing observational evidence for patients in whom no randomized and only scarce observational data existed previously.

(2) The second topic provided a comprehensive appraisal of the etiology and subsequent management of stroke despite anticoagulation, showing that (i) stroke despite anticoagulation comprises heterogeneous etiologies, including competing stroke mechanisms and insufficient anticoagulation due to inappropriate anticoagulant dosing and nonadherence, but most common is cardioembolism despite sufficient anticoagulation; (ii) after stroke despite anticoagulation, besides treatment with DOAC (as opposed to VKA) and contrary to widespread practice, no other specific prevention strategy was associated with better outcomes, including switching between DOAC and adding antiplatelets. Taken together, these findings indicate that individualized and novel preventive strategies beyond the currently available anticoagulants are needed to advance stroke prevention in these AF patients who are at high risk for recurrence and death.

(3) In the third topic, we showed (i) that electronic monitoring of medication adherence in stroke patients treated with DOAC is feasible, and (ii) can be used to obtain a nuanced evaluation of their medication-taking behaviour. The main results of the MAAESTRO study on the effectiveness of an educational, reminder-based intervention to improve adherence to DOAC in stroke patients are awaited.

(4) In the fourth topic, we used sNfL as biomarker for neuronal injury in patients with AF and (i) showed its potential as a surrogate marker for brain health in patients with heart disease, (ii) gained insights into possible mechanisms involved in cognitive dysfunction in patients with AF, and (iii) demonstrated the importance of renal function as a contributor to sNfL levels, which has implications for future sNfL-based applications in elderly cardiovascular patients.

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14. Appendix: Complete publications list of the PhD candidate

1. Polymeris AA, Helfenstein F, Benkert P, Aeschbacher S, Leppert D, Coslovsky M, et al. ***Renal function and body mass index contribute to serum neurofilament light chain levels in elderly patients with atrial fibrillation.*** Frontiers in Neuroscience. 2022 Apr 14; 16:819010. doi: 10.3389/fnins.2022.819010.
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