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Preadmission use of benzodiazepines is associated with increased post-stroke mortality.

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Key words: benzodiazepines; stroke; mortality.

Subject terms: stroke : ischemic stroke ; quality and outcomes: mortality/survival; epidemiology, lifestyle, and prevention: risk factors.

ABSTRACT.

Background and aims. We tested the hypothesis that stroke outcomes in patients with preadmission use of benzodiazepine are worse.

Method. In a prospective cohort study, we recruited patients with acute ischemic stroke. Mortality, functional outcomes and cognition were evaluated at 8 and 90 days after stroke.

Results. 370 patients were included. 62 (18.5%) of 336 remainders patients were treated with benzodiazepines when stroke occurred, and they did not receive any other psychotropic drug. There was no difference at 8-day. Benzodiazepines users had a higher mortality rate at 3 months (25.9% vs 8.1%, *p=0.0001*). After adjustment on baseline characteristics using a propensity score approach, the increase in 90-day mortality rate in benzodiazepines users remained significant, with an adjusted odds ratio (OR) of 3.24 (95% confidence interval [CI] 1.42 to 7.35). Benzodiazepines users didn't have a poorer functional outcome defined by a mRS ≥2 at 90 days (adjusted OR 1.70, 95%CI, 0.91 to 3.18) or a BI <95 (adjusted OR 1.17, 95%CI, 0.56 to 2.43). In survivors at 90 days, there was no significant difference in cognitive evaluations between benzodiazepine users and non users (adjusted OR 0.93, 95% CI, 0.31 to 2.76).

Conclusion: Our study had shown that preadmission use of benzodiazepines is associated with increased post-stroke mortality at 90 days, after adjustment on the main predictors of outcome. This finding does not support a putative neuroprotective effect of $GABA_A$ receptors agonists and should alert clinicians of their potential risks. (clinicaltrials.gov: NCT00763217)

STRENGTHS AND LIMITATIONS OF THIS STUDY

- This cohort study have a great potential with regards to public health in the field of stroke mortality, as benzodiazepines are one of the most prescribed drugs in the world.
- These results are consistent with those from the literature of a non neuroprotective effect of benzodiazepines.
- These results might be confounded by indication bias (between benzodiazepines users and non-users) but the use of a propensity score partially addresses this concern.
- There is a lack of consistency of results for mortality and functional outcomes of stroke, suggesting new experimental approaches, to provide and appropriate mechanistic explanation.

INTRODUCTION.

Knowledge of new factors that affect ischemic stroke prognosis is necessary for clinicians. Excessive chronic ethanol consumption is associated with higher stroke severity.[1] Ethanol and benzodiazepines share several central effects, especially on activation of inhibitory γ amino-butyric acid$_A$ ($GABA_A$) receptors in the brain. Because of their good efficacy and rapidity of action, benzodiazepines are one of the most prescribed drugs, widely used for anxiety and insomnia. Interestingly, the impact of preadmission benzodiazepines use in stroke had never been evaluated as stroke is emerging as a leading cause of preventable death and disability worldwide. The objective of our study was so to investigate the effect of preadmission use of benzodiazepine usage on stroke outcome.

METHODS.

Patient and public involvement statement.

Patients with ischemic stroke admitted to our university hospital's stroke unit within 48 hours of symptom onset were recruited in the prospective "Biostroke" cohort (clinicaltrials.gov: number NCT00763217).[1] The local independent ethical committee approved the study. Patients were managed according to local rules without any investigation or treatment specifically performed. Patients or close relatives gave a signed informed consent.

Data collection and clinical outcomes definition.

All patients underwent an initial standardized evaluation, including their medical history) and vascular risk factors (using a structured questionnaire), a physical examination, a routine blood biochemistry screen, and diagnostic testing. At admission patients underwent either CT scan or an MRI scan.

Patients had a follow-up examination 8 and 90 days after admission. The modified Rankin scale (mRS), the Barthel index (BI), the Mini Mental State Examination (MMSE), and all-cause mortality were recorded. A National Institutes of Health Stroke Scale (NIHSS) score ≥6 was considered as a severe clinical impairment. A mRS score ≥2 (poor functional outcome), an BI score <95 (poor functional outcome), and a MMSE score <24 (cognitive impairment) were considered as the worst possible stroke outcomes.[2] We also analysed the association between benzodiazepine use and respiratory failure and pneumonia at the acute phase of stroke.

Preadmission use of GABA receptors agonists.

Drug exposition was defined by benzodiazepine drugs administered orally for more than fifteen days before stroke regardless of length of treatment period and dosage of treatment. Hypnotic drugs were also included, since they acts on similar receptors to the benzodiazepines. Patients with concomitant use of other psychoactive drugs were excluded, because of their possible confounding effects.

Statistical analysis.

Quantitative variables are expressed as mean (standard deviation) in case of normal distribution or median (interquartile range) otherwise. Qualitative variables are expressed as numbers (percentage). Normality of distributions were assessed using histograms and Shapiro-Wilk test. Bivariate comparisons between benzodiazepine users and non-users were performed using Student's t test for quantitative variables (Mann-Whitney U test was used for non-Gaussian distribution) and Chi-squared test (Fisher's exact test was used when the expected cell frequency was <5) for categorical variables. Comparisons in clinical outcomes at 8 and 90 days (all-cause mortality, NIHSS $\geq$ 6, mRS $\geq$ 2, BI < 95 and MMSE < 24) were further adjusted for baseline between-group differences by including propensity score as a covariate into a logistic regression model. We derived from logistic regression model adjusted for propensity score, adjusted odds ratio (OR) with their 95% confidence interval (CI) as effect size measure. Propensity score was estimated using a non-parsimonious multivariable logistic regression model, with benzodiazepine use as the dependent variable and all characteristics associated with benzodiazepine exposure in bivariate analysis ($p<0.20$) as independent variables. To avoid case deletion in propensity score due to missing information for independent variables (n=38), missing values on independent variables (range, 0% to 7.1%) were imputed by multiple imputation using regression switching approach (chained equations, with n=20 imputations) with predictive mean matching method for continuous

variables, and logistic regression model for binary variables.[3] The covariates used to generate the multiple imputed data sets (using the R statistical software version 3.03) were all variables listed in table 1. Since the rate of missing data was high for 8- and 90 day MMSE (27% and 24%, respectively), we performed sensitivity analysis by also using multiple imputation approach to handle missing values. Statistical testing was done at the two-tailed α level of 0.05. Data were analyzed using SAS software (version 9.3, SAS Institute Inc., Cary, NC, USA).

RESULTS

Among the 370 patients included in the Biostroke study, 34 patients (mean age, 69.0±13.7; 14 men) were excluded because of concomitant use of other psychoactive drugs. In the 336 remainders, 62 (18.5%) were under benzodiazepines when stroke occurred. The baseline characteristics of the study population are described in Table 1. Benzodiazepine users were older (73.1±12.6 *versus* 65.4±15.0, *p=0.0002*), more likely to be women (62.9 *versus* 42.7%, *p=0.004*) and to have arterial hypertension (77.4% *versus* 56.6%, *p=0.002*), lower BMI (25.6±4.8 *versus* 27.0±4.9, *p=0.042*) and lower levels of alanine aminotransferase (18 (14-23) *versus* 21 (15-29), *p=0.029*) than benzodiazepine users.

Twenty patients were lost to follow-up between day 8 and day 90 (see supplemental table 1 for their main characteristics). Death occurred in 11 patients (3.3%) patients at day-8 and 36 (11.4%) at day-90. The mortality rate was significantly higher in benzodiazepines users than non-users at day-8 and day-90 (table 2). After adjustment on baseline differences using a propensity score approach, the increase in mortality risk for benzodiazepines users remained significant at 90 day, with an adjusted OR of 3.24 (95%CI 1.42 to 7.34).

A higher rate of poor functional outcome at day-8 defined by mRS≥2 or by the BI<95 was found in benzodiazepines users (table 2). A similar between-group difference was found for mRS ≥2 at 90-day. However, after adjustment, the difference in poor functional outcomes was not significant.

In survivors at day-8 or at day-90, there was no significant difference in MMSE. When the analyses were repeated after handling the missing data on MMSE by using multiple imputation approach (see supplemental tables 2 for main baseline characteristics in patients with and without missing values), similar non-significant differences were found : the adjusted OR (95%CI) was 0.56 (0.23-1.32) for 8-day MMSE<24 and 0.93 (0.31-2.76) for 90-

day MMSE<24.

Regarding respiratory failure or pneumonia at 8 day, benzodiazepines users have a similar early respiratory complications risk than non-users (12.9% (n=8) *vs.* 17.5% (n=48), p=0.73).

DISCUSSION

Users of benzodiazepines had a higher risk of death 90 days after stroke but benzodiazepine use was not associated with worse functional outcome.

Mortality and benzodiazepines.

GABA receptor agonists have been first considered as a type of neuroprotective agent in reducing infarct size and improving functional outcome in animal models of cerebral ischaemia.[4,5] However, in humans, a recent review does not provide the evidence to support the use of GABA receptor agonists for the treatment of patients with acute ischaemic stroke.[6] Randomized controlled trials (RCTs) investigating GABA receptor agonists versus placebo for acute stroke patients with the outcomes of death or dependency and functional independence were included. These RCTs measured death and dependency at three months in clomethiazole versus placebo or between diazepam and placebo without significant difference. In a recent non-randomized comparison, treatment with benzodiazepines after ischaemic stroke had no independent impact on stroke outcome and mortality at 90-day.[7] However, these data were registered in a trials archive and were not derived from prospective trials, with indication bias and many confounders. In our prospective study, current users of benzodiazepines have a higher rate of post-stroke mortality at 90-day. Effect of benzodiazepine drugs on mortality is still debated, but these results are consistent with those from the literature. In a large cohort of patients attending primary care, $GABA_A$ receptors agonists were associated with significantly increased risk of mortality.[8] In two other representative databases, a significant while moderate increase in all-cause mortality in relation to benzodiazepines was found, in a population of incident and mostly occasional

users.[9] A recent population-wide register-based study identified that benzodiazepines are more frequently used in patients with strokes than in controls and are associated with greater all-cause mortality in patients with stroke and matched controls.[10] The use of anti-anxiety medication and mortality risk in patients following myocardial has also been studied in a sampling database.[11] Sudden death was significantly associated with increased benzodiazepam dosage during approximately five years. For patients receiving higher doses of daily benzodiazepines, protective effects for cardiac mortality and heart failure hospitalization decreased and a J-curve dose-response relationship was seen, without providing an adequate mechanistic explanation. As with all observational findings, these results are prone to bias arising from unmeasured and residual confounding. In our study, there was no association between mortality and poor functional outcome. For the deaths, information about the underlying cause was unfortunately not obtained, but was not associated with stroke severity. Benzodiazepines have been shown to increase the occurrence of community-acquired pneumonia,[12] due to their pharmacodynamics properties. In our cohort, prior use of benzodiazepines didn't increase the incidence of respiratory depression, and cannot explain the excess mortality in these patients. Although the causes of death can't be explained, our stroke data base was prospectively collected and the study was carried out in a representative cohort of routine clinical stroke patients with an exhaustive drug history analysis. Many follow up data were available which allow adjustment on a large number of potential confounders of the benzodiazepine effects and mortality. Although, our results might be confounded by indication bias; the use of a propensity score partially addresses this concern, but benzodiazepine users could be different from non-users (unmeaseured baseline variables).

Cognition and benzodiazepines.

Benzodiazepine use was neither associated with cognitive impairment at 8 days or 90 days. However, the short term effects of benzodiazepines on impairment cognition are well known and use of benzodiazepines is also associated with increased risk of dementia, even if the nature of the link between benzodiazepines and Alzheimer's disease remains unclear.[13] In our cohort, GABA receptor agonists treatment before stroke didn't show cognitive impairment as assessed by MMSE, in these elderly patients without dementia, but a longer follow-up period may be useful. Further, although we use methods to impute for missing data, data are missing on follow-up - loss to follow-up in a set, and then lack of doing MMSE on follow-up in another set (post-stroke aphasia) - which are likely not missing at random.

Comorbid alcohol use disorder.

Prior benzodiazepine use (regardless the dosage of treatment) was not associated with higher baseline stroke severity, as excessive chronic ethanol consumption was.[1] Benzodiazepine users were also less likely to be alcohol drinkers in our study, although the difference did not reach the significance level. So, the relationship between stroke severity and alcohol consumption could not be necessarily due to a link between GABA receptors and alcohol, but due to chronic effects of ethanol consumption on other organ systems.

Unanswered questions and implication for clinical practice.

The lack of consistency of results for mortality and functional outcomes make this less

likely to explain a physiologic effect. Anyway, the increased rate of mortality after stroke found in the benzodiazepines users group add to the increasing body of evidence concerning a non-neuroprotective effect of GABA receptors agonists. Benzodiazepines reduce the cerebral metabolic rate of oxygen and cerebral blood flow and can induce post-hypoxic leukoencephalopathy.[14,15] As lack of blood flow leads to cerebral hypoxia, it results in a cascade of biological events, which facilitates glutamate release. Based upon these data, we hypothesized that chronic cerebral hypoxia could thus be induced by benzodiazepines use. Short- and long-term modulation of GABAA receptors by benzodiazepines could modulate ischaemia-induced glutamate release. Our findings generate a hypothesis that needs confirmation. As an interventional study would not be feasible, this question can be answered though experimental approaches in animals, to provide and appropriate mechanistic explanation.

This research should also not be used to condemn GABA receptors agonist drugs since their short-term use can have an important role in the management of anxiety. This study should however alert clinicians to the increased post-stroke mortality in benzodiazepine-users. As patients could also be at high risk of recurrence after stroke, use of benzodiazepines should be cautioned against.

CONCLUSION.

Preadmission use of benzodiazepines was associated with a higher medium-term post-stroke mortality. Our findings do not support a putative neuroprotective effect or these drugs. Further larges studies are warranted to confirm the association between benzodiazepines use and early post-stroke mortality.

Table 1. Comparison of baseline characteristics between benzodiazepine users and non-users.

	Benzodiazepine non-users (n=274)	Benzodiazepine users (n=62)	*P*
Demographic characteristics			
Age, y, mean±SD	65.4 ± 15.0	73.1 ± 12.6	0.0002
Men	157 (57.3)	23 (37.1)	0.004
Medical history			
Previous stroke	28 (10.3)	7 (11.3)	0.81
Previous TIA	20 (7.3)	5 (8.1)	0.79
Coronary artery disease	52 (19.0)	17 (27.4)	0.14
Sleep apnea syndrome	7 (2.6)	2 (3.2)	0.68
Heart rhythm disorders	59 (21.6)	20 (32.3)	0.075
Vascular risk factors			
Arterial hypertension	155 (56.6)	48 (77.4)	0.002
Diabetes mellitus	59 (21.5)	7 (11.3)	0.067
Hypercholesterolemia	124 (45.3)	34 (54.8)	0.17
Hypertriglyceridemia	41 (15.0)	11 (17.7)	0.58
Smoking	89 (32.5)	13 (21.0)	0.075
Chronic ethanol consumption	45 (16.5)	7 (11.5)	0.33
BMI, kg/m^2, mean±SD	27.0 ± 4.9	25.6 ± 4.8	0.042
Routine drugs			
Fibrates	17 (6.2)	6 (9.7)	0.40
Statins	85 (31.0)	22 (35.5)	0.50
Oral anticoagulants	17 (6.2)	2 (3.2)	0.54
Antiplatelet	98 (35.8)	28 (45.2)	0.17
ACE	47 (17.1)	16 (25.8)	0.12
Angiotensin II receptor antagonist	46 (16.8)	10 (16.1)	0.90

Diuretics	65 (23.7)	21 (33.9)	0.098
Calcium channel blockers	42 (15.3)	11 (17.7)	0.64
Betablockers	98 (35.9)	29 (46.8)	0.11
Oral hypoglycemic	45 (16.4)	6 (9.7)	0.18
Intravenous thrombolysis	78 (28.9)	16 (25.8)	0.63
Prehospital delay stroke, min, mean±SD	141 ± 56	160 ± 51	0.22
NIHSS ≥6	142 (51.8)	34 (54.8)	0.67
Biological characteristics			
Triglycerides, g/L, median (IQR)	1.06 (0.81-1.56)	1.01 (0.84-1.56)	0.93
Total cholesterol, g/L, mean±SD	1.96 ± 0.49	1.92 ± 0.52	0.60
HDL-cholesterol, g/L, mean±SD	0.53 ± 0.17	0.54 ± 0.13	0.86
LDL-cholesterol, g/L, mean±SD	1.17 ± 0.41	1.14 ± 0.44	0.61
Glycated hemoglobin, %, median (IQR)	5.9 (5.6-6.5)	5.9 (5.7-6.3)	0.98
Hemoglobin, g/dL, median (IQR)	13.8 (12.9-14.9)	13.5 (12.5-14.2)	0.077
Leukocytes, /mm^3, median (IQR)	8320 (6745-9865)	8335 (6700-10680)	0.55
Neutrophils, /mm^3, median (IQR)	5400 (4200-7400)	5850 (4500-8150)	0.26
Platelets, 1000/mm^3, median (IQR)	235 (197-271)	234.5 (192-274)	0.88
Prothrombin index, %, median (IQR)	96 (88-100)	94 (86-100)	0.42
Activated partial thromboplastin time, s, median (IQR)	32 (29-35)	32 (28-37)	0.52
C-reactive protein, mg/L, median (IQR)	4.7 (2.0-9.7)	5.5 (2.5-9.7)	0.65
Aspartate aminotransferase, U/L, median (IQR)	23 (19-29)	23 (20-27)	0.88
Alanine aminotransferase, U/L, median (IQR)	21 (15-29)	18 (14-23)	0.029

Data are expressed as number (%) unless otherwise indicated.

Abbreviations: ACE=angiotensin converting enzyme, BMI= body mass index, HDL= high density lipoprotein, IQR=interquartile range, LDL=low density lipoprotein, NIHSS= National Institutes of Health Stroke Scale, SD=standard deviation, TIA=transient ischemic attack.

Table 2. Outcomes at 8-day and 90-day after stroke according to prior benzodiazepine use

	Benzodiazepine users				
	No	Yes	*p*	OR (95% CI)*	*p**
Outcome at 8-day	(n=274)	(n=62)			
All-cause death	6/274 (2.2)	5/62 (8.1)	0.034	2.51 (0.67-9.32)	0.17
NIHSS ≥6	86/264 (32.58)	22/55 (40)	0.29	1.22 (0.64-2.33)	0.54
Poor outcome (mRS ≥2)	137/270 (50.7)	39/60 (65.0)	0.045	1.55 (0.84-2.87)	0.16
Poor outcome (Barthel <95)	119/265 (44.9)	35/56 (62.5)	0.017	1.78 (0.95-3.35)	0.072
Cognitive impairment (MMSE <24)	52/196 (26.5)	11/40 (27.5)	0.90	0.56 (0.23-1.32)	0.18
Outcome at 90-day	(n=258)	(n=58)			
All-cause death	21/258 (8.1)	15/58 (25.9)	0.0001	3.24 (1.42-7.35)	0.005
Poor outcome (mRS ≥2)	110/258 (42.6)	35/58 (60.3)	0.014	1.70 (0.91-3.18)	0.096
Poor outcome (Barthel <95)	72/235 (30.6)	16/43 (37.2)	0.39	1.17 (0.56-2.43)	0.68
Cognitive impairment (MMSE <24)	26/194 (13.4)	5/35 (14.3)	0.79	0.93 (0.31-2.76)	0.90

Values are n/N (%) unless otherwise indicated. * adjusted on propensity score.

Abbreviations: *MMSE: Mini* Mental State Examination, mRS: modified Rankin score, NIHSS: National Institutes of Health *S*troke Scale, OR: odds ratio, CI: confidence interval.

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AUTHORS' CONTRIBUTIONS

OC contributed to the analysis and drafting the manuscript. JL performed statistical analysis and contributed to drafting the manuscript. JD contributed to drafting the manuscript. AMM contributed to study design and data collection. VD contributed to statistical analysis and contributed to drafting the manuscript. CC contributed to study design and data collection. DD contributed to study design, data collection and analysis. DL contributed to study design, data collection and analysis. RB contributed to study design, data collection and analysis, and drafting the manuscript. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST.

The authors declare that they have no competing interests.

DATA SHARING STATEMENT

No additional data are available.

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ONLINE SUPPLEMENT.

Supplemental table 1. Main baseline characteristics of patients loss and not loss at 90-day

	90-day follow-up	
	Yes (n=316)	**No** (n=20)
Number of patients	316	20
Benzodiazepine use	58 (18.3)	4 (20.0)
Age, y, mean±SD	66.6 ± 14.9	71.5 ± 12.9
Men	170 (53.8)	10 (50.0)
Medical history		
Previous stroke	32 (10.1)	3 (15.8)
Previous TIA	24 (7.6)	1 (5.0)
Coronary artery disease	63 (19.9)	6 (30.0)
Sleep apnea syndrome	9 (2.9)	0 (0.0)
Heart rhythm disorders	75 (23.8)	4 (20.0)
Vascular risk factors		
Arterial hypertension	190 (60.1)	13 (65.0)
Diabetes mellitus	61 (19.3)	5 (25.0)
Hypercholesterolemia	149 (47.1)	9 (45.0)
Hypertriglyceridemia	51 (16.1)	1 (5.0)
Smoking	94 (19.7)	8 (40.0)
Chronic ethanol consumption	49 (15.6)	3 (15.0)
BMI, kg/m^2, mean±SD	26.8 ± 5.0	25.5 ± 3.9

Data are expressed as number (%) unless otherwise indicated.

Abbreviations: BMI= body mass index, SD=standard deviation, TIA=transient ischemic attack.

Supplemental table 2. Main baseline characteristics of alive patients with and without missing MMSE at 8- and 90-days

	8-day MMSE		90-day MMSE	
	Not missing	Missing	Not missing	Missing
Number of patients	236	89	229	71
Benzodiazepine use	40 (16.9)	17 (19.1)	35 (15.3)	12 (16.9)
Age, y, mean±SD	65.2 ± 14.4	70.3 ± 15.2	65.5 ± 14.9	69.8 ± 13.3
Men	132 (55.9)	45 (50.6)	128 (55.9)	37 (52.1)
Medical history				
Previous stroke	22 (9.3)	10 (11.4)	22 (9.6)	8 (11.4)
Previous TIA	16 (6.8)	9 (10.1)	14 (6.1)	7 (9.9)
Coronary artery disease	40 (16.9)	26 (29.2)	44 (19.2)	14 (19.7)
Sleep apnea syndrome	6 (2.6)	3 (3.4)	4 (1.8)	4 (5.7)
Heart rhythm disorders	51 (21.6)	24 (27.3)	52 (22.8)	19 (26.8)
Vascular risk factors				
Arterial hypertension	137 (58.0)	57 (64.0)	138 (60.3)	42 (59.1)
Diabetes mellitus	44 (18.6)	19 (21.3)	47 (20.5)	12 (16.9)
Hypercholesterolemia	107 (45.3)	47 (52.8)	111 (48.5)	31 (43.7)
Hypertriglyceridemia	41 (17.4)	10 (11.2)	40 (17.5)	8 (11.3)
Smoking	81 (34.3)	18 (20.2)	67 (29.3)	23 (32.4)
Chronic ethanol consumption	39 (16.7)	13 (14.6)	38 (16.6)	13 (18.6)
BMI, kg/m^2, mean±SD	26.7 ± 4.8	27.0 ± 5.1	26.8 ± 4.8	26.6 ± 5.0

Data are expressed as number (%) unless otherwise indicated.

Abbreviations: BMI= body mass index, SD=standard deviation, TIA=transient ischemic attack.

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract Page 2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found (Page 2)
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported Page 4
Objectives	3	State specific objectives, including any prespecified hypotheses Page 4
Methods		
Study design	4	Present key elements of study design early in the paper (page 5)
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection (page 5)
Participants	6	*Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up (page 5)
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed (NA)
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable (page 5)
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group (page 5-6)
Bias	9	Describe any efforts to address potential sources of bias (NA)
Study size	10	Explain how the study size was arrived (page5)
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why (page 6)
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding (page 6)
		(*b*) Describe any methods used to examine subgroups and interactions ((page 6))
		(*c*) Explain how missing data were addressed (pages 6-7)
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed (page 6)
		(*e*) Describe any sensitivity analyses (page 6)

Continued on next page

Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (page 8)
		(b) Give reasons for non-participation at each stage (page 8)
		(c) Consider use of a flow diagram (NA)
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (page 8; 15-16)
		(b) Indicate number of participants with missing data for each variable of interest (page 8; 15-16)
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount) (page 8; 15-16)
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time (page 8;17)
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (page 8; 15-17)
		(*b*) Report category boundaries when continuous variables were categorized (page 8; 15-17)
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period (NA)
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses (suppl file)
Discussion		
Key results	18	Summarise key results with reference to study objectives (page 10)
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias (page 10-12)
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence (page 13)
Generalisability	21	Discuss the generalisability (external validity) of the study results (pages 13-14)
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based (page 18)

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Preadmission use of benzodiazepines and stroke outcomes: the Biostroke prospective cohort study.

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List of figures and tables:

Key words: benzodiazepines; stroke; mortality.

Subject terms: stroke: ischemic stroke; quality and outcomes: mortality/survival; epidemiology, lifestyle, and prevention: risk factors.

ABSTRACT.

Objectives. We tested the hypothesis that stroke outcomes in patients with preadmission use of benzodiazepine are worse.

Method. In a prospective cohort study, we recruited patients with acute ischemic stroke. Mortality, functional outcomes and cognition were evaluated at 8 and 90 days after stroke.

Results. 370 patients were included. 62 (18.5%) of 336 remainders patients were treated with benzodiazepines when stroke occurred, and they did not receive any other psychotropic drug. The mortality rate was higher in benzodiazepines users than non-users at day-8 (2.2% vs. 8.1%, p=0.034) and day-90 (8.1% vs. 25.9%, p=0.0001). After controlling for baseline differences using propensity-score matching, only the difference in mortality rate at 90-day was of borderline of significance, with a matched OR of 3.93 (95%IC, 0.91-16.98). In adjusted-propensity-score cohort, this difference remained significant with a similar treatment effect size (adjusted OR, 3.50; 95%CI, 1.57-7.76). A higher rate of poor functional outcome at day-8 and day-90 defined by mRS≥2 or by the BI<95 was found in benzodiazepines users. In propensity-score adjusted cohort, only the difference in mRS ≥2 at 90-day remained significant (adjusted OR, 1.89; 95%CI, 1.02-3.48). In survivors at day-8 and at day-90, there was no significant difference in cognitive evaluation.

Conclusion: Our study has shown that preadmission use of benzodiazepines could be associated with increased post-stroke mortality at 90 days. These findings do not support a putative neuroprotective effect of $GABA_A$ receptors agonists and should alert clinicians of their potential risks. (clinicaltrials.gov: NCT00763217)

STRENGTHS AND LIMITATIONS OF THIS STUDY

- This cohort study has a great potential with regards to public health in the field of stroke outcomes, as benzodiazepines are one of the most prescribed drugs in the world.
- These results are consistent with those from the literature of a non-neuroprotective effect of benzodiazepines.
- These results might be confounded by indication bias (between benzodiazepines users and non-users) but the use of a propensity score matching/adjustment partially addresses this concern.
- There is a lack of consistency of results for mortality and functional outcomes of stroke, suggesting new experimental approaches, to provide and appropriate mechanistic explanation.
- Our results should be interpreted as hypothesis generating (without possibility of concluding that there is a causal effect) and should be replicated in further studies.

INTRODUCTION.

Stroke is the second most common cause of death and the third most common cause of disability-adjusted life-years worldwide.[1] Considering the long-term neurological disabilities which may result from acute stroke, and differences in the extent of recovery among stroke survivors, predicting the outcomes of stroke is a very important issue.[1] A wide variety of factors influence stroke prognosis, including age, stroke severity, comorbid conditions, clinical findings…[2] Knowledge of others factors - as pharmacologic and use of drugs - that could influence the severity and short-term outcomes of stroke prognosis is necessary for clinicians. For example, benzodiazepines have been first considered as a type of neuroprotective agent in reducing infarct size and improving functional outcome in animal models of cerebral ischaemia.[3,4] However, in humans, a recent review does not provide the evidence to support the use of benzodiazepines (GABA receptor agonists) for the treatment of patients with acute ischaemic stroke.[5] Benzodiazepines and ethanol share several central effects, especially on activation of inhibitory γ amino-butyric $acid_A$ ($GABA_A$) receptors in the brain. We have recently shown that excessive chronic ethanol consumption is associated with higher stroke severity.[6] Interestingly, the impact of preadmission use of benzodiazepine in stroke had never been evaluated as stroke is emerging as a leading cause of preventable death and disability worldwide. Benzodiazepines, because of their good efficacy and rapidity of action, are also one of the most prescribed drugs in the world, widely used for anxiety and insomnia. The objective of our study was so to investigate the effect of preadmission use of benzodiazepine usage on stroke outcome, to clarify their role as stroke prognostic factors

METHODS.

Patient and public involvement statement.

Patients with ischemic stroke admitted to our university hospital's stroke unit (*Lille, France*) within 48 hours of symptom onset were recruited in the prospective "Biostroke" cohort (Clinical Biological and Pharmacological Factors Influencing Stroke Outcome). The aim of the study was to understand the mechanisms of preventive neuroprotection by establishing link between biomarkers and preventive and neuroprotective measures (clinicaltrials.gov: number NCT00763217).[6] Use of benzodiazepine was one of the interests. The local independent ethical committee approved the study (*Comité de Protection des Personnes Nord-Ouest IV*). Patients were managed according to local rules without any investigation or treatment specifically performed. Patients or close relatives gave a signed informed consent.

Data collection and clinical outcomes definition.

All patients underwent an initial standardized evaluation, including their medical history) and vascular risk factors (using a structured questionnaire), a physical examination, a routine blood biochemistry screen, and diagnostic testing. At admission patients underwent either CT or MRI scan.

Patients had a follow-up examination 8 and 90 days after admission. The modified Rankin scale (mRS), the Barthel index (BI), the Mini Mental State Examination (MMSE), and all-cause mortality were recorded. Definitions used for variables included in the analysis have been previously defined.[2] A National Institutes of Health Stroke Scale (NIHSS) score ≥6 was considered as a severe clinical impairment. A mRS score ≥2 (poor functional outcome), a BI score <95 (poor functional outcome), and a MMSE score <24 (cognitive impairment) were

considered as the worst possible stroke outcomes.[2] We also analysed the association between benzodiazepine use and respiratory failure and pneumonia at the acute phase of stroke.

Preadmission use of GABA receptors agonists.

Drug exposition was defined by benzodiazepine drugs administered orally for more than fifteen days before stroke regardless of length of treatment period and dosage of treatment. Hypnotic drugs were also included, since they act on similar receptors to the benzodiazepines. Patients with concomitant use of other psychoactive drugs were excluded, because of their possible confounding effects.

Statistical analysis.

Quantitative variables are expressed as mean (standard deviation) in case of normal distribution or median (interquartile range) otherwise. Categorical variables are expressed as numbers (percentage). Normality of distributions were assessed using histograms and Shapiro-Wilk test. Bivariate comparisons between benzodiazepine users and non-users were performed using Student's t test for quantitative variables (Mann-Whitney U test was used for non-Gaussian distribution) and Chi-squared test (Fisher's exact test was used when the expected cell frequency was <5) for categorical variables

We assessed the effect of the benzodiazepine use on clinical outcomes at 8 and 90 days (all-cause mortality, NIHSS $\geq$ 6, mRS $\geq$ 2, BI < 95 and MMSE < 24) using logistic regression models and calculated the odds ratio (OR) associated with benzodiazepine use as the treatment effect size. In order to reduce the effects of potential confounding factors in the between-group comparisons, we used propensity-score methods.[7] As the main analysis, propensity score was used to assemble well-balanced groups (propensity score-matched cohort) and generalized estimating equations (GEE) models were used to take into account

the matched design. As a secondary analysis, the propensity score was used as a covariate in logistic regression models to adjust the comparisons (propensity score-adjusted cohort).

The propensity score was estimated using a non-parsimonious multivariate logistic regression model, with the benzodiazepine treatment group as the dependent variable and all of the characteristics listed in Table 1 as covariates. Benzodiazepine users were matched 1:1 to patients in the non-benzodiazepine users according to propensity score using the greedy nearest neighbor matching algorithm with a caliper width of 0.2 standard deviation of logit for propensity score.[8,9] To evaluate bias reduction using the propensity score matching method, absolute standardized differences were calculated before and after propensity-score matching; an absolute standardized difference>10% indicated a meaningful imbalance in the baseline covariate.[10]

Because of missing baseline data (see supplemental table 1), the propensity score could not be computed in 54.5% (n=183) of the study sample (61.3% in benzodiazepine users and 52.9% in non-benzodiazepine users). We therefore estimated the treatment effect size in propensity score-matched- and -adjusted cohorts after handling missing covariate values by multiple imputation[11] using a regression switching approach (chained equations with m=20 imputations obtained using the R statistical software version 3.03).[12] Imputation procedure was performed under the missing at random assumption using all variables listed in Table 1 (i.e. baseline characteristics and treatment group) with a predictive mean matching method for continuous variables and logistic regression model for categorical (all binary) variables. In each imputed dataset, we calculated the propensity score and assembled a matched cohort to provide both adjusted and matched ORs. We therefore combined the ORs from each imputed dataset using Rubin's rules.[13]

Since, among alive patients, the rate of missing data was high for 8- and 90-day MMSE (27% and 24%, respectively), we also used multiple imputation approach to handle these missing values as a sensitivity analysis. Statistical testing was done at the two-tailed α level of 0.05. Data were analyzed using SAS software (version 9.3, SAS Institute Inc., Cary, NC, USA).

RESULTS

Among the 370 patients included in the Biostroke study, 34 patients (mean age, 69.0±13.7; 14 men) were excluded because of concomitant use of other psychoactive drugs. In the 336 remainders, 62 (18.5%) were under benzodiazepines when stroke occurred.

Benzodiazepine and baseline characteristics.

The baseline characteristics of the study population according to benzodiazepine treatment are described in Table 1. Before matching, several meaningful differences (absolute standardized difference>10%) were found. In particular, benzodiazepine users were older (73.1±12.6 *versus* 65.4±15.0, *p=0.0002*), more likely to be women (62.9 *versus* 42.7%, *p=0.004*) and to have arterial hypertension (77.4% *versus* 56.6%, *p=0.002*), lower BMI (25.6±4.8 *versus* 27.0±4.9, *p=0.042*) and lower levels of alanine aminotransferase (18 (14-23) *versus* 21 (15-29), *p=0.029*) than benzodiazepine users. These differences were reduced after propensity score-matching (Figure 1 & supplemental table 2) with an absolute standardized difference >10% only for onset to admission time (22.0%), and prothrombin index (14.8%) suggesting that the two study groups were well balanced after matching.

Benzodiazepine and Outcomes

Of the 336 study patients, 20 patients were lost to follow-up between day 8 and day 90 (see supplemental table 3 for their main characteristics). Death occurred in 11 patients (3.3%) at day-8 and 36 (11.4%) at day-90 (see supplemental table 4 for main individual characteristics of mortality cases). 57.9% of survivor patients taking benzodiazepines at admission continued to take them after stroke at day-8, 46.5 % at day-90.

In unadjusted analysis, the mortality rate was higher in benzodiazepines users than non-users at day-8 (2.2% vs. 8.1%, p=0.034) and day-90 (8.1% vs. 25.9%, p=0.0001).

However, after controlling for baseline differences using propensity-score matching, only the difference in mortality rate at 90-day was of borderline of significance, with a matched OR of 3.93 (95%IC, 0.91-16.98; table 2). In adjusted-propensity-score cohort, this difference remained significant with a similar treatment effect size (adjusted OR, 3.50; 95%CI, 1.57 to 7.76; table 3).

In unadjusted analysis, a higher rate of poor functional outcome at day-8 defined by mRS≥2 or by the BI<95 was found in benzodiazepines users (table 3). A similar between-group difference was found for mRS ≥2 at 90-day. However, none of the differences were found in propensity-score matched (table 2). In propensity-score adjusted cohort, only the difference in mRS ≥2 at 90-day remained significant (adjusted OR, 1.89; 95%CI, 1.02 to 3.48).

In survivors at day-8 or at day-90, there was no significant difference in MMSE, in unadjusted, propensity-score matched and adjusted analyses. When the analyses were repeated after handling the missing data on MMSE by using multiple imputation approach (see supplemental tables 4 for main baseline characteristics in patients with and without missing values), similar non-significant differences were found. In propensity-score matched cohort, the OR (95%CI) were 0.82 (0.30 to 2.19) for 8-day MMSE<24 and 1.04 (0.23 to 4.51) for 90-day MMSE<24. In propensity-score adjusted cohort, the OR (95%CI) were 0.89 (0.43 to 1.83) for 8-day MMSE<24 and 1.04 (0.34 to 3.16) for 90-day MMSE<24.

Regarding respiratory failure or pneumonia at 8 day, benzodiazepines users have a similar early respiratory complications risk than non-users (13.8% (n=8) *vs.* 15.6% (n=48), unadjusted p=0.73).

DISCUSSION

Preadmission use of benzodiazepines could not be considered as neuroprotective, as users of benzodiazepines could have a higher risk of death at 90-days after stroke.

Mortality and benzodiazepines.

A recent review does not provide the evidence to support the use of GABA receptor agonists for the treatment of patients with acute ischaemic stroke.[5] Randomized controlled trials (RCTs) investigating GABA receptor agonists versus placebo for acute stroke patients with the outcomes of death or dependency and functional independence were included. These RCTs measured death and dependency at three months in clomethiazole versus placebo or between diazepam and placebo without significant difference. In a recent non-randomized comparison, treatment with benzodiazepines after ischaemic stroke had no independent impact on stroke outcomes and mortality at 90-day.[14] However, these data were registered in a trials archive and were not derived from prospective trials, with indication bias and many confounders. In our prospective study, current users of benzodiazepines could have a higher rate of post-stroke mortality at 90-day. Effect of benzodiazepine drugs on mortality is still debated, but these results are consistent with those from the literature. In a large cohort of patients attending primary care, $GABA_A$ receptors agonists were associated with significantly increased risk of mortality.[15] In two other representative databases, a significant while moderate increase in all-cause mortality in relation to benzodiazepines was found, in a population of incident and mostly occasional users.[16] A recent population-wide register-based study identified that benzodiazepines are more frequently used in patients with strokes than in controls and are associated with greater all-cause mortality in patients with stroke and matched controls.[17] The use of anti-anxiety medication and mortality risk in patients following

myocardial has also been studied in a sampling database.[18] Sudden death was significantly associated with increased benzodiazepam dosage during approximately five years. For patients receiving higher doses of daily benzodiazepines, protective effects for cardiac mortality and heart failure hospitalization decreased and a J-curve dose-response relationship was seen, without providing an adequate mechanistic explanation. Benzodiazepines have been shown to increase the occurrence of community-acquired pneumonia,[19] due to their pharmacodynamic properties. In our cohort, prior use of benzodiazepines didn't increase the incidence of respiratory depression and cannot explain mortality in these patients.

Cognition and benzodiazepines.

Benzodiazepine use was neither associated with cognitive impairment at 8 days or 90 days. However, the short-term effects of benzodiazepines on impairment cognition are well known and use of benzodiazepines is also associated with increased risk of dementia, even if the nature of the link between benzodiazepines and Alzheimer's disease remains unclear.[20] In our cohort, GABA receptor agonists treatment before stroke didn't show cognitive impairment as assessed by MMSE, in these elderly patients without dementia, but a longer follow-up period may be useful. Further, although we use methods to impute for missing data, data are missing on follow-up - loss to follow-up in a set, and then lack of doing MMSE on follow-up in another set (post-stroke aphasia) - which are likely not missing at random.

Comorbid alcohol use disorder.

Prior benzodiazepine use (regardless the dosage of treatment) was not associated with higher baseline stroke severity, as excessive chronic ethanol consumption was.[6] Benzodiazepine users were also less likely to be alcohol drinkers in our study, although the difference did not reach the significance level. So, the relationship between stroke severity

and alcohol consumption could not be necessarily due to a link between GABA receptors and alcohol, but due to chronic effects of ethanol consumption on other organ systems.

Strengths and limitations.

Our stroke data base was prospectively collected, and the study was carried out in a representative cohort of routine clinical stroke patients with an exhaustive drug history analysis. Dosage and compliance rate were not controlled for. More data on the length of time patients had been using the drug were not available but it is reasonable to think that they had an inadequate situation with excessive duration of prescription (demographic characteristics).[21] Potentially multi-site studies need acknowledgement, as does the need for replication in countries where there may be different practice in the prescribing on benzodiazepines. In our study, there was no clear association between mortality and poor functional outcome. For the deaths, information about the underlying cause was not obtained, but was not associated with stroke severity. It is important to acknowledge that this study is also limited to stroke outcomes in patients admitted to hospital after stroke. Unfortunately, we don't have information on premorbid functional status.

The present findings are derived from observational analyses which are subject to well-known limitations. The first is the potential for confounding by measured or unmeasured variables, which cannot be ruled out, even after propensity score matching/adjustment. It's also possible that the indication for benzodiazepines may be a causative variable, as mood (depression or anxiety) increases mortality in stroke.[22] Our results should be interpreted as hypothesis generating (without possibility of concluding that there is a causal effect).

Another limitation was the presence of missing data in some covariates, including in the propensity score calculation, as well as in MMSE outcome. Although we used multiple imputations to handle missing data as appropriate, we could not exclude that missing data

could introduce a bias in estimates. Since no formal study sample size was calculated, we could not exclude that some differences may have been overlooked due to the lack of adequate statistical power. In a posterior power calculation, we calculated the smallest significant between-group difference (expressed as effect size using odd ratio) that our study sample size allowed us to detect with a 80% power. Assuming an incidence of outcome of 10% and 50% in non-benzodiazepines users, we could detect an OR of 4.0 and 3.1 in the propensity score-matched cohort and 2.8 and 2.3 in propensity score-adjusted cohort.

Unanswered questions and implication for clinical practice.

The lack of consistency of results for moderate increase mortality and functional outcomes make this less likely to explain a physiologic effect. Anyway, the possible increased rate of mortality after stroke found in the benzodiazepines users group add to the increasing body of evidence concerning a non-neuroprotective effect of GABA receptors agonists. Benzodiazepines reduce the cerebral metabolic rate of oxygen and cerebral blood flow and can induce post-hypoxic leukoencephalopathy.[23,24] As lack of blood flow leads to cerebral hypoxia, it results in a cascade of biological events, which facilitates glutamate release. Based upon these data, we hypothesized that chronic cerebral hypoxia could thus be induced by benzodiazepine use, especially with inadequate situation with excessive duration of treatment. Long-term modulation of $GABA_A$ receptors by benzodiazepines could modulate ischaemia-induced glutamate release. Our findings generate a hypothesis that needs confirmation. As an interventional study would not be feasible, this question can be answered though experimental approaches in animals, to provide and appropriate mechanistic explanation.

This research should also not be used to condemn GABA receptors agonist drugs since their short-term use can have an important role in the management of anxiety. This study should

however alert clinicians to a possible increased post-stroke mortality in benzodiazepine-users. As patients could also be at high risk of recurrence after stroke, use of benzodiazepines should be cautioned against.

CONCLUSION.

Our findings do not support a putative neuroprotective effect of benzodiazepines. Further larger studies are warranted to confirm the association between benzodiazepine use and early post-stroke mortality.

Table 1. Comparison of baseline characteristics between benzodiazepine users and non-users.

	Benzodiazepine non-users (n=274)	Benzodiazepine users (n=62)	*P (ASD, %)*
Demographic characteristics			
Age, y, mean±SD	65.4 ± 15.0	73.1 ± 12.6	0.0002 (55.7)
Men	157 (57.3)	23 (37.1)	0.004 (41.3)
Medical history			
Previous stroke	28 (10.3)	7 (11.3)	0.81 (3.3)
Previous TIA	20 (7.3)	5 (8.1)	0.79 (2.9)
Coronary artery disease	52 (19.0)	17 (27.4)	0.14 (20.1)
Sleep apnea syndrome	7 (2.6)	2 (3.2)	0.68 (3.8)
Heart rhythm disorders	59 (21.6)	20 (32.3)	0.075 (24.2)
Vascular risk factors			
Arterial hypertension	155 (56.6)	48 (77.4)	0.002 (45.5)
Diabetes mellitus	59 (21.5)	7 (11.3)	0.067 (27.9)
Hypercholesterolemia	124 (45.3)	34 (54.8)	0.17 (19.3)
Hypertriglyceridemia	41 (15.0)	11 (17.7)	0.58 (7.5)
Smoking	89 (32.5)	13 (21.0)	0.075 (26.2)
Chronic ethanol consumption	45 (16.5)	7 (11.5)	0.33 (14.5)
BMI, kg/m^2, mean±SD	27.0 ± 4.9	25.6 ± 4.8	0.042 (29.6)
Routine drugs			
Fibrates	17 (6.2)	6 (9.7)	0.40 (12.9)
Statins	85 (31.0)	22 (35.5)	0.50 (9.5)
Oral anticoagulants	17 (6.2)	2 (3.2)	0.54 (14.1)
Antiplatelet	98 (35.8)	28 (45.2)	0.17 (19.2)
ACE	47 (17.1)	16 (25.8)	0.12 (21.2)
Angiotensin II receptor antagonist	46 (16.8)	10 (16.1)	0.90 (1.8)

Diuretics	65 (23.7)	21 (33.9)	0.098 (22.6)
Calcium channel blockers	42 (15.3)	11 (17.7)	0.64 (6.5)
Betablockers	98 (35.9)	29 (46.8)	0.11 (22.2)
Oral hypoglycemic	45 (16.4)	6 (9.7)	0.18 (20.1)
Intravenous thrombolysis	78 (28.9)	16 (25.8)	0.63 (6.9)
Onset to admission time, hours, median (IQR)	2 (1 to 7)	2 (1 to 4)	0.36 (12.8)
NIHSS, median (IQR)	6 (2-13)	7 (2-18)	0.42(12.8)
Biological characteristics			
Triglycerides, g/L, median (IQR)	1.06 (0.81-1.56)	1.01 (0.84-1.56)	0.93 (1.3)
Total cholesterol, g/L, mean±SD	1.96 ± 0.49	1.92 ± 0.52	0.60 (7.4)
HDL-cholesterol, g/L, mean±SD	0.53 ± 0.17	0.54 ± 0.13	0.86 (2.4)
LDL-cholesterol, g/L, mean±SD	1.17 ± 0.41	1.14 ± 0.44	0.61 (7.2)
Glycated hemoglobin, %, median (IQR)	5.9 (5.6-6.5)	5.9 (5.7-6.3)	0.98 (0.4)
Hemoglobin, g/dL, median (IQR)	13.8 (12.9-14.9)	13.5 (12.5-14.2)	0.077 (25.5)
Leukocytes, /mm^3, median (IQR)	8320 (6745-9865)	8335 (6700-10680)	0.55 (8.0)
Neutrophils, /mm^3, median (IQR)	5400 (4200-7400)	5850 (4500-8150)	0.26 (16.0)
Platelets, 1000/mm^3, median (IQR)	235 (197-271)	234.5 (192-274)	0.88 (2.1)
Prothrombin index, %, median (IQR)	96 (88-100)	94 (86-100)	0.42 (11.3)
Activated partial thromboplastin time, s, median (IQR)	32 (29-35)	32 (28-37)	0.52 (8.6)
C-reactive protein, mg/L, median (IQR)	4.7 (2.0-9.7)	5.5 (2.5-9.7)	0.65 (7.1)
Aspartate aminotransferase, U/L, median (IQR)	23 (19-29)	23 (20-27)	0.88 (2.2)
Alanine aminotransferase, U/L, median (IQR)	21 (15-29)	18 (14-23)	0.029 (32.2)

Data are expressed as number (%) unless otherwise indicated.

Abbreviations: ACE=angiotensin converting enzyme, ASD=absolute standardized difference, BMI= body mass index, HDL= high density lipoprotein, IQR=interquartile range, LDL=low density lipoprotein, NIHSS= National Institutes of Health Stroke Scale, SD=standard deviation, TIA=transient ischemic attack.

Table 2. Outcomes at 8-day and 90-day after stroke according to prior benzodiazepine use In Propensity-Score-Matched Cohort.

	Benzodiazepine users		Propensity-score –Matched†	
	No (n=56)	Yes (n=56)*	OR (95% CI)	*p*
Outcome at 8-day				
All-cause death	2/56 (4.3)	4/56 (6.8)	1.81 (0.23-13.90)	0.56
NIHSS ≥6	19/51 (37.3)	20/51 (39.4)	1.10 (0.46-2.60)	0.83
Poor outcome (mRS ≥2)	33/54 (60.3)	34/54 (63.2)	1.13 (0.48-2.66)	0.78
Poor outcome (Barthel <95)	29/52 (55.3)	32/52 (61.2)	1.28 (0.51-3.15)	0.59
Cognitive impairment (MMSE <24)	13/37 (34.4)	10/35 (28.0)	0.75 (0.24-2.34)	0.62
Outcome at 90-day				
All-cause death	4/53 (7.6)	12/52 (23.5)	3.93 (0.91-16.98)	0.067
Poor outcome (mRS ≥2)	25/53 (46.4)	31/52 (59.7)	1.72 (0.75-3.91)	0.19
Poor outcome (Barthel <95)	18/49 (36.0)	16/40 (39.6)	1.18 (0.47-2.94)	0.72
Cognitive impairment (MMSE <24)	6/37 (14.9)	5/33 (15.0)	1.04 (0.23-4.51)	0.96

Values are n/N (%) unless otherwise indicated, calculated after handling missing baseline data including in propensity-score calculation by multiple imputation procedure (m=20 imputed datasets; n were estimated using the combined rates and the mean number of patients without missing outcome values). * mean numbers of matched pairs among the 20-imputed datasets. † calculated using a generalized estimating equations (GEE) model (binomial distribution, logit function) take into account the propensity-score matched design.

Abbreviations: *MMSE: Mini* Mental State Examination, mRS: modified Rankin score, NIHSS: National Institutes of Health *S*troke Scale, OR: odds ratio, CI: confidence interval.

Table 3. Outcomes at 8-day and 90-day after stroke according to prior benzodiazepine use In Propensity-score adjusted Cohorts.

	Benzodiazepine users			Propensity-score –adjusted*	
	No	Yes	*p*	OR (95% CI)	*p*
Outcome at 8-day	(n=274)	(n=62)			
All-cause death	6/274 (2.2)	5/62 (8.1)	0.034	2.53 (0.68-9.27)	0.16
NIHSS ≥6	86/264 (32.58)	22/55 (40)	0.29	1.46 (0.77-2.75)	0.24
Poor outcome (mRS ≥2)	137/270 (50.7)	39/60 (65.0)	0.045	1.56(0.84-2.88)	0.15
Poor outcome (Barthel <95)	119/265 (44.9)	35/56 (62.5)	0.017	1.68 (0.90-3.14)	0.10
Cognitive impairment (MMSE <24)	52/196 (26.5)	11/40 (27.5)	0.90	0.72 (0.31-1.62)	0.42
Outcome at 90-day	(n=258)	(n=58)			
All-cause death	21/258 (8.1)	15/58 (25.9)	0.0001	3.50 (1.57-7.76)	0.002
Poor outcome (mRS ≥2)	110/258 (42.6)	35/58 (60.3)	0.014	1.89 (1.02-3.48)	0.042
Poor outcome (Barthel <95)	72/235 (30.6)	16/43 (37.2)	0.39	1.31 (0.64-2.65)	0.45
Cognitive impairment (MMSE <24)	26/194 (13.4)	5/35 (14.3)	0.79	0.87 (0.29-2.55)	0.80

Values are n/N (%) unless otherwise indicated. * logistic regression models adjusted on propensity score.

Abbreviations: *MMSE: Mini* Mental State Examination, mRS: modified Rankin score, NIHSS: National Institutes of Health *S*troke Scale, OR: odds ratio, CI: confidence interval.

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AUTHORS' CONTRIBUTIONS

OC wrote the manuscript and contributed to the analysis. JL performed statistical analysis and contributed to drafting the manuscript. JD contributed to drafting the manuscript. AMM contributed to study design and data collection. VD contributed to statistical analysis and contributed to drafting the manuscript. CC contributed to study design and data collection. DD contributed to study design, data collection and analysis. DL contributed to study design, data collection and analysis. RB contributed to study design, data collection and analysis, and drafting the manuscript. All authors have read and approved the final manuscript.

CONFLICTS OF INTEREST.

The authors declare that they have no competing interests.

DATA SHARING STATEMENT

No additional data are available.

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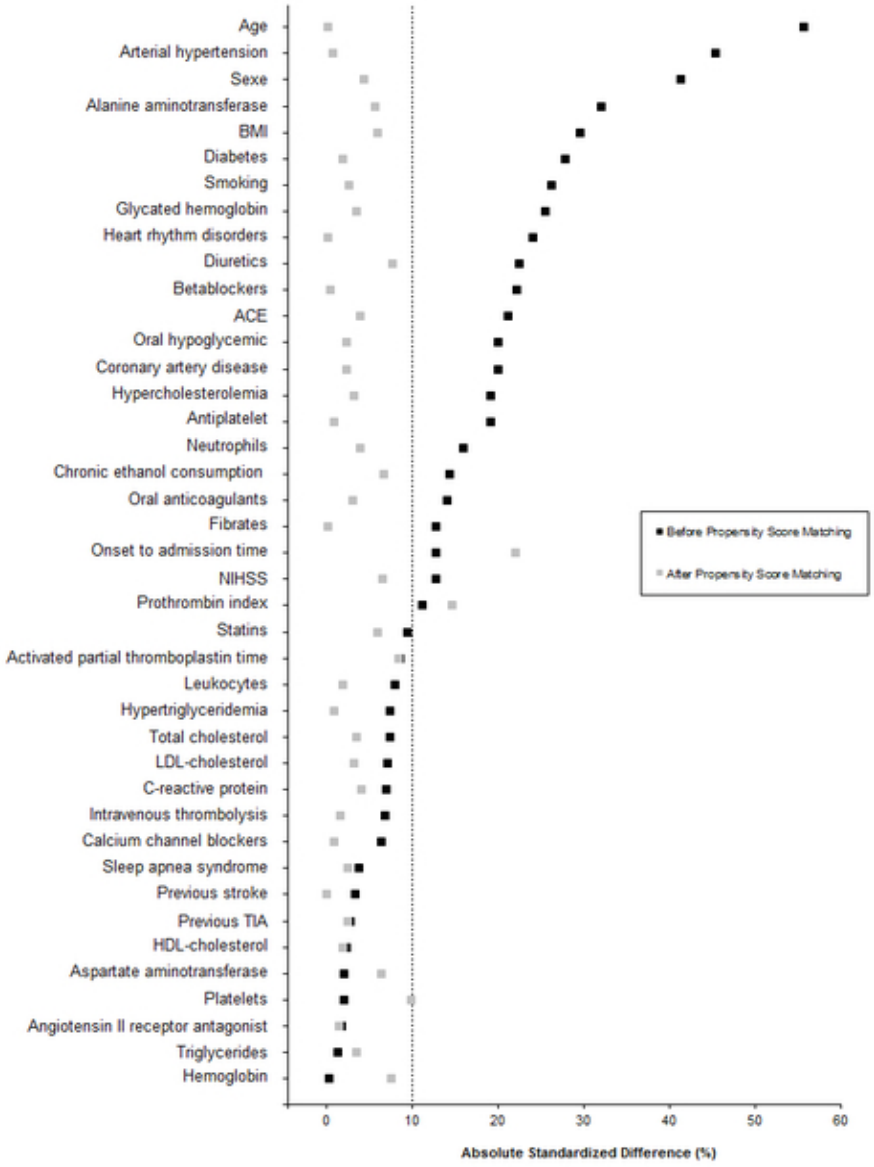

Figure 1. Absolute standardized differences between benzodiazepine users and non-users before and after propensity score matching.

40x53mm (300 x 300 DPI)

ONLINE SUPPLEMENT.

Supplemental table 1. Main baseline characteristics and Outcomes of Overall Study population (N=336)

	N	Value
Demographic characteristics		
Age, y, mean±SD	336	66.9 ± 14.9
Men	336	180 (53.6)
Medical history		
Previous stroke	335	35 (10.5)
Previous TIA	336	25 (7.4)
Coronary artery disease	336	69 (20.5)
Sleep apnea syndrome	333	9 (2.7)
Heart rhythm disorders	335	79 (23.6)
Vascular risk factors		
Arterial hypertension	336	203 (60.4)
Diabetes mellitus	336	66 (19.6)
Hypercholesterolemia	336	158 (47.0)
Hypertriglyceridemia	336	52 (15.5)
Smoking	336	102 (30.4)
Chronic ethanol consumption	334	52 (15.6)
BMI, kg/m^2, mean±SD	312	26.8 ± 4.9
Routine drugs		
Fibrates	336	23 (6.9)
Statins	336	107 (31.9)
Oral anticoagulants	336	19 (5.7)
Antiplatelet	336	126 (37.5)
ACE	336	63 (18.8)
Angiotensin II receptor antagonist	336	56 (16.7)
Diuretics	336	86 (25.6)

Calcium channel blockers	336	53 (15.8)
Betablockers	335	127 (37.9)
Oral hypoglycemic	336	51 (15.2)
Intravenous thrombolysis	332	94 (28.3)
Onset to admission time, hours, median (IQR)	332	2 (1-7)
NIHSS, median (IQR)	336	6 (2-14)
Biological characteristics		
Triglycerides, g/L, median (IQR)	329	1.1 (0.8-1.6)
Total cholesterol, g/L, mean±SD	329	1.95 ± 0.50
HDL-cholesterol, g/L, mean±SD	321	0.53 ± 0.16
LDL-cholesterol, g/L, mean±SD	319	1.16 ± 0.41
Glycated hemoglobin, %, median (IQR)	245	5.9 (5.6-6.4)
Hemoglobin, g/dL, median (IQR)	335	13.7 (12.9-14.7)
Leukocytes, /mm^3, median (IQR)	334	8320 (6730-10130)
Neutrophils, /mm^3, median (IQR)	315	5.6 (4.2-7.5)
Platelets, 1000/mm^3, median (IQR)	335	235 (195-272)
Prothrombin index, %, median (IQR)	320	95 (86-100)
Activated partial thromboplastin time, s, median (IQR)	333	32 (29-35)
C-reactive protein, mg/L, median (IQR)	280	4.9 (2-9.7)
Aspartate aminotransferase, U/L, median (IQR)	321	23 (19-28)
Alanine aminotransferase, U/L, median (IQR)	322	20 (15-28)
Outcome at 8-day		
All-cause death	336	11 (3.3)
NIHSS ≥6	319	108 (33.9)
Poor outcome (mRS ≥2)	330	176 (53.3)
Poor outcome (Barthel <95)	321	154 (48.0)
Cognitive impairment (MMSE <24)	236	63 (26.7)
Outcome at 90-day		
All-cause death	316	36 (11.4)
Poor outcome (mRS ≥2)	316	145 (45.9)

Poor outcome (Barthel <95)	278	88 (31.7)
Cognitive impairment (MMSE <24)	229	31 (13.5)

Abbreviations: ACE=angiotensin converting enzyme, ASD=absolute standardized difference, BMI= body mass index, HDL= high density lipoprotein, IQR=interquartile range, LDL=low density lipoprotein, MMSE: Mini Mental State Examination, mRS: modified Rankin score, NIHSS= National Institutes of Health Stroke Scale, SD=standard deviation, TIA=transient ischemic attack

Supplemental Table 2. Baseline characteristics in benzodiazepine users and non-users after propensity-score matching.

	Benzodiazepine non-users (n=56) *	**Benzodiazepine users** (n=56) *	***ASD, %***
Demographic characteristics			
Age, y, mean±SD	72.6 ± 14.9	72.6 ± 12.6	0.2
Men	24 (42.6)	23 (40.4)	4.5
Medical history			
Previous stroke	6 (9.9)	5 (9.7)	0.1
Previous TIA	5 (8.1)	4 (7.2)	2.6
Coronary artery disease	13 (23.7)	14 (24.6)	2.4
Sleep apnea syndrome	2 (4.2)	2 (3.6)	2.5
Heart rhythm disorders	16 (29.3)	16 (29.3)	0.2
Vascular risk factors			
Arterial hypertension	43 (77.1)	43 (76.8)	0.8
Diabetes mellitus	7 (13.2)	7 (12.3)	5.7
Hypercholesterolemia	30 (54.0)	29 (52.3)	3.3
Hypertriglyceridemia	9 (16.5)	9 (16.8)	1.0
Smoking	13 (23.3)	12 (21.9)	2.7
Chronic ethanol consumption	9 (15.6)	7 (13.0)	6.7
BMI, kg/m^2, mean±SD	26.2 ± 5.7	25.9 ± 5.3	6.0
Routine drugs			
Fibrates	6 (10.8)	6 (10.7)	0.2
Statins	19 (34.6)	18 (31.7)	6.0
Oral anticoagulants	19 (34.6)	18 (31.7)	3.1
Antiplatelet	24 (43.1)	24 (42.6)	1.1
ACE	15 (26.3)	14 (24.5)	4.0
Angiotensin II receptor antagonist	9 (16.9)	10 (17.4)	1.5
Diuretics	18 (31.9)	20 (35.4)	7.7

Calcium channel blockers	10 (18.0)	10 (17.6)	1.0
Betablockers	26 (46.0)	26 (45.8)	0.5
Oral hypoglycemic	6 (11.5)	6 (10.6)	2.4
Intravenous thrombolysis	16 (27.8)	15 (27.0)	1.6
Onset to admission time, hours, median (IQR)	2 (1-8)	2 (1-3)	22.0
NIHSS, median (IQR)	8 (2-17)	6 (2-18)	6.7
Biological characteristics			
Triglycerides, g/L, median (IQR)	1.02 (0.81-1.54)	1.02 (0.82-1.58)	3.6
Total cholesterol, g/L, mean±SD	1.93 ± 0.57	1.91 ± 0.52	3.5
HDL-cholesterol, g/L, mean±SD	0.54 ± 0.20	0.53 ± 0.14	2.0
LDL-cholesterol, g/L, mean±SD	1.14 ± 0.48	1.13 ± 0.45	3.3
Glycated hemoglobin, %, median (IQR)	6.0 (5.7-6.4)	5.9 (5.7-6.3)	3.5
Hemoglobin, g/dL, median (IQR)	13.4 (12.4-14.6)	13.5 (12.4-14.2)	7.5
Leukocytes, /mm^3, median (IQR)	8473 (6919-9884)	8276 (6590-10597)	1.9
Neutrophils, /mm^3, median (IQR)	5.7 (4.3-7.6)	5.7 (4.1-8.2)	4.0
Platelets, 1000/mm^3, median (IQR)	240 (206-277)	236 (198-274)	9.9
Prothrombin index, %, median (IQR)	95 (86-100)	92 (84-100)	14.8
Activated partial thromboplastin time, s, median (IQR)	32 (29-36)	31 (28-36)	8.5
C-reactive protein, mg/L, median (IQR)	5.7 (2.5-9.6)	5.8 (2.6-9.7)	4.2
Aspartate aminotransferase, U/L, median (IQR)	23 (19-27)	23 (20-28)	6.4
Alanine aminotransferase, U/L, median (IQR)	18 (14-24)	18 (14-23)	5.7

Data are expressed as number (%) unless otherwise indicated. Descriptive parameters and ASD were calculated after handling missing baseline data including in propensity-score calculation by multiple imputation procedure (m=20 imputed datasets). * mean numbers of matched pairs among the 20-imputed datasets.

Abbreviations: ACE=angiotensin converting enzyme, ASD=absolute standardized difference, BMI= body mass index, HDL= high density lipoprotein, IQR=interquartile range, LDL=low density lipoprotein, NIHSS= National Institutes of Health Stroke Scale, SD=standard deviation, TIA=transient ischemic attack.

Supplemental table 3. Main baseline characteristics of patients loss and not loss at 90-day

	90-day follow-up	
	Yes (n=316)	**No** (n=20)
Number of patients	316	20
Benzodiazepine use	58 (18.3)	4 (20.0)
Age, y, mean±SD	66.6 ± 14.9	71.5 ± 12.9
Men	170 (53.8)	10 (50.0)
Medical history		
Previous stroke	32 (10.1)	3 (15.8)
Previous TIA	24 (7.6)	1 (5.0)
Coronary artery disease	63 (19.9)	6 (30.0)
Sleep apnea syndrome	9 (2.9)	0 (0.0)
Heart rhythm disorders	75 (23.8)	4 (20.0)
Vascular risk factors		
Arterial hypertension	190 (60.1)	13 (65.0)
Diabetes mellitus	61 (19.3)	5 (25.0)
Hypercholesterolemia	149 (47.1)	9 (45.0)
Hypertriglyceridemia	51 (16.1)	1 (5.0)
Smoking	94 (19.7)	8 (40.0)
Chronic ethanol consumption	49 (15.6)	3 (15.0)
BMI, kg/m^2, mean±SD	26.8 ± 5.0	25.5 ± 3.9

Data are expressed as number (%) unless otherwise indicated.

Abbreviations: BMI= body mass index, SD=standard deviation, TIA=transient ischemic attack.

Supplemental table 4. Individual characteristics of the 36 mortality cases during the 90-day follow-up period after stroke onset.

Patients	Benzodiazepines	Age	Gender	NHSS	Hypertension	Diabetes	Hypercholesterolemia	Smoking	Previous Stroke
1	No	87	Women	22	Yes	Yes	No	No	No
2	No	52	Women	6	Yes	No	Yes	Yes	Yes
3	No	39	Women	15	Yes	No	No	Yes	No
4	No	80	Men	11	Yes	Yes	Yes	Yes	Yes
5	No	72	Men	10	No	No	No	Yes	No
6	No	82	Men	20	No	No	No	No	No
7	No	82	Women	21	No	Yes	No	No	No
8	No	85	Women	22	Yes	No	No	No	Yes
9	No	89	Women	26	No	No	No	No	No
10	No	81	Women	20	Yes	Yes	Yes	No	No
11	No	71	Men	8	Yes	Yes	Yes	Yes	No
12	No	63	Men	19	Yes	No	No	No	No
13	No	43	Women	11	No	No	No	Yes	No
14	No	79	Men	11	No	No	No	No	No
15	No	44	Women	7	No	No	No	Yes	No
16	No	39	Men	18	No	No	No	No	No
17	No	38	Men	0	No	No	No	Yes	No
18	No	65	Men	19	No	No	No	Yes	No
19	No	46	Men	8	Yes	No	Yes	Yes	No
20	No	70	Women	8	Yes	Yes	Yes	No	No
21	No	48	Women	17	Yes	No	No	No	No
22	Yes	85	Men	21	No	No	No	No	No
23	Yes	53	Men	27	No	No	No	Yes	No
24	Yes	84	Women	32	Yes	No	Yes	No	Yes
25	Yes	84	Men	22	Yes	No	Yes	No	No
26	Yes	85	Women	27	Yes	No	Yes	No	No
27	Yes	93	Women	21	Yes	No	Yes	No	No
28	Yes	60	Men	1	Yes	No	Yes	No	No
29	Yes	86	Women	25	Yes	No	Yes	No	No
30	Yes	76	Women	20	Yes	Yes	No	No	No
31	Yes	66	Women	17	Yes	No	No	No	No
32	Yes	71	Women	13	Yes	No	Yes	No	No
33	Yes	83	Women	1	Yes	No	Yes	No	No
34	Yes	81	Men	1	No	No	Yes	No	Yes
35	Yes	83	Women	11	Yes	No	Yes	Yes	No
36	Yes	63	Women	0	Yes	No	No	No	No

Supplemental table 5. Main baseline characteristics of alive patients with and without missing MMSE at 8- and 90-days

	8-day MMSE		90-day MMSE	
	Not missing	Missing	Not missing	Missing
Number of patients	236	89	229	71
Benzodiazepine use	40 (16.9)	17 (19.1)	35 (15.3)	12 (16.9)
Age, y, mean±SD	65.2 ± 14.4	70.3 ± 15.2	65.5 ± 14.9	69.8 ± 13.3
Men	132 (55.9)	45 (50.6)	128 (55.9)	37 (52.1)
Medical history				
Previous stroke	22 (9.3)	10 (11.4)	22 (9.6)	8 (11.4)
Previous TIA	16 (6.8)	9 (10.1)	14 (6.1)	7 (9.9)
Coronary artery disease	40 (16.9)	26 (29.2)	44 (19.2)	14 (19.7)
Sleep apnea syndrome	6 (2.6)	3 (3.4)	4 (1.8)	4 (5.7)
Heart rhythm disorders	51 (21.6)	24 (27.3)	52 (22.8)	19 (26.8)
Vascular risk factors				
Arterial hypertension	137 (58.0)	57 (64.0)	138 (60.3)	42 (59.1)
Diabetes mellitus	44 (18.6)	19 (21.3)	47 (20.5)	12 (16.9)
Hypercholesterolemia	107 (45.3)	47 (52.8)	111 (48.5)	31 (43.7)
Hypertriglyceridemia	41 (17.4)	10 (11.2)	40 (17.5)	8 (11.3)
Smoking	81 (34.3)	18 (20.2)	67 (29.3)	23 (32.4)
Chronic ethanol consumption	39 (16.7)	13 (14.6)	38 (16.6)	13 (18.6)
BMI, kg/m^2, mean±SD	26.7 ± 4.8	27.0 ± 5.1	26.8 ± 4.8	26.6 ± 5.0

Data are expressed as number (%) unless otherwise indicated.

Abbreviations: BMI= body mass index, SD=standard deviation, TIA=transient ischemic attack.

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract Page 2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found (Page 2)
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported Page 4
Objectives	3	State specific objectives, including any prespecified hypotheses Page 4
Methods		
Study design	4	Present key elements of study design early in the paper (page 5)
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection (page 5)
Participants	6	*Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up (page 5)
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed (NA)
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable (page 5)
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group (page 5-6)
Bias	9	Describe any efforts to address potential sources of bias (NA)
Study size	10	Explain how the study size was arrived (page5)
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why (page 6)
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding (page 6)
		(*b*) Describe any methods used to examine subgroups and interactions ((page 6))
		(*c*) Explain how missing data were addressed (pages 6-7)
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed (page 6)
		(*e*) Describe any sensitivity analyses (page 6)

Continued on next page

Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (page 8)
		(b) Give reasons for non-participation at each stage (page 8)
		(c) Consider use of a flow diagram (NA)
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (page 8; 15-16)
		(b) Indicate number of participants with missing data for each variable of interest (page 8; 15-16)
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount) (page 8; 15-16)
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time (page 8;17)
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (page 8; 15-17)
		(*b*) Report category boundaries when continuous variables were categorized (page 8; 15-17)
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period (NA)
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses (suppl file)
Discussion		
Key results	18	Summarise key results with reference to study objectives (page 10)
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias (page 10-12)
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence (page 13)
Generalisability	21	Discuss the generalisability (external validity) of the study results (pages 13-14)
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based (page 18)

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Preadmission use of benzodiazepines and stroke outcomes: the Biostroke prospective cohort study.

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List of figures and tables:

Key words: benzodiazepines; stroke; mortality.

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ABSTRACT.

Objectives. We tested the hypothesis that stroke outcomes in patients with preadmission use of benzodiazepine are worse.

Method. In a prospective cohort study, we recruited patients with acute ischemic stroke. Mortality, functional outcomes and cognition were evaluated at 8 and 90 days after stroke.

Results. 370 patients were included. 62 (18.5%) of 336 remainders patients were treated with benzodiazepines when stroke occurred, and they did not receive any other psychotropic drug. The mortality rate was higher in benzodiazepines users than non-users at day-8 (2.2% vs. 8.1%, p=0.034) and day-90 (8.1% vs. 25.9%, p=0.0001). After controlling for baseline differences using propensity-score matching, only the difference in mortality rate at 90-day was of borderline of significance, with a matched OR of 3.93 (95%IC, 0.91-16.98). In adjusted-propensity-score cohort, this difference remained significant with a similar treatment effect size (adjusted OR, 3.50; 95%CI, 1.57-7.76). A higher rate of poor functional outcome at day-8 and day-90 defined by mRS≥2 or by the BI<95 was found in benzodiazepines users. In propensity-score adjusted cohort, only the difference in mRS ≥2 at 90-day remained significant (adjusted OR, 1.89; 95%CI, 1.02-3.48). In survivors at day-8 and at day-90, there was no significant difference in cognitive evaluation.

Conclusion: Our study has shown that preadmission use of benzodiazepines could be associated with increased post-stroke mortality at 90 days. These findings do not support a putative neuroprotective effect of $GABA_A$ receptors agonists and should alert clinicians of their potential risks. (clinicaltrials.gov: NCT00763217)

STRENGTHS AND LIMITATIONS OF THIS STUDY

- This cohort study has a great potential with regards to public health in the field of stroke outcomes, as benzodiazepines are one of the most prescribed drugs in the world.
- These results are consistent with those from the literature of a non-neuroprotective effect of benzodiazepines.
- These results might be confounded by indication bias (between benzodiazepines users and non-users) but the use of a propensity score matching/adjustment partially addresses this concern.
- There is a lack of consistency of results for mortality and functional outcomes of stroke, suggesting new experimental approaches, to provide and appropriate mechanistic explanation.
- Our results should be interpreted as hypothesis generating (without possibility of concluding that there is a causal effect) and should be replicated in further studies.

INTRODUCTION.

Stroke is the second most common cause of death and the third most common cause of disability-adjusted life-years worldwide.[1] Considering the long-term neurological disabilities which may result from acute stroke, and differences in the extent of recovery among stroke survivors, predicting the outcomes of stroke is a very important issue.[1] A wide variety of factors influence stroke prognosis, including age, stroke severity, comorbid conditions, clinical findings…[2] Knowledge of others factors - as pharmacologic and use of drugs - that could influence the severity and short-term outcomes of stroke prognosis is necessary for clinicians. For example, benzodiazepines have been first considered as a type of neuroprotective agent in reducing infarct size and improving functional outcome in animal models of cerebral ischaemia.[3,4] However, in humans, a recent review does not provide the evidence to support the use of benzodiazepines (GABA receptor agonists) for the treatment of patients with acute ischaemic stroke.[5] Benzodiazepines and ethanol share several central effects, especially on activation of inhibitory γ amino-butyric $acid_A$ ($GABA_A$) receptors in the brain. We have recently shown that excessive chronic ethanol consumption is associated with higher stroke severity.[6] Interestingly, the impact of preadmission use of benzodiazepine in stroke had never been evaluated as stroke is emerging as a leading cause of preventable death and disability worldwide. Benzodiazepines, because of their good efficacy and rapidity of action, are also one of the most prescribed drugs in the world, widely used for anxiety and insomnia. The objective of our study was so to investigate the effect of preadmission use of benzodiazepine usage on stroke outcome, to clarify their role as stroke prognostic factors

METHODS.

Patient and public involvement statement.

Patients with ischemic stroke admitted to our university hospital's stroke unit (*Lille, France*) within 48 hours of symptom onset were recruited in the prospective "Biostroke" cohort (Clinical Biological and Pharmacological Factors Influencing Stroke Outcome). The aim of the study was to understand the mechanisms of preventive neuroprotection by establishing link between biomarkers and preventive and neuroprotective measures (clinicaltrials.gov: number NCT00763217).[6] Use of benzodiazepine was one of the interests. The local independent ethical committee approved the study (*Comité de Protection des Personnes Nord-Ouest IV*). Patients were managed according to local rules without any investigation or treatment specifically performed. Patients or close relatives gave a signed informed consent.

Data collection and clinical outcomes definition.

All patients underwent an initial standardized evaluation, including their medical history) and vascular risk factors (using a structured questionnaire), a physical examination, a routine blood biochemistry screen, and diagnostic testing. At admission patients underwent either CT or MRI scan.

Patients had a follow-up examination 8 and 90 days after admission. The modified Rankin scale (mRS), the Barthel index (BI), the Mini Mental State Examination (MMSE), and all-cause mortality were recorded. Definitions used for variables included in the analysis have been previously defined.[2] A National Institutes of Health Stroke Scale (NIHSS) score ≥6 was considered as a severe clinical impairment. A mRS score ≥2 (poor functional outcome), a BI score <95 (poor functional outcome), and a MMSE score <24 (cognitive impairment) were

considered as the worst possible stroke outcomes.[2] We also analysed the association between benzodiazepine use and respiratory failure and pneumonia at the acute phase of stroke.

Preadmission use of GABA receptors agonists.

Drug exposition was defined by benzodiazepine drugs administered orally for more than fifteen days before stroke regardless of length of treatment period and dosage of treatment. Hypnotic drugs were also included, since they act on similar receptors to the benzodiazepines. Patients with concomitant use of other psychoactive drugs were excluded, because of their possible confounding effects.

Statistical analysis.

Quantitative variables are expressed as mean (standard deviation) in case of normal distribution or median (interquartile range) otherwise. Categorical variables are expressed as numbers (percentage). Normality of distributions were assessed using histograms and Shapiro-Wilk test. Bivariate comparisons between benzodiazepine users and non-users were performed using Student's t test for quantitative variables (Mann-Whitney U test was used for non-Gaussian distribution) and Chi-squared test (Fisher's exact test was used when the expected cell frequency was <5) for categorical variables

We assessed the effect of the benzodiazepine use on clinical outcomes at 8 and 90 days (all-cause mortality, NIHSS $\geq$ 6, mRS $\geq$ 2, BI < 95 and MMSE < 24) using logistic regression models and calculated the odds ratio (OR) associated with benzodiazepine use as the treatment effect size. In order to reduce the effects of potential confounding factors in the between-group comparisons, we used propensity-score methods.[7] As the main analysis, propensity score was used to assemble well-balanced groups (propensity score-matched cohort) and generalized

estimating equations (GEE) models were used to take into account the matched design. As a secondary analysis, the propensity score was used as a covariate in logistic regression models to adjust the comparisons (propensity score-adjusted cohort).

The propensity score was estimated using a non-parsimonious multivariate logistic regression model, with the benzodiazepine treatment group as the dependent variable and all of the characteristics listed in Table 1 as covariates. Benzodiazepine users were matched 1:1 to patients in the non-benzodiazepine users according to propensity score using the greedy nearest neighbor matching algorithm with a caliper width of 0.2 standard deviation of logit for propensity score.[8,9] To evaluate bias reduction using the propensity score matching method, absolute standardized differences were calculated before and after propensity-score matching; an absolute standardized difference>10% indicated a meaningful imbalance in the baseline covariate.[10]

Because of missing baseline data (see supplemental table 1), the propensity score could not be computed in 54.5% (n=183) of the study sample (61.3% in benzodiazepine users and 52.9% in non-benzodiazepine users). We therefore estimated the treatment effect size in propensity score-matched- and -adjusted cohorts after handling missing covariate values by multiple imputation[11] using a regression switching approach (chained equations with m=20 imputations obtained using the R statistical software version 3.03).[12] Imputation procedure was performed under the missing at random assumption using all variables listed in Table 1 (i.e. baseline characteristics and treatment group) with a predictive mean matching method for continuous variables and logistic regression model for categorical (all binary) variables. In each imputed dataset, we calculated the propensity score and assembled a matched cohort to provide both adjusted and matched ORs. We therefore combined the ORs from each imputed dataset using Rubin's rules.[13]

Since, among alive patients, the rate of missing data was high for 8- and 90-day MMSE (27% and 24%, respectively), we also used multiple imputation approach to handle these missing values as a sensitivity analysis. Statistical testing was done at the two-tailed α level of 0.05. Data were analyzed using SAS software (version 9.3, SAS Institute Inc., Cary, NC, USA).

RESULTS

Among the 370 patients included in the Biostroke study, 34 patients (mean age, 69.0±13.7; 14 men) were excluded because of concomitant use of other psychoactive drugs. In the 336 remainders, 62 (18.5%) were under benzodiazepines when stroke occurred.

Benzodiazepine and baseline characteristics.

The baseline characteristics of the study population according to benzodiazepine treatment are described in Table 1. Before matching, several meaningful differences (absolute standardized difference>10%) were found. In particular, benzodiazepine users were older (73.1±12.6 *versus* 65.4±15.0, *p=0.0002*), more likely to be women (62.9 *versus* 42.7%, *p=0.004*) and to have arterial hypertension (77.4% *versus* 56.6%, *p=0.002*), lower BMI (25.6±4.8 *versus* 27.0±4.9, *p=0.042*) and lower levels of alanine aminotransferase (18 (14-23) *versus* 21 (15-29), *p=0.029*) than benzodiazepine users. These differences were reduced after propensity score-matching (Figure 1 & supplemental table 2) with an absolute standardized difference >10% only for onset to admission time (22.0%), and prothrombin index (14.8%) suggesting that the two study groups were well balanced after matching.

Benzodiazepine and Outcomes

Of the 336 study patients, 20 patients were lost to follow-up between day 8 and day 90 (see supplemental table 3 for their main characteristics). Death occurred in 11 patients (3.3%) at day-8 and 36 (11.4%) at day-90 (see supplemental table 4 for main individual characteristics of mortality cases). 57.9% of survivor patients taking benzodiazepines at admission continued to take them after stroke at day-8, 46.5 % at day-90.

In unadjusted analysis, the mortality rate was higher in benzodiazepines users than non-users at day-8 (2.2% vs. 8.1%, p=0.034) and day-90 (8.1% vs. 25.9%, p=0.0001). However, after controlling for baseline differences using propensity-score matching, only the difference in mortality rate at 90-day was of borderline of significance, with a matched OR of 3.93 (95%IC, 0.91-16.98; table 2). In adjusted-propensity-score cohort, this difference remained significant with a similar treatment effect size (adjusted OR, 3.50; 95%CI, 1.57 to 7.76; table 3).

In unadjusted analysis, a higher rate of poor functional outcome at day-8 defined by mRS≥2 or by the BI<95 was found in benzodiazepines users (table 3). A similar between-group difference was found for mRS ≥2 at 90-day. However, none of the differences were found in propensity-score matched (table 2). In propensity-score adjusted cohort, only the difference in mRS ≥2 at 90-day remained significant (adjusted OR, 1.89; 95%CI, 1.02 to 3.48).

In survivors at day-8 or at day-90, there was no significant difference in MMSE, in unadjusted, propensity-score matched and adjusted analyses. When the analyses were repeated after handling the missing data on MMSE by using multiple imputation approach (see supplemental table 5 for main baseline characteristics in patients with and without missing values), similar non-significant differences were found. In propensity-score matched cohort, the OR (95%CI) were 0.82 (0.30 to 2.19) for 8-day MMSE<24 and 1.04 (0.23 to 4.51) for 90-day MMSE<24. In propensity-score adjusted cohort, the OR (95%CI) were 0.89 (0.43 to 1.83) for 8-day MMSE<24 and 1.04 (0.34 to 3.16) for 90-day MMSE<24.

Regarding respiratory failure or pneumonia at 8 day, benzodiazepines users have a similar early respiratory complications risk than non-users (13.8% (n=8) *vs.* 15.6% (n=48), unadjusted *p*=0.73).

DISCUSSION

Preadmission use of benzodiazepines could not be considered as neuroprotective, as users of benzodiazepines could have a higher risk of death at 90-days after stroke.

Mortality and benzodiazepines.

A recent review does not provide the evidence to support the use of GABA receptor agonists for the treatment of patients with acute ischaemic stroke.[5] Randomized controlled trials (RCTs) investigating GABA receptor agonists versus placebo for acute stroke patients with the outcomes of death or dependency and functional independence were included. These RCTs measured death and dependency at three months in clomethiazole versus placebo or between diazepam and placebo without significant difference. In a recent non-randomized comparison, treatment with benzodiazepines after ischaemic stroke had no independent impact on stroke outcomes and mortality at 90-day.[14] However, these data were registered in a trials archive and were not derived from prospective trials, with indication bias and many confounders. In our prospective study, current users of benzodiazepines could have a higher rate of post-stroke mortality at 90-day. Effect of benzodiazepine drugs on mortality is still debated, but these results are consistent with those from the literature. In a large cohort of patients attending primary care, $GABA_A$ receptors agonists were associated with significantly increased risk of mortality.[15] In two other representative databases, a significant while moderate increase in all-cause mortality in relation to benzodiazepines was found, in a population of incident and mostly occasional users.[16] A recent population-wide register-based study identified that benzodiazepines are more frequently used in patients with strokes than in controls and are associated with greater all-cause mortality in patients with stroke and matched controls.[17] The use of anti-anxiety medication and

mortality risk in patients following myocardial has also been studied in a sampling database.[18] Sudden death was significantly associated with increased benzodiazepam dosage during approximately five years. For patients receiving higher doses of daily benzodiazepines, protective effects for cardiac mortality and heart failure hospitalization decreased and a J-curve dose-response relationship was seen, without providing an adequate mechanistic explanation. Benzodiazepines have been shown to increase the occurrence of community-acquired pneumonia,[19] due to their pharmacodynamic properties. In our cohort, prior use of benzodiazepines didn't increase the incidence of respiratory depression and cannot explain mortality in these patients.

Cognition and benzodiazepines.

Benzodiazepine use was neither associated with cognitive impairment at 8 days or 90 days. However, the short-term effects of benzodiazepines on impairment cognition are well known and use of benzodiazepines is also associated with increased risk of dementia, even if the nature of the link between benzodiazepines and Alzheimer's disease remains unclear.[20] In our cohort, GABA receptor agonists treatment before stroke didn't show cognitive impairment as assessed by MMSE, in these elderly patients without dementia, but a longer follow-up period may be useful. Further, although we use methods to impute for missing data, data are missing on follow-up - loss to follow-up in a set, and then lack of doing MMSE on follow-up in another set (post-stroke aphasia) - which are likely not missing at random.

Comorbid alcohol use disorder.

Prior benzodiazepine use (regardless the dosage of treatment) was not associated with higher baseline stroke severity, as excessive chronic ethanol consumption was.[6] Benzodiazepine

users were also less likely to be alcohol drinkers in our study, although the difference did not reach the significance level. So, the relationship between stroke severity and alcohol consumption could not be necessarily due to a link between GABA receptors and alcohol, but due to chronic effects of ethanol consumption on other organ systems.

Strengths and limitations.

Our stroke data base was prospectively collected, and the study was carried out in a representative cohort of routine clinical stroke patients with an exhaustive drug history analysis. Dosage and compliance rate were not controlled for. More data on the length of time patients had been using the drug were not available but it is reasonable to think that they had an inadequate situation with excessive duration of prescription (demographic characteristics).[21] Further research should definitely explore correlations between dosage or cumulative length of exposure and post-stroke mortality. Also, potentially multi-site studies need acknowledgement, as does the need for replication in countries where there may be different practice in the prescribing on benzodiazepines. In our study, there was no clear association between mortality and poor functional outcome. For the deaths, information about the underlying cause was not obtained, but was not associated with stroke severity. It is important to acknowledge that this study is also limited to stroke outcomes in patients admitted to hospital after stroke. Unfortunately, we don't have information on premorbid functional status.

The present findings are derived from observational analyses which are subject to well-known limitations. The first is the potential for confounding by measured or unmeasured variables, which cannot be ruled out, even after propensity score matching/adjustment. It's also possible that the indication for benzodiazepines may be a causative variable, as mood (depression or anxiety) increases mortality in stroke.[22] Our results should be interpreted as hypothesis generating

(without possibility of concluding that there is a causal effect).

Another limitation was the presence of missing data in some covariates, including in the propensity score calculation, as well as in MMSE outcome. Although we used multiple imputations to handle missing data as appropriate, we could not exclude that missing data could introduce a bias in estimates. Since no formal study sample size was calculated, we could not exclude that some differences may have been overlooked due to the lack of adequate statistical power. In a posterior power calculation, we calculated the smallest significant between-group difference (expressed as effect size using odd ratio) that our study sample size allowed us to detect with a 80% power. Assuming an incidence of outcome of 10% and 50% in non-benzodiazepines users, we could detect an OR of 4.0 and 3.1 in the propensity score-matched cohort and 2.8 and 2.3 in propensity score-adjusted cohort.

Unanswered questions and implication for clinical practice.

The lack of consistency of results for moderate increase mortality and functional outcomes make this less likely to explain a physiologic effect. Anyway, the possible increased rate of mortality after stroke found in the benzodiazepines users group add to the increasing body of evidence concerning a non-neuroprotective effect of GABA receptors agonists. Benzodiazepines reduce the cerebral metabolic rate of oxygen and cerebral blood flow and can induce post-hypoxic leukoencephalopathy.[23,24] As lack of blood flow leads to cerebral hypoxia, it results in a cascade of biological events, which facilitates glutamate release. Based upon these data, we hypothesized that chronic cerebral hypoxia could thus be induced by benzodiazepine use, especially with inadequate situation with excessive duration of treatment. Long-term modulation of $GABA_A$ receptors by benzodiazepines could modulate ischaemia-induced glutamate release. Our findings generate a hypothesis that needs confirmation. As an

interventional study would not be feasible, this question can be answered though experimental approaches in animals, to provide and appropriate mechanistic explanation.

This research should also not be used to condemn GABA receptors agonist drugs since their short-term use can have an important role in the management of anxiety. This study should however alert clinicians to a possible increased post-stroke mortality in benzodiazepine-users. As patients could also be at high risk of recurrence after stroke, use of benzodiazepines should be cautioned against.

CONCLUSION.

Our findings do not support a putative neuroprotective effect of benzodiazepines. Further larger studies are warranted to confirm the association between benzodiazepine use and early post-stroke mortality.

Table 1. Comparison of baseline characteristics between benzodiazepine users and non-users.

	Benzodiazepine non-users (n=274)	Benzodiazepine users (n=62)	*P (ASD, %)*
Demographic characteristics			
Age, y, mean±SD	65.4 ± 15.0	73.1 ± 12.6	0.0002 (55.7)
Men	157 (57.3)	23 (37.1)	0.004 (41.3)
Medical history			
Previous stroke	28 (10.3)	7 (11.3)	0.81 (3.3)
Previous TIA	20 (7.3)	5 (8.1)	0.79 (2.9)
Coronary artery disease	52 (19.0)	17 (27.4)	0.14 (20.1)
Sleep apnea syndrome	7 (2.6)	2 (3.2)	0.68 (3.8)
Heart rhythm disorders	59 (21.6)	20 (32.3)	0.075 (24.2)
Vascular risk factors			
Arterial hypertension	155 (56.6)	48 (77.4)	0.002 (45.5)
Diabetes mellitus	59 (21.5)	7 (11.3)	0.067 (27.9)
Hypercholesterolemia	124 (45.3)	34 (54.8)	0.17 (19.3)
Hypertriglyceridemia	41 (15.0)	11 (17.7)	0.58 (7.5)
Smoking	89 (32.5)	13 (21.0)	0.075 (26.2)
Chronic ethanol consumption	45 (16.5)	7 (11.5)	0.33 (14.5)
BMI, kg/m^2, mean±SD	27.0 ± 4.9	25.6 ± 4.8	0.042 (29.6)
Routine drugs			
Fibrates	17 (6.2)	6 (9.7)	0.40 (12.9)
Statins	85 (31.0)	22 (35.5)	0.50 (9.5)
Oral anticoagulants	17 (6.2)	2 (3.2)	0.54 (14.1)
Antiplatelet	98 (35.8)	28 (45.2)	0.17 (19.2)

ACE	47 (17.1)	16 (25.8)	0.12 (21.2)
Angiotensin II receptor antagonist	46 (16.8)	10 (16.1)	0.90 (1.8)
Diuretics	65 (23.7)	21 (33.9)	0.098 (22.6)
Calcium channel blockers	42 (15.3)	11 (17.7)	0.64 (6.5)
Betablockers	98 (35.9)	29 (46.8)	0.11 (22.2)
Oral hypoglycemic	45 (16.4)	6 (9.7)	0.18 (20.1)
Intravenous thrombolysis	78 (28.9)	16 (25.8)	0.63 (6.9)
Onset to admission time, hours, median (IQR)	2 (1 to 7)	2 (1 to 4)	0.36 (12.8)
NIHSS, median (IQR)	6 (2-13)	7 (2-18)	0.42(12.8)
Biological characteristics			
Triglycerides, g/L, median (IQR)	1.06 (0.81-1.56)	1.01 (0.84-1.56)	0.93 (1.3)
Total cholesterol, g/L, mean±SD	1.96 ± 0.49	1.92 ± 0.52	0.60 (7.4)
HDL-cholesterol, g/L, mean±SD	0.53 ± 0.17	0.54 ± 0.13	0.86 (2.4)
LDL-cholesterol, g/L, mean±SD	1.17 ± 0.41	1.14 ± 0.44	0.61 (7.2)
Glycated hemoglobin, %, median (IQR)	5.9 (5.6-6.5)	5.9 (5.7-6.3)	0.98 (0.4)
Hemoglobin, g/dL, median (IQR)	13.8 (12.9-14.9)	13.5 (12.5-14.2)	0.077 (25.5)
Leukocytes, /mm^3, median (IQR)	8320 (6745-9865)	8335 (6700-10680)	0.55 (8.0)
Neutrophils, /mm^3, median (IQR)	5400 (4200-7400)	5850 (4500-8150)	0.26 (16.0)
Platelets, 1000/mm^3, median (IQR)	235 (197-271)	234.5 (192-274)	0.88 (2.1)
Prothrombin index, %, median (IQR)	96 (88-100)	94 (86-100)	0.42 (11.3)
Activated partial thromboplastin time, s, median (IQR)	32 (29-35)	32 (28-37)	0.52 (8.6)
C-reactive protein, mg/L, median (IQR)	4.7 (2.0-9.7)	5.5 (2.5-9.7)	0.65 (7.1)
Aspartate aminotransferase, U/L, median (IQR)	23 (19-29)	23 (20-27)	0.88 (2.2)
Alanine aminotransferase, U/L, median (IQR)	21 (15-29)	18 (14-23)	0.029 (32.2)

Data are expressed as number (%) unless otherwise indicated.

Abbreviations: ACE=angiotensin converting enzyme, ASD=absolute standardized difference, BMI= body mass index, HDL= high density lipoprotein, IQR=interquartile range, LDL=low density lipoprotein, NIHSS= National Institutes of Health Stroke Scale, SD=standard deviation, TIA=transient ischemic attack.

Table 2. Outcomes at 8-day and 90-day after stroke according to prior benzodiazepine use In Propensity-Score-Matched Cohort.

	Benzodiazepine users		Propensity-score –Matched†	
	No (n=56)	Yes (n=56)*	OR (95% CI)	*p*
Outcome at 8-day				
All-cause death	2/56 (4.3)	4/56 (6.8)	1.81 (0.23-13.90)	0.56
NIHSS ≥6	19/51 (37.3)	20/51 (39.4)	1.10 (0.46-2.60)	0.83
Poor outcome (mRS ≥2)	33/54 (60.3)	34/54 (63.2)	1.13 (0.48-2.66)	0.78
Poor outcome (Barthel <95)	29/52 (55.3)	32/52 (61.2)	1.28 (0.51-3.15)	0.59
Cognitive impairment (MMSE <24)	13/37 (34.4)	10/35 (28.0)	0.75 (0.24-2.34)	0.62
Outcome at 90-day				
All-cause death	4/53 (7.6)	12/52 (23.5)	3.93 (0.91-16.98)	0.067
Poor outcome (mRS ≥2)	25/53 (46.4)	31/52 (59.7)	1.72 (0.75-3.91)	0.19
Poor outcome (Barthel <95)	18/49 (36.0)	16/40 (39.6)	1.18 (0.47-2.94)	0.72
Cognitive impairment (MMSE <24)	6/37 (14.9)	5/33 (15.0)	1.04 (0.23-4.51)	0.96

Values are n/N (%) unless otherwise indicated, calculated after handling missing baseline data including in propensity-score calculation by multiple imputation procedure (m=20 imputed datasets; n were estimated using the combined rates and the mean number of patients without missing outcome values). * mean numbers of matched pairs among the 20-imputed datasets. † calculated using a generalized estimating equations (GEE) model (binomial distribution, logit function) take into account the propensity-score matched design.

Abbreviations: *MMSE: Mini* Mental State Examination, mRS: modified Rankin score, NIHSS: National Institutes of Health *S*troke Scale, OR: odds ratio, CI: confidence interval.

Table 3. Outcomes at 8-day and 90-day after stroke according to prior benzodiazepine use In Propensity-score adjusted Cohorts.

	Benzodiazepine users			Propensity-score –adjusted*	
	No	Yes	*p*	OR (95% CI)	*p*
Outcome at 8-day	(n=274)	(n=62)			
All-cause death	6/274 (2.2)	5/62 (8.1)	0.034	2.53 (0.68-9.27)	0.16
NIHSS ≥6	86/264 (32.58)	22/55 (40)	0.29	1.46 (0.77-2.75)	0.24
Poor outcome (mRS ≥2)	137/270 (50.7)	39/60 (65.0)	0.045	1.56(0.84-2.88)	0.15
Poor outcome (Barthel <95)	119/265 (44.9)	35/56 (62.5)	0.017	1.68 (0.90-3.14)	0.10
Cognitive impairment (MMSE <24)	52/196 (26.5)	11/40 (27.5)	0.90	0.72 (0.31-1.62)	0.42
Outcome at 90-day	(n=258)	(n=58)			
All-cause death	21/258 (8.1)	15/58 (25.9)	0.0001	3.50 (1.57-7.76)	0.002
Poor outcome (mRS ≥2)	110/258 (42.6)	35/58 (60.3)	0.014	1.89 (1.02-3.48)	0.042
Poor outcome (Barthel <95)	72/235 (30.6)	16/43 (37.2)	0.39	1.31 (0.64-2.65)	0.45
Cognitive impairment (MMSE <24)	26/194 (13.4)	5/35 (14.3)	0.79	0.87 (0.29-2.55)	0.80

Values are n/N (%) unless otherwise indicated. * logistic regression models adjusted on propensity score.

Abbreviations: *MMSE: Mini* Mental State Examination, mRS: modified Rankin score, NIHSS: National Institutes of Health *S*troke Scale, OR: odds ratio, CI: confidence interval.

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AUTHORS' CONTRIBUTIONS

OC wrote the manuscript and contributed to the analysis. JL performed statistical analysis and contributed to drafting the manuscript. JD contributed to drafting the manuscript. AMM contributed to study design and data collection. VD contributed to statistical analysis and contributed to drafting the manuscript. CC contributed to study design and data collection. DD contributed to study design, data collection and analysis. DL contributed to study design, data collection and analysis. RB contributed to study design, data collection and analysis, and drafting the manuscript. All authors have read and approved the final manuscript.

CONFLICTS OF INTEREST.

The authors declare that they have no competing interests.

DATA SHARING STATEMENT

No additional data are available.

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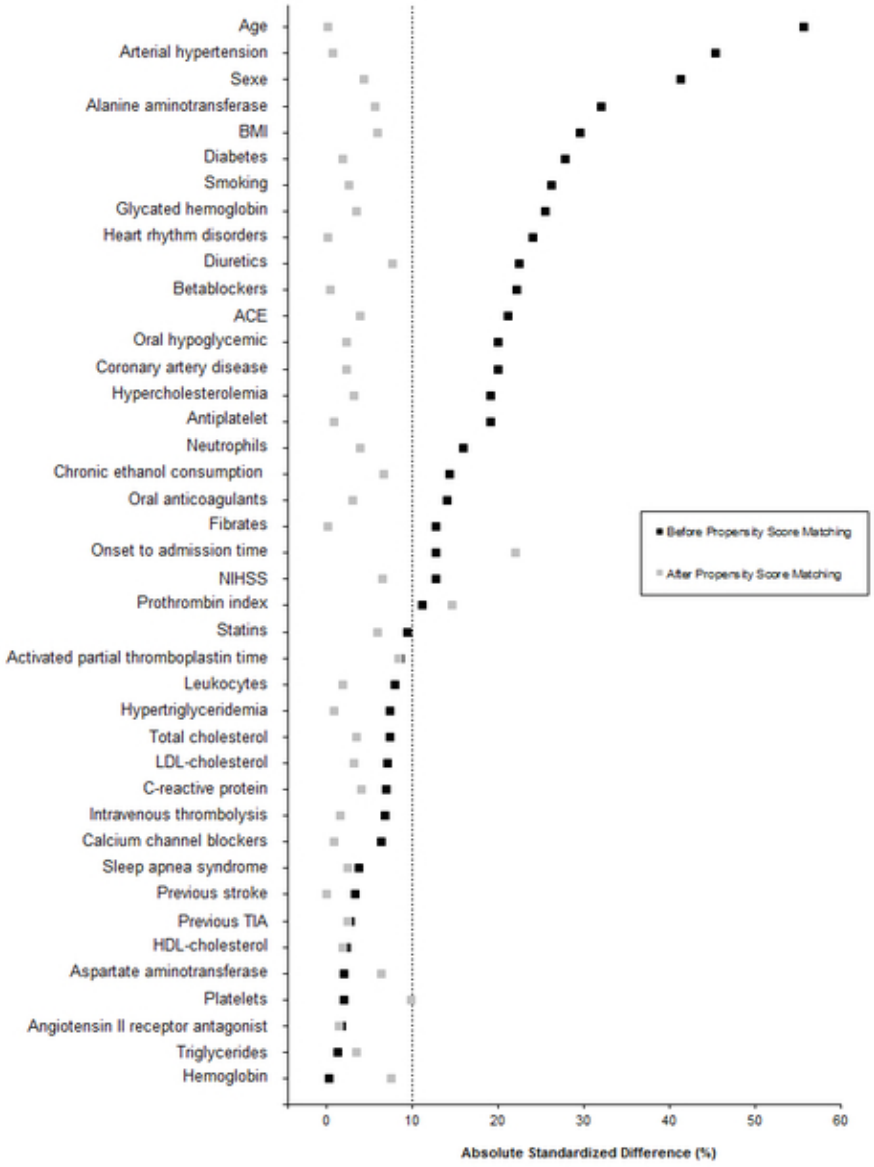

Figure 1. Absolute standardized differences between benzodiazepine users and non-users before and after propensity score matching.

40x53mm (300 x 300 DPI)

ONLINE SUPPLEMENT.

Supplemental table 1. Main baseline characteristics and Outcomes of Overall Study population (N=336)

	N	Value
Demographic characteristics		
Age, y, mean±SD	336	66.9 ± 14.9
Men	336	180 (53.6)
Medical history		
Previous stroke	335	35 (10.5)
Previous TIA	336	25 (7.4)
Coronary artery disease	336	69 (20.5)
Sleep apnea syndrome	333	9 (2.7)
Heart rhythm disorders	335	79 (23.6)
Vascular risk factors		
Arterial hypertension	336	203 (60.4)
Diabetes mellitus	336	66 (19.6)
Hypercholesterolemia	336	158 (47.0)
Hypertriglyceridemia	336	52 (15.5)
Smoking	336	102 (30.4)
Chronic ethanol consumption	334	52 (15.6)
BMI, kg/m^2, mean±SD	312	26.8 ± 4.9
Routine drugs		
Fibrates	336	23 (6.9)
Statins	336	107 (31.9)
Oral anticoagulants	336	19 (5.7)
Antiplatelet	336	126 (37.5)
ACE	336	63 (18.8)
Angiotensin II receptor antagonist	336	56 (16.7)
Diuretics	336	86 (25.6)

Calcium channel blockers	336	53 (15.8)
Betablockers	335	127 (37.9)
Oral hypoglycemic	336	51 (15.2)
Intravenous thrombolysis	332	94 (28.3)
Onset to admission time, hours, median (IQR)	332	2 (1-7)
NIHSS, median (IQR)	336	6 (2-14)
Biological characteristics		
Triglycerides, g/L, median (IQR)	329	1.1 (0.8-1.6)
Total cholesterol, g/L, mean±SD	329	1.95 ± 0.50
HDL-cholesterol, g/L, mean±SD	321	0.53 ± 0.16
LDL-cholesterol, g/L, mean±SD	319	1.16 ± 0.41
Glycated hemoglobin, %, median (IQR)	245	5.9 (5.6-6.4)
Hemoglobin, g/dL, median (IQR)	335	13.7 (12.9-14.7)
Leukocytes, /mm^3, median (IQR)	334	8320 (6730-10130)
Neutrophils, /mm^3, median (IQR)	315	5.6 (4.2-7.5)
Platelets, 1000/mm^3, median (IQR)	335	235 (195-272)
Prothrombin index, %, median (IQR)	320	95 (86-100)
Activated partial thromboplastin time, s, median (IQR)	333	32 (29-35)
C-reactive protein, mg/L, median (IQR)	280	4.9 (2-9.7)
Aspartate aminotransferase, U/L, median (IQR)	321	23 (19-28)
Alanine aminotransferase, U/L, median (IQR)	322	20 (15-28)
Outcome at 8-day		
All-cause death	336	11 (3.3)
NIHSS ≥6	319	108 (33.9)
Poor outcome (mRS ≥2)	330	176 (53.3)
Poor outcome (Barthel <95)	321	154 (48.0)
Cognitive impairment (MMSE <24)	236	63 (26.7)
Outcome at 90-day		
All-cause death	316	36 (11.4)
Poor outcome (mRS ≥2)	316	145 (45.9)

Poor outcome (Barthel <95)	278	88 (31.7)
Cognitive impairment (MMSE <24)	229	31 (13.5)

Abbreviations: ACE=angiotensin converting enzyme, ASD=absolute standardized difference, BMI= body mass index, HDL= high density lipoprotein, IQR=interquartile range, LDL=low density lipoprotein, MMSE: Mini Mental State Examination, mRS: modified Rankin score, NIHSS= National Institutes of Health Stroke Scale, SD=standard deviation, TIA=transient ischemic attack

Supplemental Table 2. Baseline characteristics in benzodiazepine users and non-users after propensity-score matching.

	Benzodiazepine non-users (n=56) *	**Benzodiazepine users** (n=56) *	***ASD, %***
Demographic characteristics			
Age, y, mean±SD	72.6 ± 14.9	72.6 ± 12.6	0.2
Men	24 (42.6)	23 (40.4)	4.5
Medical history			
Previous stroke	6 (9.9)	5 (9.7)	0.1
Previous TIA	5 (8.1)	4 (7.2)	2.6
Coronary artery disease	13 (23.7)	14 (24.6)	2.4
Sleep apnea syndrome	2 (4.2)	2 (3.6)	2.5
Heart rhythm disorders	16 (29.3)	16 (29.3)	0.2
Vascular risk factors			
Arterial hypertension	43 (77.1)	43 (76.8)	0.8
Diabetes mellitus	7 (13.2)	7 (12.3)	5.7
Hypercholesterolemia	30 (54.0)	29 (52.3)	3.3
Hypertriglyceridemia	9 (16.5)	9 (16.8)	1.0
Smoking	13 (23.3)	12 (21.9)	2.7
Chronic ethanol consumption	9 (15.6)	7 (13.0)	6.7
BMI, kg/m^2, mean±SD	26.2 ± 5.7	25.9 ± 5.3	6.0
Routine drugs			
Fibrates	6 (10.8)	6 (10.7)	0.2
Statins	19 (34.6)	18 (31.7)	6.0
Oral anticoagulants	19 (34.6)	18 (31.7)	3.1
Antiplatelet	24 (43.1)	24 (42.6)	1.1
ACE	15 (26.3)	14 (24.5)	4.0
Angiotensin II receptor antagonist	9 (16.9)	10 (17.4)	1.5
Diuretics	18 (31.9)	20 (35.4)	7.7

Calcium channel blockers	10 (18.0)	10 (17.6)	1.0
Betablockers	26 (46.0)	26 (45.8)	0.5
Oral hypoglycemic	6 (11.5)	6 (10.6)	2.4
Intravenous thrombolysis	16 (27.8)	15 (27.0)	1.6
Onset to admission time, hours, median (IQR)	2 (1-8)	2 (1-3)	22.0
NIHSS, median (IQR)	8 (2-17)	6 (2-18)	6.7
Biological characteristics			
Triglycerides, g/L, median (IQR)	1.02 (0.81-1.54)	1.02 (0.82-1.58)	3.6
Total cholesterol, g/L, mean±SD	1.93 ± 0.57	1.91 ± 0.52	3.5
HDL-cholesterol, g/L, mean±SD	0.54 ± 0.20	0.53 ± 0.14	2.0
LDL-cholesterol, g/L, mean±SD	1.14 ± 0.48	1.13 ± 0.45	3.3
Glycated hemoglobin, %, median (IQR)	6.0 (5.7-6.4)	5.9 (5.7-6.3)	3.5
Hemoglobin, g/dL, median (IQR)	13.4 (12.4-14.6)	13.5 (12.4-14.2)	7.5
Leukocytes, /mm^3, median (IQR)	8473 (6919-9884)	8276 (6590-10597)	1.9
Neutrophils, /mm^3, median (IQR)	5.7 (4.3-7.6)	5.7 (4.1-8.2)	4.0
Platelets, 1000/mm^3, median (IQR)	240 (206-277)	236 (198-274)	9.9
Prothrombin index, %, median (IQR)	95 (86-100)	92 (84-100)	14.8
Activated partial thromboplastin time, s, median (IQR)	32 (29-36)	31 (28-36)	8.5
C-reactive protein, mg/L, median (IQR)	5.7 (2.5-9.6)	5.8 (2.6-9.7)	4.2
Aspartate aminotransferase, U/L, median (IQR)	23 (19-27)	23 (20-28)	6.4
Alanine aminotransferase, U/L, median (IQR)	18 (14-24)	18 (14-23)	5.7

Data are expressed as number (%) unless otherwise indicated. Descriptive parameters and ASD were calculated after handling missing baseline data including in propensity-score calculation by multiple imputation procedure (m=20 imputed datasets). * mean numbers of matched pairs among the 20-imputed datasets.

Abbreviations: ACE=angiotensin converting enzyme, ASD=absolute standardized difference, BMI= body mass index, HDL= high density lipoprotein, IQR=interquartile range, LDL=low density lipoprotein, NIHSS= National Institutes of Health Stroke Scale, SD=standard deviation, TIA=transient ischemic attack.

Supplemental table 3. Main baseline characteristics of patients loss and not loss at 90-day

	90-day follow-up	
	Yes (n=316)	**No** (n=20)
Number of patients	316	20
Benzodiazepine use	58 (18.3)	4 (20.0)
Age, y, mean±SD	66.6 ± 14.9	71.5 ± 12.9
Men	170 (53.8)	10 (50.0)
Medical history		
Previous stroke	32 (10.1)	3 (15.8)
Previous TIA	24 (7.6)	1 (5.0)
Coronary artery disease	63 (19.9)	6 (30.0)
Sleep apnea syndrome	9 (2.9)	0 (0.0)
Heart rhythm disorders	75 (23.8)	4 (20.0)
Vascular risk factors		
Arterial hypertension	190 (60.1)	13 (65.0)
Diabetes mellitus	61 (19.3)	5 (25.0)
Hypercholesterolemia	149 (47.1)	9 (45.0)
Hypertriglyceridemia	51 (16.1)	1 (5.0)
Smoking	94 (19.7)	8 (40.0)
Chronic ethanol consumption	49 (15.6)	3 (15.0)
BMI, kg/m^2, mean±SD	26.8 ± 5.0	25.5 ± 3.9

Data are expressed as number (%) unless otherwise indicated.

Abbreviations: BMI= body mass index, SD=standard deviation, TIA=transient ischemic attack.

Supplemental table 4. Individual characteristics of the 36 mortality cases during the 90-day follow-up period after stroke onset.

Patients	Benzodiazepines	Age	Gender	NHSS	Hypertension	Diabetes	Hypercholesterolemia	Smoking	Previous Stroke
1	No	87	Women	22	Yes	Yes	No	No	No
2	No	52	Women	6	Yes	No	Yes	Yes	Yes
3	No	39	Women	15	Yes	No	No	Yes	No
4	No	80	Men	11	Yes	Yes	Yes	Yes	Yes
5	No	72	Men	10	No	No	No	Yes	No
6	No	82	Men	20	No	No	No	No	No
7	No	82	Women	21	No	Yes	No	No	No
8	No	85	Women	22	Yes	No	No	No	Yes
9	No	89	Women	26	No	No	No	No	No
10	No	81	Women	20	Yes	Yes	Yes	No	No
11	No	71	Men	8	Yes	Yes	Yes	Yes	No
12	No	63	Men	19	Yes	No	No	No	No
13	No	43	Women	11	No	No	No	Yes	No
14	No	79	Men	11	No	No	No	No	No
15	No	44	Women	7	No	No	No	Yes	No
16	No	39	Men	18	No	No	No	No	No
17	No	38	Men	0	No	No	No	Yes	No
18	No	65	Men	19	No	No	No	Yes	No
19	No	46	Men	8	Yes	No	Yes	Yes	No
20	No	70	Women	8	Yes	Yes	Yes	No	No
21	No	48	Women	17	Yes	No	No	No	No
22	Yes	85	Men	21	No	No	No	No	No
23	Yes	53	Men	27	No	No	No	Yes	No
24	Yes	84	Women	32	Yes	No	Yes	No	Yes
25	Yes	84	Men	22	Yes	No	Yes	No	No
26	Yes	85	Women	27	Yes	No	Yes	No	No
27	Yes	93	Women	21	Yes	No	Yes	No	No
28	Yes	60	Men	1	Yes	No	Yes	No	No
29	Yes	86	Women	25	Yes	No	Yes	No	No
30	Yes	76	Women	20	Yes	Yes	No	No	No
31	Yes	66	Women	17	Yes	No	No	No	No
32	Yes	71	Women	13	Yes	No	Yes	No	No
33	Yes	83	Women	1	Yes	No	Yes	No	No
34	Yes	81	Men	1	No	No	Yes	No	Yes
35	Yes	83	Women	11	Yes	No	Yes	Yes	No
36	Yes	63	Women	0	Yes	No	No	No	No

Supplemental table 5. Main baseline characteristics of alive patients with and without missing MMSE at 8- and 90-days

	8-day MMSE		90-day MMSE	
	Not missing	Missing	Not missing	Missing
Number of patients	236	89	229	71
Benzodiazepine use	40 (16.9)	17 (19.1)	35 (15.3)	12 (16.9)
Age, y, mean±SD	65.2 ± 14.4	70.3 ± 15.2	65.5 ± 14.9	69.8 ± 13.3
Men	132 (55.9)	45 (50.6)	128 (55.9)	37 (52.1)
Medical history				
Previous stroke	22 (9.3)	10 (11.4)	22 (9.6)	8 (11.4)
Previous TIA	16 (6.8)	9 (10.1)	14 (6.1)	7 (9.9)
Coronary artery disease	40 (16.9)	26 (29.2)	44 (19.2)	14 (19.7)
Sleep apnea syndrome	6 (2.6)	3 (3.4)	4 (1.8)	4 (5.7)
Heart rhythm disorders	51 (21.6)	24 (27.3)	52 (22.8)	19 (26.8)
Vascular risk factors				
Arterial hypertension	137 (58.0)	57 (64.0)	138 (60.3)	42 (59.1)
Diabetes mellitus	44 (18.6)	19 (21.3)	47 (20.5)	12 (16.9)
Hypercholesterolemia	107 (45.3)	47 (52.8)	111 (48.5)	31 (43.7)
Hypertriglyceridemia	41 (17.4)	10 (11.2)	40 (17.5)	8 (11.3)
Smoking	81 (34.3)	18 (20.2)	67 (29.3)	23 (32.4)
Chronic ethanol consumption	39 (16.7)	13 (14.6)	38 (16.6)	13 (18.6)
BMI, kg/m^2, mean±SD	26.7 ± 4.8	27.0 ± 5.1	26.8 ± 4.8	26.6 ± 5.0

Data are expressed as number (%) unless otherwise indicated.

Abbreviations: BMI= body mass index, SD=standard deviation, TIA=transient ischemic attack.

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract Page 2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found (Page 2)
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported Page 4
Objectives	3	State specific objectives, including any prespecified hypotheses Page 4
Methods		
Study design	4	Present key elements of study design early in the paper (page 5)
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection (page 5)
Participants	6	*Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up (page 5)
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed (NA)
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable (page 5)
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group (page 5-6)
Bias	9	Describe any efforts to address potential sources of bias (NA)
Study size	10	Explain how the study size was arrived (page5)
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why (page 6)
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding (page 6)
		(*b*) Describe any methods used to examine subgroups and interactions ((page 6))
		(*c*) Explain how missing data were addressed (pages 6-7)
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed (page 6)
		(*e*) Describe any sensitivity analyses (page 6)

Continued on next page

Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (page 8)
		(b) Give reasons for non-participation at each stage (page 8)
		(c) Consider use of a flow diagram (NA)
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (page 8; 15-16)
		(b) Indicate number of participants with missing data for each variable of interest (page 8; 15-16)
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount) (page 8; 15-16)
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time (page 8;17)
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (page 8; 15-17)
		(*b*) Report category boundaries when continuous variables were categorized (page 8; 15-17)
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period (NA)
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses (suppl file)
Discussion		
Key results	18	Summarise key results with reference to study objectives (page 10)
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias (page 10-12)
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence (page 13)
Generalisability	21	Discuss the generalisability (external validity) of the study results (pages 13-14)
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based (page 18)

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.