Higher anticholinergic burden from medications is associated with significant increase in markers of inflammation in the EPIC-Norfolk prospective population-based cohort study

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Abstract

Background: Higher anticholinergic burden from medications is associated with increased risk of cardiovascular disease and cognitive function decline. A mechanistic pathway has never been established. We aimed to determine whether chronic inflammation may mediate these associations. Methods: Participants were drawn from the European Prospective Investigation into Cancer, Norfolk cohort (40-79 years at baseline). The anticholinergic cognitive burden score (ACB) was calculated at baseline/first (1HC) (1993/97) and second (2HC) (1998/2000) health checks. Plasma fibrinogen and C-reactive protein (CRP) were measured during 1HC and Tumour Necrosis Factor alpha (TNF-α) and interleukin 6 (IL-6) during 2HC. Cross-sectional associations between ACB and inflammatory markers were examined for 1HC and 2HC, respectively. The prospective association was also examined between 1HC ACB and 2HC inflammatory markers. All models adjusted for age, sex, lifestyle factors, co-morbidities and medications. Results: 17,678 and 22,051 participants were included in cross-sectional analyses for CRP, and fibrinogen, respectively. A total of 5,101 participants with available data for TNF-α and IL-6 were included in the longitudinal analyses. Cross-sectionally, a point increase in the ACB was associated with a significant increase in all inflammatory markers (beta (standard error): fibrinogen – 0.035g/l (0.006), p<0.001; CRP 0.284mg/l (0.044), p<0.001; TNF-α 0.031pg/ml (0.010), p=0.002; and IL-6 0.112pg/ml (0.033), p=0.001. Longitudinally, a unit increase in the ACB was associated with a significant increase in TNF-α 0.028pg/ml (0.011), p=0.013 and IL-6 0.076 pg/ml (0.035), p=0.029. Conclusion: Higher anticholinergic burden was significantly associated with higher inflammatory markers. Inflammation may mediate the relationship between exposure to anticholinergic medications and adverse outcomes

INTRODUCTION

Anticholinergic medications block the effect of the neurotransmitter acetylcholine by inhibiting muscarinic receptors [1] and are increasingly being prescribed to older people for common conditions including asthma, urinary incontinence and dementia [2]. Anticholinergic effects are present in drugs that are extensively used, such as antiemetics, antihistamines, antihypertensives and tricyclic antidepressants [3]. If one or more of

such medications are taken, they can cause excessive anticholinergic effects due to their cumulative effects, a phenomenon also known as anticholinergic burden [4].

Older people are at a particularly higher risk of anticholinergic complications due to (a) increased risk of polypharmacy including medications with anticholinergic properties, (b) age-related reductions in central cholinergic pathways and (c) decreases renal and hepatic clearance of drugs [4]. It was suggested that 48% of the ageing population may take one or more anticholinergic medications [5]. Higher anticholinergic burden (ACB) scores are also associated with an increased risk of cardiovascular disease and mortality [6,7], dementia [8] and adverse effects in cognitive and physical function [9]. Nevertheless, potential mechanistic pathways between these associations have not been identified.

Inflammation plays an important role in the pathogenesis of cardiovascular disease [10,11,12], and dementia [13]. Raised inflammatory markers have also been linked to depression [14] and functional decline in older people [15]. The Vagus nerve may mediate inflammation through *'the inflammatory reflex'* [16]. Previous pre-clinical data suggest that central muscarinic-dependent vagal activation contributes to the downregulation of inflammatory responses [17].

Therefore, we hypothesised that increased anticholinergic burden leads to increased inflammation by blocking central muscarinic-dependent vagal activation. This may mediate the previously described relationships between anticholinergic burden and adverse outcomes. No previous investigations have assessed the relationship between antimuscarinic activity and inflammation in a population sample. In this study, we aimed to examine the cross-sectional and prospective relationships between anticholinergic burden from medications and important inflammatory markers (plasma fibrinogen, CRP, TNF-α and IL-6) in a large UK population-based study.

METHODS

Participants

The participants were drawn from 25,639 men and women who enrolled in the EPIC-Norfolk study at baseline (first health check) between 1993 and 1998. The study protocol has been previously described [18]. Study participants were followed up between 1998 and 2000 and 15,786 participants attended the second health check (2HC). The Norwich Research Ethics Committee approved the study. Figure 1 summarises the timeline of the EPIC-Norfolk study and the analyses undertaken.

Measurements

During both health checks, trained nurses measured weight, height, and body mass index (BMI) using standardized procedures. Participants also completed a health and lifestyle questionnaire that collected information on physical activity, smoking status, alcohol consumption, co-morbid conditions and medications. The Food Frequency Questionnaire (FFQ) was used to measure participants' habitual dietary pattern during the previous year. Non-fasting venous blood samples were also taken. From each individual, ˜40mls of blood was drawn and serum, plasma, erythrocytes and buffy coat were aliquoted in plastic straws of 0.5 ml each. These straws were then heat-sealed and stored under liquid nitrogen (-196°C) in a centralized biobank.

Exposure

Medications with anticholinergic properties were identified by searching the database for exact and similar entries for both generic and brand name drugs. A corresponding anticholinergic class/score was allocated to each medication: class 0 (none), class 1 (probable, score 1), classes 2 and 3 (definite, score 2 and 3, respectively). This was based on the criteria of the Anticholinergic Cognitive Burden (ACB) scale developed by Boustani et al. [19] since this is one of the most well-known scales and validated against many clinical outcomes of interest. Subsequently, the total anticholinergic burden was then calculated for each participant by adding the individual ACB scores of all their medications at baseline. Participants were divided into six groups according to their total ACB score at baseline: 0, 1, 2, 3, 4, [?]5 (due to small sample size for ACB score 6 and above) for meaningful analysis.

Outcome Measures

The inflammatory markers were the outcomes of interest: plasma fibrinogen, CRP, IL-6 and TNF-α. Fibrinogen (g/L) and CRP (mg/L) were available for a sub-sample of 1HC and IL-6 (pg/mL) and TNF-α (pg/mL) were available for a sub-sample in the 2HC. Non-fasting venous blood samples and 30ml of blood was drawn from each individual. Serum, plasma, erythrocytes and buffy coat were aliquoted in plastic straws of 0.5 ml each. These straws were then heat-sealed and stored under liquid nitrogen (-196°C) in a centralized biobank.

Statistical Analysis

Data were analysed using SPSS version 24.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics were presented for the overall sample and by ACB score groups. Differences between ACB groups were assessed using the Pearson's chi-squared test for categorical variables and analysis of variance (ANOVA) for normally distributed continuous variables or Kruskal-Wallis test for continuous variables that were not normally distributed.

Linear regression analysis was performed to determine the cross-sectional relationship of baseline/1HC ACB and 1HC inflammatory markers, fibrinogen and CRP. Linear regression was also performed to determine the cross-sectional association of 2HC ACB and 2HC inflammatory markers IL-6 and TNF-α. Longitudinal relationship between baseline/1HC ACB data and 2HC inflammatory markers IL-6 and TNF-α, was also carried out.

Four statistical models were constructed to assess the effects of potential confounding factors in a group sequential fashion. Model A was unadjusted, model B was adjusted for age and sex, model C was adjusted for age, sex and lifestyle factors (BMI, alcohol consumption, smoking status, total fruits and vegetables consumed and physical activity) and lastly, model D, the fully adjusted model was adjusted for the variables: age, sex, lifestyle factors, co-morbidities (diabetes, stroke, cancer and heart attack), medications (lipid lowering drugs, non-steroidal anti-inflammatory drugs (NSAIDs)) and total cholesterol.

RESULTS

There were 25,639 participants that attended the 1HC. A total of 3,588 participants were excluded from the fibrinogen analysis and 7,961 individuals were excluded from the CRP analysis. Therefore, a total of 22,051 and 17,678 participants were included in the fibrinogen and CRP analyses respectively. In the 2HC, out of the 15,786 participants who attended, only 5,101 men and women were included in the analysis for TNF-α and IL-6 after the exclusion of 10,685 participants with missing data for these variables. Figure 2 displays the participant population flowchart. All exclusions were made due to missing data. Online Resources 1 and 2 present the participants missing data from each variable in the first and second health check, respectively.

Descriptive Statistics

Tables 1 and 2 detail the sample characteristics at baseline/1HC by ACB group. Table 1 details the descriptive characteristics of included participants in the fibrinogen analysis. The mean (SD) age was 59.1 years (9.3) with 54% being female. The mean (SD) plasma fibrinogen was 2.9g/l (1.0). Table 2 details the descriptive statistics of included participants in the CRP analysis. The mean age (SD) was 59.1 years (9.1) for Table 2 with 55.1% being female. The median (IQR) CRP was 1.5mg/l (0.7-3.3).

There were significant differences between ACB groups for all variables considered except fruit and vegetable consumption. People in the higher ACB groups were older, had higher BMI, total cholesterol level and a lower level of physical activity. NSAIDs and lipid lowering drug usage was more prevalent in higher ACB groups. In terms of co-morbidities, high ACB score was associated with a greater percentage of individuals with a prior history of stroke, cancer, diabetes and myocardial infarction. Significantly, higher mean fibrinogen and median CRP were observed in higher ACB groups compared to ACB=0 group.

Table 3 details the characteristics of participants in the 2HC by the ACB groups. There were 5,101 participants of which 61.5% were women and the mean (SD) age was 63.1 years (8.9). The mean (SD) TNF-α was 2.0pg/ml (0.8) whilst the median (IQR) IL-6 was 0.6 (0.5-0.9). People in the higher ACB groups were

older, had higher BMI and were less likely to be active. There were no significant sex differences between ACB groups or in terms of fruit and vegetable consumption. There were higher proportions of NSAIDs and lipid lowering drugs usage in higher ACB groups. With regards to self-reported illnesses, high ACB was associated with a greater percentage of individuals with a prior history of stroke, diabetes and myocardial infarction. However, there was no significant difference between ACB groups in terms of cancer prevalence. There was a significant difference between ACB groups in terms of both TNF-α and IL-6. The levels of these inflammatory markers were higher in participants in higher ACB groups compared to ACB=0 group.

Cross-sectional analyses

Table 4 shows the results of linear regression analysis for the cross-sectional relationship between baseline ACB groups and inflammatory markers. All the adjusted models showed that a unit increase in the ACB score was associated with a significant increase in all the inflammatory markers. After complete multivariable adjustment (model 4), a unit increase in ACB score was associated with an increase of fibrinogen of 0.035g/l {(0.006) p <0.001}, CRP of 0.284mg/l {(0.044) p <0.001} (in 1HC), TNF-α of 0.031pg/ml {(0.010) p =0.002} and IL-6 of 0.112pg/ml {(0.033)p =0.001} (in 2HC), respectively.

Longitudinal analysis

Table 5 shows the results of the longitudinal analyses for prospective relationship between 1HC ACB and inflammatory markers measured at the 2HC. All models revealed a statistically significant association between ACB score at 1HC and increase in the circulating levels of inflammatory markers measured at 2HC. Upon full multivariable adjustment, a unit increase in the ACB score was associated with a significant increase in TNF-α and IL-6 of 0.028pg/ml {(0.011) p =0.013} and 0.076pg/ml {(0.035) p =0.029}, respectively.

DISCUSSION

We describe for the first time the association between anticholinergic burden and inflammation at a population level. Cross-sectionally, increases in ACB scores were independently associated with significant increases in fibrinogen, CRP, TNF-α and IL-6. Longitudinally, increases in ACB scores were independently associated with increases in TNF-α and IL-6. To the best of our knowledge, this is the first report which provides possible mechanistic link through inflammatory pathways between anticholinergic medications and adverse longer term outcomes at a population level.

Increases in such inflammatory markers in long term can lead to adverse outcomes. The meta-analysis by Kaptoge et al. [20] found a significant 17% increase (RR- 1.17 (1.09-1.25)) and 25% increase (RR- 1.25 (1.19-1.32)) in incident CHD/non-fatal MI associated with a 1-SD increase in TNF-α and IL-6 respectively. Our study suggests that 1- and 3-point ACB increases would translate into 0.646% and ˜2% relative risk increases in TNF-α- mediated incident CHD/non-fatal MI respectively [20]. Furthermore, our findings also suggest that 1- and 3-point ACB increases would translate into 1.13% and 3.39% relative risk increases in IL-6-mediated incident CHD/non-fatal MI, respectively [20]. Longitudinally, a 1-point increase in ACB would translate into a 0.595% and 0.75% increase in the relative risk of TNF-α-mediated incident CHD/non-fatal MI and IL-6-mediated incident CHD/non-fatal MI respectively [20].

The meta-analysis by Yano et al. [21] established that a 1-SD increase in fibrinogen was associated with a 30% increase (RR- 1.3 (1.2-1.4)) in all-cause mortality, a 20% increase (RR- 1.2 (1.1-1.4)) in cardiovascular disease and a 30% increase (RR-1.3 (1.2 to 1.5)) in cancer. Cross-sectionally, our study found a 1-point increase in ACB would translate into a 1.05% increase in the relative risk of all-cause mortality, a 0.7% increase in the relative risk of cardiovascular disease and a 1.05% increase in the relative risk of cancer [21]. In addition, Emerging Risk Factors Collaboration [22] also defined an increase in relative risk of CHD and ischaemic stroke associated with CRP. Our findings revealed that an increase in ACB would translate into an increase in the relative risk of CHD and ischaemic stroke.

The results of our study alongside previous investigations linking markers of chronic inflammation with a variety of adverse incident outcomes allow for the first time to quantify the contribution of inflammation in mediating the relationship between ACB and adverse outcomes. The *'inflammatory reflex'* may mediate

the described relationships between anticholinergic burden and adverse outcomes. We hypothesised that an increased anticholinergic burden may lead to raised inflammatory markers due to the 'inflammatory reflex' [16]. This mechanism also known as the cholinergic anti-inflammatory pathway suggests that the Vagus nerve, a part of the parasympathetic nervous system, has an anti-inflammatory role. Central vagus nerve stimulation downregulates inflammatory responses and thus we hypothesised that increased anticholinergic burden leads to increased inflammation by blocking central muscarinic-dependent vagal activation [17].

Based on literature, our findings can be extrapolated that a 3-point increase in ACB-related chronic inflammation can be linked to an increase in relative risk of up to ˜4% in cancer, cardiovascular disease and mortality. Furthermore, a previous study [7] including participants from the EPIC-Norfolk cohort found that people with a total ACB [?]3 from medications had a relative risk increase of mortality by 83% (HR- 1.83 (1.53-2.20)) and a 117% increase in incident CVD. The increased risk of up to 3.39% in incident CHD/non-fatal MI after a 3-point increase in ACB inferred from the study by Kaptoge et al. [20] would account for only 2.9% of the entire ACB[?]3-associated excess risk of incident CVD.

Indeed, there may be other plausible mechanisms other than chronic inflammation that may mediate the association between ACB from medications and adverse outcomes. Anticholinergic drugs have been found to suppress the parasympathetic control of heart rate, which is linked to an increased incidence of myocardial ischemia and tachyarrythmias that are known to increase the risk of embolic strokes and sudden cardiac death [23]. Anticholinergic medications can also lead to ischaemia due to their pro-ischaemic properties [24]. In addition, the arterial baroreflex is an important mechanism that protects against strokes [25]. The anticholinergic medications disrupt vagal nerve activity which is involved in the activation of arterial baroreflex therefore reducing its protective effects [25].

Our study has several strengths. The study benefited form using data from a large, prospective population-based study. We were able to delineate both the cross-sectional and longitudinal associations between ACB and markers of inflammation. The data were also prospectively collected, minimising recall bias. Additionally, a well-validated ACB score was used, and we were able to control for a variety of sociodemographic and lifestyle factors, comorbidities and relevant medications.

We acknowledge some limitations. As a volunteer study with long-term follow-up, a degree of healthy volunteer bias is possible. However, the baseline characteristics of the EPIC-Norfolk participants are similar to other UK representative population samples [18]. The participants in this study were almost completely (>99%) White British, however, it is unlikely biological mechanisms will be hugely different from other ethnicities. Potential confounders adjusted were measured at baseline, and it is possible that these may vary during the follow-up period. Although we were able to calculate the total ACB score, we were not able to identify particular drugs and the dosages that are associated to adverse outcomes. In addition, as the ACB score was calculated at baseline, we were unable to account for any changes in ACB score during the follow-up period for longitudinal analysis. Nevertheless, it is likely that individuals would either maintain similar anticholinergic exposure or be exposed to increasing anticholinergic burden during follow-up because the use of medications with anticholinergic properties would increase as participants age and accrue more disease burden and increasing polypharmacy. However, both cross-sectional and longitudinal analyses results for TNF-α and IL-6 were consistent.

This study along with future robust experimental studies may change the anticholinergic burden paradigm, by recognizing the chronic inflammatory state associated with anticholinergic medications as a therapeutic target in the era of targeted immune therapy for cardiovascular prevention. The landmark CANTOS trial used canakinumab to target the interleukin-1β innate immunity pathway, leading to a significantly lower incidence of recurrent cardiovascular events [26]. Furthermore, colchicine has been shown to be effective at preventing major adverse cardiac events after a myocardial infarction [27] and preventing cardiovascular events in patients with recent myocardial infarction [28] and stable coronary disease [29,30]. Future studies should also explore whether systematic attempts to reduce the anticholinergic burden reduces inflammation and consequently, the risk of such adverse outcomes.

In conclusion, using data from a large-scale prospective cohort study from UK, we determined the cross-sectional and longitudinal associations between the anticholinergic burden and inflammatory markers. Higher anticholinergic burden is significantly associated with higher inflammatory markers both cross-sectionally and longitudinally after multivariable adjustment. Furthermore, we underlined how chronic inflammation may be a previously unrecognized potential mechanism for the observed association between anticholinergic burden and adverse outcomes.

DECLARATIONS

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Conflict of Interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

Ethics Approval

Norwich Ethics Committee approved the study in view of the retrospective nature of the study.

Data Availability

Data available on request to EPIC-Norfolk Steering Committee for their approval.

Consent to participate and publish

Informed consent was obtained from all individual participants included in the study.

Authors' contributions

Ria Sanghavi, Tiberiu A Pana and Phyo K Myint conceived the study. Ria Sanghavi performed literature search, data analysis and writing the first draft of the paper. Ria Sanghavi, Tiberiu A Pana and Phyo K Myint verified data. Nicholas J Wareham and Kay-Tee Khaw are PIs of EPIC-Norfolk Cohort. All authors commented on previous versions of the manuscript and approved the final manuscript. Phyo K Myint is the guarantor.

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TABLES

Table 1 Baseline sample characteristics for 22,051 men and women in the EPIC-Norfolk cohort (first health check) according to the total anticholinergic burden score groups.

	All	**ACB score 0 group**	**ACB score 1 group**	**ACB score 2 group**	**ACB score 3 group**	**ACB score 4 group**	**ACB score [?]5 group**	*p* **value**
	n=22 051	n=17 609	n=2252	n=542	n=677	n=409	n=562	

Mean age (years) (SD)	59.1 (9.3)	58.2 (9.1)	62.4 (8.9)	65.5 (8.3)	62.0 (9.2)	65.0 (7.9)	62.7 (9.0)	<0.001
Sex (%)								<0.001
Men	10140 (46.0)	8065 (45.8)	1059 (47.0)	294 (54.2)	274 (40.5)	214 (52.3)	234 (41.6)	
Women	11911 (54.0)	9544 (54.2)	1193 (53.0)	248 (45.8)	403 (59.5)	195 (47.7)	328 (58.4)	
Mean BMI (kg/mˆ2) (SD)	26.3 (3.8)	26.1 (3.7)	27.0 (4.2)	27.0 (4.0)	27.0 (4.1)	27.2 (4.2)	27.2 (4.1)	<0.001
Smoking status (%)								<0.001
Current	2470 (11.2)	2012 (11.4)	215 (9.5)	54 (10.0)	72 (10.6)	34 (8.3)	83 (14.8)	
Former	9294 (42.1)	7186 (40.8)	1066 (47.3)	276 (50.9)	309 (45.6)	206 (50.4)	251 (44.7)	
Never	10287 (46.7)	8411 (47.8)	971 (43.1)	212 (39.1)	296 (43.7)	169 (41.3)	228 (40.6)	
Median alcohol consumption (units/week) (IQR)	3.5 (1.0-10.0)	4.0 (1.5-10.0)	2.5 (1.0-9.0)	2.5 (1.0-8.5)	2.5 (0.5-8.5)	2.5 (0.5-9.0)	2.0 (0.5-7.0)	<0.001
Physical activity (%)								<0.001
Inactive	6527 (29.6)	4745 (26.9)	857 (38.1)	251 (46.3)	248 (36.6)	188 (46.0)	238 (42.3)	
Moderately inactive	6351 (28.8)	5112 (29.0)	637 (28.3)	130 (24.0)	200 (29.5)	109 (26.7)	163 (29.0)	
Moderately active	5088 (23.1)	4262 (24.2)	445 (19.8)	84 (15.5)	136 (20.1)	52 (12.7)	109 (19.4)	
Active	4085 (18.5)	3490 (19.8)	313 (13.9)	77 (14.2)	93 (13.7)	60 (14.7)	52 (9.3)	
Mean total cholesterol (mmol/l) (SD)	6.2 (1.2)	6.1 (1.1)	6.3 (1.2)	6.4 (1.3)	6.3 (1.2)	6.4 (1.3)	6.4 (1.3)	<0.001

Mean total fruits and vegetables consumed (g/day) (SD)	511.3 (249.8)	509.8 (248.2)	518.8 (261.2)	504.7 (260.4)	524.0 (251.6)	514.3 (229.0)	517.6 (257.0)	0.16
NSAID (%)	3266 (14.8)	2136 (12.1)	514 (22.8)	167 (30.8)	182 (26.9)	117 (28.6)	150 (26.7)	<0.001
Lipid lowering drugs (%)	329 (1.5)	174 (1.0)	65 (2.9)	23 (4.2)	22 (3.2)	23 (5.6)	22 (3.9)	<0.001
Self-reported co-morbidities(%)								
Diabetes	489 (2.2)	290 (1.6)	77 (3.4)	38 (7.0)	26 (3.8)	23 (5.6)	35 (6.2)	<0.001
Stroke	303 (1.4)	143 (0.8)	63 (2.8)	40 (7.4)	18 (2.7)	13 (3.2)	26 (4.6)	<0.001
Cancer	1185 (5.4)	883 (5.0)	136 (6.0)	42 (7.7)	57 (8.4)	31 (7.6)	36 (6.4)	<0.001
Heart attack	693 (3.1)	262 (1.5)	162 (7.2)	93 (17.2)	72 (10.6)	47 (11.5)	57 (10.1)	<0.001
Mean plasma fibrinogen (g/l) (SD)	2.9 (1.0)	2.9 (1.0)	3.1 (0.9)	3.2 (0.9)	3.1 (0.9)	3.1 (0.9)	3.2 (0.9)	<0.001

Note: Values presented are mean (SD) for normally distributed, continuous data, median (IQR) for non-normally distributed, continuous data and number (%) for categorical data. Total ACB was calculated with the formula of ((number of class 1 anticholinergics) + (number of class 2 anticholinergics × 2) + (number of class 3 anticholinergics × 3)). Abbreviations: ACB, anticholinergic cognitive burden; BMI, body mass index; NSAID, non-steroidal anti-inflammatory drugs.

Table 2 Baseline sample characteristics for 17,678 men and women in the EPIC-Norfolk cohort (first health check) according to the total anticholinergic burden score groups.

	All	**ACB score 0 group**	**ACB score 1 group**	**ACB score 2 group**	**ACB score 3 group**	**ACB score 4 group**	**ACB score [?]5 group**	p **value**
	N= 17678	N=14161	N=1758	N=422	N=560	N=332	N=445	
Mean age (years) (SD)	59.1 (9.1)	58.2 (9.0)	62.4 (8.8)	65.4 (8.2)	62.1 (9.1)	64.8 (8.2)	62.5 (9.0)	<0.001
Sex (%)								<0.001
Men	7942 (44.9)	6343 (44.8)	805 (45.8)	232 (55.0)	226 (40.4)	168 (50.6)	168 (37.8)	

Women	9736 (55.1)	7818 (55.2)	953 (54.2)	190 (45.0)	334 (59.6)	164 (49.4)	277 (62.2)	
Mean BMI (kg/m^2) (SD)	26.2 (3.8)	26.1 (3.7)	26.8 (4.1)	27.0 (4.0)	26.6 (4.1)	27.0 (3.9)	27.2 (4.1)	<0.001
Smoking status (%)								<0.001
Current	1959 (11.1)	1590 (11.2)	168 (9.6)	44 (10.4)	65 (11.6)	23 (6.9)	69 (15.5)	
Former	7368 (41.7)	5704 (40.3)	826 (47.0)	218 (51.7)	253 (45.2)	166 (50.0)	201 (45.2)	
Never	8351 (47.2)	6867 (48.5)	764 (43.5)	160 (37.9)	242 (43.2)	143 (43.1)	175 (39.3)	
Median alcohol consumption (units/week) (IQR)	3.5 (1.0-10.0)	4.0 (1.0-10.0)	2.5 (1.0-9.0)	2.5 (1.0-9.0)	2.5 (0.5-8.5)	2.5 (1.0-9.5)	2.0 (0.5-6.8)	<0.001
Physical activity (%)								<0.001
Inactive	5216 (29.5)	3791 (26.8)	684 (38.9)	196 (46.4)	207 (37.0)	149 (44.9)	189 (42.5)	
Moderately inactive	5096 (28.8)	4111 (29.0)	489 (27.8)	108 (25.6)	172 (30.7)	89 (26.8)	127 (28.5)	
Moderately active	4053 (22.9)	3410 (24.1)	344 (19.6)	61 (14.5)	108 (19.3)	42 (12.7)	88 (19.8)	
Active	3313 (18.7)	2849 (20.1)	241 (13.7)	57 (13.5)	73 (13.0)	52 (15.7)	41 (9.2)	
Mean total cholesterol (mmol/l) (SD)	6.2 (1.2)	6.1 (1.1)	6.3 (1.2)	6.3 (1.3)	6.3 (1.2)	6.4 (1.3)	6.4 (1.3)	<0.001
Mean total fruits and vegetables consumed (g/day) (SD)	511.2 (248.0)	509.5 (246.1)	521.3 (262.8)	503.0 (250.0)	518.4 (247.1)	512.4 (221.5)	522.5 (264.4)	0.17
NSAID (%)	2592 (14.7)	1700 (12.0)	400 (22.8)	129 (30.6)	153 (27.3)	91 (27.4)	119 (26.7)	<0.001

Lipid lowering drugs (%)	256 (1.4)	135 (1.0)	49 (2.8)	18 (4.3)	17 (3.0)	18 (5.4)	19 (4.3)	<0.001
Self-reported co-morbidities (%)								
Diabetes	384 (2.2)	230 (1.6)	63 (3.6)	28 (6.6)	23 (4.1)	18 (5.4)	22 (4.9)	<0.001
Stroke	255 (1.4)	125 (0.9)	56 (3.2)	30 (7.1)	13 (2.3)	12 (3.6)	19 (4.3)	<0.001
Cancer	923 (5.2)	686 (4.8)	107 (6.1)	29 (6.9)	47 (8.4)	24 (7.2)	30 (6.7)	<0.001
Heart attack	583 (3.3)	225 (1.6)	131 (7.5)	71 (16.8)	65 (11.6)	41 (12.3)	50 (11.2)	<0.001
Median CRP (mg/l) (IQR)	1.5 (0.7-3.3)	1.4 (0.7-3.0)	2.1 (1.0-4.4)	2.4 (1.2-4.7)	2.0 (1.0-4.6)	2.3 (1.2-4.7)	2.5 (1.2-5.4)	<0.001

Note: Values presented are mean (SD) for normally distributed, continuous data, median (IQR) for non-normally distributed, continuous data and number (%) for categorical data. Total ACB was calculated with the formula of ((number of class 1 anticholinergics) + (number of class 2 anticholinergics × 2) + (number of class 3 anticholinergics × 3)). Abbreviations: ACB, anticholinergic cognitive burden; BMI, body mass index; NSAID, non-steroidal anti-inflammatory drugs; CRP, C-reactive protein.

Table 3 Characteristics from 2nd health-check for 5101 men and women in the EPIC-Norfolk cohort according to the total anticholinergic burden score groups.

	All	**ACB score 0 group**	**ACB score 1 group**	**ACB score 2 group**	**ACB score 3 group**	**ACB score 4 group**	**ACB score [?]5 group**	*p* **value**
	N=5101	N=3969	N=608	N=133	N=157	N=79	N=155	
Mean age (years) (SD)	63.1(8.9)	62.2(8.8)	65.8(8.5)	68.6(8.5)	65.6(8.3)	68.7(8.3)	65.6(8.4)	<0.001
Sex (%)								0.135
Men	1963(38.5)	1502(37.8)	247(40.6)	61(45.9)	57(36.3)	38(48.1)	58(37.4)	
Women	3138(61.5)	2467(62.2)	361(59.4)	72(54.1)	100(63.7)	41(51.9)	97(62.6)	
Mean BMI (kg/mˆ2) (SD)	26.6(3.8)	26.4(3.8)	27.2(4.1)	27.5(4.3)	27.1(4.0)	27.4(4.2)	27.8(3.8)	<0.001
Smoking status (%)								0.002
Current	351(6.9)	276(7.0)	36(5.9)	6(4.5)	12(7.6)	2(2.5)	19(12.3)	
Former	2165(42.4)	1633(41.1)	281(46.2)	58(43.6)	81(51.6)	40(50.6)	72(46.5)	

Never	2585(50.7)	2060(51.9)	291(47.9)	69(51.9)	64(40.8)	37(46.8)	64(41.3)	
Median alcohol consumption (units/week) (IQR)	3.0(1.0-9.0)	3.0(1.0-9.0)	2.5(1.0-9.0)	3.0(0.5-8.5)	3.0(1.0-8.5)	3.0(1.0-9.0)	2.0(0.5-9.0)	0.046
Physical activity (%)								<0.001
Inactive	378(7.4)	260(6.6)	49(8.1)	21(15.8)	18(11.5)	7(8.9)	23(14.8)	
Moderately inactive	1867(1238)	1412(35.6)	247(40.6)	48(36.1)	70(44.6)	27(34.2)	63(40.6)	
Moderately active	1238(24.3)	979(24.7)	142(23.4)	29(21.8)	35(22.3)	20(25.3)	33(21.3)	
Active	1618(31.7)	1318(33.2)	170(28.0)	35(26.3)	34(21.7)	25(31.6)	36(23.2)	
Mean total cholesterol (mmol/l) (SD)	6.1(1.2)	6.1(1.2)	6.1(1.1)	6.0(1.3)	6.1(1.2)	6.1(1.0)	6.4(1.3)	0.008
Mean total fruits and vegetables consumed (g/day) (SD)	538.6(254.4)	540.0(257.5)	533.6(235.4)	501.5(205.2)	534.9(270.0)	592.7(248.2)	531.9(270.6)	0.895
NSAID (%)	1100(21.6)	690(17.4)	199(32.7)	61(45.9)	60(38.2)	32(40.5)	58(37.4)	<0.001
Lipid lowering drugs (%)	275(5.4)	141(3.6)	66(10.9)	23(17.3)	20(12.7)	10(12.7)	15(9.7)	<0.001
Self-reported co-morbidities(%)								
Diabetes	161(3.2)	102(2.6)	22(3.6)	10(7.5)	10(6.4)	10(12.7)	7(4.5)	<0.001
Stroke	135(2.6)	57(1.4)	31(5.1)	15(11.3)	16(10.2)	5(6.3)	11(7.1)	<0.001
Cancer	371(7.3)	276(7.0)	48(7.9)	14(10.5)	12(7.6)	7(8.9)	14(9.0)	0.539
Heart attack	157(3.1)	46(1.2)	52(8.6)	19(14.3)	11(7.0)	11(13.9)	18(11.6)	<0.001

Μεαν TNΦ-α (πγ/μλ) (ΣΔ)	2.0(0.8)	2.0(0.8)	2.1(0.9)	2.3(1.0)	2.1(0.7)	2.1(0.7)	2.3(1.1)	<0.001
Median IL-6 (pg/ml) (IQR)	0.6(0.5-0.9)	0.6(0.5-0.9)	0.7(0.5-1.0)	0.8(0.5-1.1)	0.7(0.5-1.0)	0.8(0.6-1.1)	0.8(0.56-1.2)	<0.001

Note: Values presented are mean (SD) for normally distributed, continuous data, median (IQR) for non-normally distributed, continuous data and number (%) for categorical data. Total ACB was calculated with the formula of ((number of class 1 anticholinergics) + (number of class 2 anticholinergics × 2) + (number of class 3 anticholinergics × 3)). Abbreviations: ACB, anticholinergic cognitive burden; BMI, body mass index; NSAID, non-steroidal anti-inflammatory drugs; TNF-α, tumour necrosis factor alpha; IL-6, interleukin 6.

Table 4 Cross sectional relationship between inflammatory markers and ACB score.

INFLAMMATORY MARKER	MODEL	β VALUE for 1 point increase in inflammatory marker	β VALUE for 1-SD increase in inflammatory marker	95% CI INTERVAL	Standard Error (SE)	*p* VALUE
Fibrinogen (g/l) (1HC)	A	0.074	0.074	0.063,0.086	0.006	<0.001
	B	0.046	0.046	0.035,0.058	0.006	<0.001
	C	0.037	0.037	0.025,0.049	0.006	<0.001
	D	0.035	0.035	0.023,0.047	0.006	<0.001
CRP (mg/l)(1HC)	A	0.465	0.465	0.381,0.549	0.043	<0.001
	B	0.372	0.372	0.287,0.456	0.043	<0.001
	C	0.304	0.304	0.220,0.389	0.043	<0.001
	D	0.284	0.284	0.198,0.369	0.044	<0.001
IL-6 (pg/ml)(2HC)	A	0.129	0.052	0.068,0.190	0.031	<0.001
	B	0.113	0.045	0.051,0.174	0.031	<0.001
	C	0.107	0.043	0.045,0.169	0.032	0.001
	D	0.112	0.045	0.048,0.175	0.033	0.001
TNF-α (pg/ml)(2HC)	A	0.065	0.081	0.045,0.084	0.010	<0.001
	B	0.042	0.053	0.023,0.061	0.010	<0.001
	C	0.037	0.046	0.018,0.056	0.010	<0.001
	D	0.031	0.039	0.011,0.050	0.010	0.002

Note: This is the linear regression analysis of inflammatory markers and ACB score. Inflammatory markers fibrinogen and CRP were collected in the first health check and TNF-α and IL-6 were collected in the second health check. To demonstrate a cross-sectional relationship, the exposure for fibrinogen and CRP was baseline data from the first health check whilst for IL-6 and TNF-α, the exposure was participants' data from the second health check. The following models adjust for the ACB groups and inflammatory marker: A= univariate model, B= age and sex, C= age, sex and lifestyle factors (BMI, alcohol consumption

smoking status, total fruits and vegetables consumed and physical activity), D=age, sex, lifestyle factors, total cholesterol, medications (lipid lowering drugs, NSAIDs) and self-reported co-morbidities (stroke, cancer, heart attack and diabetes). Abbreviations: 1HC, first health check; 2HC, second health check; SD, standard deviation; CI, confidence interval.

Table 5 Longitudinal relationship of inflammatory markers IL-6 and TNF-α for 5,101 participants.

INFLAMMATORY MARKER	MODEL	β VALUE for 1 point increase in inflammatory marker	β VALUE for 1-SD increase in inflammatory marker	95% CI INTERVAL	Standard Error (SE)	*p* VALUE
IL-6 (pg/ml) (2HC)	A	0.098	0.039	0.030,0.166	0.035	0.005
	B	0.078	0.031	0.009,0.146	0.035	0.026
	C	0.079	0.032	0.013,0.145	0.034	0.020
	D	0.076	0.030	0.008,0.144	0.035	0.029
TNF-α (pg/ml) (2HC)	A	0.064	0.080	0.043,0.085	0.011	<0.001
	B	0.040	0.050	0.019,0.061	0.011	<0.001
	C	0.036	0.045	0.015,0.058	0.011	0.001
	D	0.028	0.035	0.006,0.050	0.011	0.013

Note: This is the linear regression analysis for inflammatory markers and ACB score. The exposure was baseline data from the first health check to demonstrate a longitudinal relationship because TNF-α and IL-6 were collected in the second health check.

The following models adjust for the ACB groups and inflammatory marker: A= univariate model, B= age and sex, C= age, sex and lifestyle factors (BMI, alcohol consumption, smoking status, total fruits and vegetables consumed and physical activity),D=age, sex, lifestyle factors, total cholesterol, medications (lipid lowering drugs, NSAIDs), co-morbidities (stroke, cancer, heart attack and diabetes) . Abbreviations: 1HC, first health check; 2HC, second health check; SD, standard deviation; CI, confidence interval.

FIGURES

Fig.1 The EPIC-Norfolk cohort timeline and the analyses undertaken.

Fig.2 The participant population flowchart.