BMJ Open

NSAIDs, statins and PPIs and the risk of oesophageal adenocarcinoma among patients with Barrett's oesophagus, a population based case-control study

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2014-006640
Article Type:	Research
Date Submitted by the Author:	15-Sep-2014
Complete List of Authors:	Masclee, Gwen; Erasmus University Medical Center, Medical Informatics; Gastroenterology and Hepatology Coloma, Preciosa; Erasmus MC University Medical Center, Medical Informatics Spaander, Manon; Erasmus MC University Medical Center, Gastroenterology &Hepatology Kuipers, Ernst; Erasmus MC, Gastroenterology and Hepatology Sturkenboom, Miriam; Erasmus University Medical Center, Medical Informatics; Erasmus MC University Medical Center, Epidemiology
Primary Subject Heading:	Gastroenterology and hepatology
Secondary Subject Heading:	Epidemiology, Gastroenterology and hepatology
Keywords:	EPIDEMIOLOGY, Gastrointestinal tumours < GASTROENTEROLOGY, Oesophageal disease < GASTROENTEROLOGY

NSAIDs, statins and PPIs and the risk of oesophageal adenocarcinoma among patients with Barrett's oesophagus, a population based case-control study

Short title: Risk of oesophageal adenocarcinoma

Gwen MC Masclee, Department of Medical Informatics; Department of Gastroenterology and Hepatology, Erasmus University Medical Center, Rotterdam, The Netherlands. g.masclee@erasmusmc.nl.

Preciosa M Coloma, Department of Medical Informatics, Erasmus University Medical Center, Rotterdam, The Netherlands. P.coloma@erasmusmc.nl.

Manon CW Spaander, Department of Gastroenterology and Hepatology, Erasmus University Medical Center, Rotterdam, The Netherlands. V.spaander@erasmusmc.nl.

Ernst J Kuipers, Department of Gastroenterology and Hepatology, Erasmus University Medical Center, Rotterdam, The Netherlands. E.j.kuipers@erasmusmc.nl.

Miriam CJM Sturkenboom, Department of Medical Informatics; Department of Epidemiology, Erasmus University Medical Center, Rotterdam, The Netherlands. M.sturkenboom@erasmusmc.nl.

Correspondence to:

Gwen MC Masclee, M.D.

Dept. of Medical Informatics

Erasmus University Medical Center

PO Box 2040

3000 CA Rotterdam

The Netherlands

Phone: +31 10 7044116

Fax: +31 10 7044722

E-mail: g.masclee@erasmusmc.nl

Key words: Barrett's oesophagus, oesophageal adenocarcinoma, non-steroidal anti-inflammatory drugs, statins, proton pump inhibitors

Word count (excl. references): 4112

No. of tables: 5.

No. of figures: 1.

No. of Appendices: 1.

Abbreviations

ATC	Anatomical Therapeutic Chemical
BMI	Body Mass Index
BO	Barrett's oesophagus
DDD	Defined Daily Dose
GP	General Practitioner
HGD	High-grade Dysplasia
ICPC	International Classification for Primary Care
IPCI	Integrated Primary Care Information database
IQR	Interquartile Range
NL	The Netherlands
NSAIDs	Non steroidal anti-inflammatory drugs
OAC	Oesophageal Adenocarcinoma
OR	Odds Ratios
PPIs	Proton Pump Inhibitors
THIN	The Health Improvement Network
UK	United Kingdom

ABSTRACT

Objectives: Non-steroidal anti-inflammatory drugs (NSAIDs), proton pump inhibitors (PPIs) and statins may decrease the risk of oesophageal adenocarcinoma (OAC) among Barrett's oesophagus (BO) patients. However, previous studies did not adequately address bias and confounding. Objective was to estimate the risk of OAC among BO patients exposed to NSAIDs, statins and PPIs.

Design: Case-control study nested within a BO cohort.

Setting: Two primary care databases (United Kingdom, Netherlands).

Participants: Cases were adults≥18 years with OAC or HGD diagnosis ≥1 year after BO diagnosis. Controls were matched on age, sex, year of BO diagnosis, and database.

Exposure: Drug use was assessed from BO diagnosis until matching date.

Outcome measure: Adjusted odds ratios (ORa) with 95% CI were calculated by conditional logistic regression.

Results: Within the BO cohort (n=15,134), 45 OAC (UK:40, NL:5) and 12 HGD cases (NL:12) were identified. ORa for OAC during NSAID use was 1.2 (95%CI:0.6-2.5) and during statin use for 2-3 years was 0.7 (95%CI:0.4-1.5) and >3years 0.5 (95%CI:0.1-1.7). When including HGD cases (n=57), ORa for NSAID use was 0.9 (95%CI:0.5-1.8). Statin use for 2-3 years showed ORa of 1.1 (95%CI:0.2-4.9) and >3 years 0.5 (95%CI:0.1-1.7). Statin dose was inversely associated with OAC and HGD. PPIs did not significantly decrease the risk of OAC and HGD.

Conclusion: Statins may decrease the risk of HGD and OAC up to 50% among BO patients, though we did not reach significance. These findings indicate that for an unselected group of BO patients chemoprevention by use of drugs to reduce progression to HGD and OAC should not be considered.

Article summary

Strengths:

- Within a population-based cohort of incident Barrett's oesophagus patients derived from two European countries and applying a common study protocol and drug exposure definition the risk of development of oesophageal adenocarcinoma was estimated during use of several drugs individually and concomitantly.
- We were able to minimize certain biases, for instance due to availability of drug prescription data recall bias was avoided and by using a population-based approach selection bias was minimized.

Limitations:

- The small number of oesophageal adenocarcinoma cases that was identified limited the power for the duration analyses.
- We did not have detailed pathology information on the Barrett segment length or grade of dysplasia at cohort entry.

INTRODUCTION

Barrett's oesophagus (BO) is a pre-malignant condition in which the squamous epithelium of the oesophagus is replaced by metaplastic columnar epithelium.[1] BO is considered a consequence of prolonged gastrooesophageal reflux [2] and is the most important risk factor for development of oesophageal adenocarcinoma (OAC) via a stepwise pathway of low- and high-grade dysplasia. It is estimated that the risk of OAC is increased by approximately 30-125 fold in persons with BO [3], and occurs in a small proportion of BO patients yearly.[4] Endoscopic surveillance for BO is therefore recommended.[2]

In recent decades, the incidence of BO increased, which was accompanied by a marked increase in OAC incidence in the USA and Western Europe.[5-6] However, estimates of OAC incidence among patients with BO vary substantially.[7-10] Generally, gastrointestinal cancers account for 25% of all cancers and approximately 4.9% of all deaths worldwide.[11] Death rates of most cancers decreased in recent years in contrast to the 3% increase in death rates of oesophageal cancer among males.[11] The age-standardized mortality rate for oesophageal cancer is 5.1 per 100,000 persons.[6] The need for effective prevention of oesophageal cancer is therefore warranted, particularly given the low 5-year survival rate of 13%-17%.[12]

Several studies reported that use of non-steroidal anti-inflammatory drugs (NSAIDs), statins and proton pump inhibitors (PPIs) may decrease the risk of OAC among BO patients.[13-20] However, these studies were based on small, selected samples of OAC cases. PPIs are considered standard care for symptom relief in patients with BO, thus it was suggested that PPIs may decrease the risk of progression to HGD or OAC.[20] Contrasting, other studies showed an increase in risk of OAC with PPI use, probably because the underlying treatment indication may be a risk factor for OAC rather than that PPIs are harmful for OAC among BO patients.[15 21] Nevertheless, one cannot directly assume that PPIs, which are

efficacious for treatment of erosive oesophagitis, will also be beneficial in the pathway from BO to OAC development. A meta-analysis including nine observational studies showed that the risk of oesophageal cancer among those who frequently use NSAIDs or aspirin was significantly lower compared to never users.[14] However, studies included in the meta-analysis did not specifically include patients with BO. A pooled analysis on individual patient data confirmed the significant reduction in risk of OAC in BO patients with NSAID prescriptions.[22] Two case-control studies observed an association between use of NSAIDs[15] and statins[15 23] and the risk of OAC among BO patients. Generalization and extrapolation of results from the latter studies to the general population is, however, difficult as both studies were performed in US veterans.[15 23] Additionally there was no adjustment for important risk factors of OAC progression such as alcohol use and smoking.[15]

Causality of an apparent association is generally supported by a dose- and duration-relationship.[24] However, studies to date neither reported a clear exposure definition free of recall bias [13 16 22] nor conducted dose-duration analyses. Finally, concerns have been raised about publication bias of these studies on chemoprevention of OAC in BO patients.[18]

Thus, to which extent NSAIDs, statins and PPIs may reduce the risk of oesophageal adenocarcinoma among BO patients in clinical practice remains unknown. Therefore, we conducted a matched case-control study to evaluate the risk of oesophageal adenocarcinoma among patients with BO associated with use of NSAIDs, statins and PPIs.

MATERIALS AND METHODS

Data sources

Two European population-based general practice registries served as data sources: 1) The Health Improvement Network (THIN) from the United Kingdom (UK, 1996-2011)[25] and the 2) Integrated Primary Care Information database (IPCI) from the Netherlands (1996–2012).[26] Both databases contain prospectively collected data that represents real-life practice. In the UK and in NL, all citizens are registered with a general practitioner (GP), who acts as a gatekeeper to secondary and tertiary medical care. THIN collects anonymised data on more than 3 million active patients from over 400 participating general practices, IPCI contains over 1.5 million active patients from 340 practices. For each individual patient all relevant medical information from primary and secondary care, as well as additional information, including demographics and drug prescriptions, is documented in the medical record. Both data sources comply with European Union guidelines on the use of medical data for research.

THIN employs the READ clinical terminology system for coding medical diagnosis and symptoms[27], whereas IPCI uses the International Classification for Primary Care (ICPC).[28] Information on drug prescriptions is captured in THIN with the Multilex product dictionary and British National Formulary (BNF) codes, whereas in IPCI information on drug prescriptions is coded according to the World Health Organization's Anatomical Therapeutic Chemical (ATC) classification.[29] The Scientific and Ethical Advisory Boards of both databases approved the study. Identification of the source and study population has been described previously.[10]

Source population

The source population consisted of all subjects aged ≥18 years who contributed data to the database between 1st of January 1996 and 31st of December 2011 (THIN) or March 2013 (IPCI). At least one year of available data prior to study entry was required to assess patient's medical history for exclusion criteria and risk factors. Follow-up started on 1 January 1996, date of reaching 18 years of age, or the date that one year of valid data was accrued within the database, whichever came later. Follow-up ended on the date of occurrence of study outcome (OAC), date of transfer out of the general practitioner's practice, death, or last data drawn, whichever was earliest.

Definition of Barrett's oesophagus

Patients with BO were identified using diagnosis codes; in THIN using corresponding READ codes (**Appendix Table 1**).[27] In IPCI, each potential BO case was manually validated to confirm the histological diagnosis of BO and the date of first diagnosis or mentioning of BO in the clinical record. Patients were excluded if they had a history of oesophageal cancer anytime before BO diagnosis and if they had a history of gastric cancer within 6 months after BO diagnosis.

Definition of oesophageal adenocarcinoma

In THIN, OAC cases were identified by READ codes (**Appendix Table** 1). In IPCI, all patients with a record of ICPC codes D77.1 (malignant neoplasia of the oesophagus) and D77.0 (malignant neoplasia of the digestive tract—not specified), or with a record by free text search including word combinations of 'oesophagus' 'cancer', 'carcinoma', 'malignancy' or 'neoplasia' were identified. Similar to BO, all

potential cases were manually validated for confirmation of the OAC diagnosis, date of first diagnosis and the type of carcinoma (squamous cell-, adeno-, or other types of carcinoma). Early cancer (high-grade dysplasia (HGD)) was identified in IPCI also, but could not be assessed in THIN.

Cases and controls selection

Two nested case-control studies were conducted; one including only OAC cases and a second case-control study including HGD cases from IPCI as well.

Cases were adults diagnosed with OAC ≥12 months after BO diagnosis, because cases occurring within one year of BO diagnosis were considered to be existent and related to BO diagnostic work-up (e.g. missed OAC at BO diagnosis). Index date was defined as date of first reporting of OAC diagnosis during follow-up. Controls were members of the incident BO cohort who did not develop OAC up to matching date. Controls were matched by incidence density sampling on age (± 5 years), sex, year of BO diagnosis (± 1 year), and database. We matched on year of BO diagnosis in order to account for any influence of guideline changes in endoscopic surveillance over calendar time.

Drug exposure

Drug exposures of interest were assessed in terms of outpatient prescriptions for NSAIDs (including high-dose aspirin, i.e. >325 mg/day), PPIs and statins from BO diagnosis until OAC diagnosis. In order to compare the OR of NSAIDs, PPIs and statins to other drugs, we considered another group of medications that served as control. Antidepressants (selective serotonin re-uptake inhibitors (SSRIs)) are currently not known to be either positively or negatively associated with OAC.

Duration of prescriptions was calculated based on the prescribed quantity and dosing regimen. As the most likely preventive effect of drugs on cancer progression is through a cumulative mechanism, we calculated all duration and defined daily dose (DDD) values from date of BO diagnosis until index date. Duration was classified according to never use (reference category), cumulative use of less than 1 month, between 1-12 months, > 12 months (or if applicable 1-2 years; 2-3 years and > 2 years). Considering that PPIs are indicated as treatment for BO patients, duration was classified as 0-6 months (reference category), 6-12 months, 1-2 years and > 2 years. Dose of exposure was classified using the ratio of prescribed daily dose compared to DDD using quartiles into categories (<0.8; 0.8-1.2; ≥1.2 DDD per day).

Potential confounders

We considered as potential confounders: concurrent diagnosis of oesophagitis or gastritis within 1 year before BO diagnosis; hiatal hernia; smoking habits (non-smoker, ex-smoker, current smoker) and alcohol abuse (never, current, past).

Statistical analyses

Baseline characteristics of cases and controls were described per database and compared using univariate conditional logistic regression.

To estimate the risk of HGD and OAC among patients with BO, matched and adjusted odds ratios (OR) with 95% confidence intervals (CIs) were calculated using conditional logistic regression for both databases separately and as a pooled analysis on patient-level pooled data.

Potential confounders were included in the adjusted analysis (ORa) if they resulted in a change of more than 10% of the initial estimate. Time since BO diagnosis was forced into the adjusted model.

Subsequent analyses included duration- and dose-analyses. The risk of OAC and HGD-OAC was also assessed for concomitant use of NSAIDs, statins and/or PPIs. Use of PPIs only was considered as reference category considering that PPIs are standard therapy for BO.

All analyses were performed using SAS Cary, NC version 9.2.

RESULTS

Study population

From the source population of 7,570,765 subjects in UK and 1,496,276 subjects in NL we identified 13,696 and 1,438 incident BO cases, respectively. Males accounted for 63% (UK) and 62% (NL) of BO subjects. Mean age at BO diagnosis was 64.8 (SD: 13.8) years in UK and 61.2 (SD: 13.4) years in NL.

In UK, we identified 40 incident OAC cases within the BO cohort (0.3%) to whom we could match 656 controls. Median number of controls per case was 17 (interquartile range (IQR): 9-23). In NL we identified 5 incident OAC cases among the BO cohort (0.3%). These were matched to 76 control subjects, with a median of 5 controls per case (IQR: 4-6). In addition, we identified 12 HGD cases, resulting in a second case control set of 17 cases (5 OAC + 12 HGD) matched to 753 controls (median 44 controls; IQR: 6-61). **Figure 1** shows a flowchart of the study population. **Table 1** provides baseline characteristics of cases and controls. In the UK a larger proportion of cases had a BMI over 25 kg/m²; 68% of cases and 59% of controls. In NL, only for 1 case BMI within 1 year of OAC diagnosis was available (21.3 kg/ m²). Controls had a mean BMI of 28.7 kg/m² (SD 4.7) in NL. Presence of oesophagitis or gastritis at time of BO diagnosis was more often seen in controls than in cases. In UK, a hiatal hernia was more often present among cases, whereas the opposite was found in NL. In UK, OAC cases were more likely to be current smokers than controls (OR 3.3; 95%CI: 1.4-8.0), as seen in NL though not significantly. Mean time from BO diagnosis until OAC diagnosis was 4.2 (SD: 2.5) years in UK and 3.5 (SD: 0.8) years in NL.

Table 1. Baseline Characteristics of Oesophageal Adenocarcinoma Cases and High-grade Dysplasia cases in the United Kingdom and Netherlands.

		United Kingdom				The Netherlands HGD - OAC			
		OAC Case N (%)	Control N (%)	OR (95% CI)	P-value	HGD-OAC Case N (%)	HGD-OAC control N (%)	OR (95% CI)	P-value
	Total	40 (100)	656 (100)			17 (100)	753 (100)		
	OAC					5 (29.4)			
	HGD					12 (70.6)			
Sex	male	33 (82.5)	597 (91)			11 (65)	524 (70)		
	female	7 (17.5)	59 (9)			6 (35)	229 (30)		
Mean age at index date (SD)		71.2 (10.4)	70.2 (9.0)			68.8 (8.2)	66.4 (8.8)		
Age group (years)	< 50	1 (2.5)	14 (2.1)			0 (0)	17 (2.3)		
	51-65	8 (20)	149 (23)			6 (35)	338 (45)		
	66-80	25 (62.5)	434 (66)			10 (59)	364 (48)		
	> 80	6 (15)	59 (9)			1 (5.9)	34 (4.5)		
Body Mass Index (kg/m2) mean (SD)		27.7 (4.1)	26.9 (4)	1.1 (1.0-1.1)	0.210	28.9 (6.8)	26.4 (7.4)	1.1 (0.9-1.3)	0.500
BMI categories	18-25	10 (25)	202 (31)			1 (5.9)	85 (11)		
	<18	0 (0)	7 (1.1)	-	0.989	0 (0)	22 (2.9)	-	0.997
	>25-30	19 (47.5)	269 (41)	1.5 (0. 7-3.3)	0.329	2 (12)	156 (21)	1.3 (0.1-14.7)	0.995
	>30-35	7 (17.5)	89 (14)	1.8 (0.7-5.0)	0.246	0 (0)	73 (9.7)	-	0.995
	>35	1 (2.5)	31 (4.7)	0.8 (0.1-7.0)	0.866	1 (5.9)	14 (1.9)	6.1 (0.3-112.1)	0.993
	missing	3 (7.5)	58 (8.8)	1.0 (0.3-3.8)	0.992	13 (76)	403 (54)	2.0 (0.3-16.5)	0.994
Oesophagitis at BO diagnosis	no	39 (97.5)	629 (95.9)			14 (82)	525 (70)		
	yes	1 (2.5)	27 (4.1)	0.6 (0.1-4.7)	0.633	3 (18)	228 (30)	0.5 (0.1-1.8)	0.299
Gastritis at BO diagnosis	no	38 (95)	621 (94.7)			13 (76)	582 (77)		

		United Kingdom				The Netherlands HGD - OAC			
		OAC Case N (%)	Control N (%)	OR (95% CI)	P-value	HGD-OAC Case N (%)	HGD-OAC control N (%)	OR (95% CI)	P-value
	yes	2 (5)	35 (5.3)	1.2 (0.3-5.2)	0.808	4 (24)	171 (23)	1.5 (0.5-4.9)	0.516
Hiatal Hernia at BO diagnosis	no	33 (82.5)	579 (88.3)			8 (47)	268 (36)		
	yes	7 (17.5)	77 (11.7)	1.7 (0.7-4.0)	0.259	9 (53)	485 (64)	0.7 (0.2-2.0)	0.487
Excessive alcohol use	never	17 (42.5)	370 (56)	Ref		17 (100)	713 (94.7)	-	0.991
	current	22 (55)	276 (42)	**2.0 (1.0-3.0)**	**0.048**	(0)	40 (5.3)		
	past	1 (2.5)	10 (1.5)	2.8 (0.3-23.4)	0.345				
Smoking	never	14 (35)	322 (49)	Ref		9 (53)	380 (50.5)	Ref	
	current	9 (22.5)	70 (11)	**3.3 (1.4-8.0)**	**0.009**	8 (47)	373 (49.5)	1.5 (0.5-4.5)	0.443
	past	17 (42.5)	264 (40)	1.7 (0.8-3.7)	0.155				
Index year	1998	1 (2.5)	7 (1.1)			1 (5.9)	5 (0.7)		
	2000	1 (2.5)	12 (1.8)			1 (5.9)	4 (0.5)		
	2001	3 (7.5)	24 (3.7)			1 (5.9)	7 (0.9)		
	2002	2 (5)	10 (1.5)			2 (12)	9 (1.2)		
	2003	2 (5)	15 (2.3)			1 (5.9)	3 (0.4)		
	2004	4 (10)	94 (14)						
	2005	7 (17.5)	128 (20)						
	2006	1 (2.5)	20 (3)						
	2007	2 (5)	30 (4.6)			1 (5.9)	22 (2.9)		
	2008	6 (15)	107 (16)			1 (5.9)	66 (8.8)		
	2009	4 (10)	72 (11)			1 (5.9)	49 (6.5)		
	2010	4 (10)	85 (13)			2 (12)	163 (22)		
	2011	3 (7.5)	52 (7.9)			5 (29.4)	374 (50)		
	2012					1 (5.9)	51 (6.8)		

		United Kingdom				The Netherlands HGD - OAC			
		OAC Case N (%)	Control N (%)	OR (95% CI)	P-value	HGD-OAC Case N (%)	HGD-OAC control N (%)	OR (95% CI)	P-value
Helicobacter pylori infection	no	40 (100)	603 (91.9)	-	-	17 (100)	714 (94.8)	-	
	yes	0 (0)	53 (8.1)			0 (0)	39 (5.2)		

Drug exposure

Table 2 provides characteristics of drug use from BO diagnosis until index date for cases and controls per database. Statins were used by 30% and 0% of OAC cases; and by 36% and 22% of controls in UK and NL, respectively. PPIs were used by OAC cases for a mean of 4.1 years (UK) and 2.3 years (NL) and by controls for 2.9 years (UK) and 1.9 years (NL). SSRIs were used by 12.5% of OAC cases in UK for a mean duration of 1 year, and by 7.6% of controls for a mean duration of 1.7 years.

Risk of Oesophageal Adenocarcinoma

To estimate the risk of OAC with use of NSAIDs, PPIs and statins, a nested case-control study was conducted. From the adjusted model, on patient-level pooled data, exposure to NSAIDs and PPIs did not provide a significant decrease in the risk of OAC (**Table 3**), for statins a non-significant effect was seen (ORa 0.7; 95%CI: 0.4-1.5). This was seen in both databases separately as well (data not shown).
For NSAID use, ORs ranged between 1.1 and 1.4 for all duration categories; regarding dose-analysis, no difference in risk was found between higher and lower dosages (**Table 4**). Although not significant, a dose-duration-response was seen for statins, with lower OR for longer duration of use compared to non-use of statins. Statin use ≥1.2 times higher compared to the recommended defined daily dose resulted in an OR of 0.7 (95%CI: 0.2-2.3). For PPIs an increase in OR was seen with prolonged duration, both in the matched and adjusted analyses. PPIs used at highest dose showed an OR for HGD-OAC of 0.9 (95% CI: 0.3-2.3). The ORs varied for duration categories of SSRIs. No dose-response was seen for SSRI use.

Table 2 Exposure characteristics of cases and controls in United Kingdom and the Netherlands

		United Kingdom		Netherlands	
		OAC Case	**Control**	**HGD-OAC case**	**HGD-OAC control**
		N = 40	N = 656	N = 17	N = 753
NSAIDs	Exposed - N	11	148	2	102
	Mean duration of use in days (SD)	205 (373)	218 (348)	18 (4)	49 (111)
	Mean cumulative DDD (SD)	223 (393)	232 (383)	9 (2)	31 (79)
	Median duration of use in days (IQR)	40 (20-178)	56 (28-203)	18 (15-20)	15 (10-60)
	Median cumulative DDD (IQR)	40 (30-223)	56 (28-208)	9 (7-10)	10 (5-30)
Statins	Exposed – N	12	236	3	123
	Mean duration of use in days (SD)	648 (569)	996 (913)	570 (289)	409 (300)
	Mean cumulative DDD (SD)	466 (353)	1,000 (1,258)	560 (191)	383 (331)
	Median duration of use in days (IQR)	616 (109-966)	728 (350-1,386)	450 (360-900)	330 (180-629)
	Median cumulative DDD (IQR)	504 (110-775)	625 (243-1,248)	450 (450-780)	270 (158-480)
PPIs	Exposed – N	36	570	10	389
	Mean duration of use in days (SD)	1,500 (1,134)	1,071 (978)	615 (462)	442 (372)
	Mean cumulative DDD (SD)	1,425 (1,247)	1,060 (1,123)	576 (402)	661 (1636)
	Median duration of use in days (IQR)	1,481 (644-2,017)	766 (392-1,458)	471 (240-1,020)	315 (180-630)
	Median cumulative DDD (IQR)	1,223 (644-1,772)	700 (364-1,428)	471 (300-719)	360 (180-840)
SSRIs	Exposed - N	5	50	0	15
	Mean duration of use in days (SD)	369 (280)	613 (705)	-	743 (669)
	Mean cumulative DDD (SD)	366 (283)	843 (1,430)	-	737 (670)
	Median duration of use in days (IQR)	252 (252-504)	381 (90-840)	-	600 (180-1,740)
	Median cumulative DDD (IQR)	252 (252-504)	339 (90-896)	-	596 (180-1,740)

Abbreviations: SD, standard deviation; DDD, defined daily dose; IQR, interquartile range; SSRIs, selective serotonin re-uptake inhibitors; NSAIDs, non-steroidal anti-inflammatory drugs; PPIs, proton pump inhibitors.

Table 3: Risk of Oesophageal Adenocarcinoma and High-grade Dysplasia-Oesophageal Adenocarcinoma by drug class by duration on data pooled on patient-level.

		OAC						HGD-OAC					
Exposure	**Duration category**	**OAC Case N (%)**	**OAC Control N (%)**	**ORmatched (95% CI)**	**P-value**	**ORadjusted* (95% CI)**	**P-value**	**Case N (%)**	**Control N (%)**	**ORmatched (95% CI)**	**P-value**	**ORadjusted* (95% CI)**	**P-value**
Total		**45 (100)**	**732 (100)**					**57 (100)**	**1,409 (100)**				
NSAID	None	32 (71)	566 (77)	Ref		Ref		44 (77)	1,159 (82)	Ref		Ref	
	Yes	13 (29)	166 (23)	1.3 (0.6-2.5)	0.492	1.2 (0.6-2.5)	0.532	13 (23)	250 (18)	1.0 (0.5-1.9)	1.000	0.9 (0.5-1.8)	0.876
	≤ 1 mo	6 (11)	65 (9)	1.4 (0.6-3.6)	0.454	1.4 (0.6-3.5)	0.471	6 (11)	121 (9)	1.1 (0.4-2.6)	0.882	1.0 (0.4-2.5)	0.967
	>1 mo - 1 yr	5 (9)	72 (10)	1.2 (0.4-3.1)	0.768	1.1 (0.4-3.0)	0.817	5 (9)	98 (7)	0.9 (0.3-2.4)	0.836	0.8 (0.3-2.3)	0.737
	>1 yr	2 (4)	29 (4)	1.2 (0.3-5.3)	0.837	1.1 (0.3-5.2)	0.859	2 (4)	31 (2)	1.1 (0.2-4.7)	0.934	1.0 (0.2-4.6)	0.970
Statins	None	33 (73)	479 (65)	Ref		Ref		42 (74)	1050 (75)	Ref		Ref	
	Yes	12 (27)	253 (35)	0.8 (0.4-1.5)	0.432	0.7 (0.4-1.5)	0.412	15 (26)	359 (25)	0.9 (0.5-1.7)	0.720	0.9 (0.5-1.7)	0.673
	≤ 1 mo	1 (2)	6 (1)	2.1 (0.2-20.4)	0.511	2.0 (0.2-20.1)	0.561	1 (2)	7 (0)	2.2 (0.2-20.6)	0.487	2.1 (0.2-20.5)	0.52
	>1 mo - 1 yr	3 (7)	62 (8)	0.9 (0.3-3.2)	0.908	1.0 (0.3-3.4)	0.971	4 (7)	128 (9)	0.9 (0.3-2.8)	0.914	1.0 (0.3-2.8)	0.951
	> 1 yr - 2 yrs	4 (9)	66 (9)	0.9 (0.3-2.7)	0.848	0.9 (0.3-2.6)	0.824	5 (9)	90 (6)	1.1 (0.4-2.9)	0.868	1.1 (0.4-2.8)	0.907
	> 2 yrs - 3 yrs	1 (2)	30 (4)	0.6 (0.1-4.9)	0.651	0.6 (0.1-4.7)	0.629	2 (4)	41 (3)	1.2 (0.3-5.3)	0.828	1.1 (0.2-4.9)	0.897
	> 3 yrs	3 (7)	89 (12)	0.5 (0.1-1.7)	0.259	0.5 (0.1-1.7)	0.239	3 (5)	93 (7)	0.5 (0.1-1.8)	0.276	0.5 (0.1-1.7)	0.253
PPIs	0 to ≤ 6 mo	5 (11)	103 (14)	Ref		Ref		11 (19)	450 (32)	Ref		Ref	
	Yes	40 (89)	629 (86)	1.1 (0.4-3.0)	0.814	1.1 (0.4-2.8)	0.911	46 (81)	959 (68)	1.0 (0.5-2.2)	0.917	0.9 (0.4-2.0)	0.855
	> 6 to ≤ 12 mo	6 (13)	169 (23)	1.9 (0.5-6.6)	0.502	2.0 (0.5-7.0)	0.299	7 (12)	158 (11)	1.7 (0.6-4.6)	0.293	1.7 (0.6-4.5)	0.312
	> 12 to ≤ 24 mo	9 (20)	151 (21)	1.8 (0.6-5.4)	0.672	1.7 (0.6-5.3)	0.328	10 (18)	227 (16)	1.7 (0.7-4.2)	0.255	1.6 (0.6-3.9)	0.326
	> 24 mo	5 (11)	162 (22)	2.1 (0.8-5.6)	0.476	1.9 (0.7-5.2)	0.207	27 (47)	377 (27)	1.7 (0.7-4.0)	0.204	1.5 (0.7-3.6)	0.327
SSRIs	None	40 (89)	679 (93)	Ref		Ref		52 (91)	1,344 (95)	Ref		Ref	
	Yes	5 (11)	53 (7)	1.7 (0.6-4.7)	0.281	1.7 (0.6-4.6)	0.310	5 (9)	65 (5)	1.6 (0.6-4.2)	0.356	1.5 (0.6-4.1)	0.390
	≤ 1 mo	0 (0)	3 (0)	-	0.992	0 (0-0)	0.992	0 (0)	3 (0)	-	0.988	0 (0-0)	0.988
	>1 mo - 1 yr	3 (7)	23 (3)	2.6 (0.7-9.2)	0.142	2.5 (0.7-8.9)	0.155	3 (5)	28 (2)	2.4 (0.7-8.6)	0.165	2.4 (0.7-8.4)	0.175
	>1 yr	2 (4)	27 (4)	1.2 (0.3-5.5)	0.778	1.2 (0.3-5.4)	0.815	2 (4)	34 (2)	1.1 (0.2-4.9)	0.888	1.1 (0.2-4.7)	0.931

Abbreviations: NSAID, non-steroidal anti-inflammatory drugs; PPIs, proton pump inhibitors; OAC, oesophageal adenocarcinoma; HGD, high-grade dysplasia; mo, months; yr, year.
* Adjusted for duration of follow-up since BO diagnosis.
# Cumulative use of drugs considered continuously (OR represents the change per day additional use)

Table 4. Risk of Oesophageal Adenocarcinoma and High-grade Dysplasia-Oesophageal Adenocarcinoma by drug class by daily dose on data pooled on patient-level

		OAC only				HGD-OAC			
Drug exposure	**Dose category**	**Case N(%)**	**Control N(%)**	**ORmatched (95% CI)**	**P-value**	**Case N(%)**	**Control N(%)**	**ORmatched (95% CI)**	**P-value**
Total		45 (100)	732 (100)			57 (100)	1,409 (100)		
NSAID	None	32 (71)	566 (77)	Ref	-	44 (77)	1,159 (82)	Ref	-
	<0.8 DDD per day	3 (7)	39 (5)	1.1 (0.3-3.7)	0.909	3 (5)	107 (8)	0.6 (0.2-2.2)	0.475
	≥0.8 - < 1.2 DDD per day	4 (9)	74 (10)	0.9 (0.3-2.5)	0.783	4 (7)	84 (6)	0.8 (0.3-2.3)	0.633
	≥1.2 DDD per day	6 (13)	53 (7)	2.2 (0.8-5.6)	0.111	6 (11)	59 (4)	1.9 (0.8-5.0)	0.160
Statin	None	33 (73)	479 (65)	Ref	-	42 (74)	1,050 (75)	Ref	-
	<0.8 DDD per day	8 (18)	126 (17)	0.9 (0.4-2.2)	0.880	9 (16)	174 (12)	1.0 (0.5-2.1)	0.959
	≥0.8 - < 1.2 DDD per day	1 (2)	49 (7)	0.3 (0.05-2.6)	0.305	2 (4)	62 (4)	0.7 (0.2-3.1)	0.637
	≥1.2 DDD per day	3 (7)	78 (11)	0.7 (0.2-2.3)	0.519	4 (7)	123 (9)	0.8 (0.3-2.4)	0.731
PPI	None	5 (11)	103 (14)	Ref	-	11 (19)	450 (32)	Ref	-
	<0.8 DDD per day	9 (20)	168 (23)	0.9 (0.3-3.0)	0.914	11 (19)	196 (14)	1.1 (0.4-2.8)	0.910
	≥0.8 - < 1.2 DDD per day	23 (51)	315 (43)	1.2 (0.4-3.4)	0.723	27 (47)	454 (32)	1.1 (0.5-2.6)	0.768
	≥1.2 DDD per day	8 (18)	146 (20)	1.1 (0.4-3.6)	0.822	8 (14)	309 (22)	0.9 (0.3-2.3)	0.813
SSRI	None	40 (89)	679 (93)	Ref	-	52 (91)	1,344 (95)	Ref	-
	<0.8 DDD per day	1 (2)	8 (1)	3.0 (0.4-25.4)	0.317	1 (2)	8 (1)	3 (0.3-25.1)	0.321
	≥0.8 - < 1.2 DDD per day	4 (9)	32 (4)	2.3 (0.7-7.1)	0.149	4 (7)	44 (3)	2.0 (0.7-6.0)	0.218
	≥1.2 DDD per day	0 (0)	13 (2)	-	0.987	0 (0)	13 (1)	-	0.987

Abbreviations: NSAID, non-steroidal anti-inflammatory drugs; PPIs, proton pump inhibitors; SSRIs, selective serotonin re-uptake inhibitors; DDD, defined daily dose; OAC, oesophageal adenocarcinoma; HGD, high-grade dysplasia.

Concomitant use of statins and PPIs decreased the risk of OAC with a matched OR of 0.8 (95%CI: 0.4-1.8) and an adjusted OR of 0.6 (95% CI: 0.2-1.5) (**Table 5**) compared to use of PPI only, though not significantly. Concomitant use of NSAIDs with PPIs showed a matched OR of 1.0; however, when adjusting for confounders the OR increased to 1.2. This resulted in an OR of 1.1 (95%CI: 0.4-3.0) for concomitant use of NSAIDs, statins and PPIs compared to use of PPIs only.

Table 5. Risk of Oesophageal Adenocarcinoma and High-grade Dysplasia-Oesophageal Adenocarcinoma for concomitant drug exposure of NSAIDs, statins and PPIs.

	OAC only						HGD-OAC					
Drug exposure	**Case N (%)**	**Control N (%)**	**ORmatched (95% CI)**	**P-value**	**ORadj model* (95% CI)**	**P-value**	**Case N (%)**	**Control N (%)**	**ORmatched (95% CI)**	**P-value**	**ORadj model* (95% CI)**	**P-value**
Total	45 (100)	732 (100)					57 (100)	1,409 (100)				
PPI only	22 (3)	314 (40)	Ref	-	Ref	-	25 (44)	483 (34)	Ref	-	Ref	-
NSAID only	1 (0)	15 (2)	0.8 (0.1-6.9)	0.869	0.9 (0.1-7.0)	0.881	1 (2)	16 (1)	0.8 (0.1-6.8)	0.853	0.9 (0.1-7.1)	0.890
Statin only	1 (0)	13 (2)	1.5 (0.2-12.0)	0.730	1.5 (0.2-12.7)	0.713	1 (2)	15 (1)	1.5 (0.2-12.3)	0.732	1.5 (0.2-12.6)	0.702
No NSAID or statin or PPI	3 (0)	70 (9)	0.9 (0.4-2.3)	0.846	0.8 (0.2-3.0)	0.796	9 (16)	414 (29)	0.8 (0.2-2.8)	0.715	1.0 (0.4-2.6)	0.938
NSAID + statin	(0)	5 (1)	0 (0-0)	0.989	0 (0-0)	0.989	0 (0)	5 (0)	0 (0-0)	0.989	0 (0-0)	0.989
NSAID + PPI	7 (1)	80 (10)	1.0 (0.4-2.4)	0.972	1.2 (0.5-3.0)	0.699	7 (12)	137 (10)	1.2 (0.5-3.1)	0.638	0.9 (0.4-2.2)	0.863
Statin + PPI	6 (1)	169 (22)	0.8 (0.4-1.8)	0.606	0.6 (0.2-1.5)	0.250	9 (16)	247 (18)	0.6 (0.2-1.5)	0.267	0.8 (0.4-1.8)	0.586
NSAID + Statin + PPI	5 (1)	66 (8)	1.0 (0.3-2.7)	0.934	1.1 (0.4-3.0)	0.909	5 (9)	92 (7)	1.1 (0.4-3.0)	0.895	0.9 (0.3-2.6)	0.903

Abbreviations: NSAID, non-steroidal anti-inflammatory drugs; PPIs, proton pump inhibitors; OAC, oesophageal adenocarcinoma; HGD, high-grade dysplasia.

* Adjusted for duration of follow-up since BO diagnosis.

Risk of High-Grade Dysplasia or Oesophageal Adenocarcinoma

In NL we were able to retrieve HGD cases as well. When including these in the case definition, the effects were attenuated but in the same direction as the case-control study including OAC cases only. There was no significant decrease in the risk of HGD-OAC for exposure to NSAIDs, statins and PPIs in the adjusted analysis (**Table 3**).For NSAIDs, the OR increased with use of higher dosages (**Table 4**). Again, for statins a duration-response relationship with the longest duration yielding the lowest ORa (0.5; 95% CI: 0.1-1.7) and an inverse association with increasing dose was observed, though none significant. For PPI and SSRI use, no dose-response effects were shown.

The risk of HGD-OAC was 7% lower for concomitant use of NSAIDs+PPIs (ORa 0.9; 95%CI:0.4-2.2) (**Table 5**). Concomitant use of statins with PPIs yielded an adjusted OR of 0.8 (0.4-1.8).None of the associations were statistically significant.

DISCUSSION

In this population-based case-control study nested within a cohort of Barrett's oesophagus patients, statin use may decrease the risk of both oesophageal adenocarcinoma and high-grade dysplasia by up to 50%. PPIs did not reduce the risk of HGD and OAC, however only when used at highest dose (e.g. at least 1.2 times the recommended daily dose) a non-significant reduction may be present. NSAIDs did not decrease the risk. This is the first population-based study that looked at the preventive effect of these three different drugs used individually and also concomitantly.

The mechanism of OAC-prevention is possibly related to inhibition of cyclo-oxygenase (COX)-2 production. Elevated levels of COX-2 in oesophageal epithelial cells have been observed in BO, and noted to increase with disease progression from BO to OAC.[30] In experimental studies, COX-2 inhibitors inhibited the growth of BO cells, potentially through suppression of basic fibroblast growth factor.[31] Another study confirmed that the end product of COX-2 conversion (prostaglandin E2) is reduced in BO patients without high-grade dysplasia when using esomeprazole combined with higher doses of aspirin.[32]

Statins exert anti-neoplastic properties in several ways. By inhibition of the 3-hydroxy-3-methylglutanyl coenzyme A (HMG-CoA) reductase enzyme, subsequent modulation of growth signal transduction, cellular proliferation and cell death is achieved, which affects different organs.[33] Particularly, in OAC cells statins inhibit cell proliferation and induce apoptosis [34] and limit the metastatic potential by reducing intracellular adhesion molecules.[35] However, statins also inhibit COX-2 expression in BO cells.[36]

Contrasting to other studies, we did not observe a significant preventive effect of NSAIDs and statins with respect to the risk of HGD-OAC. [13-14 22 37] Based on the biological mechanisms, combined use of statins and NSAIDs may be expected to result in a greater risk reduction compared to either drug alone. We did not observe that NSAIDs and statins combined resulted in a significant risk reduction of OAC. This may BO due to several reasons. Firstly, despite our large BO cohort the number of identified cases was smaller. Although we may have not have identified all potential OAC cases from the database, in a case-control study this is not necessary to obtain unbiased estimates. However, it limited the power of the study and resulted in statistically non-significant results. Particularly for assessment of concomitant drug exposure we did not reach statistical significance due to the lack of power, though this was not the primary aim of the study. However, given an exposure prevalence of NSAIDs of 30% among controls and a correlation of 0.5 between exposed and unexposed subjects, we had 80% power (with a type 1 error of 5%) to detect a true odds ratio of OAC of 0.34, which would be in concordance with previous studies. Our nesting cohort included all incident BO subjects from the general population and by matching on duration since BO diagnosis and excluding prevalent BO subjects, we removed any effect of selective survival bias, disease severity[38] or time window bias [39]; as those BO subjects with a longer follow-up are more likely to develop HGD or OAC. By doing so, observing any spurious associations was avoided. Secondly, we mitigated against immortal time bias[40] by defining the exposure period from BE diagnosis till matching date, and thus avoiding an overestimation of the preventive effect. The estimates from our study are likely more generalizable to the daily clinical practice in the general population, including also less severe BE subjects, i.e. those with a shorter BE segment. A potential preventive effect of NSAIDs might therefore be only observed within selected high-risk subgroups.

Secondly, the inability to show a significant decrease in HGD and OAC risk for drug use may be explained by the distinct exposure definition that we applied. Contrasting with others [13 37], we classified

exposure cumulatively and performed dose-duration-analyses rather than assessing drug exposure at a single moment. Drug exposure changes over time especially in the long time to develop cancer. Assessment of exposure on a fixed moment will result in bias that exaggerates the effect downwards; showing a protective effect while actually it has no effect.[39] A pooled analysis of observational studies demonstrated an inverse association between the risk of HGD-OAC and use of NSAIDs.[22] A prospective cohort study also showed a decreased hazard ratio of HGD-OAC for use of NSAIDs and statins, however the study results were influenced by immortal time bias.[17 41] In that study the majority of cases included HGD cases. In line with the other Dutch study [17], when we included HGD cases the risk of HGD-OAC was lower than including OAC cases only. Possibly the preventive effect is achieved in premalignant stage of dysplasia-development rather than of adenocarcinoma. It is however difficult to disentangle drug exposure effects in three different risk periods: induction (dysplasia), latent (between dysplasia and cancer) and disease period (cancer). Ideally, this requires knowledge on exact timing of the first aberrant Barrett's cell; and subsequent stages.

Third explanation for not observing a preventive effect may be the exposure prevalence. Regarding NSAID exposure prevalence, we could not capture over-the-counter use of NSAIDs. Prevalence of PPI (81%) and statin (26%) exposure in our study is however comparable to other studies and is therefore unlikely to have limited our power.[17 42]

A large prospective US cohort study showed a tremendous protective effect of NSAIDs on OAC-risk.[37] However, NSAID exposure was assessed in a personal interview and classified very broadly by NSAIDs use at least once a week for 6 months.[37] If the preventive effect of NSAIDs would be as high as reported (up to 80%), a duration and dose response effect is to be expected. This study failed to demonstrate an inverse association between duration of NSAID use and the risk of OAC. In fact, the opposite was observed; the most protective effect was seen for the shortest duration [37], contradicting a causal association.[24 43] A pooled analysis also couldn't demonstrate that prolonged duration of NSAID

use was associated with a lower risk of OAC.[22] Additionally, heterogeneity between studies was observed [22], which emphasizes the controversy around clinically effective chemoprevention with NSAIDs.

The preventive effect of statins is shown in several studies[13 17], yielding a risk reduction of OAC up to 48% for statin use >1 year.[15] Also for statins the most pronounced effect was seen when HGD was included.[16] Results from the latter study should be interpreted with caution as drug exposure was classified by self-report as 'ever' instead of a duration classification. A recent case-control study using a GP database from the UK, showed that statins may also decrease the risk of OAC and oesophagogastric junctional adenocarcinoma in the general population.[44] It could be that the preventive effect of statins is explained by other risk factors common to statin users and patients with OAC; such as cardiovascular risk factors or lifestyle changes: smoking, exercise and weight.[44] Also it may be that BO subjects died from vascular diseases rather than of cancer-related causes or before HGD or OAC developed.[45] In our study statin users were less likely to be current smokers, were of older age and more males. However, whether lifestyle changes due to co morbid cardiovascular diseases and initiating statin therapy may have resulted in healthier behavior and subsequent OAC risk reduction is open to debate.

Strengths of the current study include the scale and setting by combining healthcare data from two European countries with comparable GP databases and applying a common study protocol and drug exposure definition. The nested-case control design in a well-defined population representing the general population minimized selection bias. Whereas previous studies may have suffered from recall bias or the lack of detailed drug prescription data, we were able to estimate the risk of HGD and OAC within BO subjects during to drug use in the general population. Although our analysis may be limited by the small number of cases in the duration- and dose-analyses, partly due to the fact that we only

included incident cases (diagnosed ≥1 year after BO diagnosis), our study is unlikely to suffer from biases (immortal time bias, time window bias) and confounding (disease severity) by matching on important risk factors. Matched and adjusted analyses were in line with each other suggesting that there was little confounding.

Limitation of the study is the lack of detailed pathology information on the Barrett segment length and grade of dysplasia, as is current practice for risk stratification of BO subjects. This may have resulted in misclassification of BO and OAC, resulting in classifying subjects wrongly with BO or OAC. Assuming non-differential misclassification, this may have resulted in an underestimation. In the Dutch database we could search through all free text entered in the medical record, enabling to look for more detailed information in clinical letters, resulting in higher proportion of risk factors. We tried to address confounding-by-indication and time-window bias by matching on age, sex and year of BO diagnosis.[39] This is seen by the fact that individual risk factors did not increase the OAC risk and adjustment for these confounders did not change the estimate by ≥10%. The observation that PPIs appear to increase the risk of OAC is explained by the treatment indication being a risk factor for OAC; reverse causation and the phenomenon of 'channeling' where high-risk patients are being prescribed PPIs whereas low-risk patients not or in lower dose, [15 21 44 46-47] a phenomenon often seen with PPIs and upper gastrointestinal bleeding.[48] It could also be that the effect of PPIs is apparent after minimally 2 years of use [15 20] an observation which was not significant in our study.

In conclusion, in this population-based nested case-control study use of statins may reduce the risk of high-grade dysplasia and oesophageal adenocarcinoma among patients with Barrett's oesophagus, though we did not observe statistical significance. We did not demonstrate significant inverse associations for NSAID and PPI use and the risk of HGD and OAC. These findings indicate that for an

unselected group of patients with Barrett's oesophagus chemoprevention by use of drug to reduce progression should not be considered.

Acknowledgement section

Specific author contributions:

Gwen MC Masclee: study concept and design; acquisition of data; analysis and interpretation of data; statistical analysis; drafting of the manuscript.

Preciosa M Coloma: study concept and design; interpretation of data; drafting of the manuscript.

Manon CW Spaander: critical revision of the manuscript for important intellectual content.

Ernst J Kuipers: analysis and interpretation of data; critical revision of the manuscript for important intellectual content

Miriam CJM Sturkenboom: study concept and design; acquisition of data; analysis and interpretation of data; critical revision of the manuscript for important intellectual content; obtained funding; study supervision

All authors approved the final version of the manuscript.

Authors' declaration of competing interests:

- GMCM, PMC, MCWS do not have any conflict of interest.
- EJK has since completion of this research started working for the medical board of Erasmus University Medical Center.

MCJMS is coordinating a research group that has unconditional research grants from Pfizer, Novartis, Lilly, none related to this research.

Declaration of funding interests: None.

Data sharing statement: No additional data are available.

REFERENCES

1. Spechler SJ, Souza RF. Barrett's esophagus. *N Engl J Med* 2014;371(9):836-45.
2. Wang KK, Sampliner RE. Updated guidelines 2008 for the diagnosis, surveillance and therapy of Barrett's esophagus. *Am J Gastroenterol* 2008;103(3):788-97.
3. Solaymani-Dodaran M, Logan RF, West J*, et al.* Risk of oesophageal cancer in Barrett's oesophagus and gastro-oesophageal reflux. *Gut* 2004;53(8):1070-4.
4. de Jonge PJ, van Blankenstein M, Looman CW*, et al.* Risk of malignant progression in patients with Barrett's oesophagus: a Dutch nationwide cohort study. *Gut* 2010;59(8):1030-6.
5. Desai TK, Krishnan K, Samala N*, et al.* The incidence of oesophageal adenocarcinoma in non-dysplastic Barrett's oesophagus: a meta-analysis. *Gut* 2012;61(7):970-6.
6. Globocan 2008 Worldwide Cancer Incidence M, Prevalence and Disability-adjusted life years (DALYs). Accessed at 11th July 2014.
7. Yousef F, Cardwell C, Cantwell MM*, et al.* The incidence of esophageal cancer and high-grade dysplasia in Barrett's esophagus: a systematic review and meta-analysis. *Am J Epidemiol* 2008;168(3):237-49.
8. Sikkema M, de Jonge PJ, Steyerberg EW*, et al.* Risk of esophageal adenocarcinoma and mortality in patients with Barrett's esophagus: a systematic review and meta-analysis. *Clin Gastroenterol Hepatol* 2010;8(3):235-44; quiz e32.
9. Rastogi A, Puli S, El-Serag HB*, et al.* Incidence of esophageal adenocarcinoma in patients with Barrett's esophagus and high-grade dysplasia: a meta-analysis. *Gastrointest Endosc* 2008;67(3):394-8.
10. Masclee GM, Coloma PM, de Wilde M*, et al.* The incidence of Barrett's oesophagus and oesophageal adenocarcinoma in the United Kingdom and the Netherlands is levelling off. *Aliment Pharmacol Ther* 2014;39(11):1321-30.

11. WHO Global Health Observatory Data Repository. Cause-specific mortality, 2008: WHO region by country. Accessed 18th of October 2013. Available at: http://apps.who.int/gho/data/node.main.887?lang=en

12. Cancer Research UK. Oesophageal cancer survival statistics. Accessed 18th of October 2013. Available at: http://www.cancerresearchuk.org/cancer-info/cancerstats/types/oesophagus/survival/#Trends.

13. Beales IL, Vardi I, Dearman L. Regular statin and aspirin use in patients with Barrett's oesophagus is associated with a reduced incidence of oesophageal adenocarcinoma. *Eur J Gastroenterol Hepatol* 2012;24(8):917-23.

14. Corley DA, Kerlikowske K, Verma R, *et al.* Protective association of aspirin/NSAIDs and esophageal cancer: a systematic review and meta-analysis. *Gastroenterology* 2003;124(1):47-56.

15. Nguyen DM, Richardson P, El-Serag HB. Medications (NSAIDs, statins, proton pump inhibitors) and the risk of esophageal adenocarcinoma in patients with Barrett's esophagus. *Gastroenterology* 2010;138(7):2260-6.

16. Kantor ED, Onstad L, Blount PL, *et al.* Use of statin medications and risk of esophageal adenocarcinoma in persons with Barrett's esophagus. *Cancer Epidemiol Biomarkers Prev* 2012;21(3):456-61.

17. Kastelein F, Spaander MC, Biermann K, *et al.* Nonsteroidal anti-inflammatory drugs and statins have chemopreventative effects in patients with Barrett's esophagus. *Gastroenterology* 2011;141(6):2000-8; quiz e13-4.

18. Abnet CC, Freedman ND, Kamangar F, *et al.* Non-steroidal anti-inflammatory drugs and risk of gastric and oesophageal adenocarcinomas: results from a cohort study and a meta-analysis. *Br J Cancer* 2009;100(3):551-7.

19. Rothwell PM, Fowkes FG, Belch JF*, et al.* Effect of daily aspirin on long-term risk of death due to cancer: analysis of individual patient data from randomised trials. *Lancet* 2011;377(9759):31-41.

20. Singh S, Garg SK, Singh PP*, et al.* Acid-suppressive medications and risk of oesophageal adenocarcinoma in patients with Barrett's oesophagus: a systematic review and meta-analysis. *Gut* 2013.

21. Garcia Rodriguez LA, Lagergren J, Lindblad M. Gastric acid suppression and risk of oesophageal and gastric adenocarcinoma: a nested case control study in the UK. *Gut* 2006;55(11):1538-44.

22. Liao LM, Vaughan TL, Corley DA*, et al.* Nonsteroidal anti-inflammatory drug use reduces risk of adenocarcinomas of the esophagus and esophagogastric junction in a pooled analysis. *Gastroenterology* 2012;142(3):442-52 e5; quiz e22-3.

23. Nguyen T, Khalaf N, Ramsey D*, et al.* Statin Use is Associated with a Decreased Risk of Barrett's Esophagus. *Gastroenterology* 2014.

24. Rothman KJ, Greenland S, Lash TL. Causation and Causal Inference. *Modern Epidemiology*. 3rd ed. Philadelphia: Lippincott Williams & Wilkins, 2008.

25. Lewis JD, Schinnar R, Bilker WB*, et al.* Validation studies of the health improvement network (THIN) database for pharmacoepidemiology research. *Pharmacoepidemiol Drug Saf* 2007;16(4):393-401.

26. Vlug AE, van der Lei J, Mosseveld BM*, et al.* Postmarketing surveillance based on electronic patient records: the IPCI project. *Methods Inf Med* 1999;38(4-5):339-44.

27. Booth N. What are the Read Codes? *Health Libr Rev* 1994;11(3):177–82.

28. Lamberts H, Wood M, Hofmans-Okkes IM. International primary care classifications: the effect of fifteen years of evolution. *Fam Pract* 1992;9(3):330-9.

29. WHO Collaborating Centre for Drug Statistics Methodology. Guidelines for ATC classification and DDD assignment. Available at: http://www.whocc.no/atcddd/. (accessed April 4, 2013).

30. Wilson KT, Fu S, Ramanujam KS*, et al.* Increased expression of inducible nitric oxide synthase and cyclooxygenase-2 in Barrett's esophagus and associated adenocarcinomas. *Cancer Res* 1998;58(14):2929-34.

31. Buttar NS, Wang KK, Anderson MA*, et al.* The effect of selective cyclooxygenase-2 inhibition in Barrett's esophagus epithelium: an in vitro study. *J Natl Cancer Inst* 2002;94(6):422-9.

32. Falk GW, Buttar NS, Foster NR*, et al.* A combination of esomeprazole and aspirin reduces tissue concentrations of prostaglandin E(2) in patients with Barrett's esophagus. *Gastroenterology* 2012;143(4):917-26 e1.

33. Lochhead P, Chan AT. Statins and colorectal cancer. *Clin Gastroenterol Hepatol* 2013;11(2):109-18; quiz e13-4.

34. Ogunwobi OO, Beales IL. Statins inhibit proliferation and induce apoptosis in Barrett's esophageal adenocarcinoma cells. *Am J Gastroenterol* 2008;103(4):825-37.

35. Sadaria MR, Reppert AE, Yu JA*, et al.* Statin therapy attenuates growth and malignant potential of human esophageal adenocarcinoma cells. *J Thorac Cardiovasc Surg* 2011;142(5):1152-60.

36. Konturek PC, Burnat G, Hahn EG. Inhibition of Barret's adenocarcinoma cell growth by simvastatin: involvement of COX-2 and apoptosis-related proteins. *J Physiol Pharmacol* 2007;58 Suppl 3:141-8.

37. Vaughan TL, Dong LM, Blount PL*, et al.* Non-steroidal anti-inflammatory drugs and risk of neoplastic progression in Barrett's oesophagus: a prospective study. *Lancet Oncol* 2005;6(12):945-52.

38. Delgado-Rodriguez M, Llorca J. Bias. *J Epidemiol Community Health* 2004;58(8):635-41.

39. Suissa S, Dell'aniello S, Vahey S*, et al.* Time-window bias in case-control studies: statins and lung cancer. *Epidemiology* 2011;22(2):228-31.

40. Suissa S. Immortal time bias in pharmaco-epidemiology. *Am J Epidemiol* 2008;167(4):492-9.

41. Azoulay L, Suissa S. Immortal person-time bias in relation to the use of nonsteroidal anti-inflammatory drugs and statins in the prevention of esophageal cancer in patients with Barrett's esophagus. *Gastroenterology* 2012;142(5):e20-1; author reply e21.

42. Kastelein F, Spaander MC, Steyerberg EW*, et al.* Proton pump inhibitors reduce the risk of neoplastic progression in patients with Barrett's esophagus. *Clin Gastroenterol Hepatol* 2013;11(4):382-8.

43. Rothman KJ, Greenland S. Causation and causal inference in epidemiology. *Am J Public Health* 2005;95 Suppl 1:S144-50.

44. Alexandre L, Clark AB, Bhutta HY*, et al.* Statin Use is Associated With Reduced Risk of Histologic Subtypes of Esophageal Cancer: a Nested Case-Control Analysis. *Gastroenterology* 2014 146(3):661-68.

45. Moayyedi P, Burch N, Akhtar-Danesh N*, et al.* Mortality rates in patients with Barrett's oesophagus. *Aliment Pharmacol Ther* 2008;27(4):316-20.

46. Strom BL, Kimmel SE, editors. *Textbook of Pharmacoepidemiology*. Chichester: John Wiley & Sons, 2006.

47. Hvid-Jensen F, Pedersen L, Funch-Jensen P*, et al.* Proton pump inhibitor use may not prevent high-grade dysplasia and oesophageal adenocarcinoma in Barrett's oesophagus: a nationwide study of 9883 patients. *Aliment Pharmacol Ther* 2014.

48. Masclee GM, Valkhoff VE, Coloma PM*, et al.* Risk of Upper Gastrointestinal Bleeding from Different Drug Combinations. *Gastroenterology* 2014. doi: 10.1053/j.gastro.2014.06.007.

Figure Legends

Figure 1: Flowchart of Barrett's oesophagus and Oesophageal Adenocarcinoma cases in the United Kingdom and the Netherlands.

BO, Barrett's oesophagus; OAC, oesophageal adenocarcinoma; HGD, high-grade dypslasia.

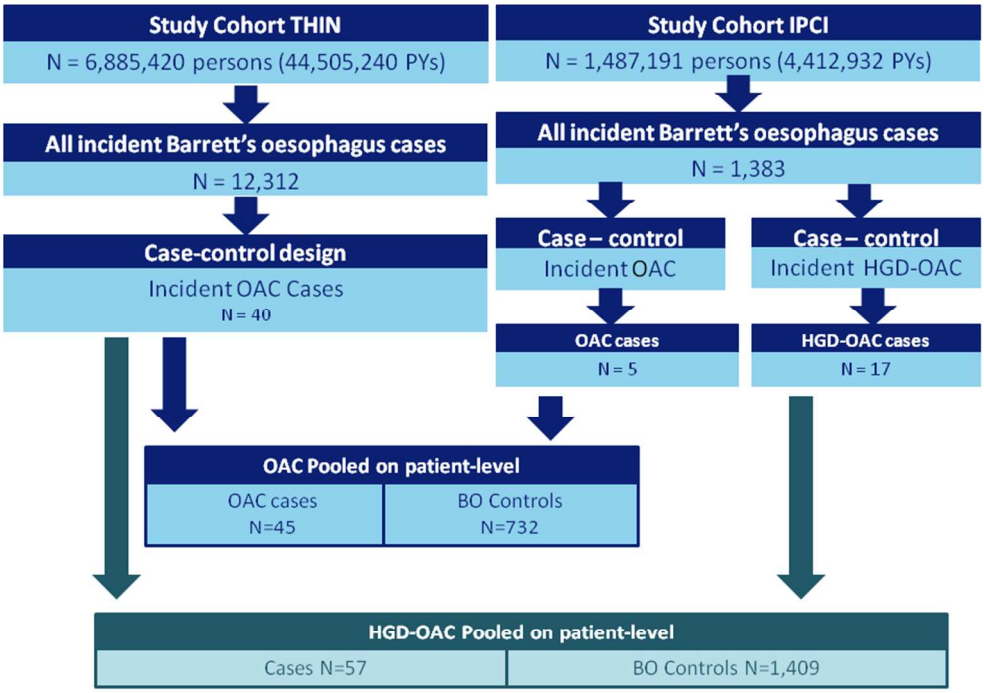

Figure 1: Flowchart of Barrett's oesophagus and Oesophageal Adenocarcinoma cases in the United Kingdom and the Netherlands.
BO, Barrett's oesophagus; OAC, oesophageal adenocarcinoma; HGD, high-grade dypslasia.
254x190mm (96 x 96 DPI)

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 Included in abstract.
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found Included in abstract. We found in a nested case-control study using primary care data from the United Kingdom and the Netherlands that statins may decrease the risk of oesophageal adenocarcinoma (OAC) and high-grrade dysplasia (HGD) among subjects with Barrett's oesophagus (BO). Proton pump inhibitors (PPIs) may reduce the risk of OAC-HGD when used at highest dose, while non-steroidal anti-inflammatory drugs (NSAIDs) did not decrease the risk of OAC-HGD.
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported Several studies reported that use of non-steroidal anti-inflammatory drugs (NSAIDs), statins and proton pump inhibitors (PPIs) may decrease the risk of OAC among BO patients. However, these studies were based on small, selected samples of OAC cases and were affected by bias and confounding. A meta-analysis including nine observational studies, (2 cohort and 7 case-control) showed that the risk of oesophageal cancer among those who frequently use NSAIDs or aspirin was significantly lower compared to never users. However, studies included in the meta-analysis did not specifically include patients with BO. Second, in a pooled analysis on individual patient data which confirmed the significant reduction in risk of OAC in BO patients with prescription of statins and NSAID US veteran were study subjects, which limits the generalization and extrapolation of results from the latter study to the general population is. Additionally there was no adjustment for important risk factors of OAC progression such as alcohol use and tobacco smoking. Causality of an apparent association is generally supported by a dose- and duration-response relationship. However, several studies neither reported a clear exposure definition free of recall bias nor conducted dose-duration analyses. Finally, concerns have been raised about publication bias of these studies on chemoprevention of OAC in BO patients. Thus, to which extent NSAIDs, statins and PPIs may reduce the risk of oesophageal adenocarcinoma among BO patients in clinical practice remains unknown. Therefore, we conducted a matched case-control study to evaluate the risk of oesophageal adenocarcinoma among patients with BO associated with use of NSAIDs, statins and PPIs.
Objectives	3	State specific objectives, including any prespecified hypotheses The aim of our study was to evaluate the risk of oesophageal adenocarcinoma among patients with BO associated with use of NSAIDs, statins and PPIs in a matched case-control study.
Methods		
Study design	4	Present key elements of study design early in the paper Nested case-control study in BO subjects identified in two primary care databases from United Kingdom and the Netherlands between 1996 and 2012. This is presented early in the Methods section.
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection Two European population-based primary care databases; The Health Improvement

		Network (THIN) database from the United Kingdom (UK, 1996–2011), and 2) the Integrated Primary Care Information database (IPCI) from the Netherlands (NL, 1996–2012). Both databases contain data that are collected prospectively and represent real-life practice. Data was collected by electronic search for diagnoses and drug prescriptions.
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls First a dynamic population-based cohort was created in which we included all patients aged 18 years and older who contributed data to the database between 1st of January 1996 and 31st of December 2011 for THIN and 2012 for IPCI and with a diagnosis of BO diagnosis. At least one year of available healthcare data prior to study entry was required in order to assess patient's medical history and to discriminate between prevalent and incident (i.e., newly-diagnosed) BO cases. Follow up started on 1 January 1996, date of reaching 18 years of age, or the date that one year of valid data was accrued within the database, whichever came later. Follow-up ended on the date of occurrence of study outcome (OAC), date of transfer out of the general practitioner's (GP) practice, death, or last data drawn, whichever was earliest. Patients were excluded if they had a history of oesophageal cancer anytime before BO diagnosis and if they had a history of gastric cancer up to 6 months after BO diagnosis. In THIN, BO and OAC cases were extracted using corresponding READ codes. In IPCI, each potential BO case was manually validated to confirm the diagnosis of BO and the date of first diagnosis or mentioning of BO in the clinical record. For OAC-HGD diagnosis, all potential cases were manually validated for confirmation of the OAC or HGD diagnosis, date of first diagnosis and the type of carcinoma (squamous cell-, adeno-, or other types of carcinoma). Early cancer (i.e., high-grade dysplasia (HGD)) was identified in IPCI as well. OAC cases were considered incident if the date of diagnosis occurred after inclusion into the BO study cohort and was at least 12 months after BO diagnosis. Cases occurring within one year from BO diagnosis were classified separately and considered to be existent in relation to the BO diagnostic work-up. The index date was defined as the date of the first reporting of OAC diagnosis during the study period. Controls were members of the incident BO cohort who did not develop OAC up to the matching date. Controls were matched by incidence density sampling on age (± 5 years), sex, year of BO diagnosis (± 1 year), and database. Consequently, the index date for controls was the date of OAC diagnosis for the corresponding case. We matched on year of BO diagnosis in order to account for any influence of guideline changes in endoscopic surveillance over calendar time.
		(*b*) For matched studies, give matching criteria and the number of controls per case Controls were matched by incidence density sampling on age (± 5 years), sex, year of BO diagnosis (± 1 year), and database. Consequently, the index date for controls was the date of OAC (or HGD) diagnosis for the corresponding case. We matched on year of BO diagnosis in order to account for any influence of guideline changes in endoscopic surveillance over calendar time. In UK, we identified 40 incident OAC cases within the BO cohort (0.3%) to whom we could match 656 controls. Median number of controls per case was 17 (interquartile range (IQR): 9-23). In NL we identified 5 incident OAC cases among the BO cohort (0.3%) and these were matched to 76 control subjects, with a median of 5 controls per case (IQR: 4-6). In

		addition, we identified 12 HGD cases, resulting in a second case control set of 17 cases (5 OAC + 12 HGD) matched to 753 controls (median 44 controls; IQR: 6-61).
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable Outcomes: In THIN, BO and OAC cases were extracted using corresponding READ codes. We applied several BO case definitions in THIN to explore outcome misclassification of BO, which did not demonstrate large differences. In IPCI the ICPC coding system is used, which does not include a diagnosis code for BO specifically. A sensitive search algorithm in free text was used including synonyms for Barrett's oesophagus ('Barrett', 'intestinal metaplasia', 'columnar epithelium'). Each potential case was manually validated to confirm the BO diagnosis and the date of first diagnosis or mentioning of BO in the clinical record. OAC cases were considered incident if the date of diagnosis occurred after inclusion into the BO study cohort and was at least 12 months after BO diagnosis. Cases occurring within one year from BO diagnosis were classified separately and considered to be existent in relation to the BO diagnostic work-up. Furthermore, in IPCI all OAC and HGD cases were manually validated. Drug exposure: Drug exposures of interest were the use of outpatient prescriptions for NSAIDs (including high-dose aspirin, i.e. >325 mg/day), PPIs and statins from BO diagnosis until OAC diagnosis. In order to compare the OR of NSAIDs, PPIs and statins to other drugs, we considered another group of medications that served as control. Antidepressants (selective serotonin re-uptake inhibitors (SSRIs)) are currently not known to be either positively or negatively associated with OAC. Duration of prescriptions was calculated based on the prescribed quantity and dosing regimen. As the most likely preventive effect of drugs on cancer progression is through a cumulative mechanism, we calculated all duration and defined daily dose (DDD) values from date of BO diagnosis until index date. Duration was classified according to never use (reference category), cumulative use of less than 1 month, between 1-12 months, > 12 months (or if applicable 1-2 years; 2-3 years and > 2 years). Considering that PPIs are indicated as treatment for BO patients, duration was classified as 0-6 months (reference category), 6-12 months, 1-2 years and > 2 years. Dose of exposure was classified using the ratio of prescribed daily dose compared to DDD using quartiles into categories (<0.8; 0.8-1.2; ≥1.2 DDD per day). Potential confounder: We considered the following as potential confounders: concurrent diagnosis of oesophagitis; gastritis within 1 year before BO diagnosis; presence of a hiatal hernia (determined from start of data entry in the database until BO diagnosis). Additionally we assessed smoking habits (non-smoker, ex-smoker, current smoker) and alcohol abuse (never, current, past).
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 For both databases, information on BO or OAC diagnosis was measured in the same manner applying the common protocol.

		As co-morbidity we determined the presence of oesophagitis, gastritis from 1 year before until the time of BO diagnosis and the presence of a hiatal hernia at any time before BO diagnosis. BO cases were classified as incident if the date of BO diagnosis occurred after inclusion in the study cohort and as prevalent if the date of BO diagnosis occurred prior to study entry.
Bias	9	Describe any efforts to address potential sources of bias The nested-case control design in a well-defined population characterizing the general population minimized selection bias. Whereas previous studies may have suffered from recall bias or the lack of detailed drug prescription data, we were able to estimate the risk of OAC and OAC-HGD within BO subjects in relation to drug prescription data in the general population. Our study is unlikely to suffer from biases (such as immortal time bias, time window bias) and confounding (such as by disease severity) by matching on important risk factors. Additionally, the adjusted analyses were in line with the matched analyses. Although we may have lacked detailed pathology information in some subjects including information on the length of the Barrett segment and the grade of dysplasia. This may have resulted in misclassification of BO and OAC, resulting in classifying subjects wrongly with BO or OAC. Outcome misclassification of OAC might have occurred, as population-based studies are challenged by the lack of detail from histology reports, which is particularly true for routinely collected GP data without free text in medical records. However, we have included only confirmed OAC/HGD cases in the study. Assuming non-differential misclassification, this may have resulted in an underestimation of the risk. In the Dutch database we could search through all free text entered in the medical record, enabling to look for more detailed information in clinical letters, resulting in higher percentages of information on oesophagitis, gastritis and hiatal hernia. We tried to address confounding-by-indication and time-window bias by matching on age, sex and year of BO diagnosis. This is seen by the fact that individual risk factors (hiatal hernia, and alcohol abuse) did not appear to increase the risk of oesophageal adenocarcinoma and adjustment for these potential confounders did not change the OR estimates by more than 10%.
Study size	10	Explain how the study size was arrived at From the source population of 7,570,765 subjects in UK and 1,496,276 subjects in NL we identified 13,696 and 1,438 incident BO cases, respectively. Males accounted for 63% in UK and 62% in NL of BO subjects. Mean age at BO diagnosis was 64.8 (SD: 13.8) years in UK and 61.2 (SD: 13.4) years in NL. In UK, we identified 40 incident OAC cases within the BO cohort (0.3%) to whom we could match 656 controls. Median number of controls per case was 17 (interquartile range (IQR): 9-23). In NL we identified 5 incident OAC cases among the BO cohort (0.3%) and these were matched to 76 control subjects, with a median of 5 controls per case (IQR: 4-6). In addition, we identified 12 HGD cases, resulting in a second case control set of 17 cases (5 OAC + 12 HGD) matched to 753 controls (median 44 controls; IQR: 6-61). A flowchart of the study population is depicted in Figure 1. In the manuscript we provide a flow chart of the study cohorts.
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why. Age at BO diagnosis among OAC cases and the mean time to OAC diagnosis was estimated by a Student's t-test.
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding

		Baseline characteristics of cases and controls were described per database and compared using univariate conditional logistic regression. To estimate the risk of OAC among patients with BO, matched and adjusted odds ratios (OR) with 95% confidence intervals (CIs) were calculated using conditional logistic regression for both databases separately and as a pooled analysis on patient-level pooled data. Potential confounders (oesophagitis, gastritis, hiatal hernia, BMI, smoking and alcohol abuse) were included in the adjusted analysis (ORa) if they resulted in a change of more than 10% of the initial estimate, whereas time since BO diagnosis was forced into the adjusted model. Subsequent analyses included duration- and dose-analyses. The risk of OAC and OAC-HGD was also assessed for concomitant use of NSAIDs, statins and/or PPIs. Use of PPIs only was considered as reference category considering that standard therapy for BO includes PPI therapy. Subgroup analyses evaluated the risk of OAC stratified by presence of risk factors: oesophagitis, gastritis or hiatal hernia at time of BO diagnosis. Multiplicative interaction was tested to identify effect modification by all of individual risk factors.
		(*b*) Describe any methods used to examine subgroups and interactions Subgroup analyses evaluated the risk of OAC stratified by presence of risk factors: oesophagitis, gastritis or hiatal hernia at time of BO diagnosis. Multiplicative interaction was tested to identify effect modification by all of individual risk factors.
		(*c*) Explain how missing data were addressed We had missing data on BMI which we report in Table 1.
		(*d*) If applicable, explain how matching of cases and controls was addressed Controls were matched by incidence density sampling on age (± 5 years), sex, year of BO diagnosis (± 1 year), and database. Consequently, the index date for controls was the date of OAC (or HGD) diagnosis for the corresponding case. We matched on year of BO diagnosis in order to account for any influence of guideline changes in endoscopic surveillance over calendar time..
		(*e*) Describe any sensitivity analyses We performed subgroup analyses by stratifying on risk factors for OAC..
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 The total study population comprised 8,372,611 persons (UK: 6,885,420; NL: 1,487,191) contributing to 48,918,172 person years (PYs) (UK: 44,505,240; NL: 4,412,932) of follow-up during the study period. We identified 12,312 and 1,383 incident BO cases in THIN and IPCI, respectively. In the manuscript we provide a flow chart of the study cohorts.
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram In the manuscript we provide a flow chart of the study cohorts.
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders Baseline characteristics of study participants are shown in Table 1 and in the first paragraph of the Results section.
		(b) Indicate number of participants with missing data for each variable of interest We had missing data on BMI which we report in Table 1.

Outcome data	15*	Report numbers in each exposure category, or summary measures of exposure The number of OAC-HGD cases and controls and the corresponding co morbid diseases and the frequency of exposure are described in Table 1 and Table 2&3 respectively.
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 In Table 3 we report matched and adjusted ORs for OAC and HGD during use of NSAIDs, statins, PPIs and SSRIs. Adjusted was for duration of follow-up since BO diagnosis, apart from the matching factors.
		(*b*) Report category boundaries when continuous variables were categorized. In Table 1 we give number of OAC cases and controls per age category (<50, 51-65, 66-80, >80 years of age).
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period Not applicable.
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses In the results section and appendix Table 2 we report the stratified analyses and interaction terms.
Discussion		
Key results	18	Summarise key results with reference to study objectives In this population-based case-control study nested within a cohort of patients with Barrett's oesophagus, the risk of both oesophageal adenocarcinoma and high-grade dysplasia may be reduced up to 50% during use of statins. PPIs reduced the risk of HGD and OAC when used at highest doses, while NSAIDs did not decrease the risk. This is the first study that looked at the chemopreventive effect of these three different drugs also when used concomitantly within a population-based study.
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 In the discussion section we have discussed the limitations of the current study. The nested-case control design in a well-defined population characterizing the general population minimized selection bias. Whereas previous studies may have suffered from recall bias or the lack of detailed drug prescription data, we were able to estimate the risk of OAC and OAC-HGD within BO subjects in relation to drug prescription data in the general population. Although our analysis may be limited by the small number of cases in the duration- and dose-analyses, our study is unlikely to suffer from biases (such as immortal time bias, time window bias) and confounding (such as by disease severity) by matching on important risk factors. Additionally, the adjusted analyses were in line with the matched analyses. An important limitation of the study is the lack of detailed pathology information in some subjects including information on the length of the Barrett segment and the grade of dysplasia. This may have resulted in misclassification of BO and OAC, resulting in classifying subjects wrongly with BO or OAC. Assuming non-differential misclassification, this may have resulted in an underestimation of the risk. In the Dutch database we could search through all free text entered in the medical record, enabling to look for more detailed information in clinical letters, resulting in higher percentages of information on oesophagitis, gastritis and hiatal hernia. We tried to address confounding-by-indication and time-window bias by matching on age, sex and year of BO diagnosis. This is seen by the fact that individual

risk factors (hiatal hernia, and alcohol abuse) did not appear to increase the risk of oesophageal adenocarcinoma and adjustment for these potential confounders did not change the OR estimates by more than 10%. The observation that PPIs appear to increase the risk of OAC may be explained by the underlying treatment indication being a risk factor for OAC, reverse causation and the phenomenon of 'channeling' where high-risk patients are being prescribed PPIs whereas low-risk patients not or in lower dose. This phenomenon is also seen for PPI use and upper gastrointestinal bleeding. It could also be that the effect of PPIs is becoming pronounced after at least 2 years of use, as is also seen from our data, showing a tendency to a lower risk of OAC-HGD with use > 2 years. That PPIs decrease the risk of OAC-HGD via gastric acid suppression is confirmed by the observation that among subjects with a hiatal hernia PPIs a reduced risk of OAC and OAC-HGD was noted whereas this was not seen in subjects without a hiatal hernia.

Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence

In this population-based case-control study nested within a cohort of patients with Barrett's oesophagus, the risk of both oesophageal adenocarcinoma and high-grade dysplasia may be reduced up to 50% during use of statins. PPIs reduced the risk of HGD and OAC when used at highest doses, while NSAIDs did not decrease the risk. This is the first study that looked at the chemopreventive effect of these three different drugs also when used concomitantly within a population-based study.

In contrast to other studies, we did not observe a significant preventive effect of NSAIDs and statins with respect to the risk of OAC and HGD. Based on the biological mechanisms, combined use of statins and NSAIDs would be expected to result in a greater risk reduction of OAC compared to either drug alone. We did not observe this synergistic protective effect. This may be due to several reasons. Firstly, despite our large BO cohort (compared to other studies), the number of identified OAC and HGD cases was smaller. This limited the power of the study and resulted in wider confidence intervals and statistically non-significant results. However, given an exposure prevalence of NSAIDs of 30% among controls and a correlation of 0.5 between exposed and unexposed subjects, we had 80% power (with a type 1 error of 5%) to detect a true odds ratio of OAC of 0.34, which would be according to prior studies. In addition, our underlying study population included all incident BO subjects from the general population. By matching on duration since BO diagnosis and excluding prevalent BO subjects, we removed any effect of selective survival bias, disease severity or time window bias; as those BO subjects with a longer follow-up are more likely to develop HGD or OAC. Secondly, we mitigated against immortal time bias by defining the exposure period from BO diagnosis till matching date, and thus avoiding an overestimation of the preventive effect. The estimates from our study are likely more generalizable to the daily clinical practice in the general population, including also less severe BO subjects. A preventive effect of NSAIDs might be therefore only applicable to selected high-risk subgroups. Secondly, the inability to show a significant decrease in OAC and OAC-HGD risk for NSAID during statin use may be explained by the distinct exposure definition that we applied. Possibly the preventive effect is achieved in the premalignant phase of development of dysplasia rather than of final adenocarcinoma, resembling different risk periods. It is however difficult to disentangle effects of drug exposure in the three different risk periods: induction (dysplasia), latent

		(between dysplasia and cancer) and disease period (cancer). Ideally, this requires knowledge on the exact timing of starting from the first aberrant Barrett's cell; and subsequent stages. A third explanation for not observing a chemopreventive effect in our study may be the exposure prevalence. The NSAID exposure prevalence was lower in our study, because we could not capture over-the-counter use of NSAIDs. Prevalence of PPI (81%) and statin (26%) exposure in our study is however comparable to other studies and is therefore unlikely to have limited our power.
Generalisability	21	Discuss the generalisability (external validity) of the study results Both two general practice databases contain a large number of patients and reflect the underlying general population. This study can be generalised to other Western European populations.
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 No specific funding for this study was obtained.

*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.

Appendix

Appendix Table 1: Corresponding Read codes for identification of Barrett's oesophagus and oesophageal adenocarcinoma in THIN database.

READ code	Description of code		READ code	Description of code	Event
J101611	Barrett's oesophagus				BO
J102500	Barrett's ulcer of oesophagus				BO
J10y600	Barrett's oesophagus				BO
B102.00	Malignant neoplasm of abdominal oesophagus				Specific algorithm OAC
B105.00	Malignant neoplasm of lower third oesophagus				Specific algorithm OAC
B106.00	Malignant neoplasm, overlapping lesion of oesophagus				Specific algorithm OAC
B10y.00	Malignant neoplasm of other specified part of oesophagus				Specific algorithm OAC
B10z.00	Malignant neoplasm of oesophagus NOS				Specific algorithm OAC
B110100	Malignant neoplasm of gastro-oesophageal junction of stomach				Specific algorithm OAC
B110111	Malignant neoplasm of gastro-oesophageal junction				Specific algorithm OAC
B10..00/B10z.11	Malignant neoplasm of oesophagus / Oesophageal cancer	Combined with:	BBB2.00	Adenocarcinoma with squamous metaplasia	Unspecific algorithm OAC *
			BB57.00	Adenocarcinoma, intestinal type	
			BB53.00	Adenocarcinoma, metastatic, NOS	
			BB5..11	Adenocarcinomas	
			BB5z.00	Adenoma or adenocarcinoma NOS	

BO, Barrett's oesophagus; OAC, oesophageal adenocarcinoma.

* Recording of the combination of the two codes should have occurred within 1 year of each other.

BMJ Open

NSAIDs, statins, low-dose aspirin and PPIs and the risk of oesophageal adenocarcinoma among patients with Barrett's oesophagus, a population based case-control study

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2014-006640.R1
Article Type:	Research
Date Submitted by the Author:	09-Nov-2014
Complete List of Authors:	Masclee, Gwen; Erasmus University Medical Center, Medical Informatics; Gastroenterology and Hepatology Coloma, Preciosa; Erasmus MC University Medical Center, Medical Informatics Spaander, Manon; Erasmus MC University Medical Center, Gastroenterology &Hepatology Kuipers, Ernst; Erasmus MC, Gastroenterology and Hepatology Sturkenboom, Miriam; Erasmus University Medical Center, Medical Informatics; Erasmus MC University Medical Center, Epidemiology
Primary Subject Heading:	Gastroenterology and hepatology
Secondary Subject Heading:	Epidemiology, Gastroenterology and hepatology
Keywords:	EPIDEMIOLOGY, Gastrointestinal tumours < GASTROENTEROLOGY, Oesophageal disease < GASTROENTEROLOGY

NSAIDs, statins, low-dose aspirin and PPIs and the risk of oesophageal adenocarcinoma among patients with Barrett's oesophagus, a population based case-control study

Short title: Risk of oesophageal adenocarcinoma

Gwen MC Masclee, Department of Medical Informatics; Department of Gastroenterology and Hepatology, Erasmus University Medical Center, Rotterdam, The Netherlands. g.masclee@erasmusmc.nl.

Preciosa M Coloma, Department of Medical Informatics, Erasmus University Medical Center, Rotterdam, The Netherlands. P.coloma@erasmusmc.nl.

Manon CW Spaander, Department of Gastroenterology and Hepatology, Erasmus University Medical Center, Rotterdam, The Netherlands. V.spaander@erasmusmc.nl.

Ernst J Kuipers, Department of Gastroenterology and Hepatology, Erasmus University Medical Center, Rotterdam, The Netherlands. E.j.kuipers@erasmusmc.nl.

Miriam CJM Sturkenboom, Department of Medical Informatics; Department of Epidemiology, Erasmus University Medical Center, Rotterdam, The Netherlands. M.sturkenboom@erasmusmc.nl.

Correspondence to:

Gwen MC Masclee, M.D.

Dept. of Medical Informatics

Erasmus University Medical Center

PO Box 2040

3000 CA Rotterdam

The Netherlands

Phone: +31 10 7044116

Fax: +31 10 7044722

E-mail: g.masclee@erasmusmc.nl

Key words: Barrett's oesophagus, oesophageal adenocarcinoma, non-steroidal anti-inflammatory drugs, statins, proton pump inhibitors

Word count (excl. references): 4649

No. of tables: 5.

No. of figures: 1.

No. of Appendices: 1.

Abbreviations

ATC	Anatomical Therapeutic Chemical
BMI	Body Mass Index
BO	Barrett's oesophagus
DDD	Defined Daily Dose
GP	General Practitioner
HGD	High-grade Dysplasia
ICPC	International Classification for Primary Care
IPCI	Integrated Primary Care Information database
IQR	Interquartile Range
NL	The Netherlands
NSAIDs	Non steroidal anti-inflammatory drugs
OAC	Oesophageal Adenocarcinoma
OR	Odds Ratios
PPIs	Proton Pump Inhibitors
THIN	The Health Improvement Network
UK	United Kingdom

ABSTRACT

Objectives: Non-steroidal anti-inflammatory drugs (NSAIDs), proton pump inhibitors (PPIs), low-dose aspirin and statins may decrease the risk of oesophageal adenocarcinoma (OAC) among Barrett's oesophagus (BO) patients. However, previous studies did not adequately address bias and confounding. Objective was to estimate the risk of OAC among BO patients exposed to NSAIDs, statins and PPIs.

Design: Case-control study nested within a BO cohort.

Setting: Two primary care databases (United Kingdom, Netherlands).

Participants: Cases were adults≥18 years with OAC or HGD diagnosis ≥1 year after BO diagnosis. Controls were matched on age, sex, year of BO diagnosis, and database.

Exposure: Drug use was assessed from BO diagnosis until matching date.

Outcome measure: Adjusted odds ratios (ORa) with 95% CI were calculated by conditional logistic regression.

Results: Within the BO cohort (n=15,134), 45 OAC (UK:40, NL:5) and 12 HGD cases (NL:12) were identified. ORa for OAC during NSAID use was 1.2 (95%CI:0.6-2.5) and during statin use for > 3years 0.5 (95%CI:0.1-1.7). When including HGD cases (n=57), ORa for NSAID use was 0.9 (95%CI:0.5-1.8) and for statin use > 3 years 0.5 (95%CI:0.1-1.7). Higher doses of statins showed lower estimates for OAC and HGD, though not statistically significant. Low-dose aspirin and PPIs did not significantly decrease the risk of OAC and HGD.

Conclusion: In this population-based nested case-control study, use of NSAIDs, PPIs, low-dose aspirin or statins did not reduce the risk of HGD and OAC among BO patients. These findings indicate that for an

unselected group of BO patients chemoprevention by use of drugs to reduce progression to HGD and OAC should not be directly considered as routine care.

Article summary

Strengths:

- Within a population-based cohort of incident Barrett's oesophagus patients derived from two European countries and applying a common study protocol and drug exposure definition the risk of development of oesophageal adenocarcinoma was estimated during use of several drugs individually and concomitantly.
- We were able to minimize certain biases, for instance due to availability of drug prescription data recall bias was avoided and by using a population-based approach selection bias was minimized.

Limitations:

- The small number of oesophageal adenocarcinoma cases that was identified limited the power for the duration analyses.
- We did not have detailed pathology information on the Barrett segment length or grade of dysplasia at cohort entry for all BO cohort members in both countries. This may have resulted by including subjects with a short segment BO whom may be at lower risk of developing HGD and OAC at start.

INTRODUCTION

Barrett's oesophagus (BO) is a pre-malignant condition in which the squamous epithelium of the oesophagus is replaced by metaplastic columnar epithelium.[1] BO is considered a consequence of prolonged gastrooesophageal reflux [2] and is the most important risk factor for development of oesophageal adenocarcinoma (OAC) via a stepwise pathway of low- and high-grade dysplasia. It is estimated that the risk of OAC is increased by approximately 30-125 fold in persons with BO [3], and occurs in a small proportion of BO patients yearly.[4] Endoscopic surveillance for BO is therefore recommended.[2]

In recent decades, the incidence of BO increased, which was accompanied by a marked increase in OAC incidence in the USA and Western Europe.[5-6] However, estimates of OAC incidence among patients with BO vary substantially.[7-10] Generally, gastrointestinal cancers account for 25% of all cancers and approximately 4.9% of all deaths worldwide.[11] Death rates of most cancers decreased in recent years in contrast to the 3% increase in death rates of all oesophageal cancer (both squamous cell as adenocarcinoma) among males.[11] The age- standardized mortality rate for oesophageal cancer overall is 5.1 per 100,000 persons.[6] The need for effective prevention of oesophageal cancer in general is therefore warranted, particularly given the low 5-year survival rate of 13%-17%.[12]

Several studies reported that use of non-steroidal anti-inflammatory drugs (NSAIDs), low-dose aspirin, statins and proton pump inhibitors (PPIs) may decrease the risk of OAC among BO patients.[13-21] However, these studies were based on small, selected samples of OAC cases. PPIs are considered standard care for symptom relief in patients with BO, thus it was suggested that PPIs may decrease the risk of progression to HGD or OAC.[20] Contrasting, other studies showed an increase in risk of OAC with PPI use, probably because the underlying treatment indication may be a risk factor for OAC rather than that PPIs are harmful for OAC among BO patients.[15 22] Nevertheless, one cannot directly assume that

PPIs, which are efficacious for treatment of erosive oesophagitis, will also be beneficial in the pathway from BO to OAC development. Two meta-analyses both including nine observational studies showed that the risk of oesophageal cancer[14] and high-grade dysplasia/oesophageal adenocarcinoma[23] among those who frequently use NSAIDs or aspirin was significantly lower compared to never users.[14] However, studies included in the earlier meta-analysis did not specifically include patients with BO. A pooled analysis on individual patient data confirmed the significant reduction in risk of OAC in BO patients with NSAID prescriptions.[24] Two case-control studies observed an association between use of NSAIDs[15] and statins[15 25] and the risk of OAC among BO patients. Generalization and extrapolation of results from the latter studies to the general population is, however, difficult as both studies were performed in US veterans.[15 25] Additionally there was no adjustment for important risk factors of OAC progression such as alcohol use and smoking.[15] Nevertheless, a recent systematic review and meta-analysis showed a risk reduction in development of oesophageal cancer in general and oesophageal adenocarcinoma among patients with BO who took statins.[26]

Causality of an apparent association is generally supported by a dose- and duration-relationship.[27] However, studies to date neither reported a clear exposure definition free of recall bias [13 16 24] nor conducted dose-duration analyses. Finally, concerns have been raised about publication bias of these studies on chemoprevention of OAC in BO patients.[18]

Thus, to which extent NSAIDs, low-dose aspirin, statins and PPIs may reduce the risk of oesophageal adenocarcinoma among BO patients in clinical practice remains unknown. Therefore, we conducted a matched case-control study to evaluate the risk of oesophageal adenocarcinoma among patients with BO associated with use of NSAIDs, low-dose aspirin, statins and PPIs.

MATERIALS AND METHODS

Data sources

Two European population-based general practice registries served as data sources: 1) The Health Improvement Network (THIN) from the United Kingdom (UK, 1996-2011)[28] and the 2) Integrated Primary Care Information database (IPCI) from the Netherlands (1996–2012).[29] Both databases contain prospectively collected data that represents real-life practice. In the UK and in NL, all citizens are registered with a general practitioner (GP), who acts as a gatekeeper to secondary and tertiary medical care. THIN collects anonymised data on more than 3 million active patients from over 400 participating general practices, IPCI contains over 1.5 million active patients from 340 practices. For each individual patient all relevant medical information from primary and secondary care, as well as additional information, including demographics and drug prescriptions, is documented in the medical record. Both data sources comply with European Union guidelines on the use of medical data for research.

THIN employs the READ clinical terminology system for coding medical diagnosis and symptoms[30], whereas IPCI uses the International Classification for Primary Care (ICPC).[31] Information on drug prescriptions is captured in THIN with the Multilex product dictionary and British National Formulary (BNF) codes, whereas in IPCI information on drug prescriptions is coded according to the World Health Organization's Anatomical Therapeutic Chemical (ATC) classification.[32] The Scientific and Ethical Advisory Boards of both databases approved the study. Identification of the source and study population has been described previously.[10]

Source population

The source population consisted of all subjects aged ≥18 years who contributed data to the database between 1st of January 1996 and 31st of December 2011 (THIN) or March 2013 (IPCI). At least one year of available data prior to study entry was required to assess patient's medical history for exclusion criteria and risk factors. Follow-up started on 1 January 1996, date of reaching 18 years of age, or the date that one year of valid data was accrued within the database, whichever came later. Follow-up ended on the date of occurrence of study outcome (OAC), date of transfer out of the general practitioner's practice, death, or last data drawn, whichever was earliest.

Definition of Barrett's oesophagus

Patients with BO were identified using diagnosis codes; in THIN using corresponding READ codes (**Appendix Table 1**).[30] In IPCI, each potential BO case was manually validated to confirm the histological diagnosis of BO and the date of first diagnosis or mentioning of BO in the clinical record. Patients were excluded if they had a history of oesophageal cancer anytime before BO diagnosis and if they had a history of gastric cancer within 6 months after BO diagnosis. In IPCI we could utilize free text from the medical record to assess the Barrett segment length and grade of dysplasia.

Definition of oesophageal adenocarcinoma

In THIN, OAC cases were identified by READ codes (**Appendix Table** 1). In IPCI, all patients with a record of ICPC codes D77.1 (malignant neoplasia of the oesophagus) and D77.0 (malignant neoplasia of the digestive tract—not specified), or with a record by free text search including word combinations of

'oesophagus' 'cancer', 'carcinoma', 'malignancy' or 'neoplasia' were identified. Similar to BO, all potential cases were manually validated for confirmation of the OAC diagnosis, date of first diagnosis and the type of carcinoma (squamous cell-, adeno-, or other types of carcinoma). Early cancer (high-grade dysplasia (HGD)) was identified in IPCI also, but could not be assessed in THIN.

We only considered incident HGD or OAC cases: i.e. if the date of diagnosis occurred after inclusion into the BO cohort and was at least 12 months after BO diagnosis. Cases occurring within 1 year from BO diagnosis were considered to be already existent at BO diagnosis date and in relation to the BO diagnostic work-up.

Cases and controls selection

Two nested case-control studies were conducted assessing the risk of OAC for use of four drugs (NSAIDs, PPIs, statins and low-dose aspirin); one including only OAC cases and a second case-control study including HGD cases from IPCI as well.

Cases were adults diagnosed with OAC ≥12 months after BO diagnosis, because cases occurring within one year of BO diagnosis were considered to be existent and related to BO diagnostic work-up (e.g. missed OAC at BO diagnosis). Index date was defined as date of first reporting of OAC diagnosis during follow-up. Controls were members of the incident BO cohort who did not develop OAC up to matching date. Controls were matched by incidence density sampling on age (± 5 years), sex, year of BO diagnosis (± 1 year), and database. We matched on year of BO diagnosis in order to account for any influence of guideline changes in endoscopic surveillance over calendar time.

Drug exposure

Drug exposures of interest included four drug groups: NSAIDs, PPIs, statins and low-dose aspirin. They were assessed in terms of outpatient prescriptions for NSAIDs (including high-dose aspirin, i.e. >325 mg/day), PPIs, statins and low-dose aspirin (up to 325 mg/day) from BO diagnosis until OAC diagnosis. In order to compare the OR of NSAIDs, PPIs and statins to other drugs, we considered another group of medications that served as control. Antidepressants (selective serotonin re-uptake inhibitors (SSRIs)) are currently not known to be either positively or negatively associated with OAC.

Duration of prescriptions was calculated based on the prescribed quantity and dosing regimen. As the most likely preventive effect of drugs on cancer progression is through a cumulative mechanism, we calculated all duration and defined daily dose (DDD) values from date of BO diagnosis until index date. Duration was classified according to never use (reference category), cumulative use of less than 1 month, between 1-12 months, > 12 months (or if applicable 1-2 years; 2-3 years and > 2 years). Considering that PPIs are indicated as treatment for BO patients, duration was classified as 0-6 months (reference category), 6-12 months, 1-2 years and > 2 years. Dose of exposure was classified using the ratio of prescribed daily dose compared to DDD using quartiles into categories (<0.8; 0.8-1.2; ≥1.2 DDD per day). As there is no DDD for low-dose aspirin, dose analysis was not performed for use of low-dose aspirin.

Potential confounders

We considered as potential confounders: concurrent diagnosis of oesophagitis or gastritis within 1 year before BO diagnosis; hiatal hernia; smoking habits (non-smoker, ex-smoker, current smoker) and alcohol abuse (never, current, past).

Statistical analyses

Baseline characteristics of cases and controls were described per database and compared using univariate conditional logistic regression.

To estimate the risk of HGD and OAC among patients with BO, matched and adjusted odds ratios (OR) with 95% confidence intervals (CIs) were calculated using conditional logistic regression for both databases separately and as a pooled analysis on patient-level pooled data.

Potential confounders were included in the adjusted analysis (ORa) if they resulted in a change of more than 10% of the initial estimate. Time since BO diagnosis was forced into the adjusted model.

Subsequent analyses included duration- and dose-analyses. The risk of OAC and HGD-OAC was also assessed for concomitant use of NSAIDs, low-dose aspirin, statins and/or PPIs. Use of PPIs only was considered as reference category considering that PPIs are standard therapy for BO.

All analyses were performed using SAS Cary, NC version 9.2.

Power Calculation

Given an exposure prevalence of NSAIDs of 30%, of statins of 22% or 36%, of PPIs of 87% or 52% and of low-dose aspirin of 25% among controls and a correlation of 0.5 between exposed and unexposed subjects, we have 80% power (with a type 1 error of 5%) to detect a true odds ratio of OAC of 0.34 for NSAIDs, around 0.38-0.40 for statins, around 0.32-0.45 for PPIs and 0.29 for low-dose aspirin which would be in concordance with previous studies.

RESULTS

Study population

From the source population of 7,570,765 subjects in UK and 1,496,276 subjects in NL we identified 13,696 and 1,438 incident BO cases, respectively. Males accounted for 63% (UK) and 62% (NL) of BO subjects. Mean age at BO diagnosis was 64.8 (SD: 13.8) years in UK and 61.2 (SD: 13.4) years in NL.

In UK, we identified 40 incident OAC cases within the BO cohort (0.3%) to whom we could match 656 controls. Median number of controls per case was 17 (interquartile range (IQR): 9-23). In NL we identified 5 incident OAC cases among the BO cohort (0.3%). These were matched to 76 control subjects, with a median of 5 controls per case (IQR: 4-6). In addition, we identified 12 HGD cases, resulting in a second case control set of 17 cases (5 OAC + 12 HGD) matched to 753 controls (median 44 controls; IQR: 6-61). **Figure 1** shows a flowchart of the study population. **Table 1** provides baseline characteristics of cases and controls. In the UK a larger proportion of cases had a BMI over 25 kg/m²; 68% of cases and 59% of controls. In NL, only for 1 case BMI within 1 year of OAC diagnosis was available (21.3 kg/ m²). Controls had a mean BMI of 28.7 kg/m² (SD 4.7) in NL. Presence of oesophagitis or gastritis at time of BO diagnosis was more often seen in controls than in cases. In UK, a hiatal hernia was more often present among cases, whereas the opposite was found in NL. In UK, OAC cases were more likely to be current smokers than controls (OR 3.3; 95%CI: 1.4-8.0), as seen in NL though not significantly. Mean time from BO diagnosis until OAC diagnosis was 4.2 (SD: 2.5) years in UK and 3.5 (SD: 0.8) years in NL.

Table 1. Baseline Characteristics of Oesophageal Adenocarcinoma Cases and High-grade Dysplasia cases in the United Kingdom and Netherlands.

		United Kingdom				The Netherlands HGD - OAC			
		OAC Case N (%)	Control N (%)	OR (95% CI)	P-value	HGD-OAC Case N (%)	HGD-OAC control N (%)	OR (95% CI)	P-value
	Total	40 (100)	656 (100)			17 (100)	753 (100)		
	OAC					5 (29.4)			
	HGD					12 (70.6)			
Sex	male	33 (82.5)	597 (91)			11 (65)	524 (70)		
	female	7 (17.5)	59 (9)			6 (35)	229 (30)		
Mean age at index date (SD)		71.2 (10.4)	70.2 (9.0)			68.8 (8.2)	66.4 (8.8)		
Age group (years)	< 50	1 (2.5)	14 (2.1)			0 (0)	17 (2.3)		
	51-65	8 (20)	149 (23)			6 (35)	338 (45)		
	66-80	25 (62.5)	434 (66)			10 (59)	364 (48)		
	> 80	6 (15)	59 (9)			1 (5.9)	34 (4.5)		
Body Mass Index (kg/m2) mean (SD)		27.7 (4.1)	26.9 (4)	1.1 (1.0-1.1)	0.210	28.9 (6.8)	26.4 (7.4)	1.1 (0.9-1.3)	0.500
BMI categories	18-25	10 (25)	202 (31)			1 (5.9)	85 (11)		
	<18	0 (0)	7 (1.1)	-	0.989	0 (0)	22 (2.9)	-	0.997
	>25-30	19 (47.5)	269 (41)	1.5 (0. 7-3.3)	0.329	2 (12)	156 (21)	1.3 (0.1-14.7)	0.995
	>30-35	7 (17.5)	89 (14)	1.8 (0.7-5.0)	0.246	0 (0)	73 (9.7)	-	0.995
	>35	1 (2.5)	31 (4.7)	0.8 (0.1-7.0)	0.866	1 (5.9)	14 (1.9)	6.1 (0.3-112.1)	0.993
	missing	3 (7.5)	58 (8.8)	1.0 (0.3-3.8)	0.992	13 (76)	403 (54)	2.0 (0.3-16.5)	0.994
Oesophagitis at BO diagnosis	no	39 (97.5)	629 (95.9)			14 (82)	525 (70)		
	yes	1 (2.5)	27 (4.1)	0.6 (0.1-4.7)	0.633	3 (18)	228 (30)	0.5 (0.1-1.8)	0.299
Gastritis at BO diagnosis	no	38 (95)	621 (94.7)			13 (76)	582 (77)		

		United Kingdom				The Netherlands HGD - OAC			
		OAC Case N (%)	Control N (%)	OR (95% CI)	P-value	HGD-OAC Case N (%)	HGD-OAC control N (%)	OR (95% CI)	P-value
	yes	2 (5)	35 (5.3)	1.2 (0.3-5.2)	0.808	4 (24)	171 (23)	1.5 (0.5-4.9)	0.516
Hiatal Hernia at BO diagnosis	no	33 (82.5)	579 (88.3)			8 (47)	268 (36)		
	yes	7 (17.5)	77 (11.7)	1.7 (0.7-4.0)	0.259	9 (53)	485 (64)	0.7 (0.2-2.0)	0.487
Excessive alcohol use	never	17 (42.5)	370 (56)	Ref		17 (100)	713 (94.7)	-	0.991
	current	22 (55)	276 (42)	**2.0 (1.0-3.0)**	**0.048**	(0)	40 (5.3)		
	past	1 (2.5)	10 (1.5)	2.8 (0.3-23.4)	0.345				
Smoking	never	14 (35)	322 (49)	Ref		9 (53)	380 (50.5)	Ref	
	current	9 (22.5)	70 (11)	**3.3 (1.4-8.0)**	**0.009**	8 (47)	373 (49.5)	1.5 (0.5-4.5)	0.443
	past	17 (42.5)	264 (40)	1.7 (0.8-3.7)	0.155				
Index year	1998	1 (2.5)	7 (1.1)			1 (5.9)	5 (0.7)		
	2000	1 (2.5)	12 (1.8)			1 (5.9)	4 (0.5)		
	2001	3 (7.5)	24 (3.7)			1 (5.9)	7 (0.9)		
	2002	2 (5)	10 (1.5)			2 (12)	9 (1.2)		
	2003	2 (5)	15 (2.3)			1 (5.9)	3 (0.4)		
	2004	4 (10)	94 (14)						
	2005	7 (17.5)	128 (20)						
	2006	1 (2.5)	20 (3)						
	2007	2 (5)	30 (4.6)			1 (5.9)	22 (2.9)		
	2008	6 (15)	107 (16)			1 (5.9)	66 (8.8)		
	2009	4 (10)	72 (11)			1 (5.9)	49 (6.5)		
	2010	4 (10)	85 (13)			2 (12)	163 (22)		
	2011	3 (7.5)	52 (7.9)			5 (29.4)	374 (50)		
	2012					1 (5.9)	51 (6.8)		

		United Kingdom				The Netherlands HGD - OAC			
		OAC Case N (%)	Control N (%)	OR (95% CI)	P-value	HGD-OAC Case N (%)	HGD-OAC control N (%)	OR (95% CI)	P-value
Helicobacter pylori infection	no	40 (100)	603 (91.9)	-	-	17 (100)	714 (94.8)	-	
	yes	0 (0)	53 (8.1)			0 (0)	39 (5.2)		

Drug exposure

Table 2 provides characteristics of drug use from BO diagnosis until index date for cases and controls per database. Statins were used by 30% and 0% of OAC cases; and by 36% and 22% of controls in UK and NL, respectively. PPIs were used by OAC cases for a mean of 4.1 years (UK) and 2.3 years (NL) and by controls for 2.9 years (UK) and 1.9 years (NL). SSRIs were used by 12.5% of OAC cases in UK for a mean duration of 1 year, and by 7.6% of controls for a mean duration of 1.7 years. Low-dose aspirin was used by 26% of BO subjects in UK and 6% of BO subjects in NL.

Risk of Oesophageal Adenocarcinoma

To estimate the risk of OAC with use of NSAIDs, PPIs, statins and low-dose aspirin a nested case-control study was conducted. From the adjusted model, on patient-level pooled data, exposure to NSAIDs and PPIs did not provide a significant decrease in the risk of OAC (**Table 3**), for statins a non-significant effect was seen (ORa 0.7; 95%CI: 0.4-1.5). This was seen in both databases separately as well (data not shown). For NSAID use, ORs ranged between 1.1 and 1.4 for all duration categories; regarding dose-analysis, no difference in risk was found between higher and lower dosages (**Table 4**). Although not significant, a dose-duration-response was seen for statins, with lower OR for longer duration of use compared to non-use of statins. Statin use ≥1.2 times higher compared to the recommended defined daily dose resulted in an OR of 0.7 (95%CI: 0.2-2.3). For PPIs an increase in OR was seen with prolonged duration, both in the matched and adjusted analyses. PPIs used at highest dose showed an OR for HGD-OAC of 0.9 (95% CI: 0.3-2.3). The ORs varied for duration categories of SSRIs. No dose-response was seen for SSRI use. Use of low-dose aspirin provided ORs below 1 for OAC for matched and adjusted analysis when considering the exposure at any time between BO diagnosis and OAC diagnosis; however the 95%

confidence limits still included the 1. When considering duration analysis, the adjusted model provided for the prolonged duration of use (> 1 year) an OR of 0.9 (95%CI 0.4-2.1).

Concomitant use of drugs of interest did not decrease the risk of OAC (**Table 5**) compared to use of PPIs only, probably due to the smaller number of cases.

Table 2 Exposure characteristics of cases and controls in United Kingdom and the Netherlands

		United Kingdom		Netherlands	
		OAC Case	**Control**	**HGD-OAC case**	**HGD-OAC control**
		N = 40	N = 656	N = 17	N = 753
NSAIDs	Exposed - N	11	148	2	102
	Mean duration of use in days (SD)	205 (373)	218 (348)	18 (4)	49 (111)
	Mean cumulative DDD (SD)	223 (393)	232 (383)	9 (2)	31 (79)
	Median duration of use in days (IQR)	40 (20-178)	56 (28-203)	18 (15-20)	15 (10-60)
	Median cumulative DDD (IQR)	40 (30-223)	56 (28-208)	9 (7-10)	10 (5-30)
Statins	Exposed – N	12	236	3	123
	Mean duration of use in days (SD)	648 (569)	996 (913)	570 (289)	409 (300)
	Mean cumulative DDD (SD)	466 (353)	1,000 (1,258)	560 (191)	383 (331)
	Median duration of use in days (IQR)	616 (109-966)	728 (350-1,386)	450 (360-900)	330 (180-629)
	Median cumulative DDD (IQR)	504 (110-775)	625 (243-1,248)	450 (450-780)	270 (158-480)
PPIs	Exposed – N	36	570	10	389
	Mean duration of use in days (SD)	1,500 (1,134)	1,071 (978)	615 (462)	442 (372)
	Mean cumulative DDD (SD)	1,425 (1,247)	1,060 (1,123)	576 (402)	661 (1,636)
	Median duration of use in days (IQR)	1,481 (644-2,017)	766 (392-1,458)	471 (240-1,020)	315 (180-630)
	Median cumulative DDD (IQR)	1,223 (644-1,772)	700 (364-1,428)	471 (300-719)	360 (180-840)
SSRIs	Exposed - N	5	50	0	15
	Mean duration of use in days (SD)	369 (280)	613 (705)	-	743 (669)
	Mean cumulative DDD (SD)	366 (283)	843 (1,430)	-	737 (670)
	Median duration of use in days (IQR)	252 (252-504)	381 (90-840)	-	600 (180-1,740)
	Median cumulative DDD (IQR)	252 (252-504)	339 (90-896)	-	596 (180-1,740)
Low-dose Aspirin	Exposed - N	10	173	1	47
	Mean duration of use in days (SD)	796 (606)	804 (733)	360	391 (301)
	Mean cumulative DDD (SD)*	-	-		-
	Median duration of use in days (IQR)	672 (448-1,344)	600 (280-1,096)		270 (180-540)
	Median cumulative DDD (IQR)	-	-		

Abbreviations: SD, standard deviation; DDD, defined daily dose; IQR, interquartile range; SSRIs, selective serotonin re-uptake inhibitors; NSAIDs, non-steroidal anti-inflammatory drugs; PPIs, proton pump inhibitors.
* Low-dose aspirin (≤325 mg/day) has no defined daily dose value

Table 3: Risk of Oesophageal Adenocarcinoma and High-grade Dysplasia-Oesophageal Adenocarcinoma by drug class by duration on data pooled on patient-level.

		OAC						HGD-OAC					
Exposure	**Duration category**	**OAC Case N (%)**	**OAC Control N (%)**	**ORmatched (95% CI)**	**P-value**	**ORadjusted* (95% CI)**	**P-value**	**Case N (%)**	**Control N (%)**	**ORmatched (95% CI)**	**P-value**	**ORadjusted* (95% CI)**	**P-value**
Total		**45 (100)**	**732 (100)**					**57 (100)**	**1,409 (100)**				
NSAID	None	32 (71)	566 (77)	Ref		Ref		44 (77)	1,159 (82)	Ref		Ref	
	Yes	13 (29)	166 (23)	1.3 (0.6-2.5)	0.492	1.2 (0.6-2.5)	0.532	13 (23)	250 (18)	1.0 (0.5-1.9)	1.000	0.9 (0.5-1.8)	0.876
	≤ 1 mo	6 (11)	65 (9)	1.4 (0.6-3.6)	0.454	1.4 (0.6-3.5)	0.471	6 (11)	121 (9)	1.1 (0.4-2.6)	0.882	1.0 (0.4-2.5)	0.967
	>1 mo - 1 yr	5 (9)	72 (10)	1.2 (0.4-3.1)	0.768	1.1 (0.4-3.0)	0.817	5 (9)	98 (7)	0.9 (0.3-2.4)	0.836	0.8 (0.3-2.3)	0.737
	>1 yr	2 (4)	29 (4)	1.2 (0.3-5.3)	0.837	1.1 (0.3-5.2)	0.859	2 (4)	31 (2)	1.1 (0.2-4.7)	0.934	1.0 (0.2-4.6)	0.970
Statins	None	33 (73)	479 (65)	Ref		Ref		42 (74)	1050 (75)	Ref		Ref	
	Yes	12 (27)	253 (35)	0.8 (0.4-1.5)	0.432	0.7 (0.4-1.5)	0.412	15 (26)	359 (25)	0.9 (0.5-1.7)	0.720	0.9 (0.5-1.7)	0.673
	≤ 1 mo	1 (2)	6 (1)	2.1 (0.2-20.4)	0.511	2.0 (0.2-20.1)	0.561	1 (2)	7 (0)	2.2 (0.2-20.6)	0.487	2.1 (0.2-20.5)	0.52
	>1 mo - 1 yr	3 (7)	62 (8)	0.9 (0.3-3.2)	0.908	1.0 (0.3-3.4)	0.971	4 (7)	128 (9)	0.9 (0.3-2.8)	0.914	1.0 (0.3-2.8)	0.951
	> 1 yr - 2 yrs	4 (9)	66 (9)	0.9 (0.3-2.7)	0.848	0.9 (0.3-2.6)	0.824	5 (9)	90 (6)	1.1 (0.4-2.9)	0.868	1.1 (0.4-2.8)	0.907
	> 2 yrs - 3 yrs	1 (2)	30 (4)	0.6 (0.1-4.9)	0.651	0.6 (0.1-4.7)	0.629	2 (4)	41 (3)	1.2 (0.3-5.3)	0.828	1.1 (0.2-4.9)	0.897
	> 3 yrs	3 (7)	89 (12)	0.5 (0.1-1.7)	0.259	0.5 (0.1-1.7)	0.239	3 (5)	93 (7)	0.5 (0.1-1.8)	0.276	0.5 (0.1-1.7)	0.253
PPIs	0 to ≤ 6 mo	5 (11)	103 (14)	Ref		Ref		11 (19)	450 (32)	Ref		Ref	
	Yes	40 (89)	629 (86)	1.1 (0.4-3.0)	0.814	1.1 (0.4-2.8)	0.911	46 (81)	959 (68)	1.0 (0.5-2.2)	0.917	0.9 (0.4-2.0)	0.855
	> 6 to ≤ 12 mo	6 (13)	169 (23)	1.9 (0.5-6.6)	0.502	2.0 (0.5-7.0)	0.299	7 (12)	158 (11)	1.7 (0.6-4.6)	0.293	1.7 (0.6-4.5)	0.312
	> 12 to ≤ 24 mo	9 (20)	151 (21)	1.8 (0.6-5.4)	0.672	1.7 (0.6-5.3)	0.328	10 (18)	227 (16)	1.7 (0.7-4.2)	0.255	1.6 (0.6-3.9)	0.326
	> 24 mo	5 (11)	162 (22)	2.1 (0.8-5.6)	0.476	1.9 (0.7-5.2)	0.207	27 (47)	377 (27)	1.7 (0.7-4.0)	0.204	1.5 (0.7-3.6)	0.327
SSRIs	None	40 (89)	679 (93)	Ref		Ref		52 (91)	1,344 (95)	Ref		Ref	
	Yes	5 (11)	53 (7)	1.7 (0.6-4.7)	0.281	1.7 (0.6-4.6)	0.310	5 (9)	65 (5)	1.6 (0.6-4.2)	0.356	1.5 (0.6-4.1)	0.390
	≤ 1 mo	0 (0)	3 (0)	-	0.992	-	0.992	0 (0)	3 (0)	-	0.988	-	0.988
	>1 mo - 1 yr	3 (7)	23 (3)	2.6 (0.7-9.2)	0.142	2.5 (0.7-8.9)	0.155	3 (5)	28 (2)	2.4 (0.7-8.6)	0.165	2.4 (0.7-8.4)	0.175
	>1 yr	2 (4)	27 (4)	1.2 (0.3-5.5)	0.778	1.2 (0.3-5.4)	0.815	2 (4)	34 (2)	1.1 (0.2-4.9)	0.888	1.1 (0.2-4.7)	0.931

		OAC						HGD-OAC					
Exposure	Duration category	OAC Case N (%)	OAC Control N (%)	ORmatched (95% CI)	P-value	ORadjusted* (95% CI)	P-value	Case N (%)	Control N (%)	ORmatched (95% CI)	P-value	ORadjusted* (95% CI)	P-value
Total		**45 (100)**	**732 (100)**					**57 (100)**	**1,409 (100)**				
Low-dose Aspirin	None	35 (78)	553 (76)	Ref		Ref		46 (81)	1,189 (84)	Ref		Ref	
	Yes	10 (22)	179 (24)	0.9 (0.4-1.8)	0.702	0.8 (0.4-1.8)	0.662	11 (19)	220 (16)	0.9 (0.4-1.9)	0.799	0.9 (0.4-1.8)	0.764
	≤ 6 mo	2 (4)	33 (5)	1.0 (0.2-4.2)	0.954	1.0 (0.2-4.3)	0.970	2 (4)	49 (3)	0.9 (0.2-3.7)	0.840	0.9 (0.2-3.8)	0.847
	>6 mo - 1 yr	0 (0)	26 (4)	-		-		1 (2)	36 (3)	-		-	
	>1 yr	8 (18)	120 (16)	1.0 (0.4-2.2)	0.920	0.9 (0.4-2.1)	0.844	8 (14)	135 (10)	0.9 (0.4-2.1)	0.867	0.9 (0.4-2.1)	0.805

Abbreviations: NSAID, non-steroidal anti-inflammatory drugs; PPIs, proton pump inhibitors; OAC, oesophageal adenocarcinoma; HGD, high-grade dysplasia; mo, months; yr, year.

* Adjusted for duration of follow-up since BO diagnosis.

Cumulative use of drugs considered continuously (OR represents the change per day additional use)

Table 4. Risk of Oesophageal Adenocarcinoma and High-grade Dysplasia-Oesophageal Adenocarcinoma by drug class by daily dose on data pooled on patient-level

		OAC only				HGD-OAC			
Drug exposure	**Dose category**	**Case N(%)**	**Control N(%)**	**ORmatched (95% CI)**	**P-value**	**Case N(%)**	**Control N(%)**	**ORmatched (95% CI)**	**P-value**
Total		45 (100)	732 (100)			57 (100)	1,409 (100)		
NSAID	None	32 (71)	566 (77)	Ref	-	44 (77)	1,159 (82)	Ref	-
	<0.8 DDD per day	3 (7)	39 (5)	1.1 (0.3-3.7)	0.909	3 (5)	107 (8)	0.6 (0.2-2.2)	0.475
	≥0.8 - < 1.2 DDD per day	4 (9)	74 (10)	0.9 (0.3-2.5)	0.783	4 (7)	84 (6)	0.8 (0.3-2.3)	0.633
	≥1.2 DDD per day	6 (13)	53 (7)	2.2 (0.8-5.6)	0.111	6 (11)	59 (4)	1.9 (0.8-5.0)	0.160
Statin	None	33 (73)	479 (65)	Ref	-	42 (74)	1,050 (75)	Ref	-
	<0.8 DDD per day	8 (18)	126 (17)	0.9 (0.4-2.2)	0.880	9 (16)	174 (12)	1.0 (0.5-2.1)	0.959
	≥0.8 - < 1.2 DDD per day	1 (2)	49 (7)	0.3 (0.05-2.6)	0.305	2 (4)	62 (4)	0.7 (0.2-3.1)	0.637
	≥1.2 DDD per day	3 (7)	78 (11)	0.7 (0.2-2.3)	0.519	4 (7)	123 (9)	0.8 (0.3-2.4)	0.731
PPI	None	5 (11)	103 (14)	Ref	-	11 (19)	450 (32)	Ref	-
	<0.8 DDD per day	9 (20)	168 (23)	0.9 (0.3-3.0)	0.914	11 (19)	196 (14)	1.1 (0.4-2.8)	0.910
	≥0.8 - < 1.2 DDD per day	23 (51)	315 (43)	1.2 (0.4-3.4)	0.723	27 (47)	454 (32)	1.1 (0.5-2.6)	0.768
	≥1.2 DDD per day	8 (18)	146 (20)	1.1 (0.4-3.6)	0.822	8 (14)	309 (22)	0.9 (0.3-2.3)	0.813
SSRI	None	40 (89)	679 (93)	Ref	-	52 (91)	1,344 (95)	Ref	-
	<0.8 DDD per day	1 (2)	8 (1)	3.0 (0.4-25.4)	0.317	1 (2)	8 (1)	3 (0.3-25.1)	0.321
	≥0.8 - < 1.2 DDD per day	4 (9)	32 (4)	2.3 (0.7-7.1)	0.149	4 (7)	44 (3)	2.0 (0.7-6.0)	0.218
	≥1.2 DDD per day	0 (0)	13 (2)	-	0.987	0 (0)	13 (1)	-	0.987

Abbreviations: NSAID, non-steroidal anti-inflammatory drugs; PPIs, proton pump inhibitors; SSRIs, selective serotonin re-uptake inhibitors; DDD, defined daily dose; OAC, oesophageal adenocarcinoma; HGD, high-grade dysplasia.

Table 5. Risk of Oesophageal Adenocarcinoma and High-grade Dysplasia-Oesophageal Adenocarcinoma for concomitant drug exposure of NSAIDs, low-dose aspirin, statins and PPIs.

	OAC only						HGD-OAC					
Drug exposure#	**Case N (%)**	**Control N (%)**	**ORmatched (95% CI)**	**P-value**	**ORadj model* (95% CI)**	**P-value**	**Case N (%)**	**Control N (%)**	**ORmatched (95% CI)**	**P-value**	**ORadj model* (95% CI)**	**P-value**
Total	45 (100)	732 (100)					57 (100)	1,409 (100)				
PPI only	19 (42)	284 (39)	Ref	-	Ref	-	22 (39)	441 (31)	Ref	-	Ref	-
No NSAID or LDA or statin or PPI	3 (7)	65 (9)	0.9 (0.2-3.2)	0.837	0.9 (0.3-3.4)	0.919	9 (16)	407 (29)	1.0 (0.4-2.4)	0.947	1.1 (0.4-2.8)	0.839
NSAID + PPI	6 (13)	72 (10)	1.2 (0.5-3.2)	0.700	1.1 (0.4-3.0)	0.773	6 (11)	124 (9)	0.9 (0.4-2.4)	0.898	0.9 (0.3-2.2)	0.774
Statin + PPI	5 (11)	85 (12)	1.0 (0.4-2.9)	0.963	1.0 (0.3-2.8)	0.988	7 (12)	143 (10)	1.2 (0.5-3.1)	0.630	1.2 (0.5-3.0)	0.674
Low-dose aspirin + PPI	3 (7)	30 (4)	1.4 (0.4-5.5)	0.597	1.3 (0.4-5.2)	0.655	3 (5)	42 (3)	1.3 (0.4-4.9)	0.691	1.2 (0.3-4.7)	0.742
LDA + PPI + Statin							2 (4)	104 (7)	0.4 (0.1-1.7)	0.202	0.4 (0.1-1.7)	0.198
NSAID + LDA + Statin + PPI	4 (9)	41 (6)	1.2 (0.4-3.8)	0.744	1.2 (0.4-3.8)	0.760	4 (7)	43 (3)	1.2 (0.4-3.9)	0.727	1.2 (0.4-3.8)	0.745

Abbreviations: NSAID, non-steroidal anti-inflammatory drugs; PPIs, proton pump inhibitors; LDA, low-dose aspirin OAC, oesophageal adenocarcinoma; HGD, high-grade dysplasia.

* Adjusted for duration of follow-up since BO diagnosis.

Numbers do not add up due to drug exposure categories with only one exposed case are not shown in the Table.

Risk of High-Grade Dysplasia or Oesophageal Adenocarcinoma

In NL we were able to retrieve HGD cases as well. When including these in the case definition, the effects were attenuated but in the same direction as the case-control study including OAC cases only. There was no significant decrease in the risk of HGD-OAC for exposure to NSAIDs, statins, PPIs and low-dose aspirin in the adjusted analysis (**Table 3**).For NSAIDs, the OR increased with use of higher dosages (**Table 4**). Again, for statins a duration-response relationship with the longest duration yielding the lowest ORa (0.5; 95% CI: 0.1-1.7) and an inverse association with increasing dose was observed, though none significant. For low-dose aspirin, PPI and SSRI use, no dose-response effects were shown.

The risk of HGD-OAC was 13% lower for concomitant use of NSAIDs+PPIs (ORa 0.9; 95%CI:0.3-2.2) (**Table 5**). None of the associations were statistically significant.

DISCUSSION

In this population-based case-control study nested within a cohort of Barrett's oesophagus patients, statin use may decrease the risk of both oesophageal adenocarcinoma and high-grade dysplasia by up to 50%. PPIs did not reduce the risk of HGD and OAC, however only when used at highest dose (e.g. at least 1.2 times the recommended daily dose) a non-significant reduction may be present. In this unselected group of BO patients use of low-dose aspirin or NSAIDs was not associated with a decrease in risk of OAC. This is the first population-based study that looked at the preventive effect of these four different drugs used individually and also concomitantly.

The mechanism of OAC-prevention is possibly related to inhibition of cyclo-oxygenase (COX)-2 production. Elevated levels of COX-2 in oesophageal epithelial cells have been observed in BO, and noted to increase with disease progression from BO to OAC.[33] In experimental studies, COX-2 inhibitors inhibited the growth of BO cells, potentially through suppression of basic fibroblast growth factor.[34] Another study confirmed that the end product of COX-2 conversion (prostaglandin E2) is reduced in BO patients without high-grade dysplasia when using esomeprazole combined with higher doses (up to 325 mg/day) of cardiovascular aspirin.[35]

Statins exert anti-neoplastic properties in several ways. By inhibition of the 3-hydroxy-3-methylglutanyl coenzyme A (HMG-CoA) reductase enzyme, subsequent modulation of growth signal transduction, cellular proliferation and cell death is achieved, which affects different organs.[36] Particularly, in OAC cells statins inhibit cell proliferation and induce apoptosis [37] and limit the metastatic potential by reducing intracellular adhesion molecules.[38] However, statins also inhibit COX-2 expression in BO cells.[39]

Contrasting to other studies, we did not observe a significant preventive effect of NSAIDs, low-dose aspirin and statins with respect to the risk of HGD-OAC. [13-14 24 40] Based on the biological mechanisms, combined use of statins and NSAIDs or statins with low-dose aspirin may be expected to result in a greater risk reduction compared to either drug alone. We did not observe that NSAIDs or low-dose aspirin with statins combined resulted in a significant risk reduction of OAC. This may be due to several reasons. Firstly, despite our large BO cohort the number of identified cases was smaller. Although we may have not have identified all potential OAC cases from the database, in a case-control study this is not necessary to obtain unbiased estimates. However, it limited the power of the study and resulted in statistically non-significant results. Particularly for assessment of concomitant drug exposure we did not reach statistical significance due to the lack of power, though this was not the primary aim of the study. Our nesting cohort included all incident BO subjects from the general population and by matching on duration since BO diagnosis and excluding prevalent BO subjects, we removed any effect of selective survival bias, disease severity[41] or time window bias [42]; as those BO subjects with a longer follow-up are more likely to develop HGD or OAC. By doing so, observing any spurious associations was avoided. Secondly, we mitigated against immortal time bias[43] by defining the exposure period from BE diagnosis till matching date, and thus avoiding an overestimation of the preventive effect. The estimates from our study are likely more generalizable to the daily clinical practice in the general population, including also less severe BO subjects, i.e. those with a shorter BO segment. A potential preventive effect of NSAIDs might therefore be only observed within selected high-risk subgroups.

Secondly, the inability to show a significant decrease in HGD and OAC risk for drug use may be explained by the distinct exposure definition that we applied. Contrasting with others [13 40], we classified exposure cumulatively and performed dose-duration-analyses rather than assessing drug exposure at a single moment. This however, also limited the analyses by creating multiple exposure categories. Drug

exposure changes over time especially in the long time to develop cancer. Assessment of exposure on a fixed moment will result in bias that exaggerates the effect downwards; showing a protective effect while actually it has no effect.[42] A pooled analysis of observational studies demonstrated an inverse association between the risk of HGD-OAC and use of NSAIDs.[24] A prospective cohort study also showed a decreased hazard ratio of HGD-OAC for use of NSAIDs and statins, however the study results were influenced by immortal time bias.[17 44] In that study the majority of cases included HGD cases. In line with the other Dutch study [17], when we included HGD cases the risk of HGD-OAC was lower than including OAC cases only. Possibly the preventive effect is achieved in premalignant stage of dysplasia-development rather than of adenocarcinoma. It is however difficult to disentangle drug exposure effects in three different risk periods: induction (dysplasia), latent (between dysplasia and cancer) and disease period (cancer). Ideally, this requires knowledge on exact timing of the first aberrant Barrett's cell; and subsequent stages.

Third explanation for not observing a preventive effect may be the exposure prevalence. Regarding NSAID exposure prevalence, we could not capture over-the-counter use of NSAIDs. During the study period NSAIDs and PPIs were reimbursable in the Netherlands and United Kingdom, and thus we assume that over-the-counter use of NSAIDs and PPIs did not confound the results to a great extent. Prevalence of PPI (81%) and statin (26%) exposure in our study is however comparable to other studies and is therefore unlikely to have limited our power.[17 45]

A large prospective US cohort study showed a tremendous protective effect of NSAIDs on OAC-risk.[40] However, NSAID exposure was assessed in a personal interview and classified very broadly by NSAIDs use at least once a week for 6 months.[40] If the preventive effect of NSAIDs would be as high as reported (up to 80%), a duration and dose response effect is to be expected. This study failed to demonstrate an inverse association between duration of NSAID use and the risk of OAC. In fact, the opposite was observed; the most protective effect was seen for the shortest duration [40], contradicting a

causal association.[27 46] A pooled analysis also couldn't demonstrate that prolonged duration of NSAID use was associated with a lower risk of OAC.[24] Additionally, heterogeneity between studies was observed [24], which emphasizes the controversy around clinically effective chemoprevention with NSAIDs.

The preventive effect of statins is shown in several studies[13 17], yielding a risk reduction of OAC up to 48% for statin use >1 year.[15] However, in a meta-analysis the risk reduction of OAC among BO patients was only seen when studies were included that assessed drug exposure by patient interview, which may be prone to recall bias, whereas the risk reduction was not significant including studies that assessed drug exposure by use of prescription/dispensing data in electronic medical records.[26] Also for statins the most pronounced effect was seen when HGD was included.[16] Results from the latter study should be interpreted with caution as drug exposure was classified by self-report as 'ever' instead of a duration classification. A recent case-control study using a GP database from the UK, showed that statins may also decrease the risk of OAC and oesophagogastric junctional adenocarcinoma in the general population.[47] The chemopreventive action of statins was more pronounced when combined with low-dose aspirin in a previous study.[13] It could be that the preventive effect of statins is explained by other risk factors common to statin users and patients with OAC; such as cardiovascular risk factors or lifestyle changes: smoking, exercise and weight.[47] Also it may be that BO subjects died from vascular diseases rather than of cancer-related causes or before HGD or OAC developed.[48] In our study statin users were less likely to be current smokers, were of older age and more males. However, whether lifestyle changes due to co morbid cardiovascular diseases and initiating statin therapy may have resulted in healthier behavior and subsequent OAC risk reduction is open to debate.

Strengths of the current study include the scale and setting by combining healthcare data from two European countries with comparable GP databases and applying a common study protocol and drug exposure definition. The nested-case control design in a well-defined population representing the general population minimized selection bias. Whereas previous studies may have suffered from recall bias or the lack of detailed drug prescription data, we were able to estimate the risk of HGD and OAC within BO subjects during to drug use in the general population. Although our analysis may be limited by the small number of cases in the duration- and dose-analyses, partly due to the fact that we only included incident cases (diagnosed ≥1 year after BO diagnosis), our study is unlikely to suffer from biases (immortal time bias, time window bias) and confounding (disease severity) by matching on important risk factors. Matched and adjusted analyses were in line with each other suggesting that there was little confounding.

Limitation of the study is the lack of detailed pathology information on the Barrett segment length and grade of dysplasia, as is current practice for risk stratification of BO subjects. This may have resulted in misclassification of BO and OAC. However, the 1-year risk of OAC after BO diagnosis, excluding OAC cases within 1 year after BO diagnosis, was 0.086% (95% CI: 0.04–0.17) in the current study[10], which is similar to other population-based studies.[4 49-50] Because we could not verify the diagnosis of Barrett's oesophagus against a clinical pre-specified standard and did not review biopsy specimens, it is also possible that we inadvertently included patients at very low risk of developing OAC. In the Dutch database we could search through the medical records and noted that 8% had a segment length < 2cm, 13.7% between 2 and 3 cm, 11.8% longer than 3 cm, whereas for 60% of BO controls the length was not mentioned. Regarding the grade of dysplasia at time of Barrett's oesophagus diagnosis, 45% of controls had no dysplasia; there was low grade dysplasia in 6% of BO subjects, indefinite for dysplasia in 1.8%, whereas no information on dysplasia grade was available in 46% of controls. Of the cases that developed HGD or EAC, 24% had a prior histology report of low-grade dysplasia. In the Dutch

database we could utilize all free text entered in the medical record, enabling to look for more detailed information in clinical letters, resulting in higher proportion of risk factors, such as presence of oesophagitis and a hiatal hernia at time of BO diagnosis as compared to the UK database in which we relied on diagnosis codes. We tried to address confounding-by-indication and time-window bias by matching on age, sex and year of BO diagnosis.[42] This is seen by the fact that individual risk factors did not increase the OAC risk and adjustment for these confounders did not change the estimate by ≥10%. The observation that PPIs appear to increase the risk of OAC is explained by the treatment indication being a risk factor for OAC; reverse causation and the phenomenon of 'channeling' where high-risk patients are being prescribed PPIs whereas low-risk patients not or in lower dose, [15 22 47 51-52] a phenomenon often seen with PPIs and upper gastrointestinal bleeding.[53] It could also be that the effect of PPIs is apparent after minimally 2 years of use [15 20] an observation which was not significant in our study.

In conclusion, in this population-based nested case-control study use of NSAIDs, PPIs, low-dose aspirin or statins did not reduce the risk of high-grade dysplasia and oesophageal adenocarcinoma among patients with Barrett's oesophagus. These findings indicate that for an unselected group of patients with Barrett's oesophagus chemoprevention by use of drug to reduce progression should not be considered directly as routine care.

Acknowledgement section

Specific author contributions:

Gwen MC Masclee: study concept and design; acquisition of data; analysis and interpretation of data; statistical analysis; drafting of the manuscript.

Preciosa M Coloma: study concept and design; interpretation of data; drafting of the manuscript.

Manon CW Spaander: critical revision of the manuscript for important intellectual content.

Ernst J Kuipers: analysis and interpretation of data; critical revision of the manuscript for important intellectual content

Miriam CJM Sturkenboom: study concept and design; acquisition of data; analysis and interpretation of data; critical revision of the manuscript for important intellectual content; obtained funding; study supervision

All authors approved the final version of the manuscript.

Authors' declaration of competing interests:

- GMCM, PMC, MCWS do not have any conflict of interest.
- EJK has since completion of this research started working for the medical board of Erasmus University Medical Center.

MCJMS is coordinating a research group that has unconditional research grants from Pfizer, Novartis, Lilly, none related to this research.

Declaration of funding interests: None.

Data sharing statement: No additional data are available.

REFERENCES

1. Spechler SJ, Souza RF. Barrett's esophagus. *N Engl J Med* 2014;371(9):836-45.

2. Wang KK, Sampliner RE. Updated guidelines 2008 for the diagnosis, surveillance and therapy of Barrett's esophagus. *Am J Gastroenterol* 2008;103(3):788-97.

3. Solaymani-Dodaran M, Logan RF, West J*, et al.* Risk of oesophageal cancer in Barrett's oesophagus and gastro-oesophageal reflux. *Gut* 2004;53(8):1070-4.

4. de Jonge PJ, van Blankenstein M, Looman CW*, et al.* Risk of malignant progression in patients with Barrett's oesophagus: a Dutch nationwide cohort study. *Gut* 2010;59(8):1030-6.

5. Desai TK, Krishnan K, Samala N*, et al.* The incidence of oesophageal adenocarcinoma in non-dysplastic Barrett's oesophagus: a meta-analysis. *Gut* 2012;61(7):970-6.

6. Globocan 2008 Worldwide Cancer Incidence M, Prevalence and Disability-adjusted life years (DALYs). Accessed at 11th July 2014.

7. Yousef F, Cardwell C, Cantwell MM*, et al.* The incidence of esophageal cancer and high-grade dysplasia in Barrett's esophagus: a systematic review and meta-analysis. *Am J Epidemiol* 2008;168(3):237-49.

8. Sikkema M, de Jonge PJ, Steyerberg EW*, et al.* Risk of esophageal adenocarcinoma and mortality in patients with Barrett's esophagus: a systematic review and meta-analysis. *Clin Gastroenterol Hepatol* 2010;8(3):235-44; quiz e32.

9. Rastogi A, Puli S, El-Serag HB*, et al.* Incidence of esophageal adenocarcinoma in patients with Barrett's esophagus and high-grade dysplasia: a meta-analysis. *Gastrointest Endosc* 2008;67(3):394-8.

10. Masclee GM, Coloma PM, de Wilde M*, et al.* The incidence of Barrett's oesophagus and oesophageal adenocarcinoma in the United Kingdom and the Netherlands is levelling off. *Aliment Pharmacol Ther* 2014;39(11):1321-30.

11. WHO Global Health Observatory Data Repository. Cause-specific mortality, 2008: WHO region by country. Accessed 18th of October 2013. Available at: http://apps.who.int/gho/data/node.main.887?lang=en

12. Cancer Research UK. Oesophageal cancer survival statistics. Accessed 18th of October 2013. Available at: http://www.cancerresearchuk.org/cancer-info/cancerstats/types/oesophagus/survival/#Trends.

13. Beales IL, Vardi I, Dearman L. Regular statin and aspirin use in patients with Barrett's oesophagus is associated with a reduced incidence of oesophageal adenocarcinoma. *Eur J Gastroenterol Hepatol* 2012;24(8):917-23.

14. Corley DA, Kerlikowske K, Verma R*, et al.* Protective association of aspirin/NSAIDs and esophageal cancer: a systematic review and meta-analysis. *Gastroenterology* 2003;124(1):47-56.

15. Nguyen DM, Richardson P, El-Serag HB. Medications (NSAIDs, statins, proton pump inhibitors) and the risk of esophageal adenocarcinoma in patients with Barrett's esophagus. *Gastroenterology* 2010;138(7):2260-6.

16. Kantor ED, Onstad L, Blount PL*, et al.* Use of statin medications and risk of esophageal adenocarcinoma in persons with Barrett's esophagus. *Cancer Epidemiol Biomarkers Prev* 2012;21(3):456-61.

17. Kastelein F, Spaander MC, Biermann K*, et al.* Nonsteroidal anti-inflammatory drugs and statins have chemopreventative effects in patients with Barrett's esophagus. *Gastroenterology* 2011;141(6):2000-8; quiz e13-4.

18. Abnet CC, Freedman ND, Kamangar F*, et al.* Non-steroidal anti-inflammatory drugs and risk of gastric and oesophageal adenocarcinomas: results from a cohort study and a meta-analysis. *Br J Cancer* 2009;100(3):551-7.

19. Rothwell PM, Fowkes FG, Belch JF*, et al.* Effect of daily aspirin on long-term risk of death due to cancer: analysis of individual patient data from randomised trials. *Lancet* 2011;377(9759):31-41.

20. Singh S, Garg SK, Singh PP*, et al.* Acid-suppressive medications and risk of oesophageal adenocarcinoma in patients with Barrett's oesophagus: a systematic review and meta-analysis. *Gut* 2013.

21. Sivarasan N, Smith G. Role of aspirin in chemoprevention of esophageal adenocarcinoma: a meta-analysis. *J Dig Dis* 2013;14(5):222-30.

22. Garcia Rodriguez LA, Lagergren J, Lindblad M. Gastric acid suppression and risk of oesophageal and gastric adenocarcinoma: a nested case control study in the UK. *Gut* 2006;55(11):1538-44.

23. Zhang S, Zhang XQ, Ding XW*, et al.* Cyclooxygenase inhibitors use is associated with reduced risk of esophageal adenocarcinoma in patients with Barrett's esophagus: a meta-analysis. *Br J Cancer* 2014;110(9):2378-88.

24. Liao LM, Vaughan TL, Corley DA*, et al.* Nonsteroidal anti-inflammatory drug use reduces risk of adenocarcinomas of the esophagus and esophagogastric junction in a pooled analysis. *Gastroenterology* 2012;142(3):442-52 e5; quiz e22-3.

25. Nguyen T, Khalaf N, Ramsey D*, et al.* Statin Use is Associated with a Decreased Risk of Barrett's Esophagus. *Gastroenterology* 2014;147(2):314-23.

26. Singh S, Singh AG, Singh PP*, et al.* Statins are associated with reduced risk of esophageal cancer, particularly in patients with Barrett's esophagus: a systematic review and meta-analysis. *Clin Gastroenterol Hepatol* 2013;11(6):620-9.

27. Rothman KJ, Greenland S, Lash TL. Causation and Causal Inference. *Modern Epidemiology*. 3rd ed. Philadelphia: Lippincott Williams & Wilkins, 2008.

28. Lewis JD, Schinnar R, Bilker WB*, et al.* Validation studies of the health improvement network (THIN) database for pharmacoepidemiology research. *Pharmacoepidemiol Drug Saf* 2007;16(4):393-401.

29. Vlug AE, van der Lei J, Mosseveld BM*, et al.* Postmarketing surveillance based on electronic patient records: the IPCI project. *Methods Inf Med* 1999;38(4-5):339-44.

30. Booth N. What are the Read Codes? *Health Libr Rev* 1994;11(3):177–82.

31. Lamberts H, Wood M, Hofmans-Okkes IM. International primary care classifications: the effect of fifteen years of evolution. *Fam Pract* 1992;9(3):330-9.

32. WHO Collaborating Centre for Drug Statistics Methodology. Guidelines for ATC classification and DDD assignment. Available at: http://www.whocc.no/atcddd/. (accessed April 4, 2013).

33. Wilson KT, Fu S, Ramanujam KS*, et al.* Increased expression of inducible nitric oxide synthase and cyclooxygenase-2 in Barrett's esophagus and associated adenocarcinomas. *Cancer Res* 1998;58(14):2929-34.

34. Buttar NS, Wang KK, Anderson MA*, et al.* The effect of selective cyclooxygenase-2 inhibition in Barrett's esophagus epithelium: an in vitro study. *J Natl Cancer Inst* 2002;94(6):422-9.

35. Falk GW, Buttar NS, Foster NR*, et al.* A combination of esomeprazole and aspirin reduces tissue concentrations of prostaglandin E(2) in patients with Barrett's esophagus. *Gastroenterology* 2012;143(4):917-26 e1.

36. Lochhead P, Chan AT. Statins and colorectal cancer. *Clin Gastroenterol Hepatol* 2013;11(2):109-18; quiz e13-4.

37. Ogunwobi OO, Beales IL. Statins inhibit proliferation and induce apoptosis in Barrett's esophageal adenocarcinoma cells. *Am J Gastroenterol* 2008;103(4):825-37.

38. Sadaria MR, Reppert AE, Yu JA*, et al.* Statin therapy attenuates growth and malignant potential of human esophageal adenocarcinoma cells. *J Thorac Cardiovasc Surg* 2011;142(5):1152-60.

39. Konturek PC, Burnat G, Hahn EG. Inhibition of Barret's adenocarcinoma cell growth by simvastatin: involvement of COX-2 and apoptosis-related proteins. *J Physiol Pharmacol* 2007;58 Suppl 3:141-8.

40. Vaughan TL, Dong LM, Blount PL, *et al.* Non-steroidal anti-inflammatory drugs and risk of neoplastic progression in Barrett's oesophagus: a prospective study. *Lancet Oncol* 2005;6(12):945-52.

41. Delgado-Rodriguez M, Llorca J. Bias. *J Epidemiol Community Health* 2004;58(8):635-41.

42. Suissa S, Dell'aniello S, Vahey S, *et al.* Time-window bias in case-control studies: statins and lung cancer. *Epidemiology* 2011;22(2):228-31.

43. Suissa S. Immortal time bias in pharmaco-epidemiology. *Am J Epidemiol* 2008;167(4):492-9.

44. Azoulay L, Suissa S. Immortal person-time bias in relation to the use of nonsteroidal anti-inflammatory drugs and statins in the prevention of esophageal cancer in patients with Barrett's esophagus. *Gastroenterology* 2012;142(5):e20-1; author reply e21.

45. Kastelein F, Spaander MC, Steyerberg EW, *et al.* Proton pump inhibitors reduce the risk of neoplastic progression in patients with Barrett's esophagus. *Clin Gastroenterol Hepatol* 2013;11(4):382-8.

46. Rothman KJ, Greenland S. Causation and causal inference in epidemiology. *Am J Public Health* 2005;95 Suppl 1:S144-50.

47. Alexandre L, Clark AB, Bhutta HY, *et al.* Statin Use is Associated With Reduced Risk of Histologic Subtypes of Esophageal Cancer: a Nested Case-Control Analysis. *Gastroenterology* 2014 146(3):661-68.

48. Moayyedi P, Burch N, Akhtar-Danesh N, *et al.* Mortality rates in patients with Barrett's oesophagus. *Aliment Pharmacol Ther* 2008;27(4):316-20.

49. Bhat S, Coleman HG, Yousef F, *et al.* Risk of malignant progression in Barrett's esophagus patients: results from a large population-based study. *J Natl Cancer Inst* 2011;103(13):1049-57.

50. Hvid-Jensen F, Pedersen L, Drewes AM, *et al.* Incidence of adenocarcinoma among patients with Barrett's esophagus. *N Engl J Med* 2011;365(15):1375-83.

51. Strom BL, Kimmel SE, editors. *Textbook of Pharmacoepidemiology*. Chichester: John Wiley & Sons, 2006.

52. Hvid-Jensen F, Pedersen L, Funch-Jensen P*, et al.* Proton pump inhibitor use may not prevent high-grade dysplasia and oesophageal adenocarcinoma in Barrett's oesophagus: a nationwide study of 9883 patients. *Aliment Pharmacol Ther* 2014.

53. Masclee GM, Valkhoff VE, Coloma PM*, et al.* Risk of Upper Gastrointestinal Bleeding from Different Drug Combinations. *Gastroenterology* 2014. doi: 10.1053/j.gastro.2014.06.007;147(4):784-92.e9, quiz e13-4.

Figure Legends

Figure 1: Flowchart of Barrett's oesophagus and Oesophageal Adenocarcinoma cases in the United Kingdom and the Netherlands.

BO, Barrett's oesophagus; OAC, oesophageal adenocarcinoma; HGD, high-grade dypslasia.

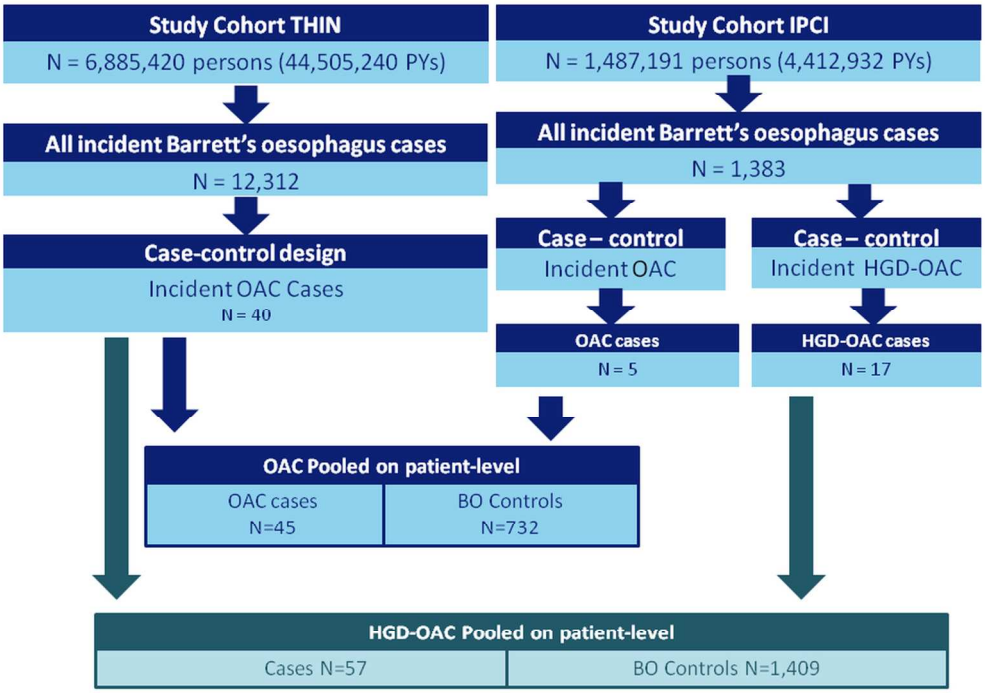

Figure 1: Flowchart of Barrett's oesophagus and Oesophageal Adenocarcinoma cases in the United Kingdom and the Netherlands.
BO, Barrett's oesophagus; OAC, oesophageal adenocarcinoma; HGD, high-grade dypslasia.
119x90mm (300 x 300 DPI)

Appendix

Appendix Table 1: Corresponding Read codes for identification of Barrett's oesophagus and oesophageal adenocarcinoma in THIN database.

READ code	Description of code		READ code	Description of code	Event
J101611	Barrett's oesophagus				BO
J102500	Barrett's ulcer of oesophagus				BO
J10y600	Barrett's oesophagus				BO
B102.00	Malignant neoplasm of abdominal oesophagus				Specific algorithm OAC
B105.00	Malignant neoplasm of lower third oesophagus				Specific algorithm OAC
B106.00	Malignant neoplasm, overlapping lesion of oesophagus				Specific algorithm OAC
B10y.00	Malignant neoplasm of other specified part of oesophagus				Specific algorithm OAC
B10z.00	Malignant neoplasm of oesophagus NOS				Specific algorithm OAC
B110100	Malignant neoplasm of gastro-oesophageal junction of stomach				Specific algorithm OAC
B110111	Malignant neoplasm of gastro-oesophageal junction				Specific algorithm OAC
B10..00/B10z.11	Malignant neoplasm of oesophagus / Oesophageal cancer	Combined with:	BBB2.00	Adenocarcinoma with squamous metaplasia	Unspecific algorithm OAC *
			BB57.00	Adenocarcinoma, intestinal type	
			BB53.00	Adenocarcinoma, metastatic, NOS	
			BB5..11	Adenocarcinomas	
			BB5z.00	Adenoma or adenocarcinoma NOS	

BO, Barrett's oesophagus; OAC, oesophageal adenocarcinoma.

* Recording of the combination of the two codes should have occurred within 1 year of each other.

BMJ Open: first published as 10.1136/bmjopen-2014-006640 on 29 January 2015. Downloaded from

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 Included in abstract.
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found Included in abstract. We found in a nested case-control study using primary care data from the United Kingdom and the Netherlands that statins may decrease the risk of oesophageal adenocarcinoma (OAC) and high-grrade dysplasia (HGD) among subjects with Barrett's oesophagus (BO). Proton pump inhibitors (PPIs) may reduce the risk of OAC-HGD when used at highest dose, while non-steroidal anti-inflammatory drugs (NSAIDs) did not decrease the risk of OAC-HGD.
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported Several studies reported that use of non-steroidal anti-inflammatory drugs (NSAIDs), statins and proton pump inhibitors (PPIs) may decrease the risk of OAC among BO patients. However, these studies were based on small, selected samples of OAC cases and were affected by bias and confounding. A meta-analysis including nine observational studies, (2 cohort and 7 case-control) showed that the risk of oesophageal cancer among those who frequently use NSAIDs or aspirin was significantly lower compared to never users. However, studies included in the meta-analysis did not specifically include patients with BO. Second, in a pooled analysis on individual patient data which confirmed the significant reduction in risk of OAC in BO patients with prescription of statins and NSAID US veteran were study subjects, which limits the generalization and extrapolation of results from the latter study to the general population is. Additionally there was no adjustment for important risk factors of OAC progression such as alcohol use and tobacco smoking. Causality of an apparent association is generally supported by a dose- and duration-response relationship. However, several studies neither reported a clear exposure definition free of recall bias nor conducted dose-duration analyses. Finally, concerns have been raised about publication bias of these studies on chemoprevention of OAC in BO patients. Thus, to which extent NSAIDs, statins and PPIs may reduce the risk of oesophageal adenocarcinoma among BO patients in clinical practice remains unknown. Therefore, we conducted a matched case-control study to evaluate the risk of oesophageal adenocarcinoma among patients with BO associated with use of NSAIDs, statins and PPIs.
Objectives	3	State specific objectives, including any prespecified hypotheses The aim of our study was to evaluate the risk of oesophageal adenocarcinoma among patients with BO associated with use of NSAIDs, statins and PPIs in a matched case-control study.
Methods		
Study design	4	Present key elements of study design early in the paper Nested case-control study in BO subjects identified in two primary care databases from United Kingdom and the Netherlands between 1996 and 2012. This is presented early in the Methods section.
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection Two European population-based primary care databases; The Health Improvement

		Network (THIN) database from the United Kingdom (UK, 1996–2011), and 2) the Integrated Primary Care Information database (IPCI) from the Netherlands (NL, 1996–2012). Both databases contain data that are collected prospectively and represent real-life practice. Data was collected by electronic search for diagnoses and drug prescriptions.
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls First a dynamic population-based cohort was created in which we included all patients aged 18 years and older who contributed data to the database between 1st of January 1996 and 31st of December 2011 for THIN and 2012 for IPCI and with a diagnosis of BO diagnosis. At least one year of available healthcare data prior to study entry was required in order to assess patient's medical history and to discriminate between prevalent and incident (i.e., newly-diagnosed) BO cases. Follow up started on 1 January 1996, date of reaching 18 years of age, or the date that one year of valid data was accrued within the database, whichever came later. Follow-up ended on the date of occurrence of study outcome (OAC), date of transfer out of the general practitioner's (GP) practice, death, or last data drawn, whichever was earliest. Patients were excluded if they had a history of oesophageal cancer anytime before BO diagnosis and if they had a history of gastric cancer up to 6 months after BO diagnosis. In THIN, BO and OAC cases were extracted using corresponding READ codes. In IPCI, each potential BO case was manually validated to confirm the diagnosis of BO and the date of first diagnosis or mentioning of BO in the clinical record. For OAC-HGD diagnosis, all potential cases were manually validated for confirmation of the OAC or HGD diagnosis, date of first diagnosis and the type of carcinoma (squamous cell-, adeno-, or other types of carcinoma). Early cancer (i.e., high-grade dysplasia (HGD)) was identified in IPCI as well. OAC cases were considered incident if the date of diagnosis occurred after inclusion into the BO study cohort and was at least 12 months after BO diagnosis. Cases occurring within one year from BO diagnosis were classified separately and considered to be existent in relation to the BO diagnostic work-up. The index date was defined as the date of the first reporting of OAC diagnosis during the study period. Controls were members of the incident BO cohort who did not develop OAC up to the matching date. Controls were matched by incidence density sampling on age (± 5 years), sex, year of BO diagnosis (± 1 year), and database. Consequently, the index date for controls was the date of OAC diagnosis for the corresponding case. We matched on year of BO diagnosis in order to account for any influence of guideline changes in endoscopic surveillance over calendar time.
		(*b*) For matched studies, give matching criteria and the number of controls per case Controls were matched by incidence density sampling on age (± 5 years), sex, year of BO diagnosis (± 1 year), and database. Consequently, the index date for controls was the date of OAC (or HGD) diagnosis for the corresponding case. We matched on year of BO diagnosis in order to account for any influence of guideline changes in endoscopic surveillance over calendar time. In UK, we identified 40 incident OAC cases within the BO cohort (0.3%) to whom we could match 656 controls. Median number of controls per case was 17 (interquartile range (IQR): 9-23). In NL we identified 5 incident OAC cases among the BO cohort (0.3%) and these were matched to 76 control subjects, with a median of 5 controls per case (IQR: 4-6). In

		addition, we identified 12 HGD cases, resulting in a second case control set of 17 cases (5 OAC + 12 HGD) matched to 753 controls (median 44 controls; IQR: 6-61).
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable Outcomes: In THIN, BO and OAC cases were extracted using corresponding READ codes. We applied several BO case definitions in THIN to explore outcome misclassification of BO, which did not demonstrate large differences. In IPCI the ICPC coding system is used, which does not include a diagnosis code for BO specifically. A sensitive search algorithm in free text was used including synonyms for Barrett's oesophagus ('Barrett', 'intestinal metaplasia', 'columnar epithelium'). Each potential case was manually validated to confirm the BO diagnosis and the date of first diagnosis or mentioning of BO in the clinical record. OAC cases were considered incident if the date of diagnosis occurred after inclusion into the BO study cohort and was at least 12 months after BO diagnosis. Cases occurring within one year from BO diagnosis were classified separately and considered to be existent in relation to the BO diagnostic work-up. Furthermore, in IPCI all OAC and HGD cases were manually validated. Drug exposure: Drug exposures of interest were the use of outpatient prescriptions for NSAIDs (including high-dose aspirin, i.e. >325 mg/day), PPIs and statins from BO diagnosis until OAC diagnosis. In order to compare the OR of NSAIDs, PPIs and statins to other drugs, we considered another group of medications that served as control. Antidepressants (selective serotonin re-uptake inhibitors (SSRIs)) are currently not known to be either positively or negatively associated with OAC. Duration of prescriptions was calculated based on the prescribed quantity and dosing regimen. As the most likely preventive effect of drugs on cancer progression is through a cumulative mechanism, we calculated all duration and defined daily dose (DDD) values from date of BO diagnosis until index date. Duration was classified according to never use (reference category), cumulative use of less than 1 month, between 1-12 months, > 12 months (or if applicable 1-2 years; 2-3 years and > 2 years). Considering that PPIs are indicated as treatment for BO patients, duration was classified as 0-6 months (reference category), 6-12 months, 1-2 years and > 2 years. Dose of exposure was classified using the ratio of prescribed daily dose compared to DDD using quartiles into categories (<0.8; 0.8-1.2; ≥1.2 DDD per day). Potential confounder: We considered the following as potential confounders: concurrent diagnosis of oesophagitis; gastritis within 1 year before BO diagnosis; presence of a hiatal hernia (determined from start of data entry in the database until BO diagnosis). Additionally we assessed smoking habits (non-smoker, ex-smoker, current smoker) and alcohol abuse (never, current, past).
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 For both databases, information on BO or OAC diagnosis was measured in the same manner applying the common protocol.

		As co-morbidity we determined the presence of oesophagitis, gastritis from 1 year before until the time of BO diagnosis and the presence of a hiatal hernia at any time before BO diagnosis. BO cases were classified as incident if the date of BO diagnosis occurred after inclusion in the study cohort and as prevalent if the date of BO diagnosis occurred prior to study entry.
Bias	9	Describe any efforts to address potential sources of bias The nested-case control design in a well-defined population characterizing the general population minimized selection bias. Whereas previous studies may have suffered from recall bias or the lack of detailed drug prescription data, we were able to estimate the risk of OAC and OAC-HGD within BO subjects in relation to drug prescription data in the general population. Our study is unlikely to suffer from biases (such as immortal time bias, time window bias) and confounding (such as by disease severity) by matching on important risk factors. Additionally, the adjusted analyses were in line with the matched analyses. Although we may have lacked detailed pathology information in some subjects including information on the length of the Barrett segment and the grade of dysplasia. This may have resulted in misclassification of BO and OAC, resulting in classifying subjects wrongly with BO or OAC. Outcome misclassification of OAC might have occurred, as population-based studies are challenged by the lack of detail from histology reports, which is particularly true for routinely collected GP data without free text in medical records. However, we have included only confirmed OAC/HGD cases in the study. Assuming non-differential misclassification, this may have resulted in an underestimation of the risk. In the Dutch database we could search through all free text entered in the medical record, enabling to look for more detailed information in clinical letters, resulting in higher percentages of information on oesophagitis, gastritis and hiatal hernia. We tried to address confounding-by-indication and time-window bias by matching on age, sex and year of BO diagnosis. This is seen by the fact that individual risk factors (hiatal hernia, and alcohol abuse) did not appear to increase the risk of oesophageal adenocarcinoma and adjustment for these potential confounders did not change the OR estimates by more than 10%.
Study size	10	Explain how the study size was arrived at From the source population of 7,570,765 subjects in UK and 1,496,276 subjects in NL we identified 13,696 and 1,438 incident BO cases, respectively. Males accounted for 63% in UK and 62% in NL of BO subjects. Mean age at BO diagnosis was 64.8 (SD: 13.8) years in UK and 61.2 (SD: 13.4) years in NL. In UK, we identified 40 incident OAC cases within the BO cohort (0.3%) to whom we could match 656 controls. Median number of controls per case was 17 (interquartile range (IQR): 9-23). In NL we identified 5 incident OAC cases among the BO cohort (0.3%) and these were matched to 76 control subjects, with a median of 5 controls per case (IQR: 4-6). In addition, we identified 12 HGD cases, resulting in a second case control set of 17 cases (5 OAC + 12 HGD) matched to 753 controls (median 44 controls; IQR: 6-61). A flowchart of the study population is depicted in Figure 1. In the manuscript we provide a flow chart of the study cohorts.
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why. Age at BO diagnosis among OAC cases and the mean time to OAC diagnosis was estimated by a Student's t-test.
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding

		Baseline characteristics of cases and controls were described per database and compared using univariate conditional logistic regression. To estimate the risk of OAC among patients with BO, matched and adjusted odds ratios (OR) with 95% confidence intervals (CIs) were calculated using conditional logistic regression for both databases separately and as a pooled analysis on patient-level pooled data. Potential confounders (oesophagitis, gastritis, hiatal hernia, BMI, smoking and alcohol abuse) were included in the adjusted analysis (ORa) if they resulted in a change of more than 10% of the initial estimate, whereas time since BO diagnosis was forced into the adjusted model. Subsequent analyses included duration- and dose-analyses. The risk of OAC and OAC-HGD was also assessed for concomitant use of NSAIDs, statins and/or PPIs. Use of PPIs only was considered as reference category considering that standard therapy for BO includes PPI therapy. Subgroup analyses evaluated the risk of OAC stratified by presence of risk factors: oesophagitis, gastritis or hiatal hernia at time of BO diagnosis. Multiplicative interaction was tested to identify effect modification by all of individual risk factors.
		(*b*) Describe any methods used to examine subgroups and interactions Subgroup analyses evaluated the risk of OAC stratified by presence of risk factors: oesophagitis, gastritis or hiatal hernia at time of BO diagnosis. Multiplicative interaction was tested to identify effect modification by all of individual risk factors.
		(*c*) Explain how missing data were addressed We had missing data on BMI which we report in Table 1.
		(*d*) If applicable, explain how matching of cases and controls was addressed Controls were matched by incidence density sampling on age (± 5 years), sex, year of BO diagnosis (± 1 year), and database. Consequently, the index date for controls was the date of OAC (or HGD) diagnosis for the corresponding case. We matched on year of BO diagnosis in order to account for any influence of guideline changes in endoscopic surveillance over calendar time..
		(*e*) Describe any sensitivity analyses We performed subgroup analyses by stratifying on risk factors for OAC..
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 The total study population comprised 8,372,611 persons (UK: 6,885,420; NL: 1,487,191) contributing to 48,918,172 person years (PYs) (UK: 44,505,240; NL: 4,412,932) of follow-up during the study period. We identified 12,312 and 1,383 incident BO cases in THIN and IPCI, respectively. In the manuscript we provide a flow chart of the study cohorts.
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram In the manuscript we provide a flow chart of the study cohorts.
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders Baseline characteristics of study participants are shown in Table 1 and in the first paragraph of the Results section.
		(b) Indicate number of participants with missing data for each variable of interest We had missing data on BMI which we report in Table 1.

Outcome data	15*	Report numbers in each exposure category, or summary measures of exposure The number of OAC-HGD cases and controls and the corresponding co morbid diseases and the frequency of exposure are described in Table 1 and Table 2&3 respectively.
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 In Table 3 we report matched and adjusted ORs for OAC and HGD during use of NSAIDs, statins, PPIs and SSRIs. Adjusted was for duration of follow-up since BO diagnosis, apart from the matching factors.
		(*b*) Report category boundaries when continuous variables were categorized. In Table 1 we give number of OAC cases and controls per age category (<50, 51-65, 66-80, >80 years of age).
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period Not applicable.
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses In the results section and appendix Table 2 we report the stratified analyses and interaction terms.
Discussion		
Key results	18	Summarise key results with reference to study objectives In this population-based case-control study nested within a cohort of patients with Barrett's oesophagus, the risk of both oesophageal adenocarcinoma and high-grade dysplasia may be reduced up to 50% during use of statins. PPIs reduced the risk of HGD and OAC when used at highest doses, while NSAIDs did not decrease the risk. This is the first study that looked at the chemopreventive effect of these three different drugs also when used concomitantly within a population-based study.
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 In the discussion section we have discussed the limitations of the current study. The nested-case control design in a well-defined population characterizing the general population minimized selection bias. Whereas previous studies may have suffered from recall bias or the lack of detailed drug prescription data, we were able to estimate the risk of OAC and OAC-HGD within BO subjects in relation to drug prescription data in the general population. Although our analysis may be limited by the small number of cases in the duration- and dose-analyses, our study is unlikely to suffer from biases (such as immortal time bias, time window bias) and confounding (such as by disease severity) by matching on important risk factors. Additionally, the adjusted analyses were in line with the matched analyses. An important limitation of the study is the lack of detailed pathology information in some subjects including information on the length of the Barrett segment and the grade of dysplasia. This may have resulted in misclassification of BO and OAC, resulting in classifying subjects wrongly with BO or OAC. Assuming non-differential misclassification, this may have resulted in an underestimation of the risk. In the Dutch database we could search through all free text entered in the medical record, enabling to look for more detailed information in clinical letters, resulting in higher percentages of information on oesophagitis, gastritis and hiatal hernia. We tried to address confounding-by-indication and time-window bias by matching on age, sex and year of BO diagnosis. This is seen by the fact that individual

		risk factors (hiatal hernia, and alcohol abuse) did not appear to increase the risk of oesophageal adenocarcinoma and adjustment for these potential confounders did not change the OR estimates by more than 10%. The observation that PPIs appear to increase the risk of OAC may be explained by the underlying treatment indication being a risk factor for OAC, reverse causation and the phenomenon of 'channeling' where high-risk patients are being prescribed PPIs whereas low-risk patients not or in lower dose. This phenomenon is also seen for PPI use and upper gastrointestinal bleeding. It could also be that the effect of PPIs is becoming pronounced after at least 2 years of use, as is also seen from our data, showing a tendency to a lower risk of OAC-HGD with use > 2 years. That PPIs decrease the risk of OAC-HGD via gastric acid suppression is confirmed by the observation that among subjects with a hiatal hernia PPIs a reduced risk of OAC and OAC-HGD was noted whereas this was not seen in subjects without a hiatal hernia.
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence In this population-based case-control study nested within a cohort of patients with Barrett's oesophagus, the risk of both oesophageal adenocarcinoma and high-grade dysplasia may be reduced up to 50% during use of statins. PPIs reduced the risk of HGD and OAC when used at highest doses, while NSAIDs did not decrease the risk. This is the first study that looked at the chemopreventive effect of these three different drugs also when used concomitantly within a population-based study. In contrast to other studies, we did not observe a significant preventive effect of NSAIDs and statins with respect to the risk of OAC and HGD. Based on the biological mechanisms, combined use of statins and NSAIDs would be expected to result in a greater risk reduction of OAC compared to either drug alone. We did not observe this synergistic protective effect. This may be due to several reasons. Firstly, despite our large BO cohort (compared to other studies), the number of identified OAC and HGD cases was smaller. This limited the power of the study and resulted in wider confidence intervals and statistically non-significant results. However, given an exposure prevalence of NSAIDs of 30% among controls and a correlation of 0.5 between exposed and unexposed subjects, we had 80% power (with a type 1 error of 5%) to detect a true odds ratio of OAC of 0.34, which would be according to prior studies. In addition, our underlying study population included all incident BO subjects from the general population. By matching on duration since BO diagnosis and excluding prevalent BO subjects, we removed any effect of selective survival bias, disease severity or time window bias; as those BO subjects with a longer follow-up are more likely to develop HGD or OAC. Secondly, we mitigated against immortal time bias by defining the exposure period from BO diagnosis till matching date, and thus avoiding an overestimation of the preventive effect. The estimates from our study are likely more generalizable to the daily clinical practice in the general population, including also less severe BO subjects. A preventive effect of NSAIDs might be therefore only applicable to selected high-risk subgroups. Secondly, the inability to show a significant decrease in OAC and OAC-HGD risk for NSAID during statin use may be explained by the distinct exposure definition that we applied. Possibly the preventive effect is achieved in the premalignant phase of development of dysplasia rather than of final adenocarcinoma, resembling different risk periods. It is however difficult to disentangle effects of drug exposure in the three different risk periods: induction (dysplasia), latent

		(between dysplasia and cancer) and disease period (cancer). Ideally, this requires knowledge on the exact timing of starting from the first aberrant Barrett's cell; and subsequent stages. A third explanation for not observing a chemopreventive effect in our study may be the exposure prevalence. The NSAID exposure prevalence was lower in our study, because we could not capture over-the-counter use of NSAIDs. Prevalence of PPI (81%) and statin (26%) exposure in our study is however comparable to other studies and is therefore unlikely to have limited our power.
Generalisability	21	Discuss the generalisability (external validity) of the study results Both two general practice databases contain a large number of patients and reflect the underlying general population. This study can be generalised to other Western European populations.
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 No specific funding for this study was obtained.

*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.