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Do P2Y12 receptor inhibitors prescribed poststroke modify the risk of cognitive disorder or dementia? Protocol for a target trial using multiple national Swedish registries

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ABSTRACT

Introduction The target of a class of antiplatelet medicines, P2Y12R inhibitors, exists both on platelets and on brain immune cells (microglia). This protocol aims to describe a causal (based on a counterfactual model) approach for analysing whether P2Y12R inhibitors prescribed for secondary prevention poststroke may increase the risk of cognitive disorder or dementia via their actions on microglia, using real-world evidence.

Methods and analysis This will be a cohort study nested within the Swedish National Health and Medical Registers, including all people with incident stroke from 2006 to 2016. We developed directed acyclic graphs to operationalise the causal research question considering potential time-independent and time-dependent confounding, using input from several experts. We developed a study protocol following the components of the target trial approach described by Hernan *et al* and describe the data structure that would be required in order to make a causal inference. We also describe the statistical approach required to derive the causal estimand associated with this important clinical question; that is, a time-to-event analysis for the development of cognitive disorder or dementia at 1, 2 and 5-year follow-up, based on approaches for competing events to account for the risk of all-cause mortality. Causal effect estimates and the precision in these estimates will be quantified.

Ethics and dissemination This study has been approved by the Ethics Committee of the University of Gothenburg and Confidentiality Clearance at Statistics Sweden with Dnr 937-18, and an approved addendum with Dnr 2019-0157. The analysis and interpretation of the results will be heavily reliant on the structure, quality and potential for bias of the databases used. When we implement the protocol, we will consider and document any biases specific to the dataset and conduct appropriate sensitivity analyses. Findings will be disseminated to local stakeholders via conferences, and published in appropriate scientific journals.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This protocol describes an approach using design principles from randomised trials to the analysis of observational data, which allowed us to identify an appropriate analytic approach to answer our research question.

⇒ The structural approach to identifying potential confounders described in this protocol incorporated input from experts in preclinical neuroscience and practicing neurologists to capture relationships between relevant variables to allow us to make robust conclusions about the direction of causal effects.

⇒ The statistical approach described in this protocol describes the most appropriate method for handling causal inference in the presence of the competing risks of mild cognitive impairment or dementia and all-cause mortality.

⇒ The implementation of the protocol will be limited by the structure and availability of variables in the proposed dataset.

INTRODUCTION

Stroke is associated with an elevated risk of incident dementia and cognitive impairment, which is highest in the first months after stroke[1] and remains elevated for years.[2 3] Our understanding of the mechanisms that lead to the development of dementia and cognitive impairment following stroke is presently incomplete and is likely to be dependent on a complex interplay between several factors. Animal models of ischaemic stroke have provided vital new information around neurodegenerative mechanisms associated with cognitive decline[4]; however, it is notoriously difficult to translate this evidence into clinically relevant information. One consistent

finding has been that the vascular injury caused by stroke promotes a neurodegenerative process, referred to as secondary neurodegeneration (SND). In both human and animal models, SND involves the progressive death of neurons in anatomically distinct regions, but functionally connected, to the initial core and later established total infarction zone but were not initially damaged by the stroke.[5] SND occurs over a significantly longer time scale than the initial infarction and recovery processes (at a minimum over several months).[5 6] Recent human and animal studies have provided compelling evidence that SND is associated with loss of viable central nervous system (CNS) tissue and neurological deficits, in particular worsening poststroke memory impairments and general cognitive decline.[5]

Microglia are the resident immune cells of the CNS. In the resting CNS, their most prominent feature is their fine processes, which engage in constitutive immunosurveillance.[7] Although initially thought to be harmful to the CNS due to their capacity for producing inflammatory markers in response to danger signals,[8] multiple studies that either conditionally removed or blocked microglial activity after experimental stroke showed an exacerbation of neuronal loss and increased infarct size.[9–11] Altogether, this suggests that microglia play a vital beneficial role in brain repair and limit the extent of SND after stroke.[9–11] Putative mechanisms for the neuroprotective functions of microglia have been explored in recent years.[12 13] One of these is the P2Y12 receptor (P2Y12R), which is a purinoceptor located across the surface of ramified microglial processes, where it plays a central role in facilitating microglial interaction with their microenvironment.[14] Importantly, P2Y12R has been shown to mediate the activation of microglia by injury or disease, including the microglial repair and regeneration response to tissue insult.[15] A recent study showed that peripheral administration of the P2Y12R inhibitor clopidogrel in mice with vascular damage reduced movement of juxtavascular microglial processes when compared with control animals.[16] Similarly, when the P2Y12R inhibitor PSB0739 was administered into the CNS immediately poststroke in mice, microglial sensing was blocked, and these cells no longer displayed a typical response to the injury, leading to a larger lesion and disrupted functional connectivity.[15] Further, microglia from mice deficient in P2Y12R, when exposed to an excitotoxic CNS insult, displayed significantly reduced chemotactic responses to injury.[14] Collectively, these results demonstrate that in rodents, P2Y12R engagement is necessary for microglial repair responses to occur, and inhibition of this receptor negatively impacts brain repair and regeneration responses.

In the CNS, P2Y12R is highly, and virtually exclusively, expressed on microglia,[17] while peripherally, P2Y12R is primarily located on platelets where it is an important regulator of platelet activation and aggregation during the blood clotting process.[18] As such, the P2Y12R is the target of several antiplatelet drugs, including clopidogrel, prasugrel, ticagrelor and ticlopidine, which inhibit P2Y12R. These drugs are commonly prescribed for the prevention of thrombotic vascular events.[19] The most commonly prescribed P2Y12R inhibitor is clopidogrel, a drug with few known side effects except prolongation of bleeding time and haemorrhagic risk ascribable to its antiplatelet actions, which has contributed to its popularity.[18] Since its approval in 1997 by the US Food and Drug Administration, clopidogrel has been prescribed to millions of patients worldwide.

The active metabolites of P2Y12R inhibitors have a low blood–brain barrier (BBB) permeability,[20] and thus would not be expected to interfere greatly with microglial activity under conditions where the BBB is largely intact, and CNS immune privilege is maintained. However, BBB dysfunction, characterised by structural disruption of endothelial cell junctions and increased permeability, is a prominent pathological characteristic of both ischaemic and haemorrhagic stroke in humans.[21 22] In the study by Lou *et al*[16] discussed previously, the vascular injury mouse model was associated with extravascular leakage, suggesting compromise of the BBB. In this model, microglial responses to vascular damage in single capillaries in the CNS were inhibited in the presence of peripherally administered P2Y12R inhibitors. The time course of BBB disruption after stroke has been difficult to precisely characterise; it appears to occur predominantly in the hyperacute and acute stages of stroke and may be correlated with the severity of stroke.[23] The permeability of the BBB has been observed to be significantly reduced by 6 weeks poststroke compared with the acute phases; however, some degree of BBB dysfunction appears to persist during the chronic phases of stroke recovery.[24] This may also be dependent on other comorbidities, in particular, studies using serum biomarkers to detect BBB dysfunction suggest that small-vessel disease (SVD) burden may be associated with chronic BBB injury injuries.[25]

The compromise of the BBB which occurs in ischaemic stroke may allow P2Y12R inhibitors and their active metabolites to gain entry into the affected CNS, resulting in suppression of P2Y12R-mediated microglial activation. Given that microglial cells are the only cell type within the CNS to express P2Y12R constitutively, and that microglia play a key role in brain repair, memory and functional recovery, which is at least partly mediated through this receptor, it is possible that if their actions are inhibited we might observe substantially worse outcomes concerning tissue loss and neurodegeneration within the CNS, which correlates with cognitive impairment. We do not propose that administration of P2Y12R inhibitors is the sole cause of poststroke mild cognitive disorder and dementia; however, there is a significant possibility that their use may increase the risk of these outcomes. Even if this risk is modest, the use of clopidogrel and similar agents is so widespread that the repercussions would potentially be significant at a population level. It is generally assumed that controlling vascular risk factors, including the use of antiplatelet medication, will decrease the risk of cognitive impairment in stroke survivors. However, clinical studies

examining the effect of secondary prevention strategies of vascular risk via antiplatelet regimens on long-term cognitive impairment have provided mixed results. The Prevention Regimen for Effectively Avoiding Second Strokes (PRoFESS) trial, which examined the neuroprotective effects of antiplatelet compounds and the angiotensin II receptor antagonist telmisartan showed no significant differences in the proportion of patients with cognitive impairment or dementia between those given aspirin and dipyridamole, or clopidogrel.[26] In a large cohort study of stroke survivors, a combination of aspirin and dipyridamole, but not clopidogrel monotherapy, was associated with a reduction in risk of cognitive impairment.[27] Given that the prescription of P2Y12R inhibitors could be considered on an individual basis poststroke based on risk, the investigation of this relationship is warranted.

Causal effect estimates derived from observational data

Ideally, questions about the comparative efficacy and safety of a drug, such as the risk of developing cognitive decline or dementia associated with the use of P2Y12R inhibitors poststroke, would be answered using an appropriately designed and conducted randomised controlled trial (RCT). When treatment is randomised, generally an unbiased estimate of the population average causal effect of that treatment can be generated. However, given that dementia and cognitive impairment have long lead times, a clinical trial aimed at detecting an increase of these outcomes would require large numbers of participants over a long duration of follow-up. More evidence is required to conduct such a study, particularly given that based on current evidence and guidelines, also it would be unethical to withhold clopidogrel from poststroke patients.[28 29]

The increasing availability of large pharmacoepidemiological datasets generated for administrative purposes means that real-world evidence is increasingly being used as a source of information for comparative effectiveness research conducted outside of clinical trials. Although these data sources have enormous potential for answering causal questions, historically these analyses have been prone to substantial biases in their effect estimates.[30] In recent years, considerable efforts have been made to improve causal inference assumptions, methods and frameworks, and several initiatives have shown that the causal estimand can be recovered or complemented in observational datasets by ensuring the application of principles of causal inference, in particular by explicitly attempting to emulate a target randomised trial, and ensuring the correct population is recruited into the analysis.[30–33] Marginal structural models, typically fit with inverse probability weights (such as treatment or censoring weights), are an established causal inference method to address time-varying treatment and confounding.[34 35] Well designed and executed observational studies in pharmacoepidemiology, where the aim is to assess the impact of a known biological pathway being targeted with a defined therapy, may therefore be used to

Table 1 Key components of the research question addressed in the protocol

Component	Description
Population	People aged over 55 years with the first stroke between 2006 and 2016 in Sweden
Intervention	Initiates treatment with P2Y12R inhibitors during the acute poststroke period
Comparator	No treatment with P2Y12R inhibitors poststroke
Outcomes	Mild cognitive disorder or dementia; all-cause mortality

answer causal questions.[36] This may be particularly useful where a clinical trial may not be feasible to conduct. We understand that significant challenges remain, however, by attempting to emulate the ideal target trial using a large national dataset, and registering a peer-reviewed study protocol prior to calculating any effect estimates or 95% CIs, we aim to produce a high-quality observational study within the methodological boundaries of available evidence, which aims to answer a causal question. Here, we outline the planned structural and statistical causal methods based on the target trial approach, which will allow us to draw causal inferences about the risk of poststroke mild cognitive disorder or dementia associated with P2Y12R inhibitor use.[37] The approach described in this protocol may be applied to other similar translational research questions in the future. Data cleaning, linkage and transformation for this study commenced in May 2021 and is expected to finish in March 2022. The analyses described in this protocol are expected to be conducted between mid-March and June 2022.

METHODS AND ANALYSIS

The operationalised research question is outlined using the population, intervention, comparison and outcome (PICO) structure (table 1).

Data sources

We propose using the Swedish National Health and Medical Registers. The national Swedish registers represent a highly validated individual-level epidemiological dataset, which essentially covers an entire population. All Swedish residents have a unique 12-digit personal identification number, making the linkage between national registers possible. This study proposes to link information from the Swedish National Inpatient Register (IPR), the Swedish Stroke Register (Riks-Stroke), the Longitudinal Integrated Database for Health Insurance and Labor Market Studies (Swedish acronym LISA; from 'Longitudinell integrationsdatabas för sjukförsäkrings- och arbetsmarknadsstudier'), the Cause of Death Register (CDR), and the Swedish Prescribed Drug Register (SPDR). This provides an unprecedented opportunity to establish a comprehensive database of stroke survivors,

including medical diagnoses and hospital admissions, pharmaceuticals and socioeconomic information. Importantly, Riks-Stroke, as a clinical quality and outcomes register, includes several clinical characteristics of stroke, including stroke type and severity (as indexed by the US National Institutes of Health Stroke Scale score).

Structural approach
Directed acyclic graph

Directed acyclic graphs (DAGs) are non-parametric graphical models that provide a visual representation of assumed causal relationships between variables, based on prior causal knowledge. DAGs are used to aid in the assessment of bias in epidemiological studies and formalise the minimal sufficient adjustment sets to estimate an internally valid population average treatment effect. DAGs also guide the choice of an appropriate statistical analytic technique for the dataset. We created a causal DAG based on literature review and expert opinion, to provide a basis for the selection of confounders that would need to be controlled in a causal analysis based on known common causes of exposure and outcome. The DAG includes variables that may mediate the relationship between the exposure (P2Y12R inhibitors) and the outcome (diagnosis of mild cognitive disorder or dementia) and the competing outcome of all-cause mortality. The assumed associations were based on published literature, including preclinical studies explaining the putative underlying neurobiology of SND that occurs poststroke,[38–42] and clinical risk evaluations for poststroke dementia.[43 44] We also consulted published treatment guidelines[45 46] and asked clinical experts how they make decisions around the prescribing of P2Y12R inhibitors poststroke. Some factors, such as birth date, sex and level of education may not change after the treatment decision has been made and will be included in the model as baseline variables. However, other variables, in particular those related to the likelihood of ongoing treatment and the risk of dementia, may change over time and will be therefore included as time-varying covariates in a per-protocol analysis.

The causal DAG shows that confounding can be controlled by blocking the backdoor paths from treatment with P2Y12R inhibitors poststroke to mild cognitive disorder or dementia (figure 1); we include CVD risk and microglial activity as latent variables that cannot be directly measured. The socioeconomic status will be modelled using variables recorded in the LISA database, including education and income. We used the online software dagitty[47] to identify the minimal sufficient adjustment set for estimating the total effect of P2Y12R inhibitors on mild cognitive disorder/dementia, which includes acute coronary syndromes, age, aspirin, depression, inflammation, prior transient ischaemic attack (TIA), small vessel disease, socioeconomic status, statins and stroke severity.

The treatment variable (P2Y12R inhibitors), cardiovascular risk and events, and the number of (recurrent) strokes may change over time and affect exposure and outcome variables at future time points (figure 2). These will be included in a per-protocol analysis adjusting for time-varying treatment and covariates (see the Analysis section).
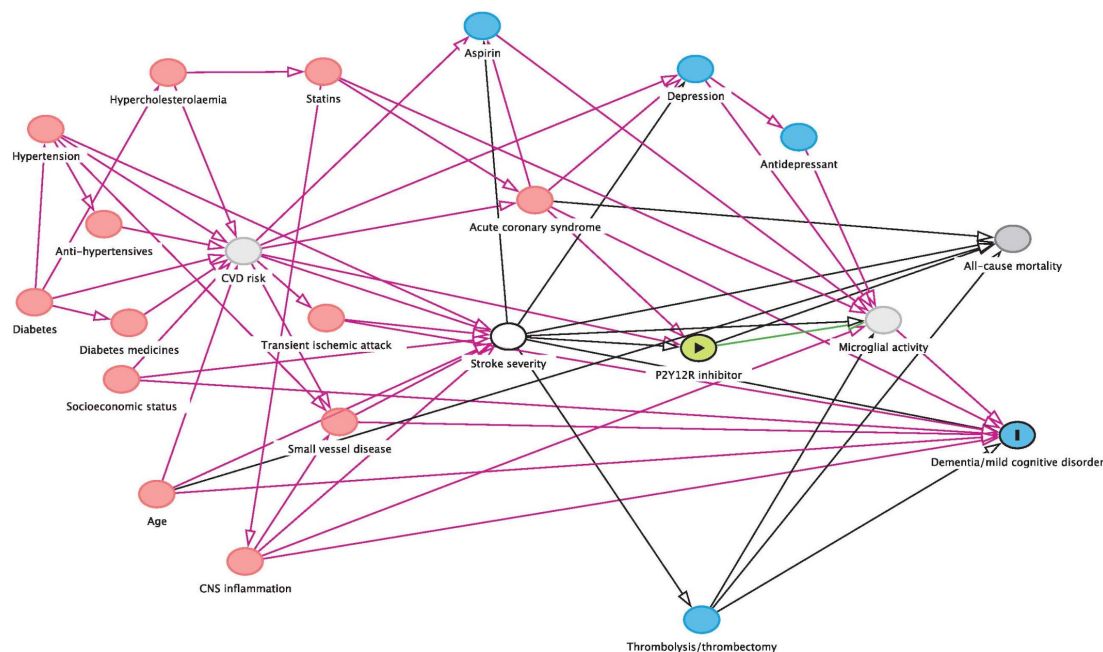
Figure 1 A causal directed acyclic graph (DAG) representing the assumed relationships (edges) between variables of interest (nodes) at baseline.[47] This DAG represents variables that may affect the relationship between treatment (P2Y12R inhibitors) and outcome (mild cognitive disorder/dementia), and the competing event (death). Green with arrow denotes exposure; blue with I denotes outcome; other blue denotes ancestors of outcome; white denotes adjusted; dark grey denotes other variable; pale grey denotes unobserved (latent) variable; red denotes ancestors of exposure and outcome. CNS, central nervous system; P2Y12R, P2Y12 receptor.
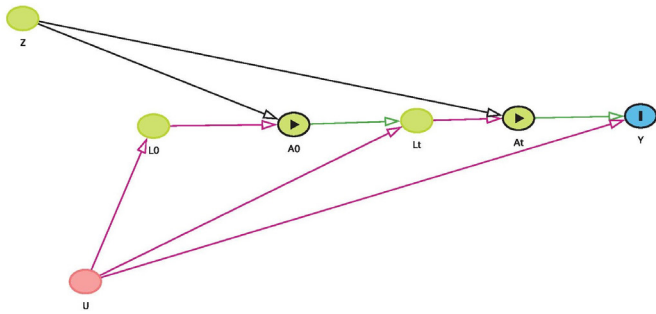
Figure 2 A causal directed acyclic graph (DAG) representing the assumed relationships (edges) between time-varying variables of interest (nodes) to be taken into account in the per-protocol analysis.[47] This DAG represents variables that may affect the likelihood of receiving treatment (P2Y12 receptor (P2Y12R) inhibitors) over time, including treatment allocation (Z), time-varying covariates (L) and treatment (A), to follow-up time (t) and incorporates the possible influence of unmeasured confounding bias (U). In this analysis, prior treatment adherence to P2Y12R inhibitors may affect measured covariates (such the occurrence of acute coronary syndromes, including recurrent stroke), and the probability of future treatment. This treatment-confounder feedback will require the use of g-methods such as inverse probability weighting,[72] to calculate the per-protocol effect of treatment with P2Y12R inhibitors over time on the outcome of dementia/mild cognitive disorder.

Explicitly defining the target trial

Target trial emulation refers to the application of randomised trial design principles to the analysis of observational data, which explicitly links the analysis to features of the hypothetical trial it is imitating. The purpose is to improve the quality of observational epidemiology through the application of trial design principles. The target trial methodology described by Hernán and Robins as well as Labrecque and Swanson,[37 48] describes a structural approach to the design of causal studies using observational data, alongside advanced statistical methods which can control for time-dependent exposure and confounding variables. The target trial approach suggests designing any observational data analysis as if one were designing an RCT. This approach is consistent with counterfactual theory in the analysis of causality, and its application, with a thorough identification of the factors summarised in table 2, has been shown to overcome biases historically associated with the analysis of observational data, as well as improve the accuracy of estimates of comparative effectiveness when replicating clinical trials.[49] Further, as clinicians and other researchers are familiar with the design principles of RCTs, it facilitates the interpretation and communication of study methods and results. This approach has been shown to improve causal effect estimates made using observational data.[49 50] In particular, organising the analysis of observational data as if it were a randomised trial, particularly by clarifying eligibility criteria and entry periods into the target trial, supports the use of analytic approaches that prevent apparent paradoxes and common biases.[51]

Hernán and Robins[37] outline seven key components of the target trial protocol: the eligibility criteria, treatment strategies being compared (including their start and end times), assignment procedures, follow-up period, the outcome of interest, causal contrast of interest and analysis plan (table 2).

Study population and eligibility criteria

The sampling frame will be drawn from the Swedish National Health Registers. The planned inclusion and exclusion criteria are summarised in table 3.

Briefly, the study population includes the older adult population (aged 55 years and over) of Sweden who have experienced incident ischaemic stroke between 2006 and 2016, with no record of any stroke in the previous 8 years, recorded as the primary diagnosis via International Classification of Diseases (ICD) codes in the IPR. We plan to include individuals who either follow the intervention treatment strategy (P2Y12R inhibitors), including clopidogrel, ticagrelor and prasugrel, or the comparator (non-P2Y12R inhibitors). Individuals will be excluded with a diagnosis of a mild cognitive disorder or dementia prior to first stroke, or which is diagnosed in the initial 6 months poststroke to minimise reverse causation by reducing the risk of including patients with the pre-existing mild cognitive disorder or dementia.[52] Individuals who experience a fatal stroke (death within 28 days of the stroke event) will be randomised between treatment arms. Furthermore, patients with atrial fibrillation and/or with non-vitamin K antagonist oral anticoagulants use, will be excluded as these populations are unlikely to be treated with P2Y12R inhibitors. Including patients who are unlikely to ever receive the exposure treatment would violate the positivity assumption, where causal estimates may be poorly identified if certain included subgroups would never receive treatment.[53] Finally, any patients recorded in the stroke quality register who do not also appear in the IPR will be excluded. The codes we plan to use to define variables are summarised in online supplemental file. A diagram of the longitudinal study design is included in figure 3.

Treatment strategies

Two treatment strategies will be compared, essentially a P2Y12R inhibitor treatment strategy, and the comparator, a non-P2Y12R inhibitor treatment strategy. All individuals will enter the study at a similar time point, that is, from the time they experience their first stroke (time 0). As treatment may not commence precisely at time 0, we will allow a 3-month grace period for treatment to begin poststroke to include individuals in the 'treated' arm.[37] Therefore, participants in the intervention group need to start treatment with any P2Y12R inhibitor within the early acute stroke recovery period (within the first 3 months poststroke, with 6 months also tested in sensitivity analysis[54]); participants in the control group should not commence treatment with a P2Y12R inhibitor during the same period. Currently, clopidogrel is relatively commonly prescribed after stroke, but not routinely recommended,

Table 2 A summary of the protocol of a target trial to estimate the effect of thienopyridine use poststroke on the risk of mild cognitive disorder and dementia

Component	Description
Population and eligibility criteria	People aged 55 years and over with first incident ischaemic stroke, with no evidence of use of P2Y12R inhibitors during the previous 6 months and no history of dementia or mild cognitive disorder (see table 3)
Treatment strategies	1. Initiate P2Y12R inhibitor therapy during the acute phase of stroke (0–3 months poststroke[54]) 2. Refrain from taking any P2Y12R inhibitor
Assignment procedures	'Randomly' prescribed P2Y12R inhibitor during the subacute phase of stroke, or not
Follow-up period	Starts at assignment to treatment (stroke(time 0)±prescription of P2Y12R inhibitor) and ends at diagnosis of dementia/cognitive disorder, death or censoring (eg, due to migration, loss to follow-up or administrative censor/end of follow-up, whichever occurs earlier) Follow-up periods of 12 months, 24 months and 5 years will be investigated Allow a grace period at baseline (ie, those assigned to the treatment strategy to initiate treatment within 3 months of being eligible)
Outcome	Dementia or mild cognitive disorder All-cause mortality
Causal contrasts of interest	1. Intention-to-treat (ITT; adjustment for baseline confounding). All those who initiate P2Y12R inhibitor therapy within the window period (regardless of compliance), compared with those who do not initiate the treatment strategy within the window period 2. Per-protocol analysis (as for ITT analysis with adjustment for time-varying treatment adherence and confounders)
Analysis plan	Intention-to-treat effect estimated via comparison of dementia/cognitive disorder risks among individuals assigned to each treatment strategy, adjusting for baseline confounders only Per-protocol effect estimated via comparison of dementia/cognitive disorder risks among individuals who comply with each treatment strategy with adjustments for prebaseline and postbaseline prognostic factors associated with adherence to the treatment strategies of interest Direct and controlled direct effects will be calculated, taking into account the competing events of dementia/all-cause mortality. An exploratory analysis will be conducted using the recently proposed separable effects estimators[70]

ITT, intention-to-treat[37 48]; P2Y12R, P2Y12 receptor.

as several non-P2Y12R antiplatelet treatments are available; therefore, we expect to be able to identify patients assigned to both treatment strategies. In the intention-to-treat (ITT) analysis, randomisation to treatment will be emulated via a comparison of initiators of the two treatment strategies of interest. Treatment initiation will be defined by prescription of any available P2Y12R inhibitor

Table 3 Inclusion and exclusion criteria

Inclusion	Exclusion
Initial stroke from 2006 onwards Aged 55 years or over at the time of stroke	Diagnosis of dementia or mild cognitive disorder prior to, or within 6 months of, stroke Oral anticoagulant use (warfarin, NOACs) Atrial fibrillation Patients recorded in the stroke quality register, but not the IPR Stroke within 8 years prior Use of P2Y12R inhibitors 6 months prior to stroke

IPR, Swedish National Inpatient Register; NOAC, non-vitamin K antagonist oral anticoagulants.

identified in the pharmaceutical database within the first 3 months poststroke, the Prescribed Drug Register. Using the prescription of any P2Y12R inhibitors as the indicator of P2Y12R inhibitor use will be analogous to treatment assignment at baseline in a randomised trial, regardless of whether the treatment strategy continues to be followed after baseline.

The time course and dose–response of P2Y12R inhibitor treatment on mild cognitive disorder or dementia will be considered in a per-protocol analysis, which will take into account the average causal effect in people who fully comply with their assigned treatment strategy during follow-up. For each patient, all prescriptions for P2Y12R inhibitors will be identified, and treatment episodes will be constructed based on prescription data. A treatment episode is defined as a series of subsequent prescriptions. The duration of each prescription will be estimated based on the number of tablets prescribed and the prescribed dosage regimen. The dose will be calculated using prescribed defined daily doses (DDDs; assumed average maintenance dose per day for a drug based on its main indication in adults), which are available in the SPDR. Clopidogrel is typically prescribed for a 30-day period, in which case patients will be considered to have
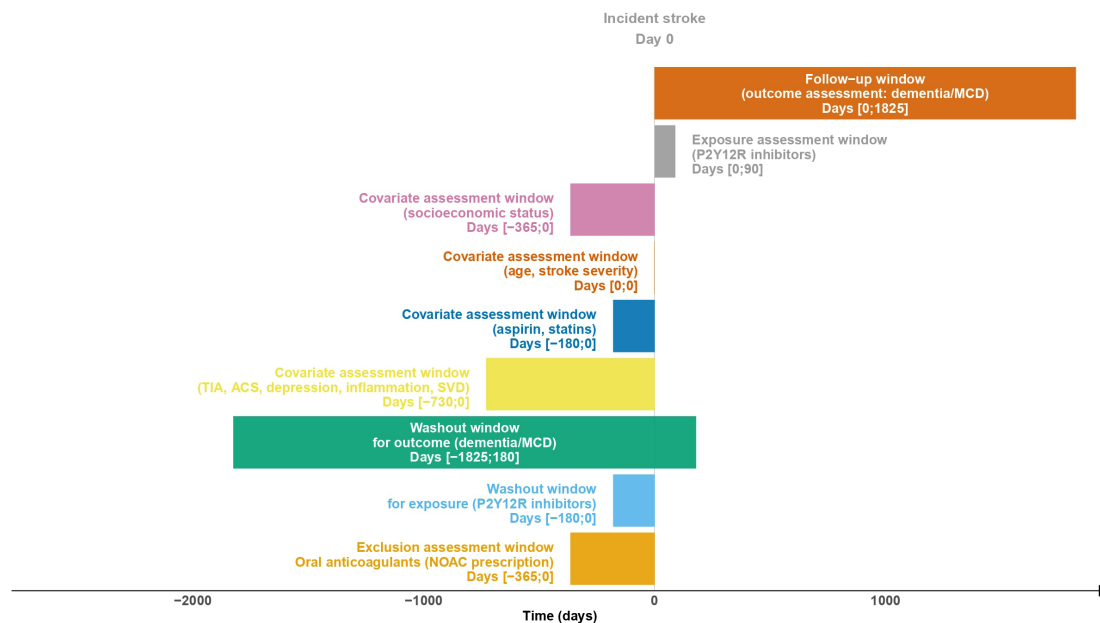
Figure 3 A graphical depiction of the study design for the main analysis, depicting baseline confounders. Based on the recommendations of the article by Schneeweiss et al.[73] ACS, acute coronary syndrome; MCD, mild cognitive disorder; NOAC, non-vitamin K antagonist oral anticoagulants; P2Y12R, P2Y12 receptor; SVD, small-vessel disease; TIA, transient ischaemic attack.

discontinued therapy if 30 days or more have elapsed between the theoretical end date of one prescription and the dispensing date of the subsequent prescription. Patients who comply with their assigned strategy (P2Y12R inhibitor or no P2Y12R inhibitor) for the duration of follow-up (1, 2 or 5 years) will be included in the per-protocol analysis.

Assignment procedure
In Sweden, medical therapy is usually dispensed in standard 3-month prescription intervals.[55] Usually, four iterations are initiated per occasion of initiation, but being discharged from a hospital may involve only one iteration, with continued iterations made by primary healthcare centres. Subsequent prescriptions are considered to be valid for inferring compliance to medications.[55 56] Participants will be assigned to a treatment arm at the beginning of the study to emulate randomisation. We will allow a grace period of 3 months from time 0 (first stroke) during which anyone prescribed a P2Y12R inhibitor will be deemed an initiator of treatment, as treatment may not commence precisely at time 0 (immediately following the stroke). The impact of the length of the grace period on the results will be tested in sensitivity analyses, using a 6-month window. The individuals in the no-treatment arm must not initiate treatment with a P2Y12R inhibitor treatment at any time during the subacute (3 months) period in the ITT analysis, or any time during follow-up in the per-protocol analysis. Allowing for this grace period means that any person who did not initiate treatment prior to 3 months, but died or was censored prior to this, will be consistent with both treatment arms. Therefore, we will randomly assign these patients to either treatment

arm using a random number generator, rather than excluding them from the analysis.

Follow-up periods
The study baseline (time 0) is the time of the first stroke. The follow-up period starts at baseline (date of the first stroke) and finishes at the end of the 5-year follow-up time. Follow-up will cease at the occurrence of the outcome, death, migration or administrative censoring, whichever occurs first.

Outcomes
The primary outcome is the diagnosis of dementia or mild cognitive disorder during the follow-up period. We will use information from multiple sources to identify those with the outcome. Initially, diagnostic codes recorded in the IPR at any level will be used to identify the presence of mild cognitive disorder or dementia via ICD codes. The first onset of dementia after 6 months of follow-up of several dementia disorders will be defined using ICD codes for a number of dementia disorders including Alzheimer's disease, vascular dementia and dementia as a result of other diseases (ICD-9: 290, 294, 331; ICD-10: F00-F03, G30, G31, G91.2), which have previously been used in another cohort study using Swedish health and medical registers.[57] Similarly, the mild cognitive disorder will be operationalised using ICD codes for the mild cognitive disorder (ICD-10: F06.7), subjective complaints of mild cognitive disorder (ICD-10: R41.8A), and mild memory disturbance (ICD-9: 310W). These data will be triangulated using prescription data for dementia drugs. There are four dementia drugs available in Sweden, including three cholinesterase inhibitors (donepezil, rivastigmine

and galantamine), and the N-methyl-D-aspartate receptor (NMDA) receptor (NMDAR) antagonist memantine. In cases where no diagnosis data (eg, ICD-10 code) exists to indicate the presence of mild cognitive disorder or dementia, but the prediction based on medication indicates a probability of disease, it will be assumed that cognitive disorder or dementia is present. The reported validity of dementia diagnosis in Sweden, with respect to IPR, is reported to have a high positive predictive value of 72%, but with a lower sensitivity of 26%–42%.[58] The latter will be considerably increased by capturing antidementia medications. A sensitivity analysis will be conducted whereby diagnoses of the mild cognitive disorder are excluded from the outcome, given that it is potentially distinct from dementia, to evaluate whether this affects study findings. All-cause mortality during follow-up will be collected as a competing event.

Causal contrasts of interest

We will estimate the ITT effect (ie, the comparative effect of being assigned to the treatment strategies at baseline, regardless of whether the individuals continue following the strategies after baseline). The causal per-protocol effect of each assigned treatment strategy will also be estimated, adjusting for adherence to the sustained treatment strategies over time.

Statistical analysis plan

Descriptive statistics will be used to summarise the data, including a flow chart illustrating the number of individuals assigned to each treatment arm, those who follow the protocol, and those who are censored or excluded, and reasons for exclusion. Patient characteristics to be descriptively summarised include age, sex, presence of baseline confounders, dementia or mild cognitive disorder, and survival.

ITT population

In the ITT population, we will test the effect of being assigned to treatment (initiators vs non-initiators), based on the prescription of P2Y12R inhibitors to mimic treatment assignment.[37] However, those included may not necessarily adhere to their assigned treatment over time. We will adjust for baseline (time-fixed) covariates required to estimate the total effect of P2Y12R inhibitors on the cognitive disorder or dementia as identified in the DAG presented in figure 1. These include acute coronary syndrome, age, aspirin, depression, inflammation, prior TIA, small vessel disease, socioeconomic status, statins and stroke characteristics (primarily stroke severity). These will be included as covariates in a logistic regression model with treatment strategy as the outcome to estimate propensity weights. The treatment weight for each patient will be the inverse of the probability that the patient had the treatment that she or he actually received, given their set of time-fixed confounders. Inverse probability of treatment weights will be assigned based on propensity scores, to achieve the balance between the two

treatment strategies. The weights will be applied in the survival (time to event) models as described later.

Per-protocol population

The per-protocol analysis will aim to estimate the treatment effect taking into account persistence and adherence to treatment and prognostic factors for treatment over time. This analysis will therefore include weighting for baseline confounders as well as time-varying treatment, confounding and censoring. Treatment will be based on dispensing data, rather than prescription data.[37] Time-varying exposure to treatment will be calculated using dispensing dates, DDDs per package and the number of dispensed packages. The duration of treatment will be calculated using DDDs per pack multiplied by the number of dispensed packages. Cessation of treatment will be defined as the absence of a new dispensation of the medication within 60 days after the calculated last day of supply from the previous dispensation. Time-dependent confounders include variables that may change after baseline and may affect treatment adherence, making them both confounders and mediators. Recurrent stroke, aspirin, acute coronary syndromes, depression, statins and inflammatory disorders reported during follow-up may be time-dependent confounders in the proposed study. In particular, recurrent stroke events will increase both the risk of dementia or mild cognitive disorder (MCD) developing and the likelihood that P2Y12R inhibitors will be prescribed. Conventional survival models are unable to appropriately account for time-varying confounding variables that lie on the causal pathway from exposure to an outcome event. Here, we propose using marginal structural models based on inverse probability weighting to account for such variables.[34] In addition to a treatment weight generated for baseline confounders, for each subject within the study sample, weights will be assigned based on the inverse of the probability of the individual's actual treatment at a given time point proportional to the inverse of the probability of treatment received, conditional on given time-dependent confounders and previous treatment.[59] The status of time-varying confounders based on relevant diagnosis or prescription will be updated every 3 months. Weights will also be estimated based on the probability that an individual was censored, due to migration or death, where relevant.[60] Individuals will be weighted using the predicted probability of not being censored in a 3-month interval in each assigned treatment arm. Where death is treated as a censoring event such as in the controlled direct effect as described later, the censoring weight is the product of the inverse probability of surviving in each interval prior to the current one, conditional on measured shared causes of death and dementia.[61] The inverse of that probability is the assigned weight for that interval. As very high weights may generate unstable results, the distribution of estimated weights will be assessed (eg, by comparing different specifications of the propensity score model), and stabilised weights used where appropriate.[62] To calculate the weights, logistic

regression models will be fit to estimate the probabilities of being treated and censored. The final weights, defined as the product of the baseline, 3-month specific treatment and 3-month specific censoring weights, will be applied to all observations. To generate the stabilised weights in this study, we will use the marginal probability of treatment instead of 1 in the weight numerator. Finally, survival models as described later will be fitted using P2Y12R inhibitor use as the only covariate in the pseudo-population created using the cumulative weights.

Analysis of primary outcome

The primary outcome is the time from baseline (first stroke, time 0) until the first diagnosis of mild cognitive disorder or dementia during the defined follow-up periods. In all analyses, censoring may occur due to administrative censor, or migration. In this study, careful consideration of competing risks will be required. During follow-up, individuals may die before developing cognitive disorder and dementia, and treatment with P2Y12R inhibitors may affect the risk of death in the analyses, via their antiplatelet effects. Estimating causal effects in the presence of competing risks has been contentious, and at present, there is no universally recommended approach. Previously studied exposures associated with dementia risk, which also increase the risk of death, have not always considered competing risks.[63–67] This has resulted in counterintuitive results in some studies, where exposures known to contribute to mortality risk, such as smoking[64 66] or cancer,[65 67] appear protective for the risk of dementia as individuals die before developing this outcome. Although treatment with P2Y12R inhibitors may increase the risk of cognitive disorder or dementia via their effects on brain repair mechanisms, it may also decrease mortality via their antiplatelet effects. Therefore, we will estimate the risk of dementia while taking into account that individuals can also progress to death and render dementia or mild cognitive disorder unobservable.

Young *et al*[68] developed a comprehensive framework for modelling causal treatment effects in the presence of competing events. One of the primary findings derived in this paper is that differences in cause-specific and subdistribution hazards, which are frequently reported as the sole causal effect estimate in epidemiological papers, cannot be interpreted as causal effects because hazards may differ due to differences in individuals who survive until a specific time point because of treatment effects before this time point.[68] This echoes a point made by Hernán,[69] who argues that HRs are biased as they are conditional on survival up to a given point in time, which may differ between treatment arms. Young *et al*[68] specify that causal treatment effects may be defined by differences in the respective cumulative incidence functions; Hernán[69] also suggested the use of survival curves over time as opposed to HRs.

Therefore, in both the ITT and per-protocol analyses, we will consider the total treatment effect of P2Y12R inhibitor use defined by the contrast of the cumulative incidences (risk) of dementia or MCI under each treatment strategy, at 1, 2 and 5 years of follow-up to estimate the total effect of treatment on the outcome. In competing event settings, a counterfactual contrast of cause-specific cumulative incidences will quantify the total causal effect of a treatment on the event of interest. To estimate the total effect of P2Y12R inhibitor use post-stroke on dementia risk, we will compare a weighted Aalen-Johansen estimator based on the cumulative incidences, in both the ITT and per-protocol populations. However, the effects of treatment on the competing event (death) may indirectly contribute to this total effect, complicating its interpretation. This means that the effect on our main outcome is through all pathways between the intervention and the outcome, including those possibly mediated by the competing event. For example, in our analysis exposure to P2Y12R inhibitors may appear to increase the risk of dementia, but some of this effect may be because they are delaying death via their antiplatelet effects. Therefore, we will also calculate the controlled direct effect, using the marginal cumulative incidence of dementia risk under elimination of death (considering death as a censoring event). To estimate the controlled direct effect, we will compare the complement of a weighted Kaplan-Meier survival estimator in treated vs untreated individuals, with time indexed in months. In this analysis, death is essentially treated as a censoring event. Although this will provide a causal estimate of the treatment effect on our outcome of interest, the interpretation in this setting emulates a counterfactual world in which death could be entirely prevented. As an investigation of a potential biological mechanism, this may be a reasonable estimator; however, the use of a population in which death is prevented may not be realistic and relies on additional strong no-unmeasured-confounding assumptions. Further, if the exposure is likely to affect the risk of death (which it may via its antiplatelet effects), censoring will be informative and bias the sample.

Estimates of the total and controlled direct effect at 1, 2 and 5 years of follow-up will be presented as risk differences and risk ratios. For all analyses, 95% CIs will be constructed using 500 non-parametric bootstrap samples. All analyses will be performed using R. For all models we will assess linear (or non-linear) annual calendar time trends by including a restricted cubic spline term as a covariate.

Exploratory analysis

Given that the total effect and controlled direct effect are both subject to certain assumptions and can only be interpreted in specific populations, we will also attempt to perform an exploratory analysis using a relatively novel estimand referred to as separable effects.[70] While identifying these effects similarly relies on strong assumptions, including measuring multiple shared causes of dementia and death, estimates of these effects could conceivably be confirmed with future studies on these modified treatments. The high dimensionality of the data set proposed

to be used in this study is also likely to allow for the measurement of these variables.

In this analysis, we would need to assume that treatment with P2Y12R inhibitors can be decomposed into their effects on brain repair by inhibiting microglial cell activation and movement towards sites of injury, and their remaining components as antiplatelet therapies, which leads to reductions in clotting and associated disorders. Each mechanism of action is likely to have differential effects on the competing risks of dementia and all-cause mortality. To date, this has only been demonstrated using data from RCTs. For the study proposed here, we would need to extend the separable effects theory to time-varying treatments using observational data, for effects that may only be partially isolated. Therefore, this analysis, based on the cause-specific cumulative incidences calculated using inverse probability weighted pooled logistic regression models, is proposed to be exploratory only.

Subgroup and sensitivity analysis
Because we believe that the mechanism by which P2Y12R inhibitors influence cognition is only after BBB disturbance, which cannot be directly observed in this dataset, we will run subgroup analyses examining the effect of other conditions known to be associated with BBB compromises such as multiple sclerosis, traumatic brain injury or SVD. We will also consider the effect of age on outcomes, as age is also known to be associated with BBB breakdown.

Sensitivity analyses will be conducted to assess the effects of decisions made during data cleaning and variable transformation. Although we hypothesise that the subacute period of stroke will be most important, we will also investigate critical periods of exposure, based on the duration of exposure to P2Y12R inhibitors in the post-stroke period. Further analyses will be conducted to assess sensitivity to unmeasured confounding. E-values will be generated for the estimates and the CIs, along with a discussion around potential unmeasured confounders.

Handling missing data
The outcome of mild cognitive disorder or dementia will be primarily based on ICD-10 codes, which will have no indicator as to whether they contain missing variables or not. Therefore, we will use additional variables, particularly prescription data, to predict and impute diagnoses. In cases where no diagnosis data (eg, ICD-10 code) exist to indicate the presence of mild cognitive disorder or dementia, but the prediction based on prescribed medication indicates a probability of disease, it will be assumed that mild cognitive disorder or dementia is present. This assumption will be tested in sensitivity analyses.

For all covariates with missing data, we will use a fully conditional approach to imputation in the primary analyses, whereby conditional distributions or regression models are specified for each missing value in a variable, conditional on values of the other variables in the imputation model.[71]

Ethics and dissemination
This study has been approved by the Ethics Committee of the University of Gothenburg and Confidentiality Clearance at Statistics Sweden with Dnr 937-18, and an approved addendum with Dnr 2019-0157. The code generated in the analysis of the dataset will be shared on a browsable website. The data that will be used in the planned study is subject to all appropriate Swedish approvals, and will be available by application to Statistics Sweden. The data will not be made publicly available due to privacy and ethical restrictions. Any changes to the protocol made in the course of analysis, in particular, due to issues arising in regard to data availability and quality, will be documented and described in the final report. The study will be published in an academic journal.

Patient and public involvement
There was no patient or public involvement in the development of this protocol.

DISCUSSION
Safety signals cannot be assessed in trials; frequently neurocognitive adverse events of drugs take many years to develop and are not identified until they have been marketed for some time. However, the potential for real-world data to address these questions is increasingly being recognised. Here, we describe an approach to use real-world evidence to answer the causal question of whether treatment with P2Y12R inhibitors may be harmful for some period of time following a stroke in some patients, in order to avoid potential neurocognitive impacts. We described the data necessary for a causal analysis of observational data. These analyses are only valid if complete information around the baseline and time-dependent confounders are available. The quality of the databases used to draw inputs will play an essential role in the validity and reliability of the results. The proposed study protocol has some limitations that need to be acknowledged when applying it to any data and interpreting the results. Appropriate sensitivity analyses will be conducted to determine the robustness of results, and potential bias must be carefully considered.

The proposed study was designed to provide insight into a potential neuroimmune mechanism, which may contribute towards the development of post-stroke dementia or mild cognitive disorder. The role of microglial cells in mediating neurodegenerative outcomes has been increasingly under investigation in recent years, with both clinical and preclinical findings suggesting that microglia are vital for brain repair and functional improvement. This dataset can also be used to examine other modifiers of microglial activity including the history of stress-related and inflammatory/ infectious disorders, immune-modifying drugs, statins and antidepressants.

There are a number of potential limitations that may arise in the proposed study, including but not limited to unmeasured confounding, underdiagnosis of dementia or mild cognitive disorder due to reliance on hospital admissions

data, and other sources of bias associated with observational cohort studies. However, we plan to conduct comprehensive sensitivity analyses to explore these limitations and quantify the effect of biases on the effect estimates generated in the study. Further, by using a national-level dataset based on administrative datasets, we hope to minimise the effect of missing data.

We have developed a study protocol that follows the target trial approach to allow drawing causal conclusions from real-world observational databases; we seek to extend observations from animal models to humans regarding the potential adverse effects of P2Y12R inhibitors on the risk of mild cognitive disorder and dementia in stroke survivors.

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