Baseline anticholinergic burden from medications predicts incident fatal and non-fatal stroke in the EPIC-Norfolk general population

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Keywords

- Anticholinergic burden
- Stroke
- Mortality
- Incidence
- Epidemiology

Key Points

- Few studies have been conducted to examine the relationship between anticholinergic burden (ACB) and stroke in a general population.
- In this prospective cohort study including 21,722 participants, we demonstrated an increased in risk of incident stroke and stroke mortality with increasing anticholinergic burden.
- Our results provide incentive to clinicians to cautiously use medications with anticholinergic properties to reduce the global burden of stroke.

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Abstract

Background: Stroke is primarily a disease of older age with a substantial impact on global mortality and morbidity. Whilst medications with anticholinergic effects are widely used, no studies have been conducted to examine the relationship between anticholinergic burden (ACB) and stroke in a general population.

Method: The sample was drawn from the EPIC-Norfolk cohort. Baseline assessments were carried out during 1993-1997 and participants were followed up until March 2016. Participants were divided into 4 groups according to their total ACB score at baseline, these groups were those with a total ACB score of 0, 1, 2-3 and >3. After exclusion, Cox-proportional hazards models were constructed to determine the associations between the ACB score groups and the risk of incident stroke and stroke mortality. Sensitivity analysis and propensity score matched analyses were performed.

Results: In total 25,639 participants attended the first health check, 3,917 participants were excluded, leaving 21,722 participants to be included. Participants had a mean age (SD) of 58.9 (9.2) years (54.4% women). Of these, 2,131 suffered incident stroke and 562 died from stroke. Mean follow up was approximately 18 years for both outcomes. In the fully adjusted model, those with an ACB of >3 had 59% relative risk of incident stroke (HR (95% CI) 1.59 (1.34 - 1.89)) and 86% relative risk of stroke mortality (1.86 (1.37 - 2.53)) compared to those in ACB 0 category. Sensitivity analyses and propensity score matched analyses showed similar results.

Conclusions: Our results provide an incentive for the cautious use of medications with anticholinergic properties to help reduce the global burden of stroke.

Introduction

Polypharmacy is a common factor in ageing populations. As such, the appropriate use of medications with anticholinergic (antimuscarinic) adverse effects is of particular interest due to their additive effects, age related changes to pharmacokinetic and pharmacodynamics properties and the wide range of therapeutic categories in which they occur. These medications are prescribed frequently, with studies in older adults reporting the prevalence of anticholinergic medications ranging from 8-37% depending on the study setting (1, 2, 3).

Recently, there have been studies published examining a link between anticholinergic burden (ACB) and health outcomes in general populations. We have recently shown an association between ACB and risk of all-cause mortality and incident cardiovascular disease, describing a class effect as well as a dose response relationship (4). A large study, part of the Medical Research Council Cognitive Function and Ageing Study (MRC CFAS), showed an increase in the cumulative risk of cognitive impairment and mortality in approximately 13,000 participants aged 65 and over (5). Another study in approximately 2,600 participants aged over 65, part of The Irish Longitudinal Study on Ageing (TILDA) showed an association with subsequent injurious falls in older men (6). In addition, other studies have been carried out examining for a link between ACB and health outcomes, but these have primarily focused on specific patient populations (7, 8, 9, 10).

Importantly, previous studies in general populations have not examined for stroke outcomes and whilst some studies have been published on the link between ACB and stroke, these have been in specific patient populations, focusing primarily on inhaled anticholinergics in COPD patients producing mixed results (11, 12). An important gap in the literature exists regarding risk of stroke and ACB in a general population.

Stroke represents one of the most common and significant life changing events, affecting 15 million people worldwide leading to substantial mortality and morbidity, with an estimated annual cost to the European economy of €64.1 billion (13). Stroke is primarily a disease of older age, and with the increase in ageing populations the global incidence of stroke is expected to rise across the world's regions despite improved risk factor management. As stroke is a potentially preventable condition, the link between ACB and risk of stroke would have an important impact on clinical practice globally as it represents an easily identifiable and potentially modifiable risk factor.

Therefore, in this study, we examined the prospective relationship between total ACB from medications at baseline and incident stroke and stroke mortality in a UK population based study, European Prospective Investigation into Cancer (EPIC)-Norfolk.

Methods

Population

The study population was drawn from men and women participants aged between 39 - 79 years at the baseline (1993-1997) who took part in the EPIC-Norfolk prospective population-based study. The study protocol of EPIC-Norfolk has been described previously (14). In brief, participants were invited from general practice age-sex registers between 1993-1997 and followed up to March 2016. In total 25,639 participants (99·6% White British) attended a baseline health examination during 1993-1997.

Ethics

Ethical approval was obtained from the Norwich Ethics Committee and all participants gave informed signed consent for the examination of medical records and use of data.

Measurement methods

Details of data collection and measurement methods are described in full as part of the online content. In summary, at baseline, participants completed a health and lifestyle questionnaire in which information such as educational status, social class, physical activity, smoking status, alcohol consumption, prevalent illness and medications were asked. Other physiological and biological parameters such as height, weight, blood pressure and non-fasting venous blood samples were collected by trained research nurses at clinic visits.

Drugs associated with anti-cholinergic burden (**Appendix 1**) were identified by searching the database for exact and similar entries for both generic and brand name drugs. Each medication was assigned to the corresponding anti-cholinergic score and the total

anticholinergic burden (ACB) was calculated using the formula: {[number of class 1 anti-cholinergic drugs] + [the number of class 2 anti-cholinergic drugs x 2] + [the number class 3 anti-cholinergic drugs x 3]}.

The development of the anti-cholinergic burden (ACB) scale used in this study has been previously reported. Classification of drugs with ACB was class 0 (none), class 1 (mild), 2 and 3 (severe). Examples of drugs with include atenolol, ranitidine, codeine (class 1), amantadine, carbamazepine, pethidine (class 2) and amitriptyline, oxybutynin, olanzapine (class 3). The score's predictive validity in cognitive decline has been shown in three large scale studies and a score of 2 or more was associated with increased mortality in an older population (15).

Participants were divided into 4 groups according to their ACB score at baseline. These groups were those with baseline ACB score of 0, 1, 2-3 and >3.

Case ascertainment

Stroke mortality of participants was identified from the Office of National Statistics and admission episodes were identified from the NHS hospital information system and ENCORE (East Norfolk COmmission Record). Stroke mortality and incident stroke were identified from the death certificates or hospital discharge code ICD 9, 430-438, or ICD 10, I60-I69 for stroke incidence. The follow up protocol of EPIC-Norfolk had been previously validated using incident stroke cases which showed high sensitivity and specificity (16).

Follow up time for this study started at the date of study enrolment and ended in March 2016 for both incident stroke events and stroke mortality outcomes.

Exclusion criteria

As shown in **Appendices 2 and 3**, of 25,639 participants attended first health check, 3,917 participants were excluded from analysis due to prevalent stroke and missing data, leaving 21,722 participants included in the analysis.

Statistical analysis

Data were analysed using SPSS version 24.0 (SPSS Inc., Chicago, IL, USA) and Stata 14.0 (StataCorp LP, TX, USA). Descriptive statistics were presented for the overall sample and by ACB score groups and compared using one-way analysis of variance for means, and Pearson's Chi-squared test for categorical measures.

Cox-proportional hazards models were constructed to determine the associations between ACB and the risk of incident stroke and incident stroke mortality using ACB score 0 group as the reference category. Adjusted analyses were undertaken to account for potential confounding factors such as age, sex, lifestyle, socioeconomic, co-morbidities, stroke risk factors such as blood pressure and cholesterol levels, use of primary prevention medications such as aspirin. A variety of adjusted models were used to assess the effects of these potential confounding factors in a group sequential fashion. Model A adjusted for age and sex; model B adjusted for age, sex, smoking, alcohol consumption, physical activity level, education level, occupational social class, systolic blood pressure, cholesterol level and body mass index; model C adjusted for variables in model B plus prevalent asthma, COPD, diabetes, MI and cancer; model D adjusted for variables in model B as well as excluding people with prevalent asthma, COPD, diabetes, MI and cancer; model E adjusted for variables in model C as well as excluding all events occurring within first two

years of follow up; model F adjusted for variables in model C plus aspirin use; model G adjusted for variables in model B truncated at 5 years follow-up; model H adjusted for variables in model B truncated at 10 years follow-up; model I was propensity adjusted, based on propensity score estimated from factors in model B. Cox-proportional Hazards Ratios (HR) for incident stroke subdivided into those with ischaemic strokes (ICD category I63) and those with haemorrhagic strokes (ICD category I61) adjusted for variables in model C was also performed.

As ACB was calculated from the baseline we examined the relationship using shorter follow up periods (models G and H) to determine if the outcomes varied depending on follow up duration and repeated the analyses after exclusion of individuals with strokes occurring within the first two years of follow up to account for reverse causality (model E) as well as after excluding other prevalent illness (model D).

An alternative analysis was performed using propensity score analysis (model I). Individuals were matched using estimated propensity scores from a logistic regression model based on covariates that predict ACB group. This was done 3 times, once for each comparison for ACB score of 0. The matched pairs were then entered into an unadjusted Cox-regression model.

Kaplan-Meier curves were constructed for the 4 different ACB score groups. The end-point was incident stroke, which included both fatal and non-fatal strokes. All statistical tests were assessed at two-sided significance level of 0.05.

Results

In total 25,639 participants attended the first health check, 3,917 participants were excluded due to prevalent stroke at the baseline (n= 388) and those with any missing data on the variables included in the analysis, leaving 21,722 study participants eligible to be included in the analysis. Most variables with missing data has less then <1% missing, with the highest missing data exclusion for missing cholesterol levels. The mean age of participants (SD) was 58.9 (9.2) years and 54.4% were women. Of these, 2,131 suffered an incident stroke and 562 participants died from stroke. The mean follow up (SD) for incident stroke was 17.77(4.91) years (total person years 385,979) and 18.05 (4.73) years (total person years 392,176) for stroke mortality.

Table 1 details sample characteristics at baseline and crude outcome rates during the whole follow up period by total ACB score groups. Differences were observed across all ACB groups for all variables aside from sex. The participants in the higher ACB score groups at study baseline had a greater age, were physically less active, had higher total cholesterol levels, higher systolic blood pressure and were more likely to be on primary prevention medications for stroke such as aspirin, lipid lowering drugs and antihypertensive drugs. They were more likely to have had a diagnosis of COPD, asthma and diabetes. Higher ACB is also associated with higher proportion of participants in lower occupational social classes and those with lower educational attainment.

Higher crude rates of events for both incident stroke and stroke mortality occurred in higher ACB score groups. Over the entire follow up the overall crude stroke event rates were

8.5%, 14.4%, 15.8% and 16.0% and the crude stroke mortality rates were 2.1%, 4.6%, 3.4% and 5.3% for ACB score 0, 1, 2-3 and >3 groups, respectively.

Table 2 details the Cox-proportional Hazards Ratios (HR) and corresponding 95% confidence intervals (95% CI) for incident stroke and stroke mortality across the 4 ACB score groups using ACB score of 0 group as the reference category. Higher ACB score groups were associated with a higher risk of incident stroke and stroke death. The models of adjustment with incremental inclusion of various confounders were associated with attenuation in HRs but the risk remained high. The participants in highest ACB group (ACB >3) had increase in relative risk of 59% and 86% for incident stroke and incident stroke mortality, respectively, compared to those with ACB of 0 in the fully adjusted model (model C). Excluding participants with prevalent medical conditions, events occurring within the first two years of follow up, truncation of follow-up at shorter time frames of 5 and 10 years and propensity score analysis only slightly attenuated the results.

Table 3 details the Cox-proportional Hazards Ratios (HR) and corresponding 95% confidence intervals (95% CI) for incident stroke subdivided into those with ischaemic strokes (ICD category I63) and those with haemorrhagic strokes (ICD category I61) adjusted for variables in model C. Higher ACB groups were associated with higher risk of incident ischaemic stroke but not for haemorrhagic stroke. Stroke mortality was not calculated due to small samples sizes (see Table 1 for participant numbers).

Figure 1 shows Kaplan-Meier curves for time to incident stroke for ACB score groups 0, 1, 1-2 and >3. The end-point was incident stroke, which included both fatal and non-fatal strokes. Higher ACB groups had a shorter time to incident stroke ($p<0.001$).

Table 1 here.

Table 2 here.

Table 3 here

Figure 1 here.

Discussion

To the best of our knowledge, this is the first study to examine the association between ACB from medications and the subsequent risk of stroke in an apparently healthy general population. Our results show that participants with higher baseline ACB scores were at an increased risk of stroke incidence and stroke related mortality compared to the ACB 0 reference group. Participants with higher anticholinergic burden were older, more likely to have prior co-morbidities and cerebrovascular risk factors, but adjustment for these variables and other potential confounders yielded similar results.

In the fully adjusted model (model C) those with an ACB of greater than 3 had a HR (95% CI) of 1.59 (1.34 - 1.89) for incident stroke and 1.86 (1.37 - 2.53) for stroke mortality. Importantly, a reduction from one ACB category to another also represents an important reduction in risk. Given the wide variety of medications that exhibit anticholinergic properties a vitally important message to prescribers is that even small changes in a patient's drug regimen can result in a considerable risk reduction.

Little research has been published examining a link between ACB and stroke risk in general population with most studies focusing on inhaled anticholinergic medication in COPD patients, with mixed results. One population-based nested case–control study in 15,396 newly-diagnosed COPD patients showed an increased risk (Adjusted Odds Ratio, 2.02; 95% CI, 1.71 - 2.41) of stroke in those treated with ipratropium within 6 months (17), whereas another study showed no increase in risk of all-cause stroke from inhaled anticholinergics in a UK primary care derived population (18). A meta-analysis of 17 randomised placebo or active controlled trials showed that there was no increased in the risk of stroke (RR, 1.46 [95% CI, 0.81-2.62]; P=.20, I^2=0%) in patients with COPD taking

inhaled tiotropium or ipratropium (11). These findings were echoed by another meta-analysis of 19 randomised control (placebo or salmeterol) trials showed that tiotropium did not increase the risk of non-fatal stroke (RR = 1.04; 95% CI, 0.78-1.39, I^2 0%) (12).

It can be observed that the 95% CIs overlap for ACB score groups 1, 2-3 and > 3 for models B-D. This may suggest that the major jump in risk is between those with no ACB and those with ACB of at least 1. However, this maybe a reflection of the sample size and we would expect that these 95% CIs to be narrower in a larger population.

Mechanistically, potential explanations exist for why there is a plausible causal link between ACB from medications and increased incidence of stroke and stroke related death. It has been suggested that anticholinergic medications have pro-arrhythmic and pro-ischaemic properties, which may lead to cerebral ischaemia (19). Anticholinergic drugs act to suppress parasympathetic stimulation to the heart, which is associated with tachyarrhythmias. These arrhythmias confer an increased risk of embolic strokes as well as cardiac ischaemia and sudden death in susceptible cardiac patients (20). It has been shown previously that the arterial baroreflex is a vital mechanism that plays an important role in determining many stroke outcomes, including prognosis (21). It has been suggested that vagal nerve activity may be involved in the protective effects of the baroreflex in stroke and that loss of parasympathetic activation in increasing age and cardiovascular disease attenuates this protective effect (22). It stands to reason that interference of vagal tone by antimuscarinic medication could have a similar effect.

Effects through immunomodulation by the cholinergic system may also serve as a potential mechanism. Both experimental and clinical evidence has demonstrated inflammation as an

important component of stroke aetiology and pathophysiology (23). The cholinergic system regulates immune response as nicotinic receptor activation leads to inhibition of the innate and adaptive immune systems and muscarinic receptor antagonists have been shown to inhibit T-cell proliferation, response and migration. Interference of these systems may lead to an inappropriate inflammatory response (24, 25).

As shown in table 3, an increase in risk of incident stroke was demonstrated in higher ACB categories for those with ischaemic stroke but not for those with haemorrhagic stroke. This may be due to the fact that the above mechanisms are more likely to be relevant to the pathophysiology of the ischaemic stroke. However, the numbers of haemorrhagic strokes were relatively small in our data set and it is likely this was under powered to detect a meaningful difference. Future population studies should be conducted to examine this in a larger population.

Our study has several strengths. We used a large population based sample, which improves the generalisability of our findings. As a prospective study, with robust case ascertainment, we introduce less bias. We used a well validated ACB score. Further, we were able to control for a wide range of demographic, lifestyle and socioeconomic factors, as well as for medical co-morbidities and concomitant primary preventive medications for stroke, and additionally performed propensity score matched analysis. There is no reason to believe potential mechanistic link between ACB and stroke risk would differ in different races and thus our study has global implication with regard to reducing global stroke burden.

There are some limitations worth discussing. It could be argued that we introduced healthy volunteer bias as this was a volunteer study which required long-term follow up. Nevertheless, the baseline characteristics of the EPIC-Norfolk have been shown to be similar to other UK representative population samples (14). Approximately 3,500 participants were excluded due to missing data, however the examination of percentage missing demonstrated most missing variables were <1% missing. This is unlikely to have significantly attenuated the observed associations. EPIC-Norfolk participants are mainly Caucasians (>99.6%) but as eluded earlier in the strengths of this study, the biological mechanism between the link between anticholinergic and risk of outcomes examined is unlikely to be different between different races.

Reverse causality is a major factor in measuring the association between medications and health outcomes. To further understand this effect, we excluded events that occurred within the first two years and the observed associations remained after this adjustment. As ACB was calculated at baseline, we do not know whether participants continued this medication regimen throughout the follow up period. To account for this, we truncated our analysis using shorter follow up periods of 5 and 10 years, shown in Table 2 as models G and H. The results were broadly similar for these different follow up periods. Furthermore, it is likely ACB burden increases with increasing age and this applies to all ACB groups.

Potential confounders were measured at baseline and it is possible that these may vary during the follow up period. However, truncation of the analysis at 5, 10 and 15 years only slightly attenuated the results. Whilst both multivariable adjustment and propensity score analyses were performed to assess the impact of known available confounders we recognise that residual and unmeasured confounding could not be ruled out.

Those in higher ACB groups had a higher cardiovascular risk profile and hence were potentially more likely to be followed up thus introducing possible surveillance bias. However, endpoint ascertainment was based on validated record linkage system within UK NHS, which limits any potential surveillance bias.

Implications

International guidance on the management of polypharmacy recommends ‘medicines optimisation’, in partnership with patients, to attain the best possible outcomes and avoid inappropriate polypharmacy (26, 27). A multidisciplinary approach is required to tackle this issue with pharmacists and primary care providers being in an ideal position to monitor and adjust medicine usage. It is important to note, polypharmacy can be appropriate and anticholinergic medicines often play a central role in disease management. Of note, polypharmacy does not necessarily equate to high anticholinergic burden and it is possible to reduce anticholinergic burden through processes such as switching to medications with similar pharmacological effects but without anticholinergic properties. Where polypharmacy does occur, we encourage clinicians to carefully consider prescribing medications with ACB and offer alternatives when feasible. As stroke is one of the leading causes of mortality and morbidity, reduction of ACB at the population level has huge potential impact on reducing the growing global burden of stroke.

Conclusion

Our study has shown a dose-response relationship between ACB from medications and risk of incident stroke and stroke mortality in a large, general population. In absence of long term population based clinical trials which examine the impact of reducing ACB at the general population level, our results provide incentive to patients, public, and clinicians to use medications with anticholinergic properties cautiously to reduce risk of stroke and subsequent global burden of stroke.

Conflict of interest statement and funding

All authors declare: no support from any organisation for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work; no other relationships or activities that could appear to have influenced the submitted work.

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Table 1 Baseline sample characteristics of 21,722 men and women of the EPIC-Norfolk and crude outcome rates during the whole follow up according to the total anticholinergic burden score groups

	All (n= 21,722)	**ACB score 0 group (n= 17,467)**	**ACB score 1 group (n= 2,179)**	**ACB score 2-3 group (n=1,157)**	**ACB score >3 group (n=919)**	**P***
Mean age years (SD)	58.9 (9.2)	58.0 (9.1)	62.23 (8.8)	63.30 (9.02)	63.35 (8.6)	<0.001
Sex (%)						0.79
Men	9913 (45.6)	7946 (45.5)	1016 (46.6)	531 (45.9)	420 (45.7)	
Women	11809 (54.4)	9521 (54.5)	1163 (53.4)	626 (54.1)	499 (54.3)	
Social class (%)						<0.001
Professional	1546 (7.1)	1291 (7.4)	138 (6.3)	64 (5.5)	53 (5.8)	
Manager	8048 (37.0)	6595 (37.8)	767 (35.2)	408 (35.3)	278 (30.3)	
Skilled non-manual	3621 (16.7)	2859 (16.4)	385 (17.7)	198 (17.1)	179 (19.5)	
Skilled manual						
Semi-skilled	4973 (22.9)	3962 (22.7)	496 (22.8)	276 (23.9)	238 (25.9)	
Unskilled	2817 (13.0)	2224 (12.7)	309 (14.2)	161 (13.9)	133 (14.5)	
	707 (3.3)	535 (3.1)	84 (3.9)	50 (4.3)	38 (4.1)	
Smoking (%)						<0.001
Current-smoker	2421 (11.1)	1988 (11.4)	199 (9.1)	120 (10.4)	114 (12.4)	
Ex-smoker	9227 (42.5)	7195 (41.2)	1044 (47.9)	559 (48.3)	429 (46.7)	
Never smoker	10074 (46.4)	8284 (47.4)	936 (43.0)	478 (41.3)	376 (40.9)	
Alcohol use (units/week) (SD)	7.15 (9.35)	7.34 (9.44)	6.73 (9.53)	6.18 (8.30)	5.77 (8.20)	<0.001
Education level (%)						<0.001
No qualification	7698 (35.4)	5859 (33.5)	905 (41.5)	505 (43.6)	429 (46.7)	
0-Level	2269 (10.4)	1882 (10.8)	210 (9.6)	97 (8.4)	80 (8.7)	
A-Level	8915 (41.0)	7282 (41.7)	845 (38.8)	455 (39.3)	333 (36.2)	
Higher degree	2840 (31.1)	2444 (14.0)	219 (10.1)	100 (8.6)	77 (8.4)	
Physical activity (%)						<0.001
Inactive	6319 (29.1)	4636 (26.5)	816 (37.4)	468 (40.4)	399 (43.4)	
Moderately inactive	6294 (29.0)	5096 (29.2)	618 (28.4)	320 (27.7)	260 (28.3)	
Moderately active	5044 (23.2)	4252 (24.3)	434 (19.9)	205 (17.7)	153 (16.6)	
Active	4065 (18.7)	3483 (19.9)	311 (14.3)	164 (14.2)	107 (11.6)	
Cholesterol (mmol/L) (SD)	6.17 (1.16)	6.13 (1.15)	6.31 (1.21)	6.30 (1.21)	6.42 (1.24)	<0.001
Systolic BP (mmHg) (SD)	135.05 (18.2)	134.11 (17.90)	139.24 (18.47)	136.76 (18.81)	140.78 (19.43)	<0.001
BMI (kg/m^2) (SD)**	26.26 (3.8)	26.10 (3.72)	26.88 (4.20)	26.78 (4.03)	27.19 (4.16)	<0.001
COPD (%)	2008 (9.2)	1437 (8.2)	301 (13.8)	160 (13.8)	110 (12)	<0.001
Asthma (%)	1809 (8.3)	923 (5.3)	587 (26.9)	173 (15.0)	126 (13.7)	<0.001
Diabetes* (%)**	460 (2.1)	286 (1.6)	70 (3.2)	52 (4.5)	52 (5.7)	<0.001

MI (%)	656 (3.0)	250 (1.4)	156 (7.2)	149 (12.9)	101 (11.0)	<0.001
Cancer (%)	1168 (5.4)	886 (5.1)	131 (6.0)	91 (7.8)	60 (6.5)	<0.001
Aspirin use # (%)	1920 (10.4)	1168 (7.7)	325 (18.3)	236 (24.2)	191 (25.8)	<0.001
Antihypertensive use (%)	3882 (17.9)	1595 (9.1)	1106 (50.8)	557 (48.1)	295 (32.1)	<0.001
Lipid lowering medication use (%)	314 (1.4)	166 (1)	61 (2.8)	43 (3.7)	44 (4.8)	<0.001
New incident stroke (%)	2131 (9.8)	1487 (8.5)	314 (14.4)	183 (15.8)	147 (16.0)	
Haemorrhage	374 (1.7)	281 (1.6)	49 (2.3)	22 (1.9)	22 (2.4)	
Infarct	956 (4.4)	667 (3.8)	132 (6.1)	90 (7.8)	67 (7.3)	
Stroke Deaths (%)	562 (2.6)	374 (2.1)	100 (4.6)	39 (3.4)	49 (5.3)	
Haemorrhage	156 (0.7)	108 (0.6)	26 (1.2)	14 (1.2)	8 (0.9)	
Infarct	79 (0.4)	54 (0.3)	12 (0.6)	3 (0.3)	10 (1.1)	

Values presented are mean (SD) for continuous and number (%) for categorical data. *overall P value. BP=blood pressure, BMI = body mass index, COPD= chronic obstructive pulmonary disease and MI=myocardial infarction. Total anticholinergic burden (ACB) calculated as a score which is the sum of the [number of class 1 anticholinergic drugs, the number of class 2 anticholinergic drugs x2 and the number class 3 anticholinergic drugs x3]. Classification of drugs with ACB class 1, 2 and 3 based on criteria of Anticholinergic Cognitive Burden Scale[15]. ** *n = 21,707,* *** *n = 21,713,* # 1,920 events total 18,532.

Table 2: Hazard ratios and their corresponding 95%CI of incident stroke and stroke mortality according to total anticholinergic burden score groups during follow up in EPIC-Norfolk

Models	Incident Stroke (Events (n=2,131)/Total N=21,722)			
	ACB score 0 group	**ACB score 1 group**	**ACB score 2-3 group**	**ACB score >3 group**
A	1.00	1.39 (1.23 – 1.58)	1.58 (1.35 – 1.84)	1.72 (1.45 – 2.04)
B	1.00	1.36 (1.20 – 1.53)	1.60 (1.37 – 1.87)	1.68 (1.42 – 1.99)
C	1.00	1.33 (1.17 – 1.51)	1.54 (1.31 – 1.80)	1.59 (1.34 – 1.89)
D	1.00	1.32 (1.13 – 1.55)	1.61 (1.32 – 1.95)	1.88 (1.53 – 2.30)
E	1.00	1.32 (1.16 – 1.50)	1.55 (1.32 – 1.81)	1.57 (1.32 – 1.87)
F	1.00	1.28 (1.11 – 1.48)	1.34 (1.11 – 1.61)	1.53 (1.26 – 1.86)
G	1.00	1.27 (0.81 – 1.98)	1.27 (0.71 – 2.27)	2.08 (1.26 – 3.45)
H	1.00	1.58 (1.25 – 2.00)	1.84 (1.38 – 2.45)	2.46 (1.86 – 3.25)
I	1	1.35 (1.19 – 1.52)	1.56 (1.38 – 1.83)	1.64 (1.38 – 1.94)
Models	**Stroke Mortality (114) 160 – 169 (Events (n=562)/Total N=21,722)**			
	ACB score 0 group	**ACB score 1 group**	**ACB score 2-3 group**	**ACB score >3 group**
A	1.00	1.57 (1.25 – 1.96)	1.13 (0.81 – 1.58)	1.95 (1.44 – 2.63)
B	1.00	1.55 (1.24 – 1.94)	1.19 (0.85 – 1.66)	1.94 (1.44 – 2.62)
C	1.00	1.58 (1.25 – 1.99)	1.16 (0.83 – 1.62)	1.86 (1.37 – 2.53)
D	1.00	1.56 (1.17 – 2.07)	1.03 (0.66 – 1.62)	2.24 (1.58 – 3.18)
E	1.00	1.57 (1.24 – 1.98)	1.19 (0.85 – 1.67)	1.87 (1.37 – 2.54)

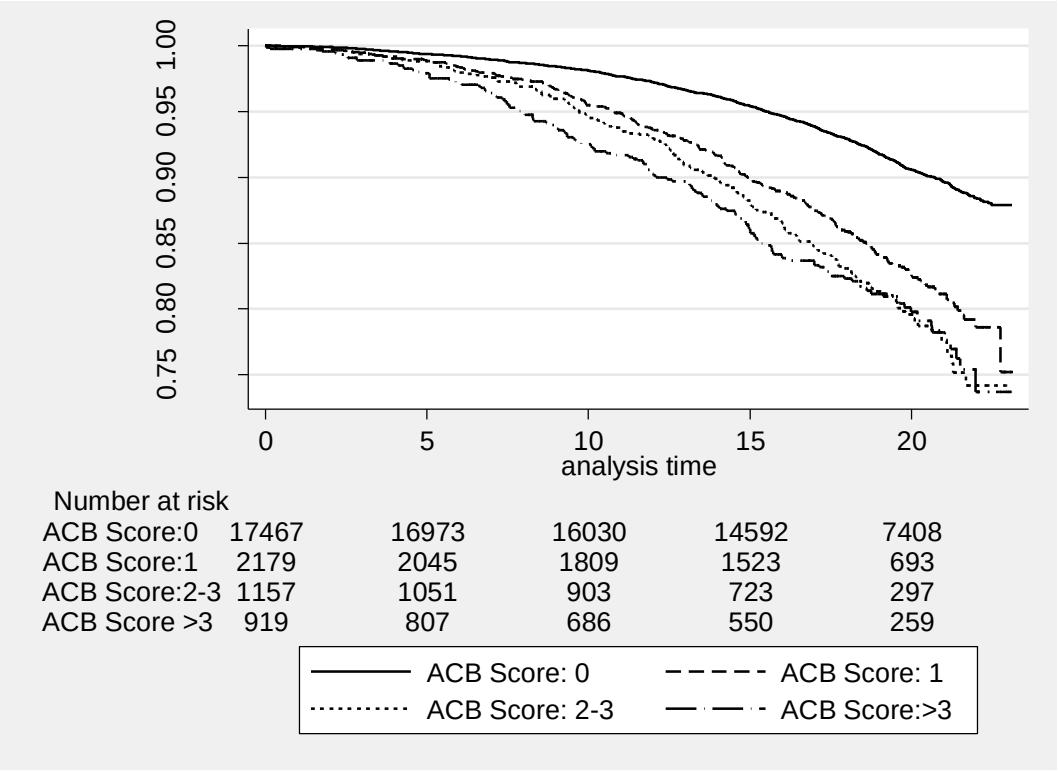
F	1.00	1.56 (1.21 – 2.02)	0.97 (0.65 – 1.45)	1.84 (1.31 – 2.59)
G	1.00	1.37 (0.59 – 3.15)	1.10 (0.33 – 3.64)	2.56 (1.05 – 6.25)
H	1.00	1.76 (1.15 – 2.71)	1.38 (0.75 – 2.54)	3.11 (1.92 – 5.04)
I	1.00	1.54 (1.23 – 1.93)	1.14 (0.81 – 1.59)	1.90 (1.40 – 2.57)

ACB = Anticholinergic burden score.
Model A: n/N=2,131/21,722 for incident stroke, n/N= 562/21,722 for stroke mortality analysis; adjusted for age and sex.
Model B: n/N=2,129/21,707 for incident stroke, n/N= 562/21,707 for stroke mortality analysis; Model A plus smoking, alcohol consumption, physical activity level, education level, occupational social class, systolic blood pressure, cholesterol level and body mass index.
Model C: n/N=2,123/21,685 for incident stroke, n/N= 561/21,685 for stroke mortality events analysis; Model B plus prevalent conditions asthma, COPD, diabetes, MI and cancer.
Model D: n/N=1,544/16,547 for incident stroke, n/N= 400/16,547 for stroke mortality analysis; as in Model B excluding people with prevalent asthma, COPD, diabetes, MI and cancer.
Model E: n/N=2,095/21,448 for incident stroke, n/N= 549/21,448 for stroke mortality events analysis; as in Model C excluding all events occurring within first two years of follow up.
Model F: n/N=1,704/18,500 for incident stroke, n/N= 454/18,500 for stroke mortality events; Model C plus aspirin use.
Model G: n/N=161/ 21,707 for incident stroke, n/N= 44 / 21,707 for stroke mortality events analysis; Model B truncated at 5 years follow-up
Model H: n/N= 524/ 21,707 for incident stroke, n/N= 148/ 21,707 for stroke mortality events analysis; Model B truncated at 10 years follow-up
Model I: Propensity adjusted: Based on propensity score estimated from factors in Model B.

Table 3: Hazard ratios and their corresponding 95%CI of incident stroke according to total anticholinergic burden score groups during follow up in EPIC-Norfolk subdivided into ischaemic and haemorrhagic stroke sub types adjusted for age, sex, smoking, alcohol consumption, physical activity level, education level, occupational social class, systolic blood pressure, cholesterol level, body mass index, asthma, COPD, diabetes, MI and cancer (model C).

Stroke subtype	**Incident Stroke (Total N=21,722)**			
	ACB score 0 group	**ACB score 1 group**	**ACB score 2-3 group**	**ACB score >3 group**
Ischaemic (total events = 956)	1.00	1.29 (1.07 – 1.57)	1.73 (1.38 – 2.17)	1.60 (1.23 – 2.07)
Haemorrhagic (total events = 374)	1.00	1.13 (0.82 – 1.56)	1.03 (0.66 – 1.60)	1.38 (0.88 – 2.14)

Figure 1: Kaplan-Meier curves for time to incident stroke for ACB score categories during follow up in EPIC-Norfolk



X-axis - Analysis time in years
Y-axis – Cumulative probability of not having incident stroke, which includes both fatal and non-fatal strokes.
Number at risk – number of participants at risk of incident stroke per time interval
ACB = Anticholinergic burden score

Appendix 1: Anticholinergic Cognitive Burden scoring of drugs

Score 1	Score 2	Score 3
Alimemazine	Amantadine	Amitriptyline
Alverine	Belladone alkaloids	Amoxapine
Alprazolam		Atropine
Atenolol	Cyclobenzaprine	Benztropine
Brompheniramine maleate	Cyproheptadine	Brompheniramine
Bupropion hydrochloride	Empracet	Carbinoxamine
Captopril	Loxapine	Chlorpheniramine
Chlorthalidone	Meperidine	Chlorpromazine
Cimetidine hydrochloride	Methotrimeprazine	Clemastine
Ranitidine	Molindone	Clomipramine
Clorazepate	Oxcarbazepine	Clozapine
Codeine	Pethidine hydrochloride	Darifenacin
Colchicine	Pimozide	Desipramine
Coumadin		Dicyclomine

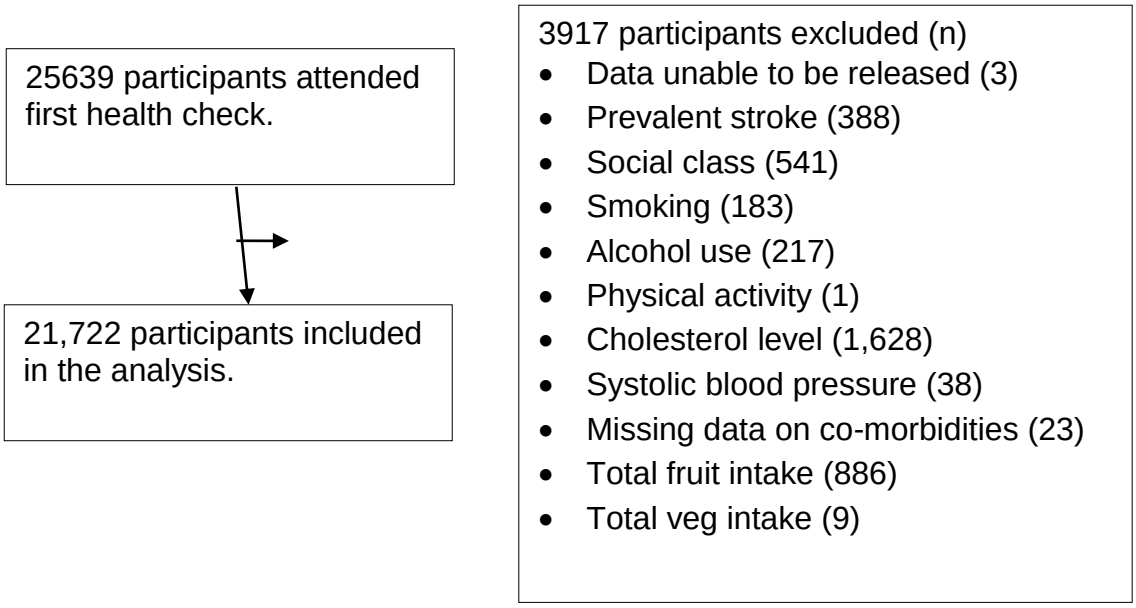
Score 1	Score 2	Score 3
Diazepam		Dimenhydrinate
Digoxin		Diphenhydramine
Dipyridamole		Doxepin
Disopyramide phosphate		Flavoxate
Fentanyl		Hydroxyzine
Furosemide		Hyoscyamine
Fluvoxamine		Imipramine
Haloperidol		Meclizine
Hydralazine		Nortriptyline
Hydrocortisone		Olanzapine
Isosorbide		Orphenadrine
Loperamide		Oxybutynin
Metoprolol		Paroxetine
Morphine		Perphenazine
Nifedipine		Procyclidine

Score 1	Score 2	Score 3
Prednisone		Promazine
Quinidine		Promethazine
Risperidone		Propentheline
Theophylline		Pyrilamine
Trazodone		Quetiapine
Triamterene		Scopolamine
		Thioridazine
		Tolterodine
		Trifluoperazine
		Trihexyphenidy
		Trimipramine

Appendix 2 Table showing numbers and proportions of participants with missing data

Variable	**Missing data**	**Proportion (%)**
Data unable to be released	3	0.012
Prevalent stroke	388	1.51
Social class	541	2.11
Smoking	183	0.71
Alcohol use	217	0.85
Physical activity	1	0.0039
Cholesterol level	1,628	6.35
Systolic blood pressure	38	0.15
Missing data on co-morbidities	23	0.09
Total fruit intake	886	3.46
Total veg intake	9	0.035

Appendix 3 Flow diagram of participants included in the study with reasons and numbers of those excluded



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Online only content

Measurement methods

Trained nurses examined individuals at clinic visit. Weight was measured with participants wearing light clothing without shoes. Height was measured up to the nearest 0·1 cm using a stadiometer with shoes removed. Body mass index (BMI) was calculated as weight (kilogram) divided by height in metres squared (m^2). Blood pressure (BP) was measured with an Accutorr monitor (Datascope, Huntingdon, UK) after the participant had been seated for 5 min. We used the mean of two BP measurements for analysis. Non-fasting venous blood samples were taken into plain and citrate bottles. We measured serum total cholesterol with the RA 1000 (Bayer Diagnostics, Basingstoke, UK).
At the baseline participants completed a detailed health and lifestyle questionnaire. Participant's educational status, occupational social class, and physical activity were obtained from the baseline health and lifestyle questionnaire. Educational status was recorded as no qualification, O- level, A-level, degree or higher qualification. Social class was classified according to the Registrar General's occupation-based classification scheme. A four-level physical activity index was derived from the validated EPIC short physical activity questionnaire designed to assess combined work and leisure activity. For stratified analyses, social class was re-categorised into manual (III-manual, IV and V) and non-manual (III-non-manual, II and I), educational attainment was re-categorised as low educational attainment (no or O level) and high educational attainment (at least A level) and physical activity was re-categorised as high (active and moderately active) and low (inactive and moderately inactive) physical activity categories.

Smoking status was categorised as current smoker, ex-smoker and those who have never-smoked. *"Current smokers"* were defined as those who answered *"yes"* to the question *"Do you smoke cigarettes now?"*. *"Never smokers"* were defined as those who answered *"no"* to the question *"Have you ever smoked as much as one cigarette a day for as long as a year?"* All others were classed as *"former smokers"*. Average alcohol consumption (units/week) was derived from a food frequency questionnaire (FFQ) completed at the baseline. Prevalent illnesses were determined by a positive response to the question "*Has a doctor ever told you that you have any of the following?"* followed by a list of options including asthma, COPD, cancer, stroke, heart attack, and diabetes.

Aspirin, steroid tablets or injections and diuretics use was ascertained by a question "Have you taken (aspirin, steroid tablets or injections and diuretics) continuously for three months or more?". Other medications were identified by participant's response to the question "In the last week have you taken any drugs or medicines either prescribed by your doctors or bought from the chemist? If YES, please name them." The medication name or brand, dose and frequency of administration were recorded and each medication was coded exactly as written in the baseline survey into a database.

Patient and public engagement in research project
We have engaged with general practitioners and participants throughout the study design, from first inception to final conduct of the study. We conduct regular advisory meetings 3-4 times a year with our advisory panel of participants. We seek advice from the panel on study design and conduct. Components such as questionnaire development and outcome measures were informed by participants' priorities, preferences and experiences. It is our policy to acknowledge the participants in every publication and dissemination of materials (posters and PowerPoint presentations). We regularly disseminate the research findings to participants through newsletters, participants' meetings and public engagement events. EPIC-Norfolk celebrated its 20th anniversary recently and participants received personalised information.

Data sharing statement
Patient level data, full dataset, technical appendix and statistical codes are available from the EPIC-Norfolk Management Committee upon request.

Transparency statement
I, Phyo Kyaw Myint, lead author (the manuscript's guarantor) of the manuscript affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

Contributorship
PKM conceived the study. DTG performed literature review, data analysis under supervision by ABC. ABC performed propensity score matched analyses. RNL is responsible for data linkage. NJW and KTK are PIs of EPIC-Norfolk Study. DTG, ABC and PKM drafted the manuscript and all authors contributed to the writing of the paper. PKM is the guarantor.

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