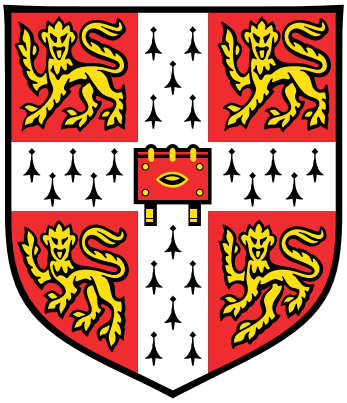
Integrating Epidemiological and Genomic Factors to Inform Outcomes in Barrett's Oesophagus and Oesophageal Adenocarcinoma



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Dedicated to the memory of Alena Eftihia Frey.

Declaration

This thesis is the result of my own work and includes nothing which is the outcome of work done in collaboration except as declared in the preface and specified in the text. I further state that no substantial part of my thesis has already been submitted, or, is being concurrently submitted for any such degree, diploma or other qualification at the University of Cambridge or any other University or similar institution except as declared in the Preface and specified in the text. It does not exceed the prescribed word limit for the Clinical Medicine and Veterinary Medicine Degree Committee.

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Abstract

The incidence of oesophageal adenocarcinoma (OAC) has rapidly increased, and its prognosis remains poor. Barrett's oesophagus (BO) is considered the precursor to OAC, however, BO is not apparent adjacent to tumour in nearly half of OAC patients. We have previously demonstrated that patients with BO-adjacent tumours (BO+ve OAC phenotype) have a favourable prognosis compared to those without evidence of BO adjacent to the tumour (BO-ve OAC phenotype). It has been suggested that the BO-ve OAC tumour phenotype may arise independently of BO. Recent experimental and computational studies show that all OACs likely arise from BO, even if this precursor lesion is not histologically apparent adjacent to the tumour. However, there is a lack of consolidated clinical, epidemiological and molecular data to examine this question.

In this thesis, I used orthogonal approaches to examine the overlap between the BO+ve OAC and BO-ve OAC phenotypes and explain the aetiology and the observed altered prognosis. To achieve this, I assembled a large cohort (n=4,695) comprising BO+ve OAC cases (n=1,235), BO-ve OAC cases (n= 880), OAC cases with an unascertainable BO status (BO(?) OAC; n= 985), cancer-free BO cases (n=1,091) and reflux controls (n=554). A subset of the OAC cases (n=950) with available clinical, epidemiological and whole-genome sequencing data was also examined.

There was little to no association between most of the 34 clinical and epidemiological factors and the OAC phenotypes. Weak associations were observed for cigarette smoking and gender with self-reported ever-smoking and female cases being more likely in the BO-ve OAC phenotype group relative to the BO+ve OAC phenotype group. However, tumour stage, lymph node spread and metastasis (TNM) was strongly associated with increased risk of BO-ve OAC. Higher TNM stage was strongly correlated with BO-ve OAC with an adjusted odds ratio of 2.4 (95% CI:1.8-3.3), 2.9 (95% CI:2.2-3.9) and 3.2 (95% CI: 1.7-5.9) for stages II, III and IV, compared to stage I. The improved survival associated with BO+ve OAC persisted in survival analyses adjusted for the tumour stage and location as well as the effects of smoking, obesity and heartburn (adjusted hazard ratio=0.88, 95% CI: 0.77-0.95, p=0.015). Of note, the BO-ve OAC phenotype was reported for 21 OAC cases with a history of undergoing endoscopic surveillance for BO.

Seven different types of genomic alterations were examined in relation to the OAC phenotypes. BO+ve OAC tumours had a slightly higher tumour mutation load relative to BO-ve OAC tumours, which was not explained by the effects of ageing and cigarette smoking. There were no differences in the distribution of driver gene alterations, frequency of whole-genome doubling events, or rates of aneuploidy between the OAC phenotypes. Similarly, the prevalence of complex events such as breakage-fusion-bridges and extrachromosomal DNA did not differ according to OAC phenotype. Importantly, signature 17, which is shown to be preserved across the BO-OAC continuum, was equally enriched among the BO+ve OAC and BO-ve OAC tumours.

This thesis presents the first comprehensive evaluation of epidemiological, clinical and molecular factors between the two defined OAC phenotypes. While certain epidemiological factors differ between the phenotypes, they do not explain the observed prognostic difference. The genomic landscapes of these tumour phenotypes were remarkably similar. Taken together, it is likely that all OACs arise from BO even if this precursor lesion is no longer apparent at the time of diagnosis or resection pathology. The advanced-stage tumours suggest that the precursor lesion is overgrown in BO-ve OACs. This work contributes evidence for screening strategies focused on identifying individuals with BO to reduce the public health burden of OAC.

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Glossary

Acronyms / Abbreviations

AJCC American Joint Committee on Cancer

BEACON Barrett's and Esophageal Adenocarcinoma Consortium

BEST2 Barrett's oEsophagus Screening Trial 2

BFB Breakage-Fusion-Bridge

BH Benjamini-Hochberg Procedure

BMI Body mass index

BO Barrett's oesophagus

BSG British Society of Gastroenterology

BWA Burrows-Wheeler Aligner

CI Confidence interval

CMR Crude mortality rate

CNA Copy number alteration

COSMIC Catalogue Of Somatic Mutations In Cancer

CRF Clinical Research Form

DDR DNA damage repair

ecDNA Extrachromosomal DNA

FFPE Formalin-Fixed, Paraffin-Embedded

GEL Genomics England (private limited company

GI Gastrointestinal

GM Gastric metaplasia

GORD Gastro-oesophageal reflux disease

GP General Practitioner

H2RA Histamine type 2 receptor antagonists

H&E Haematoxylin & Eosin

HGD High grade dysplasia

HR Hazard ratio

ICGC International Cancer Genome Consortium

IM Intestinal metaplasia

IND Insertion/deletion

IQR Interquartile range

LGD Low grade dysplasia

MI Multiple imputation

MMR Mismatch repair

Mut/Mb Mutations per megabase

NDBO Non-dysplastic Barrett's oesophagus

NHS National Health Service

NIH National Institutes of Health

NMF Non-negative matrix factorisation

NSAID Non-steroidal anti-inflammatory drug

OAC Oesophageal adenocarcinoma

OCCAMS Oesophageal Cancer Clinical and Molecular Stratification consortium

OGD Oesophagogastroduodenoscopy

OR Odds ratio

OSCC Oesophageal squamous cell carcinoma

OS Overall survival

OTC Over the Counter (as in OTC medications

PCAWG Pan-Cancer Analysis of Whole Genomes

PH Proportional hazards

PPI Proton Pump Inhibitor

ROS Reactive oxygen species

RR Relative risk

SBS Single-base substitution

SD Standard deviation

SNV Single nucleotide variant

TCGA The Cancer Genome Atlas

TNM Tumor, Node, Metastasis

UICC Union for International Cancer Control

UK United Kingdom of Great Britain and Northern Ireland

US United States of America

WGD Whole genome duplication

WGS Whole genome sequencing

WHR Waist-to-hip ratio

Chapter 1

Introduction

1.1 Overview of oesophageal cancer

Oesophageal cancer is the seventh most common cancer type and the sixth leading cause of cancer-associated mortality worldwide (Bray et al., 2018; Smyth et al., 2017). The two main histological subtypes of this cancer include oesophageal squamous cell carcinoma (OSCC), which generally occurs in the proximal and middle segments of the oesophagus, and oesophageal adenocarcinoma (OAC), which typically develops near the gastro-oesophageal junction (Pennathur et al., 2013). Globally, OSCC is the predominant subtype with the highest incidence seen along the two so-called oesophageal cancer belts, one that stretches from central China to northern Iran and one from eastern to southern Africa (Abnet et al., 2018). The incidence of OAC has dramatically increased (>6-fold) over the past five decades, especially among men, and is now the most common subtype of oesophageal cancer diagnosed in the United Kingdom, the United States and most Western populations (Coleman et al., 2018). While the incidence of OSCC is projected to decline, the number of new OAC cases is expected to continue to rise (Arnold et al., 2017; Offman et al., 2018). Despite their distinct biology and epidemiology, the aggressive nature of these tumour subtypes coupled with advanced-stage presentation leads to a poor prognosis with less than 20% of patients surviving beyond five years after diagnosis (Gavin et al., 2012). This thesis will focus on OAC within the geographic scope of the United Kingdom (UK).

1.2 Epidemiology and overview of oesophageal adenocarcinoma

In the UK, the incidence of OAC increased by more than five-fold between 1971 to 2009 (Edgren et al., 2013). Recent studies estimate a total of 6,562 new cases were diagnosed in 2018 which is expected to grow to about 8,600 cases per year by 2030 (Arnold et al., 2017, 2020). This incidence is among the highest in the world and is predicted to be exceeded only by The Netherlands. There is a striking male predominance in the incidence of OAC with a male-to-female ratio of 6:1 and as high as 9:1 in North America (Xie & Lagergren, 2016; Xie et al., 2016). The reasons for this difference are not fully understood but likely involve a complex interaction between environmental, hormonal and host factors. Furthermore, the incidence of OAC is highest among individuals with European/white ancestry compared to other racial and ethnic groups. For example, the incidence of OAC is five times greater among non-Hispanic white men relative to Black men in the US (Cook et al., 2009). Similar to most other epithelial cancers, the incidence of OAC increases with age such that 99% of cases occur among individuals over the age of 40 in most Western countries (Edgren et al., 2013). Between 2001-2009 in England, the mean age at diagnosis remained stable at 68 years old (Gajperia et al., 2009).

Patients with OAC typically present with swallowing difficulties referred to as dysphagia, and significant weight loss. Other symptoms may include odynophagia (pain on swallowing), worsening heartburn and anaemia. When patients present with these so-called "alarm symptoms" an oesophagogastroduodenoscopy (OGD) procedure with biopsies is indicated to make a diagnosis. A histopathological diagnosis is essential to confirm whether the tumour is OAC or OSCC and to determine the grade of differentiation, meaning the extent to which the tissue architecture is preserved, graded as well, moderate or poor. Subsequently, tumour staging is performed to determine the extent of the tumour. Tumours are assessed for the depth of invasion through the walls of the oesophagus (T stage), lymph node involvement (N stage) and distant metastatic spread to more distant lymph nodes or other organs (M stage) (Figure 1.1). These individual stages are then combined into an overall staging (Table 1.1) according to the International Cancer Control (UICC) Tumour, Nodes and Metastases (TNM) Guidelines which is the same methodology as the Eighth Edition of the American Joint Committee on Cancer (AJCC) staging manual (Rice et al., 2017).

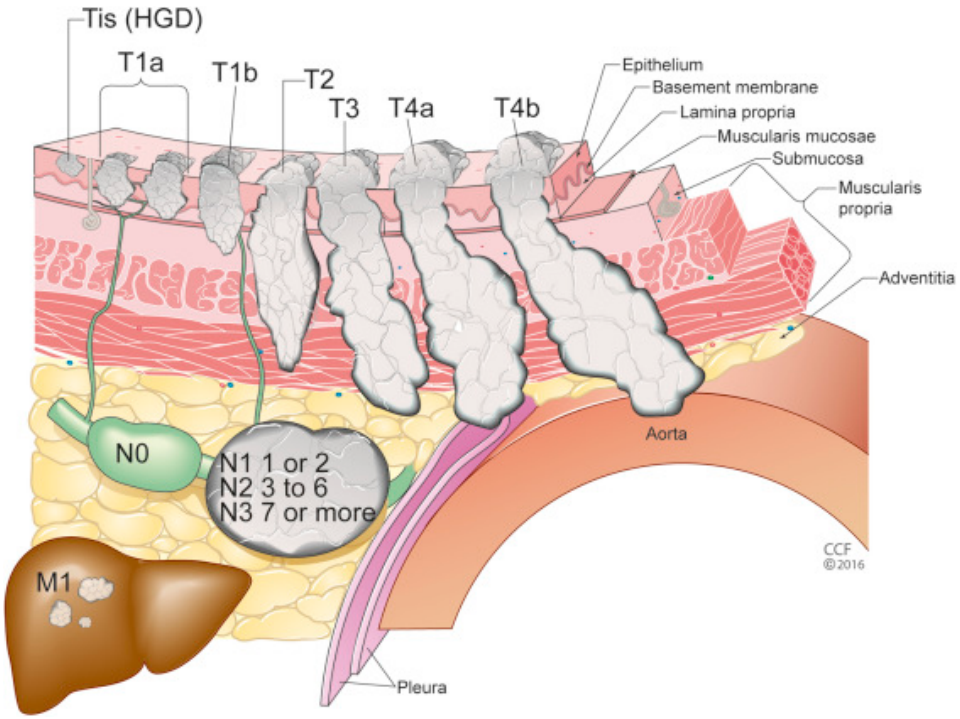

Figure 1.1 – Depiction of tumour, node and metastasis stages (adapted from Rice *et al*., 2017). Tumour (T) stage is determined by the extent of tumour invasion as shown using Tis-T4b. Lymph node involvement (N) is measured according to the number of nodes with metastasis as defined by N0-N3. The presence of distant metastasis (M) is denoted using M1. Abbreviations: HGD, high-grade dysplasia.

Table 1.1 – TNM staging of oesophageal adenocarcinoma. Stages are defined according to the eighth edition of the AJCC/UICC cancer staging manual.

	N0	**N1**	**N2**	**N3**	**M1**
T1	I	IIA	IVA	IVA	IVB
T2	IIB	III	IVA	IVA	IVB
T3	III	III	IVA	IVA	IVB
T4a	III	III	IVA	IVA	IVB
T4b	IVA	IVA	IVA	IVA	IVB

Tumour stage and grade are strongly associated with survival. An analysis from the Northern Ireland Cancer Registry demonstrated that patients with stage I tumours had a five-year survival rate of 80.5%, while patients with stage II, III, and IV tumours had lower survival rates of 45.1%, 17.6%, and 2.1%, respectively (Coleman et al., 2018). Further, staging is used to guide the treatment and management of OAC. Stage I or earlier-stage tumours are generally resected using endoscopic procedures such as endoscopic mucosal or submucosal resection. Patients with stage II or III typically receive neoadjuvant chemotherapy and/or radiotherapy followed by oesophagectomy which is then often followed by further chemotherapy. At advanced stage IV with metastases, palliative chemotherapy or best supportive care are used to manage symptoms and prolong life. Increasingly targeted therapies are being offered in the palliative setting for patients over-expressing growth factors such as HER-2 or the PD-L1 receptor which is an indication for immunotherapy (Smyth et al., 2017).

1.3 Epidemiology and overview of Barrett's oesophagus

Barrett's oesophagus (BO) is considered the precursor to OAC and is named after Norman Rupert Barrett, a surgeon who described the condition in the 1950s (Barrett, 1950). Given the poor outcomes associated with OAC, there has been considerable interest in characterising the epidemiology of BO in order to better identify those at risk of OAC and improve outcomes. However, the population prevalence of BO remains largely unknown because most individuals with heartburn are not offered an endoscopy and some individuals may have silent reflux, meaning that they do not experience heartburn and so again do not receive an endoscopy (Fitzgerald et al., 2001). Few studies have quantified the prevalence of BO in unselected populations and the estimated prevalence ranges from 0.5% to 1.6% in the general population (Cameron et al., 1990; Ronkainen et al., 2005; Zagari et al., 2008). Not unexpectedly, the prevalence of BO in selected groups such as those undergoing endoscopy is estimated to be higher at 10-15% (Qumseya et al., 2019; Westhoff et al., 2005). Given the substantial number of undiagnosed BO cases, the incidence of BO may not be accurately estimated. The best possible estimates are based on data from gastroenterology clinics and should be viewed as a proxy for the relative incidence and the number of undiagnosed cases. Using primary care data, the most recent estimate of the incidence of BO in the UK was 12,312 cases from 2000 to 2011 which translates to approximately 32 BO cases per 1,000 endoscopies (Masclee et al., 2014). Similar to OAC, the incidence of BO is higher among men, with a male-to-female ratio of approximately 2:1 (Cook et al., 2005). Additionally, BO predominantly affects individuals of European/white ancestry (Abrams et al., 2008), and the incidence of the disease increases with age, peaking in the fifth to seventh decades of life (Runge et al., 2015). Most BO patients do not progress to OAC, however, one population-based study has estimated a 10 to 55-fold increased risk of OAC for BO patients compared to the generation population (Cook et al., 2018).

The management of BO involves controlling gastro-oesophageal reflux disease (GORD), often experienced as heartburn, using acid-suppressing medications to lower further damage to the oesophageal lining. In addition, individuals diagnosed with BO in the UK are offered endoscopic surveillance accompanied by biopsies at regular intervals as outlined in the most recent British Society of Gastroenterology (BSG) guidelines (Fitzgerald et al., 2014). In these guidelines, the endoscopic surveillance intervals for patients with non-dysplastic BO range from every 2 to 5 years depending on the clinical assessment of the BO lesion as well as patient fitness and preference. The objective of BO surveillance is to identify any evidence of dysplastic progressive cases and intervene in a timely manner. For patients with dysplastic BO or stage I OAC, endoscopic treatment options such as endoscopic mucosal resection may be used to remove the abnormal tissue (Haidry et al., 2013).

1.4 Multi-stage model of pathogenesis

BO is characterised by the transformation of the normal squamous epithelial lining into a metaplastic columnar mucosa which typically occurs in the distal oesophagus (Spechler, 2002). This metaplastic transformation and the presence of goblet cells indicate intestinal metaplasia (IM) which is a histological hallmark of BO that resembles the cells which line the intestine. When goblet cells are absent, the transformed tissue is referred to as gastric metaplasia (GM). Although current guidelines of the BSG regard both IM and GM as BO, because IM may be missed due to sampling bias (Fitzgerald et al., 2014), all other Gastroenterology Societies require IM for diagnosing BO. This thesis focuses on BO defined by the presence of IM and the use of BO in the text means containing IM.

The development of BO is strongly associated with long-standing GORD which is typically experienced as heartburn by affected individuals (Cook et al., 2005). Chronic GORD causes gastric acid and bile to flow backwards into the oesophagus, leading to chronic inflammation, oxidative stress and DNA damage injury in the epithelial lining (Cook et al., 2014; Smith et al., 2005). Therefore, GORD is considered a key risk factor for BO, and most screening guidelines rely on GORD-associated symptoms to determine eligibility for BO screening (Sawas et al., 2022). The role of GORD/heartburn will be discussed in detail in the chapter on risk factors for BO and OAC. At the metaplastic stage, indolent non-dysplastic BO (NDBO) can further progress through low-grade dysplasia (LGD) and high-grade dysplasia (HGD) stages which involve progressively higher cellular abnormalities and architectural disorganisation (Peters et al., 2019). At each stage, the risk of progression to OAC increases: patients with NDBO have an annual progression risk of 0.1-0.3% (Desai et al., 2012; Hvid-Jensen et al., 2011); while the patients with LGD and HGD have a progression risk of 1-10% and 13-30% per year, respectively (Bhat et al., 2011; Duits et al., 2015; Phoa et al., 2014; Rastogi et al., 2008).

1.5 Risk factors for oesophageal adenocarcinoma and Barrett's oesophagus

1.5.1 Gastro-oesophageal reflux

As described, gastro-oesophageal reflux disease (GORD) is the primary risk factor for BO and OAC. GORD is a common chronic condition characterised by regular and persistent reflux of gastric contents into the oesophagus that can cause acid-induced damage to the epithelial lining of the oesophagus. GORD may be experienced as symptoms of heartburn and regurgitation, or it can be 'silent' where the burning sensation is absent.

In a meta-analysis of five case-control studies conducted in Australia, Northern Ireland, the Republic of Ireland and the US, weekly symptoms of GORD increased the risk of OAC by nearly five-fold (odds ratio (OR)=4.92, 95% CI: 3.90-6.22) and daily frequency of symptoms increased the risk seven-fold (OR=7.40, 95% CI: 4.94-11.10) compared to those not experiencing symptoms (Rubenstein & Taylor, 2010). Data from Barrett's Esophagus and Esophageal Adenocarcinoma Consortium (BEACON) confirmed these associations by demonstrating that those with at least weekly had five-fold (OR=4.81, 95% CI: 3.39-6.82) and those with daily symptoms eight-fold (OR=7.96, 95% CI:4.51-14.04) higher risk of OAC compared to individuals with infrequent or no GORD symptoms (Cook et al., 2014). In the same study, it was also demonstrated that the duration since the onset of heartburn symptoms was associated with OAC risk in a linear manner with ORs of 2.80, 3.85, and 6.24 for symptom durations of <10 years, 10 to <20 years, and $\geq$20 years. A meta-analysis of studies of GORD in relation to BO found that GORD is strongly associated with long-segment BO ($\geq$3 cm in length; OR=4.92, 95% CI: 2.01-12.0) but not associated with short-segment BO (OR=1.15, 95% CI: 0.76-1.73) (Taylor & Rubenstein, 2010).

Despite the association of GORD with BO and OAC, approximately 30-50% of OAC occurs among persons without chronic heartburn and regurgitation (Farrow et al., 2000; Lagergren et al., 1999; Vaughan & Fitzgerald, 2015).

1.5.2 Demographic factors

The risk of developing OAC has been associated with several demographic factors, such as age, gender, ethnicity, family history and socioeconomic status. Similar to other epithelial cancers, the incidence of OAC rises gradually with increasing age, with the highest rates reported among individuals between 70-80 years old (Falk, 2009; A. P. Thrift, 2018). Data from Sweden show that among men, the incidence rate in this age group was 7.10 (95% CI:

6.21-8.08) per 100,000 person-years, whereas among women, the corresponding rate was 1.98 (95% CI: 1.61-2.40) (Rutegård et al., 2010).

The epidemiology of OAC is characterised by a remarkably strong male predominance which remains unexplained. In Western populations, the male:female incidence ratio ranges from 6.1 in Europe to as high as 9 in the US (Thrift, 2021; Xie & Lagergren, 2016). Likewise, BO is more common among older individuals, and nearly twice as prevalent among men (Cook et al., 2005). For both BO and OAC, incidence rates are 3-5-fold higher in white individuals than in non-white individuals (Runge et al., 2015; Xie & Lagergren, 2016). A family history of BO or OAC among first or second-degree relatives also increases the risk of developing BO and progressing to OAC. In a study of a family with extensive BO and OAC history, the odds of BO or OAC among family members were 12.23 (95% CI: 3.34-44.76) times greater than among individuals without a family history of either condition (Groves et al., 2005).

Income, education and socioeconomic status are associated with risk of OAC. A nationwide study in Sweden found that compared to primary school education level, the highest level of education (university level) was associated with a 33% decrease in relative risk (RR=0.67, 95% CI: 0.56-0.79) of OAC among men and a non-significant 26% reduction among women (RR=0.74, 95% CI: 0.49-1.11) (Lagergren et al., 2016). Additionally, compared to the lowest quintile of income, the highest quintile was associated with a 17% reduction (RR=0.83, 95% CI: 0.71-0.97) among men and a non-significant 7% reduction among women (RR=0.93, 95% CI: 0.64-1.36).

1.5.3 Obesity, diet and physical activity

Elevated body mass index (BMI, defined as weight in kilograms/height in meters squared) and measures of central adiposity, such as waist circumference or waist-to-hip ratio (WHR), have been associated with increased risk of both BO and OAC.

In a meta-analysis of 22 studies, a five kg/m^2 increase in BMI was associated with increased risk of OAC (RR=2.73, 95% CI: 2.16-3.46) (Turati et al., 2013). A pooled analysis of 12 case-control studies from BEACON demonstrated that obese individuals with a BMI of 30.0-34.9 kg/m^2 (class I obesity) and $\geq$40.0 kg/m^2 (class III obesity) had a two-fold (OR=2.39, 95% CI: 1.86-3.06) and a nearly five-fold (OR=4.76, 95% CI: 2.96-7.66) higher risk of OAC, respectively, in comparison to those with BMI 18.5-<25.0 kg/m^2 (normal BMI) (Hoyo et al., 2012). Additionally, in the prospective NIH-AARP cohort, a BMI of $\geq$35.0 kg/m^2 was associated with a hazard ratio (HR) of 2.11 (95% CI: 1.09-4.09) compared to a normal BMI (Abnet et al., 2008). In the same study, compared to the lowest quartile, the highest quartile of WHR was associated with increased risk of OAC (HR=1.81, 95% CI:

1.24-2.64). The association of BMI and waist circumference with OAC risk was also observed in the European Prospective Investigation into Cancer and Nutrition cohort study (Steffen et al., 2015). In this study, mutual adjustment for BMI and waist circumference attenuated the association of BMI while waist circumference showed a strong positive association (highest vs. lowest quintile HR_{BMI}=1.19, 95% CI: 0.63-2.22 and $HR_{\text{waist circumference}}$=3.76, 95% CI: 1.72-8.22). Lastly, a meta-analysis of seven studies examining elevated BMI during early life stages (age $\leq$30 years) with OAC has shown that each five kg/m^2 increase in BMI was associated with increased risk of OAC (RR=1.88, 95% CI 1.37-2.56) although the effect size was larger (RR=3.13) when restricted to only case-control studies (Hidayat et al., 2018).

In BO, a meta-analysis showed that the highest category of central adiposity measured using visceral adipose tissue area, WHR or waist circumference was associated with increased risk (OR=1.98, 95% CI: 1.52-2.57) compared to the lowest category (Singh et al., 2013a). This association persisted after adjusting for BMI (OR=1.88) and BMI alone was associated with a borderline significant risk of BO (highest vs. lowest quartile OR=1.24, 95% CI: 1.02-1.52). Further, a pooled analysis from the BEACON Consortium showed a similar increased risk association between high waist circumference and BO (highest vs. lowest quartile OR=1.87, 95% CI: 1.22-2.87) and no association between BMI (as a continuous measure) and risk of BO (OR=0.95, 95% CI: 0.88-1.03) (Kubo et al., 2013). Taken together, elevated body fatness is a risk factor for both BO and OAC. The processes linked to this association are not fully understood, however, possible mechanisms may include obesity-induced GORD and molecular processes related to metabolism and systemic inflammation (Lagergren, 2011; Thrift et al., 2014).

Systematic reviews and meta-analyses examining the role of diet in OAC development have primarily focused on meat, vegetable and micronutrient intake, yielding results with varying degrees of heterogeneity. Of the studies investigating meat consumption, all have reported increased risk of OAC related to processed meat consumption. However, the association of total red meat with OAC risk has been less consistent in these studies. The most recent meta-analysis of meat consumption estimated a 23% increased risk of OAC comparing the highest category of intake to the lowest (RR=1.23, 95% CI: 1.01-1.50) (Huang et al., 2013; Salehi et al., 2013; Zhu et al., 2014).

The association of vegetable and fruit intake in relation to BO and OAC risk has been evaluated in three recent reviews and meta-analyses (Vingeliene et al., 2017; Vingeliene et al., 2016; Zhao et al., 2016). In the 2017 review by Vingeliene *et al.*, diets high in vegetable intake were correlated with an 11% reduced risk of OAC (highest vs. lowest quartile RR=0.89, 95% CI: 0.80-0.99). Reviews of fruit intake and OAC risk have produced less consistent results without an apparent effect on OAC risk.

Micronutrients may play a role in OAC development. In one systematic review and meta-analysis of vitamin intake in a Northern European population, reduced risk of OAC incidence was associated with the highest quartile compared to the lowest for intake of Vitamin A/β carotene (OR=0.46, 95% CI: 0.36-0.59), Vitamin C (OR=0.49, 95% CI: 0.39-0.62) and Vitamin E (OR=0.80, 95% CI: 0.63-1.03) (Kubo & Corley, 2007). In a review of ten case-control studies, dietary fibre intake was associated with a 34% reduction in risk of BO (OR=0.66, 95% CI: 0.44-0.98) for the highest quartile compared to the lowest quartile of intake (Coleman et al., 2013).

Limited studies exist for the association between physical activity and risk of OAC and no studies have examined this in BO. A meta-analysis of nine studies demonstrated that physical activity was associated with a 29% reduced risk of OAC comparing the most physically active individuals compared to the least active (OR=0.71, 95% CI: 0.57-0.89) (Singh et al., 2014).

Lastly, while drinking hot tea and other high-temperature beverages has been associated with increased risk of OSCC, they are unrelated to OAC. In two small meta-analyses, no association was seen between the consumption of hot foods and drinks or consumption of tea, coffee or tea and coffee and risk of OAC (Andrici & Eslick, 2015; Turati et al., 2011).

1.5.4 Alcohol

Alcohol consumption is an established risk factor for many cancers, but it is not associated with risk of OAC or BO (Boffetta et al., 2006; Whiteman & Wilson, 2016). The most recent systematic review and meta-analysis update from the World Cancer Research Fund/American Institute for Cancer Research confirmed that alcohol consumption measured in 10 grams/day increments was not associated with OAC risk (RR=1.00, 95% CI: 0.98-1.02) (Vingeliene et al., 2017). A pooled analysis of nine case-control studies and seven cohort studies from the BEACON found no association between any type of alcoholic beverage consumption (beer, wine and spirits) and risk of OAC development comparing the highest frequency category ($\geq$7 drinks per day) to lowest (OR=0.97, 95% CI: 0.68-1.36) (Freedman et al., 2011). A separate analysis in BEACON also showed no relation between alcohol consumption and risk of BO (Thrift et al., 2014). It is important to note that while no associations have been observed for OAC, alcohol consumption is a risk factor for the development of OSCC (Armstrong et al., 2018).

1.5.5 Cigarette smoking

Cigarette smoking is a risk factor for BO, OAC and neoplastic progression. A meta-analysis of 23 studies reported that compared to non-smokers, the risk of OAC was more than doubled among current cigarette smokers (RR=2.34, CI: 2.04-2.69) and only marginally lower among former smokers (RR=1.66, CI: 1.48-1.85) (Wang et al., 2017). A pooled analysis of ten case-control and four cohort studies from the BEACON Consortium found that the risk of OAC was nearly doubled in ever-smokers compared to never-smokers (OR=1.96, 95% CI: 1.64-2.34) and confirmed a dose-response relationship with pack-years of smoking (Cook et al., 2010). A separate analysis also from BEACON showed that ever-smoking was associated with increased risk of BO, whether compared to controls with heartburn (OR=1.61, 95% CI: 1.33-1.96) or to population controls (OR=1.67, 95% CI: 1.04-2.67), but risk of BO was not related to increasing pack-years of smoking (Cook et al., 2012). In a large registry-based study (n=3,167) of BO patients, cigarette smoking was correlated with increased risk of progression to dysplasia and OAC (HR=2.03, 95% CI:1.29-3.17) regardless of smoking intensity (Coleman et al., 2012).

1.5.6 Medication use

Proton pump inhibitors (PPIs) and histamine H2-receptor antagonists (H2RAs) are widely used acid-suppressant medications in the management of GORD. In a systematic review and meta-analysis of five cohort studies and two case-control studies of BO patients, PPI use was associated with a lower risk of BO-OAC progression risk (OR=0.29, 95% CI:0.12-0.79) compared to no PPI use (Singh et al., 2013b). Only two studies, one cohort and one case-control, reported on H2RA use and both demonstrated no significant effect; these were not included in the meta-analysis due to reporting limitations. The association between the use of non-steroidal anti-inflammatory drugs (NSAIDs) and of BO or OAC is unclear. Several observational studies have reported an inverse relation between NSAID use and risk of OAC, prompting a meta-analysis that showed NSAID use was associated with a 32% reduced risk of OAC (OR=0.68, 95% CI: 0.56-0.83) compared to no NSAID use (Liao et al., 2012). However, in BO, no association was observed between NSAID use and risk of BO in a pooled analysis from BEACON (Thrift et al., 2016). Findings from the AspECT trial, a multicentre randomised controlled trial that allocated BO patients to high-or low-dose PPI with or without aspirin, did not show a benefit for aspirin alone in preventing OAC (Jankowski et al., 2018). The role of aspirin and NSAIDs in chemoprevention of BO and OAC remains uncertain.

1.6 Genomic characteristics of oesophageal adenocarcinoma and Barrett's oesophagus

Studies of germline genetic variants (inherited) and somatic genomic alterations (acquired) have revealed molecular changes associated with risk of BO and OAC. It has been estimated that approximately 7% of BO and OAC cases are familial (Chak et al., 2006; Verbeek et al., 2014). Genome-wide association studies have identified 24 germline variants of small effect that are associated with risk of BO and OAC (Gharahkhani et al., 2016). Furthermore, such studies have shown BO/OAC are highly polygenic with many shared germline variants associated with disease susceptibility. In parallel to germline studies, the advent of next-generation sequencing technologies has enabled in-depth characterisation of somatic alterations in BO and OAC genomes. Overall, the somatic alteration landscapes of BO and OAC are highly heterogenous with limited commonly occurring mutations seen across patients.

1.6.1 Point Mutations and copy number alterations

Human tumour development, as described by Hanahan and Weinberg (2011), is a multistage process where cells acquire the hallmarks of cancer amidst a background of genomic instability. Large international research projects, such as the International Cancer Genome Consortium (ICGC) and The Cancer Genome Atlas (TCGA), have provided comprehensive, high-resolution analyses of tumour genomes, leading to the discovery of a plethora of genomic alterations, such as somatic point mutations. By combining both ICGC and TCGA resources, the Pan-Cancer Analysis of Whole Genomes (PCAWG) Consortium has revealed substantial insights across a range of tumours. The PCAWG analysis of OAC genomes further confirmed the significantly mutated and heterogenous landscape of this disease.

Whole-genome sequencing (WGS) studies in OAC have revealed an abundance of single nucleotide variants (SNVs) and small insertions or deletions (INDs) in OAC (Dulak et al., 2013; Frankell et al., 2019; Weaver et al., 2014). The median number of SNV/IND is 6.4 per megabase (Mut/Mb) in OAC and similar to that seen in melanoma and lung cancer which are strongly associated with environmental mutagens (Akdemir et al., 2020). Despite a high frequency of point mutations in OAC, few genes are recurrently mutated across tumours. These genes, called 'driver genes', promote tumorigenesis and cancer progression (Martínez-Jiménez et al., 2020). Mutations outside of such genes are typically referred to as 'passenger' mutations and are generally inconsequential (Stratton et al., 2009). WGS data show that SNVs drive the mutations of *TP53* which is recurrently found in 72% of OAC tumours (Frankell et al., 2019). Besides this gene, SNV-driven mutations of other genes were found

in under 15% of patients. In BO, the SNV/IND mutation load gradually increase across the progression continuum with non-dysplastic BO exhibiting the lowest (~1.5 Mut/Mb) and dysplastic BO having a mutation load (~4.0 Mut/Mb) similar to OAC (Katz-Summercorn et al., 2022; Newell et al., 2019). In non-dysplastic BO, *CDKN2A* is the most frequently altered driver gene, occurring in approximately 50% of cases, and TP53 mutations are typically absent and mark progression to dysplasia (Ross-Innes et al., 2015; Stachler et al., 2015, 2018; Weaver et al., 2014).

The highly heterogeneous landscape of point mutations presents challenges in identifying genomic variations between tumours based solely on SNV/IND mutations. Therefore, it is important to also examine copy number alterations (CNAs) as these mutations have been found to play a significant role in the genomic landscape of OAC (Noorani et al., 2017; Secrier et al., 2016). CNAs are characterised by an increase (gain) or decrease (loss) in the number of copies of a genomic region such as a protein-coding region. In OAC, recurrent CNAs have been identified, involving gains in regions containing oncogenes (e.g., *ERBB2*, *VEGFA* and *MYC*) and losses in regions harbouring tumour suppressor genes (e.g., *CDKN2A*, *SMAD4* and *TP53*) (Frankell et al., 2019). Both SNVs and CNAs result in altered cellular states via different mechanisms: SNVs can affect the function of genes, while CNAs can lead to changes in the dosage of genes. SNVs and CNAs are analysed in tandem to address the heterogeneity of mutations and identify driver events. For example, SNV/IND mutations alone caused *CDKN2A* mutations among 12% of tumours but with copy number losses also included this gene was altered in 28% of tumours. To date, 77 driver genes have been identified in 551 OAC genomes analysed with a median of 5 events in driver genes per genome (Table 1.2).

Table 1.2 – Driver genes in oesophageal adenocarcinoma. Only genes recurrently mutated in ten percent or more of cases are presented.

Gene	% mutated	Gene	% mutated
TP53	72	*GATA6*	14
CDKN2A	28	*SMAD4*	14
KRAS	19	*CDK6*	14
MYC	19	*ARID1A*	13
ERBB2	18	*EGFR*	12
GATA4	15	*CCNE1*	10
CCND1	14	*CCND3*	10

Data from Frankell *et al.* (2019).

1.6.2 Mutational signatures

First described in 2013, mutational signatures are unique patterns of somatic mutations that arise from the effects of various endogenous and exogenous processes, such as DNA repair mechanisms and exposure to environmental mutagens (Alexandrov et al., 2013). Mutational signatures include all mutations and not are confined to gene regions alone, thus enabling a holistic view of mutations across the genome. Given the highly heterogeneous landscape of mutations in OAC, mutational signatures provide an ideal approach to understanding the role of point mutations and their related aetiology in this disease.

Single base substitutions (SBS) can be represented using six major types (C>A, C>G, C>T, T>A, T>C, T>G) and according to the immediate flanking bases (5' and 3' positions) which results in 96 possible mutation combinations. The relative frequency of mutations within each trinucleotide context is used to identify SBS signatures present in a tumour. A recent PCAWG analysis of 23,829 tumour samples, including 4,645 whole-genome and 19,184 whole-exome sequences, revealed a total of 49 SBS signatures with a likely biological origin as catalogued in the COSMIC database (Alexandrov et al., 2020; Tate et al., 2019). The aetiology of most signatures remains unknown; however, a collection of signatures has been consistently associated with ageing, reactive oxygen species (ROS), tobacco smoking and anti-cancer drugs. The PCAWG analysis demonstrated that SBS1, 2, 5, 13, 17a, 17b, 18 and 40 were predominant across 347 OAC tumours (Figure 1.2).

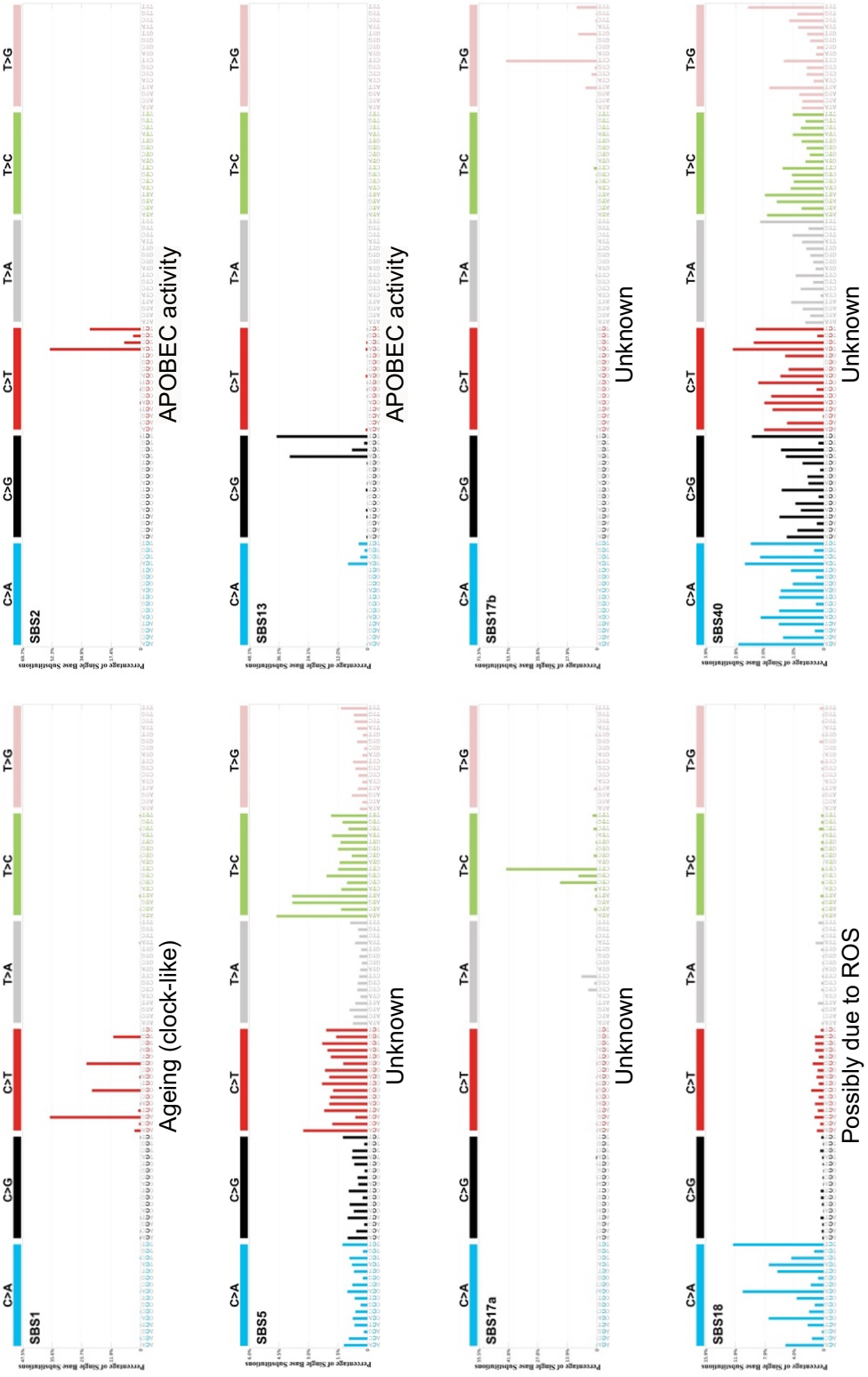

Figure 1.2 – Mutational signatures prevalent in oesophageal adenocarcinoma. Peaks show the proportion of mutations in each trinucleotide context and the proposed aetiology is listed below each plot. Mutational profiles and the associated proposed aetiology taken from COSMIC v.97, June 2022 (cancer.sanger.ac.uk/signatures). Abbreviation: ROS, reactive oxygen species.

In early studies of mutational signatures in OAC as well as in PCAWG (347 OAC tumours), SBS17a/b emerged as hallmark signatures of OAC as these were relatively specific to this disease with a prevalence of 50-65% across the analysed tumours (Alexandrov et al., 2020; Dulak et al., 2013; Secrier et al., 2016). As the same exposure may lead to mutational processes with highly correlated downstream effects, some signatures may be split to reflect such a possibility. This is true for SBS17 which was split into two signals of SBS17a (T>C in a CTT context) and SBS17b (T>G in a CTT context) which are speculated to be associated with gastric acid reflux and mutations related to ROS (Pich et al., 2019; Secrier et al., 2016). Importantly, in a cross-sectional WGS study of pre-cancer BO samples, SBS17a/b were uniformly distributed across all samples ranging from non-dysplastic BO to low and high-grade dysplasia and intramucosal carcinoma (Katz-Summercorn et al., 2022). This suggests that the mutational processes related to SBS17a/b are active from early in BO, irrespective of progression to dysplasia.

SBS1 is correlated with the age at cancer diagnosis and is seen in >95% of OAC tumours as catalogued among 347 OAC tumours in PCAWG. This signature is characterised by spontaneous deamination of 5-methylcytosine to thymine, which can occur due to errors in DNA replication. The aetiology of SBS5 and 40 is largely unknown, but these signatures are correlated with age in about half of all examined cancer types and 20% of OAC tumours from PCAWG. In a study from our group under submission at the point of writing, we found that SBS1 was already present in 95% of all pre-cancer BO (n=161) and similarly prevalent in OAC genomes (n=777) (Abbas et al., 2023). Furthermore, the mutational contribution of SBS5 decreased while SBS40 increased between matched BO and OAC samples.

Lastly, SBS2 and 13 are related to a high level of APOBEC enzyme activity and have been observed in about 30% of OAC genomes in PCAWG. SBS18 is observed in nearly 20% of OAC tumours and is possibly associated with ROS. Overall, mutational signatures can help to reduce the heterogeneity of point mutations in BO and OAC. So far, the analyses of mutational signatures in BO and OAC have generated important insights across the progression continuum and the possible underlying mutational processes.

1.6.3 Large-scale and catastrophic events

In addition to point mutations and copy number alterations, catastrophic and large-scale structural events have also emerged as important features in BO and OAC. These events involve genomic regions larger than 50 basepairs and can modulate the expression of oncogenes or tumour suppressor genes thus causing genomic instability and leading to carcinogenesis. Structural variations (SVs), whole-genome duplication (WGD), breakage–fusion–bridge (BFB) processes and the formation of extrachromosomal DNA (ecDNA) are commonly

found large-scale genomic events in BO/OAC (Hadi et al., 2020; Ng et al., 2022; Nones et al., 2014; Stachler et al., 2015; Turner et al., 2017).

OAC tumours exhibit a notable abundance of structural variations (SVs), with a median count of 289 per tumour (Ng et al., 2022). SVs are genomic events that can lead to large-scale structural rearrangements spanning individual genes up to entire chromosome arms (Li et al., 2020). SVs are also present in NDBO (median=43 per sample) which accumulate across dysplasia grades and are significantly enriched in dysplastic samples (median=141 per tumour) (Katz-Summercorn et al., 2022).

WGD is characterised by a complete duplication of a cell's genome, resulting in tetraploid cells that have double the complete set of chromosomes. In OAC, WGD is observed in 50-65% of tumours and has been associated with increased chromosomal instability and modified gene expression patterns (Campbell et al., 2020; Secrier et al., 2016; Stachler et al., 2015). WGD often follows the loss of *TP53* and is significantly more common in dysplastic (18%) than non-dysplastic BO (4%) (Newell et al., 2019; Paulson et al., 2022).

The BFB cycle process typically initiates with the loss of telomeres, which can cause unprotected chromosomal ends to fuse (Murnane, 2012). During anaphase, these fused chromosomes are torn apart, which can repeat over several cell cycles resulting in inverted duplications with significant copy number increases (Murnane, 2012). In cases where the amplified regions contain oncogenes, this can provide a growth advantage to tumour cells. BFB events have been identified in 25-55% of OAC and are generally not present in NDBO (Hadi et al., 2020; Luebeck et al., 2023; Newell et al., 2019; Nones et al., 2014).

ecDNA is circular DNA that is detached from chromosomal DNA and can result in oncogene activation thus accelerating tumorigenesis (Wu et al., 2022). The mechanism of ecDNA generation has not been fully characterised but may involve mechanisms such as DNA replication errors, chromosomal breakage and replication stress (Wu et al., 2021). Furthermore, ecDNA-mediated oncogene amplification has been associated with poor survival across various cancers, including oesophageal cancer (Turner et al., 2017). In a recent study of ecDNA in BO and OAC, ecDNA was absent in NDBO/LGD samples (0/42) while it was found in 25% of early-stage (T I, 13/51) and 43% in late-stage (T II-IV, 38/88) OAC tumours (Luebeck et al., 2023).

To summarise, large-scale and catastrophic genomic events are frequent in OAC and generally accumulate across the BO-OAC continuum, becoming most prevalent in late-stage OAC tumours. Investigating these events adds important information to understanding the molecular mechanisms related to the aetiology and outcomes of OAC.

1.7 Prognostic phenotypes in oesophageal adenocarcinoma

In a collaborative study from our group, it was demonstrated that OAC cases had improved survival if BO tissue was present adjacent to the tumour compared to cases without apparent BO tissue next to the tumour (Figure 1.3) (Sawas et al., 2018). These tumour phenotypes will be referred to as BO+ve OAC and BO-ve OAC hereafter, respectively.

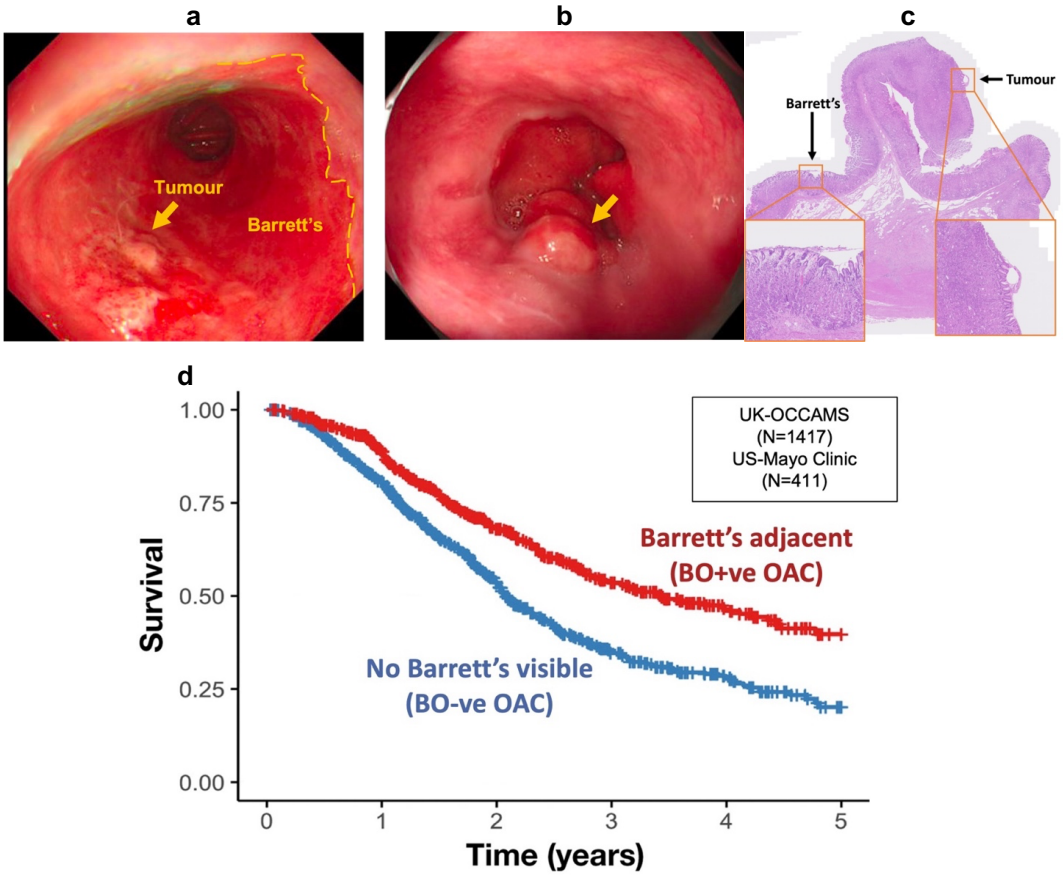

Figure 1.3 – Prognostic phenotypes of oesophageal adenocarcinoma. a, endoscopic image showing Barrett's oesophagus (dashed border) and tumour (arrow). b, endoscopic image of a tumour with no visible Barrett's oesophagus segment. c, microscopic visualisation of an H&E stained slide of a tumour with adjacent Barrett's and tumour histology highlighted. d, survival benefit observed for patients with BO +ve compared to BO-ve OAC tumours in the UK cohort (adapted from Sawas *et al* ., 2018).
Abbreviation: H&E, heaematoxylin and eosin.

In the study by Sawas *et al.* (2018), the UK cohort (n=1,417; 634 BO+ve OAC) and the US cohort (n=411; 204 BO+ve OAC) were analysed separately, yet the findings were similar. In the UK cohort, patients with BO+ve OAC tumours had a reduced risk of mortality compared to those with BO-ve OAC tumours (HR=0.59, 95% CI: 0.50-0.69) and a similar association was observed in the US cohort (HR=0.44, 95% CI: 0.34-0.57). In the UK cohort, this association remained significant after adjusting for patient age, gender, tumour stage and tumour location (HR=0.77, 95% CI: 0.64-0.93), and in the US cohort, the association also persisted after adjusting for the same factors as well as tumour length (HR=0.66, 95% CI: 0.50-0.88).

Several aetiologic possibilities may explain this survival difference. First, some OAC tumours may overgrow extant BO. While OAC overgrowth was observed in specific tumour stage subgroup analysis, the notation of BO was independent of tumour length and stage. Second, sudden and complex somatic alterations trigger genomic instability that rapidly transmutes BO to OAC thus inhibiting endoscopic detection. To date, genomic studies of BO/OAC have produced mixed and inconclusive findings regarding the existence of an 'aggressive' aetiology, likely due to the heterogonous nature of both conditions. Third and finally, some OACs may progress through a different pathway that does not involve BO.

A recent experimental study from our group investigated if all OACs, regardless of BO+ve or BO-ve OAC phenotype, originate from BO cells (Nowicki-Osuch et al., 2021). Single-cell RNA-seq profiles of NDBO were used to interrogate bulk RNA-seq data from OAC phenotypes. Both OAC phenotypes showed equal expression of undifferentiated BO cell markers, which were genes specific to BO undifferentiated and endocrine-like cells, while the expression of differentiated markers, BO columnar and goblet cell markers, was lower in both phenotypes. Thus, suggesting a shared metaplastic origin even when BO was not detected adjacent to the tumour (Figure 1.4).

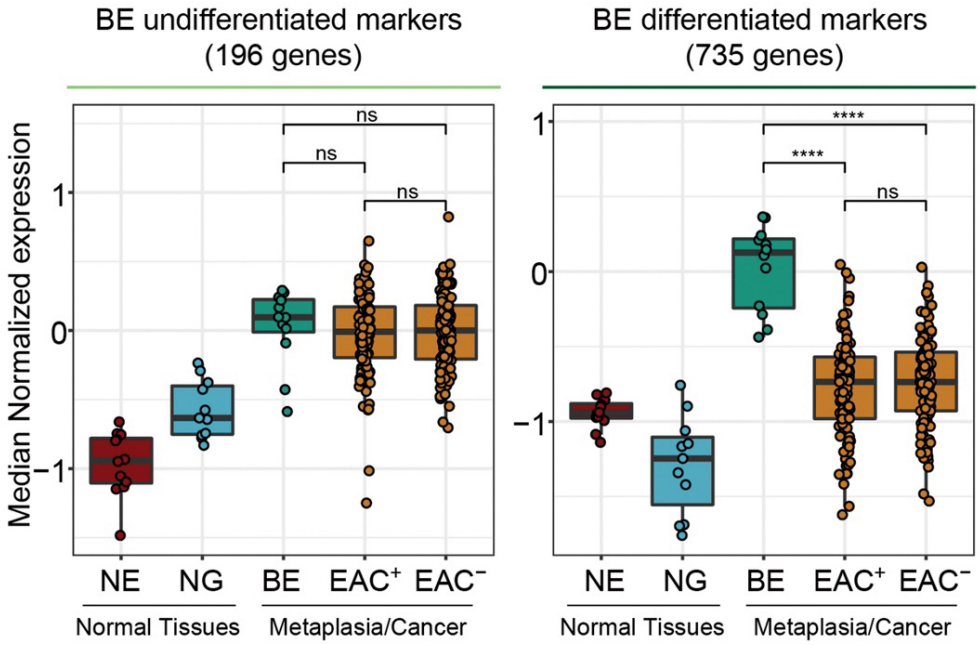

Figure 1.4 – Contribution of undifferentiated or differentiated markers of Barrett's oesophagus to phenotypes of oesophageal adenocarcinoma (adapted from Nowicki-Osuch *et al*., 2021). Undifferentiated and endocrine-like (left) or differentiated (foveolar-like/goblet; right) phenotypes of BE to transcriptomes of NE, NG, BE, and EAC samples with or without adjacent BE (EAC^+ and EAC^-). Abbreviations: BE, Barrett's esophagus; NE, normal esophagus; NG, normal gastric. EAC^+ and EAC^- correspond to BO+ve and BO-ve OAC.

Curtis *et al*. (2020) used a mathematical modelling approach to evaluate if the estimated prevalence of BO could explain the incidence of OAC in the US. Given the prevalence of GORD and the incidence of OAC in the US, a stochastic model was calibrated to estimate the expected number of OAC cases in defined populations and time periods. Additionally, the outputs included the prevalence of BO and its progression rates as extrapolated using the published epidemiology of GORD and BO/OAC. The estimated number of OAC cases (n=9,970, 95% CI: 9,140-11,980) closely approximated the incidence reported in the Surveillance, Epidemiology and End Results registry (n=9,400) during the study period, as well as previous prior estimations of 10,000 incidence OAC cases annually. These findings suggest that it is improbable that OAC tumours can arise from a pathogenic pathway which is independent of BO.

The prognostic difference observed for OAC phenotypes is an important finding that raises questions about the aetiology and outcomes of OAC. Recent evidence suggests that all OACs arise from BO. However, epidemiological and genomic investigations are needed to further elucidate the characteristics of these tumour phenotypes and understand factors that may explain the altered prognosis between these two phenotypes.

Chapter 2

Hypothesis and aims

Epidemiological studies have identified major risk factors for Barrett's oesophagus (BO) and oesophageal adenocarcinoma (OAC) including increasing age, male gender, obesity, cigarette smoking and chronic heartburn. Furthermore, genomic investigations have revealed important molecular changes across the BO-OAC progression continuum. The identification of prognostic phenotypes of OAC has raised questions about the aetiology of OAC, which have been scrutinised in recent experimental and computational studies. The current knowledge base lacks consolidated epidemiological, clinical and molecular data in the context of OAC phenotypes. To address this, I formulated the hypothesis that an epidemiological and molecular investigation would further determine whether all OAC arises from BO or whether there are additional pathogenic pathways. To evaluate this hypothesis, I assembled a large molecular epidemiological cohort, characterised by OAC phenotype as determined by the appearance or lack of visible BO adjacent to OAC (BO+ve OAC or BO-ve OAC) to address the following aims:

1. Confirm the prognostic difference between BO+ve and BO-ve OAC phenotypes in a larger cohort and comprehensively examine other potential prognostic factors.

2. Elucidate the association of epidemiological factors with BO+ve and BO-ve OAC phenotypes compared to BO and GORD cases.

3. Evaluate the genomic landscape of mutations, mutational signatures, copy number alterations and other genomic events in BO+ve and BO-ve OAC phenotypes.

4. Determine whether other epidemiological and/or molecular factors influence the altered prognosis in these phenotypes.

Chapter 3

Methods

3.1 Cohort design

3.1.1 Overview

All cases of OAC were recruited through the Oesophageal Cancer Classification and Molecular Stratification (OCCAMS) study, a UK-based consortium with 25 study centres that was created in 2010 to develop a robust clinical and molecular prospective dataset for prevention, detection and treatment of oesophageal cancer (occams.org.uk). Data from OCCAMS was used in the initial study of prognostic OAC phenotypes by Sawas *et al.* in 2018 and this dataset is independent of the US-based Mayo Clinic cohort which is not included in this thesis. Since the 2018 study, the OCCAMS study has expanded in size and nearly 4,500 oesophageal cancer patients have been recruited at the time of writing. The clinical centres that span the UK and constitute OCCAMS ensure the patient cohort is representative on a regional level.

Cases of BO and reflux controls were ascertained from Barrett's oEsophagus Screening Trial 2 (BEST2) which was a multi-centre case-control study examining the performance, safety, sensitivity and specificity of the Cytosponge™–Trefoil Factor 3 test in detecting BO compared to the standard-of-care endoscopy and biopsy (Ross-Innes et al., 2017). The trial aimed to recruit 500-700 cases and 500-700 controls across 11 study centres in the UK between June 2011 to September 2013. Similar to OCCAMS, the BEST2 study centres were distributed across the country to recruit a representative group of patients.

The OCCAMS study was registered and approved by relevant research ethics entities (UKCRNID-8880, REC 07/H0305/52 and 10/H0305/1). The BEST2 trial was approved by the East of England–Cambridge Central Research Ethics Committee (10/H0308/71) and registered in the UK Clinical Research Network Study Portfolio (No. 9461). All participants

provided individual informed consent and all data was anonymised. All studies were initiated and coordinated by the Fitzgerald Research Group at the University of Cambridge.

Using the methodology detailed below, an overall cohort of 4,695 cases and controls with epidemiological data, pathology annotation and a sub-cohort with whole genome sequencing data was assembled as shown in Figure 3.1.

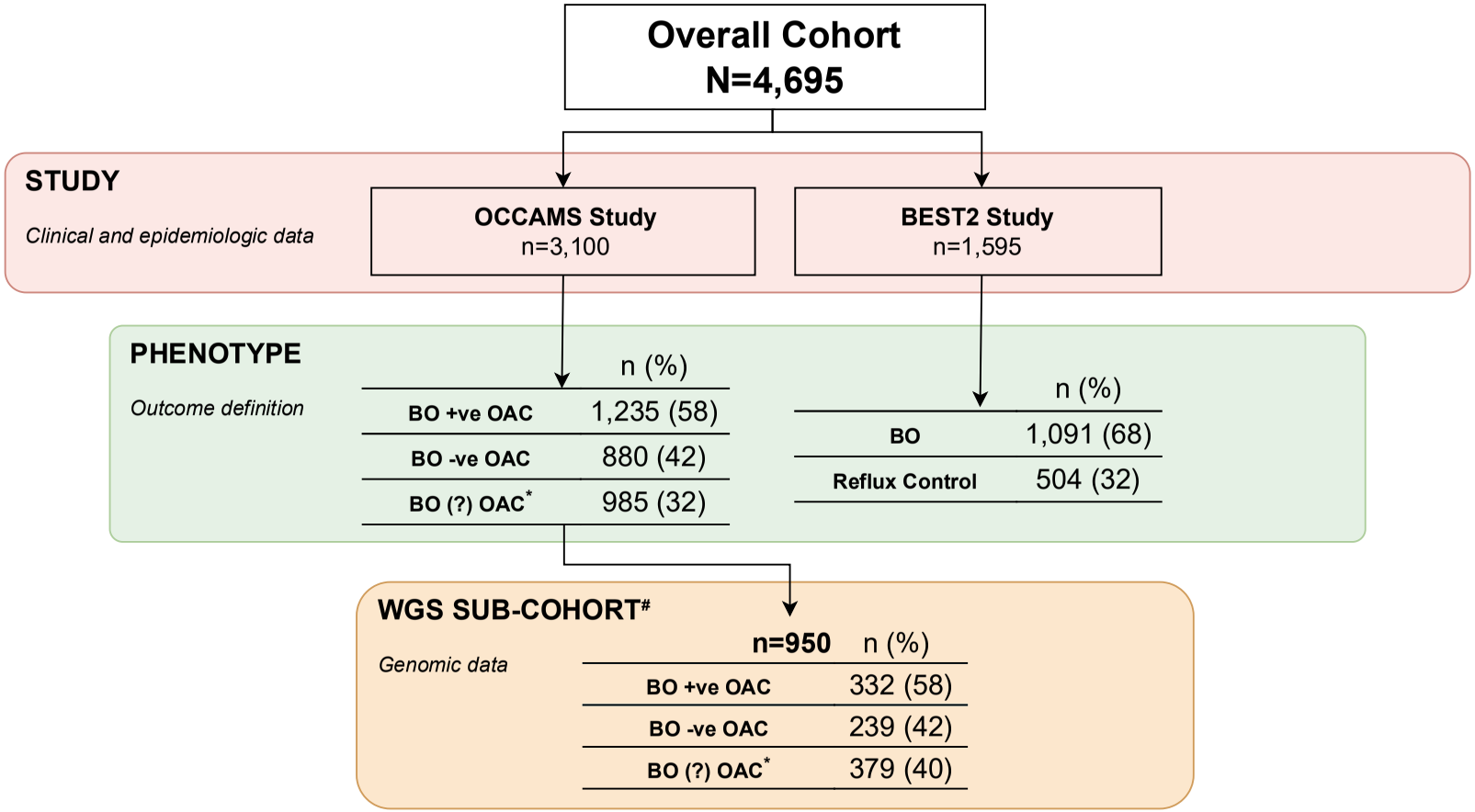

Figure 3.1 – Overview of the study cohort. The number of cases from each study along with the phenotype definition and samples with whole-genome sequencing data are outlined. *The percentage of BO(?) OAC is based on all cases from OCCAMS, while BO+ve OAC and BO-ve OAC proportions are derived only from cases with an ascertainable phenotype. #The entire sub-cohort (n=950) is used only in mutation burden analysis, but a subset of 710 WGS tumours comprised of 252 BO+ve OAC (58%), 183 BO-ve OAC (42%) and 275 BO(?) OAC (39%) is used in all analyses beyond mutation burden. Abbreviations: OCCAMS, Oesophageal Cancer Clinical and Molecular Stratification; BEST2, Barrett's oEsophagus Screening Trial 2; BO, Barrett's oesophagus; OAC, oesophageal adenocarcinoma; WGS, whole-genome sequencing.

3.1.2 Oesophageal adenocarcinoma cohort

Selection of cases

The inclusion criteria for the OCCAMS study select patients with a confirmed diagnosis of adenocarcinoma of the oesophagus, stomach and gastro-oesophageal junction suitable for therapy which was generally neo-adjuvant chemotherapy and surgery (oesophagostomy or extended total gastrectomy). For these cases, we aimed to collect pre-treatment samples for sequencing but where this was not possible a sample was taken from the surgical resection specimen. For patients with early disease, treatment comprised endoscopic therapy (endoscopic mucosal resection with or without radiofrequency ablation). A small number of advanced-stage patients were included who were initially deemed to be curative but in whom the full staging showed more advanced disease not suitable for a curative pathway.

Comprehensive clinical research guidelines were developed in the Fitzgerald Research Group and followed by trained clinical and research staff at all OCCAMS study centres. At each study centre, eligible OAC patients were identified and approached regarding participation in the OCCAMS study and their desire to join as a participant. Alternatively, an OCCAMS research staff in the clinic asked the patient's permission to be contacted with more information via mail and a follow-up phone call. Patients had the opportunity to ask questions, to think about their involvement by talking to their GP, for example, and could return the signed form later. Consent was obtained from patients to contact their GP to inform them of their participation in OCCAMS and to obtain relevant medical information from the cancer registries and other NHS data controllers.

Figure 3.2 describes the flow of OAC cases from the initial database to the final study sample included here. Patients with pathologically assessed tumours and diagnosed with any adenocarcinoma of the stomach, oesophagus and gastro-oesophageal junction between 2002-2022 were included – these were classified according to their Siewert type. The Siewert-Stein classification system provides the anatomical location of OAC tumours relative to gastric cardia (Siewert & Stein, 2003). Patients with tumour histology other than OAC were excluded, and a majority (n=233, 67%) were OSCC cases. Furthermore. Patients with 'open & shut' surgery with more advanced disease than expected were also excluded. This was because a tumour sample was generally not collected for these patients, therefore tumour phenotype ascertainment would have not been possible. In addition, little data was recorded on the baseline questionnaire forms for such patients. A small number of cases (n=22, <1%) were missing age or gender and these were excluded. Of note, the cohort included 214 (5%) patients with a history of undergoing BO surveillance. This information was either self-reported or abstracted from medical records by OCCAMS research staff. These cases

are later excluded as part of sensitivity analyses and also examined separately in survival analysis.

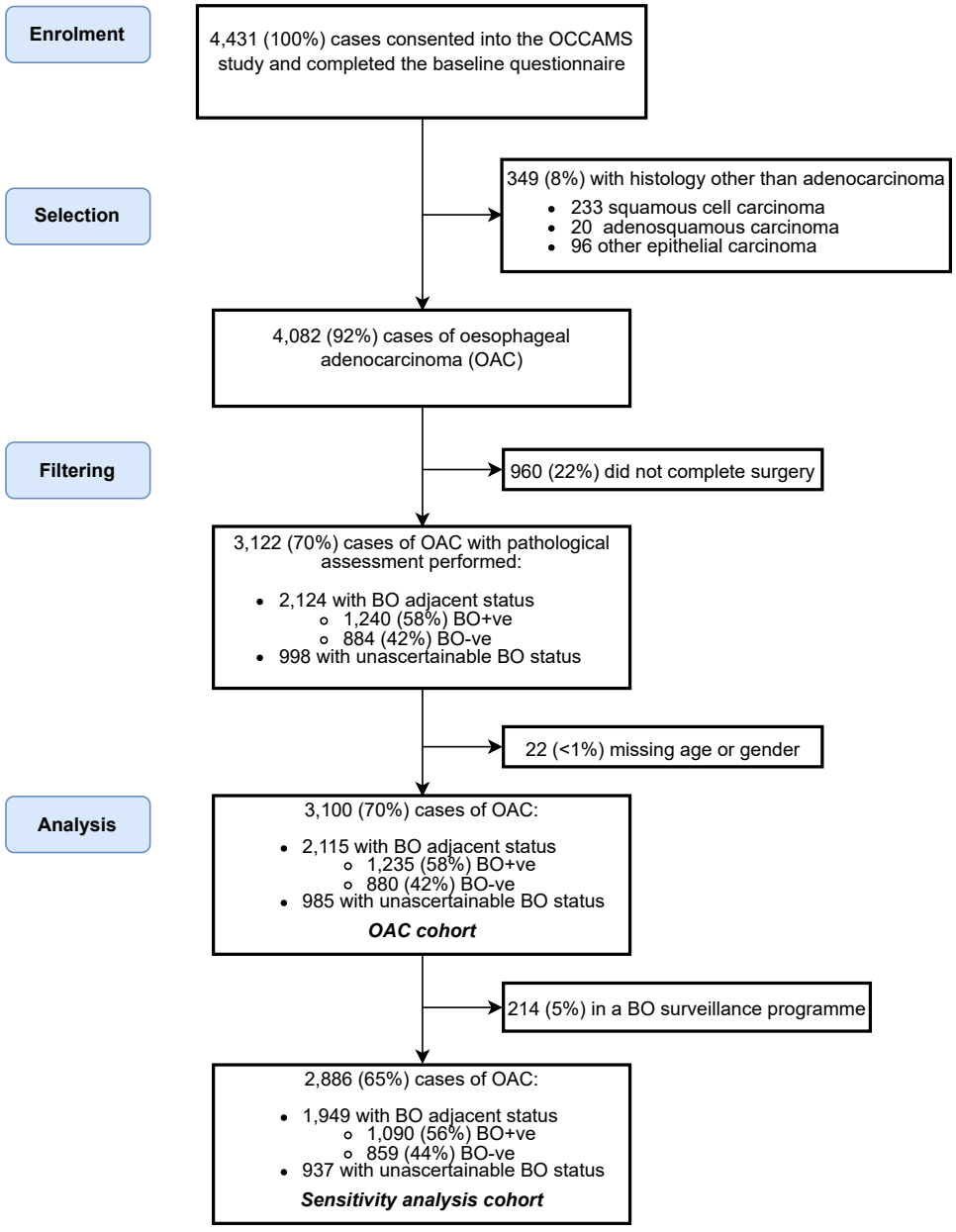

Figure 3.2 – Flow diagram for selecting oesophageal adenocarcinoma cases. All cases originated from the OCCAMS database. Abbreviations: OCCAMS, Oesophageal Cancer Classification and Molecular Stratification; BO, Barrett's oesophagus.

Pathology review

A strict expert pathology review was performed for all cases. At least two pathologists reviewed each OAC case: one pathologist from the referring study centre and another pathologist from the OCCAMS central study centre at Cambridge University Hospitals who had more than 20 years of experience in upper GI cancer. Tumours were staged based on the UICC/AJCC Tumour, Nodes and Metastases (TNM) Guidelines (7th edition) (Edge & Compton, 2010). The T, N and M stages were assigned using the available information in the patient's medical records including clinical chart notes, endoscopic ultrasound, positron emission tomography, endoscopic mucosal resection and histopathological reports following surgical resection. We used the most advanced stage prior to or at the time of surgery for patients that received neoadjuvant therapy.

The presence of BO adjacent to OAC for OCCAMS cases based on endoscopic (macroscopic) visual changes observed at pre-staging evaluation with pathology review showing IM at the time of surgical resection which was assessed by expert GI pathologists of the recruiting OCCAMS sites. IM was also identified in cases without macroscopic evidence of BO upon expert review of the pathology specimen. All pathologists followed a specific synoptic report proposed by the College of American Pathologists. Additionally, pathologists followed the OCCAMS study protocol which required thorough assessment for BO in the proximal and distal resection margins and tumour. Tumour sampling was done for all borders of the resected tumour and the tumour bed to minimise sampling error. The number of biopsy specimens varied based on tumour size.

From the 3,100 cases, a total of 2,115 (68%) tumours could be evaluated for BO adjacent status according to the outlined pathology review process. Among these tumours, 1,235 (58%) were classified as BO+ve OAC if IM was detected and confirmed and 880 (42%) were classified as BO-ve OAC if IM was not detected as outlined above. Although we performed a thorough evaluation for IM, it is important to note the possibility that extant IM may have been overgrown by the tumour or very small IM segments may have been missed and indeed this is the hypothesis being tested. Hence, these tumours may be more appropriately described as having no visible adjacent BO present rather than being BO/IM-absent tumours.

A total of 985 tumours comprising 32% of 3,100 cases in the analysis cohort were unascertainable for BO adjacent status. This was because tumours were not evaluated for IM during pre-staging, did not have information regarding IM on the pathology report or did not have pathology reports on file. These tumours were defined as BO(?) OAC tumours meaning BO adjacent status was unascertainable. This group of tumours likely represents a mixture of tumours with adjacent BO and tumours without visible adjacent BO so these were kept in the analysis to use as a comparison group.

Baseline data collection

Trained research staff collected baseline characteristics using chart review or during structured face-to-face interviews using a uniform case report form (CRF) across the 25 sites in the OCCAMS Consortium. All covariates used in the analysis originated from the study CRF. Patient baseline characteristics were collected on demographic, anthropometric and environmental exposures. Weight and height were measured objectively at the baseline visit or the from the next closest record to baseline. Overall survival time (in years) was calculated from the date of diagnosis to the date of death or the date patient was last seen in the clinic. Vital status was ascertained from all-cause mortality. All patients who consented to participate provided the minimum reporting standard which required demographic and clinical details.

Research staff transcribed and entered data captured on the OCCAMS CRFs into the study database. These data were anonymised and stored in a secure central database hosted on Cambridge University Hospitals NHS Foundation Trust servers. Several data management issues should be noted as the data collection process may introduce errors or biases. Errors during the baseline interview (e.g., failure to ask questions or record a response) or lack of information in the case notes/electronic records may contribute to missing data. As many patients are of advanced age, recall bias may also introduce discrepancies (e.g., answers to history of heaving drinking or smoking).

A history of undergoing BO surveillance was assessed using self-report or medical chart review by study staff, however, the source of this information was not recorded.

Nested questionnaire cohort

In February 2018, an additional questionnaire instrument was introduced as part of the baseline enrolment data collection when the OCCAMS study began contributing to the Cancer Grand Challenges Mutographs project (mutographs.org). As part of this effort, a subset of patients recruited to the OCCAMS study was asked to complete an extensive questionnaire on their demographics, lifestyle and environmental exposure factors. This questionnaire was originally developed by researchers involved in the Mutographs project. I revised the questionnaire in 2020 to include more detailed questions and simplify the questions to reduce respondent burden. The questionnaire collects information about patients in 12 domains including demographics, body size, medical history, family medical history, smoking history, alcohol use patterns, consumption of hot drinks, dietary patterns, occupational history, non-occupational physical activity, oral health and reproductive history.

The questionnaire was completed 1) during the same visit where patients were enrolled in the OCCAMS study and completed the baseline OCCAMS CRF, 2) during a later visit within the first month following the initial diagnosis, or 3) mailed to patients within a month following the initial diagnosis and returned to the OCCAMS central study centre in Cambridge. OCCAMS patients who completed this questionnaire were included in a sub-cohort called the Mutographs Questionnaire Cohort (MQC). Similar to the data entry process for OCCAMS CRFs, paper questionnaires for the MQC were transcribed into an electronic form per patient. Data entry was completed by multiple research staff including myself between 2018 to 2023 and different versions of the questionnaire were used. Therefore, there was significant variation in the raw data that needed to be standardised before any analysis could be conducted. I exported the raw data from the electronic forms into a master dataset (comma-separated values file format, .csv) using Adobe Acrobat Pro software (Adobe Inc., Mountain View, California, US). Following data export, I completed pre-processing steps to clean the raw dataset. The pre-processing step cleaned all variable values by isolating the response and denoting the units in the variable name (a total of 313 variables). For example, as many fields allowed free-text entry, variables such as height were recorded as a numeric value followed by different abbreviations of the unit (e.g., feet, f, ft, ‘, etc.). Following the pre-processing step and development of a processed dataset, variables were further recoded, converted or calculated. For example, height was self-reported using metric or imperial system measures and it was converted into meters for all patients. After data cleaning, 10% of questionnaires were randomly selected and manually corroborated against the cleaned dataset to ensure data quality and integrity. This process was completed per each batch of exports. All data pre-processing and processing steps were carried out using R Version 4.2.3 on macOS Ventura 13.3.1.

3.1.3 Barrett’s oesophagus cases and reflux controls

Selection of cases and controls

Cases and controls were recruited across 11 BEST2 study centres in the UK. At these centres, consecutive patients who were clinically indicated for an endoscopy procedure were asked to participate. Cases were defined as patients with a previous diagnosis of BO undergoing endoscopic surveillance. Controls were patients who were referred for endoscopy due to dyspepsia and/or reflux symptoms. Incident BO cases among controls were crossed over to the case arm. Patients with bleeding diatheses, using an anticoagulant medication, or with known cirrhosis, oesophageal varices or dysphagia were excluded.

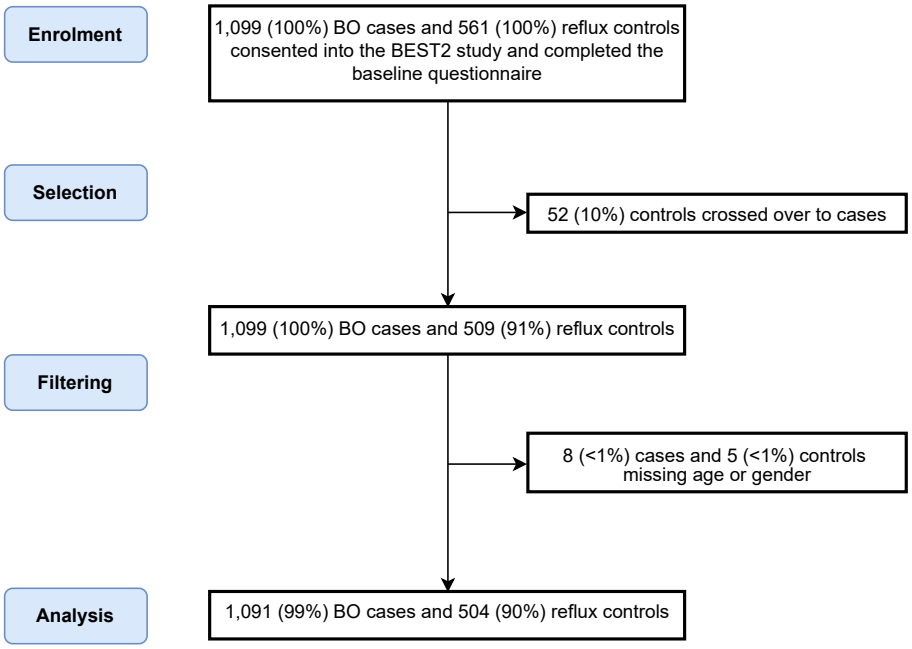

Figure 3.3 – Flow diagram for selecting Barrett's oesophagus and reflux controls. All cases and controls originated from the BEST2 database. Abbreviation: BO, Barrett's oesophagus.

The flowchart for selecting cases and controls for the cohort used here is described in Figure 3.3. Controls that were found to have BO and crossed over to cases were excluded (10%) to avoid introducing bias, as they may have different characteristics or risk factors than other controls that did not cross over. Few cases and controls (<1%) were missing age or gender and these were also excluded.

Pathology review

BO was defined as an endoscopically visible columnar-lined oesophagus that measured at least 1 cm circumferentially or at least 3 cm in non-circumferential tongues (Prague classification $\geq$ C1 or $\geq$ M3), with documented histopathological evidence of IM on at least one biopsy in the course of their endoscopic history.

Baseline data collection

At each study centre and during a single office visit, trained research staff administered the uniform CRF to cases and controls to collect patient baseline characteristics on demographics, risk factors exposures and reflux symptoms using a validated reflux questionnaire (Jones et al., 2007). Each paper CRF was then entered by the study centre's research nurse using a secured web-based application with the primary dataset hosted on Cambridge University Hospitals NHS Foundation Trust servers.

3.2 Data preparation and variable construction

3.2.1 Processing of baseline clinical and epidemiological data

The raw and fully anonymised baseline data for OCCAMS (R Data file format, .Rdata) and BEST2 (comma-separated values file format, .csv) were exported to my university-furnished computing device in June 2022. The files containing the data for OCCAMS and BEST2 were collated, processed and screened for completeness, accuracy and consistency. Data were cleaned, removing or correcting any inconsistencies, inaccuracies or implausible values. All pragmatic strategies to minimise missing data were implemented. The cleaned dataset was then carefully checked against the raw data to ensure quality data pre-processing. The datasets and data-cleaning code were saved as plain-text files and tracked and managed using version control software (Git/Subversion). All data processing was carried out using R Version 4.2.3 on macOS Ventura 13.3.1.

The following common methodology was used to clean data for both OCCAMS and BEST2 studies. Due to the inclusion criteria, age at diagnosis and gender were complete. Ethnicity was recoded into white or other as there were too few observations in other ethnicity codes which is not unexpected for BO/OAC patient population.

The age at diagnosis for OAC and BO cases and age at recruitment for reflux controls, as well as BMI at baseline, were categorised into groups. This was done to create a more meaningful comparison for these measures. Age at diagnosis was categorised into four groups of under 50 years old, 50-59 years old, 60-69 years old and 70 years or older. BMI was calculated using the baseline weight and height (weight in kg/height in m squared) and BMI categories were defined according to standard ranges of underweight (<18.5 kg/m^2), normal (18.5-24.9 kg/m^2), overweight (25-29.9 kg/m^2) and obese ($\geq$30 kg/m^2). Underweight cases were included among normal weight due to very small frequencies in the cohort (<2.5%). The continuous distribution and the grouped frequencies were used in descriptive analyses and only the categorical variables for age and BMI were included in regression models.

Cigarette smoking was collapsed into a binary variable with ‘former’ and ‘current’ recoded as ‘ever’ smoker and ‘never’ remaining as defined. Additionally, if the average number of cigarettes per day was recorded as zero, smoking status was set to ‘never’ and if it was a non-zero value then smoking was recoded to ‘ever’. The number of pack-years was calculated by multiplying the number of packs of cigarettes smoked per day by the number of years of smoking.

The self-reported responses for medication use frequency of aspirin, NSAIDs, PPIs, H2RAs and over-the-counter acid (OTC) suppressants included ‘Never,’ ‘No,’ ‘Past Use,’ ‘Occasional Use,’ and ‘Current Use.’ However, responses such as ‘Past Use’ and ‘Occasional

Use' are open-ended, so to mitigate this issue, responses were recoded to binary 'Ever Use' and 'Never Use'. The duration of medication use was recorded in years, months, weeks and days. The total duration of use (in years) was calculated for each medication type by summing the individual measures. Additionally, if the frequency was set to 'Never' and a non-zero total duration of use was reported, the total was set to null. Conversely, if a non-zero duration was recorded, then the frequency of use was set to 'Ever Use'. Aspirin and NSAID frequency of use were combined into a single variable measuring use of either medication. Similarly, a single variable for the use of any acid suppressant medication was derived using the frequency of use of PPIs, H2RAs and OTC acid suppressants.

Alcohol intake was recorded as the number of units of beer, wine and spirits consumed per week. These individual measures were summed into a single continuous variable for the total number of alcoholic drink units consumed per week. Heavy drinking status was self-reported by patients in both studies.

In both studies, frequency of reflux symptoms was reported as 'Never', 'Sometimes', 'Often', 'Daily' and 'Unknow/sporadic'. Duration of reflux symptoms was harmonised into an ordinal variable with four ranges (Never, 5 years, 5-10 years, > 10 years and unknown/sporadic). A single variable was created that combined all measures related to reflux symptoms and acid-suppressant medication use. This variable is referend to as the "derived heartburn symptoms status" variable (Figure 3.4). In addition, a single variable for use of any acid-suppressant medications was derived based only on the acid-suppressant medication use variables (patient on acid suppressant and use or duration of PPIs, OTC acid suppressant medications or H2RAs).

For variables that contained responses with undefined free text or numeric ranges instead of a single value, the response was either set to missing or the mid-range was calculated. For example, a free text input of 'undistilled only' for total alcohol unit intake was set to missing and a response of '3-5' cigarettes per day was recalculated to '4' per day. Continuous variables where a negative numeric value was recorded were recoded to missing as per CRF instructions.

The UK regions for OCCAMS and BEST2 study centres were determined based on their locations and classified using the International Territorial Level 1 (Office of National Statistics). Finally, for OAC cases only, combined TNM staging was created according to the UICC/AJCC 7th edition guidelines (Edge & Compton, 2010).

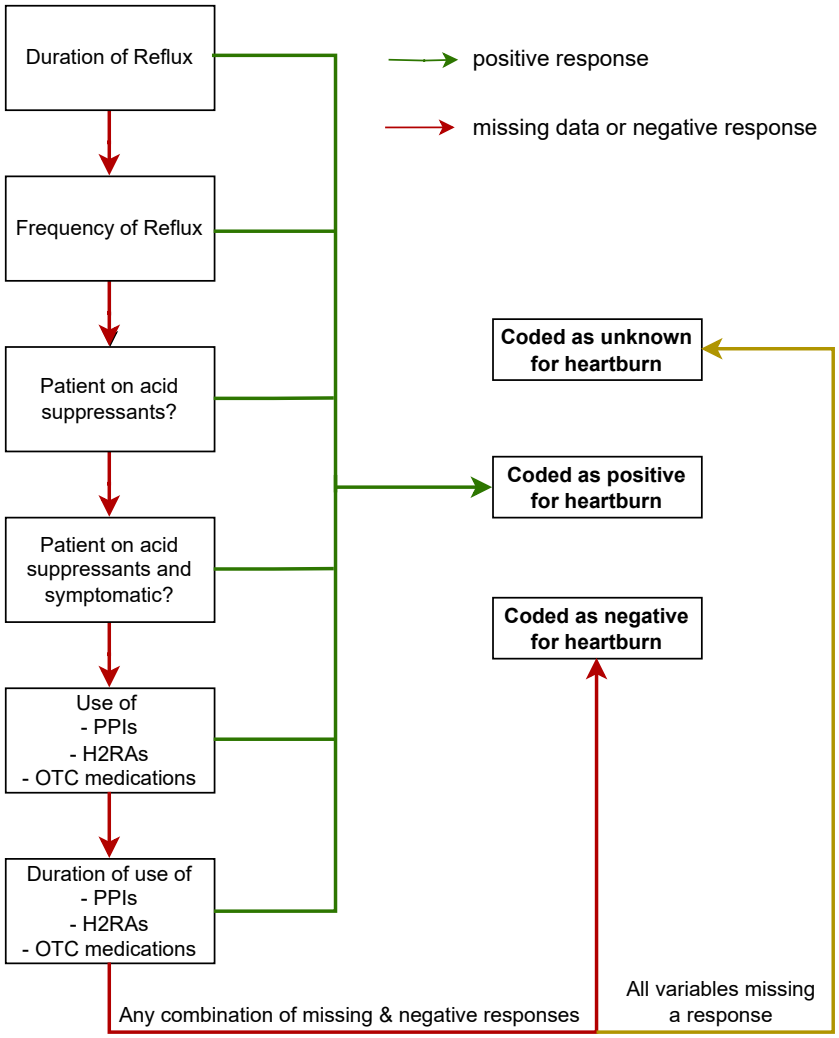

Figure 3.4 – Schematic for deriving the heartburn variable using a combination of reflux-related variables. Abbreviations: PPIs, proton pump inhibitors; H2RAs, histamine H2-receptor antagonists; OTC, over-the-counter.

3.2.2 Variable selection

Following baseline data cleaning and screening in the OCCAMS cohort and as informed by the results of the literature review, a total of 34 variables across five domains were deemed relevant and included (Table 3.1). To select variables for inferential analysis, a purposeful selection process was followed:

1. Unconditional logistic regression was used to obtain univariable ORs and 95% CIs for the association of each variable with BO-ve OAC compared to BO+ve OAC cases.

2. Variables with a p-value <0.25 and missing data <60% overall were pre-selected and included in a multivariable logistic regression model with BO-ve OAC as the outcome compared to BO+ve OAC.

3. Only variables with a p-value <0.05 or those deemed to have epidemiological or clinical importance were selected in the final stage. Directed acyclic graphs were also used to determine which variables should be included as potential confounders.

4. Variables from the BEST2 study were then harmonised to match the selected variables from the OCCAMS data as outlined.

Table 3.1 – List of variables included for the analysis from the OCCAMS study. Bold indicates variables which were selected for further analysis using the selection process.

Domain	Variable
Demographics	**Age at diagnosis**
	Gender
	Ethnicity
Risk factor exposures	**BMI at baseline; *kg/m²***
	BMI five years prior to diagnosis; *kg/m²*
	BMI difference (prior to baseline); *kg/m²*
	Cigarette smoking status
	Number of cigarettes smoked per day
	Years of smoking cigarettes
	Number of pack-years of smoking
	Heavy alcohol drinking status
	Units of alcohol intake per week
Anti-inflammatory medications	Aspirin use status
	Years of aspirin use
	NSAID use status
	Years of NSAID use
	Any use of aspirin or NSAID
Reflux symptoms & acid suppressant medications	Frequency of reflux symptoms
	Duration since reflux symptoms began
	Currently taking acid suppressant medications
	Currently symptomatic for reflux while on acid-suppressants
	PPI medication use status
	Years of PPI medication use
	OTC acid suppressant medication use status
	Years of OTC medication use status
	H2RA medication use
	Years of H2RA medication use
	Use of any acid suppressant/reducing medications status
	Derived heartburn symptom status
Clinical factors	Tumour length, *cm*
	Siewert-Stein classification
	Tumour location (resection pathology)
	Tumour growth (T stage pre-op to T stage post-op)
	TNM

3.3 Whole-genome sequencing

3.3.1 Cohort, processing and sequencing of samples

A total of 950 tumours had undergone WGS through the OCCAMS study. Of these, a total of 496 (52%) were part of the ICGC sequencing project, 214 (23%) were from the Mutographs study and 240 (25%) were included in the Exploring oeSophageal CAncer using informative popuLATion sEquencing study (ESCALATE). Strict pathology consensus review was observed for these samples, with a minimum 70% cellularity requirement before inclusion in the ICGC or Mutographs projects or a minimum 50% cellularity for ESCALATE. All tissue samples were snap frozen. Peripheral blood was used as the germline reference and in cases where this was not possible, a sample of normal squamous epithelium located at least 5 cm away from the tumour was used as the germline reference.

Methods for sample quality control, DNA extraction and WGS were as previously described (Abbas et al., 2023; Frankell et al., 2019). Briefly, samples in the ICGC/Mutographs projects underwent WGS at an average depth of 50X under contracts by Illumina (San Diego, US) and ESCALATE samples were sequenced at an average depth of 150X by Genomics England (GEL, London, UK). Matched tumour samples were sequenced at 30X and 75X for samples in the ICGC/Mutographs or ESCALATE projects, respectively. Reads were then aligned with BWA-MEM to GRCh37 (ICGC/Mutographs) or GRCh38 (ESCALATE) (Li, 2013). Quality checks were conducted using the FastQC package (bioinformatics.babraham.ac.uk/projects/fastqc) and duplicated reads were removed using Picard (broadinstitute.github.io/picard).

3.3.2 Single nucleotide and copy number variant calling

Somatic variants were called using Strelka version 2.0.15 (Kim et al., 2018) and Variant Effect Predictor (VEP) version 78 (McLaren et al., 2016). For ICGC/Mutographs samples, mutation burden was derived from each VEP file by summing the number of SNVs and INDs across the genome. Multiple samples per case were sequenced in ESCALATE and mutation burden was calculated by taking the average across each case's VEP file. Mutations per megabase were calculated using the length of the reference genome (3,137,454,505 bp). GISTIC2.0 was used to detect recurrently deleted or amplified regions of the genome using raw copy number values obtained from ASCAT (Mermel et al., 2011; Van Loo et al., 2010).

3.3.3 Selection and calling of driver genes

Previously reported driver genes in OAC were derived from genes listed in Frankell *et al*. (2019) and genomic regions were identified using Ensembl BioMart (Cunningham et al., 2022). These gene regions were then used to extract alterations from the outputs of VEP and GISTIC2.0. Driver mutation status was determined based on the alteration type (e.g., missense, nonsense or frameshift) using Strelka and VEP. One or more affected copies were deemed as a mutation.

3.3.4 Mutational signatures

Mutational signatures discovery within the cohort was carried out using SigProfilerExtractor (Alexandrov et al., 2013). The optimal signature configuration was determined by selecting from a range of signature combinations (from 5 to 17) based on the highest stability and lowest Frobenius reconstruction error for a signature combination. The optimal configuration was composed of 14 signatures, and its validity was confirmed by independent analysis using Bayesian methodology from Sigminer (Wang et al., 2021). Subsequently, deconstructSigs was employed to deduce the mutational contributions of these processes to each sample, following the identification of the primary mutational processes in the cohort (Rosenthal et al., 2016).

3.3.5 Whole-genome duplication and aneuploidy

Raw copy number values from ASCAT and the PCAWG-11 consensus purity pipeline (github.com/PCAWG-11) were used to determine samples with whole-genome duplication based on tumour ploidy and the extent of loss of heterozygosity (Dentro et al., 2021). Per sample ploidy and purity were also inferred using this method.

3.3.6 Identification and classification of amplicon events

Copy number segments were called using CNVkit version 0.9.8 and regions of amplifications of size 50kb, copy number > 4.5 were used as input for the identification of amplified regions and reconstructed using Amplicon Architect (Deshpande et al., 2019; Talevich et al., 2016). The classification of amplicons into ecDNA and BFB events was done using Amplicon Classifier (github.com/jluebeck/AmpliconClassifier).

3.4 Study design

3.4.1 Comparsion of phenotypes

The baseline characteristics were compared between pairs of OAC phenotypes, as well as between each OAC phenotype and BO cases and reflux controls. This resulted in nine comparison pairs (Table 3.2). The cases-case and case-control analyses aimed to investigate the degree of similarity or difference between the baseline characteristics of OAC phenotypes and their association with BO and reflux controls. BO cases were not compared to reflux controls as this was not an aim of the study. The analysis of genomic characteristics was only conducted for the first three comparison sets and used a different number of samples as previously outlined (3.1.1).

Table 3.2 – Comparison sets and group sizes used for the analysis of baseline characteristics.

Reference group	Outcome group	Comparison set	$n_{reference}$ vs. $n_{outcome}$
BO+ve OAC	BO-ve OAC	Primary	1,235 vs. 880
BO(?) OAC	BO+ve OAC	2	985 vs. 1,235
	BO-ve OAC	3	985 vs. 880
BO cases	BO+ve OAC	4	1,091 vs. 1,235
	BO-ve OAC	5	1,091 vs. 880
	BO(?) OAC	6	1,091 vs. 985
Reflux controls	BO+ve OAC	7	504 vs. 1,235
	BO-ve OAC	8	504 vs. 880
	BO(?) OAC	9	504 vs. 985

3.4.2 Statistical analysis

Logistic regression

Since the outcome in each comparison was dichotomous and the association of covariates was non-linear, logistic regression was appropriate. Multinomial logistic regression was also considered; however, it was determined that binary logistic regression would be more appropriate due to its simpler interpretation. The statistical independence of the outcomes

was assumed based on the absence of repeated events and the binomial distribution of the residual variation. It is rare for this assumption of logistic regression to be violated.

The process of selecting variables for these comparisons was described in 3.2.2 (Table 3.1). To ensure that the assumption of multiplicativity was satisfied, effect measure modification was assessed between BMI and heartburn and aspirin/NSAID use and heartburn. A priori it was known that BMI may modulate heartburn. The latter interaction was tested because the heartburn variable was partly derived from PPI use and NSAIDs may modify the effects of PPI in relation to OAC (Jankowski et al., 2018).

For each comparison set, crude and adjusted OR and 95%CI were obtained for the association of the age group, gender, BMI group, cigarette smoking, aspirin/NSAID use, heartburn symptoms and TNM (OAC only) with the outcome phenotype. We performed three separate adjusted analyses per comparison: 1) minimally adjusted for age and gender only, 2) fully adjusted for all covariates and 3) fully adjusted model eliminating heartburn as a covariate. As heartburn may be on the causal pathway, if its elimination as a covariate changed the log odds ratio by more than 10%, then it could be considered a confounder. Missing data were coded as indicator variable.

Three sensitivity analyses were performed to assess the robustness of the estimates obtained using the fully adjusted model for each comparison set. The first sensitivity analysis involved excluding any observations with missing data for the variables in the fully adjusted model, adopting a complete case approach. The second sensitivity analysis utilised estimates derived from multiple imputation data (detailed below). Lastly, a sensitivity analysis was conducted by excluding OAC cases with a history of undergoing BO surveillance.

Missing data

Missing data for baseline characteristics was calculated as a percentage of the total number of cases. The percentage of the recorded values is reported as a fraction of complete cases. For variables dependent on the response to other variables, the missing percentage was calculated as a fraction of cases where the first response variable was available. For example, the proportion of missing data for the duration of cigarette smoking was based on the total number of cases who self-reported current or former cigarette smoking.

Multiple imputation (MI) was performed on the datasets corresponding to each comparison group to assess how missing data might bias the observed associations. Age and gender were complete and therefore not included in MI. BMI group, cigarette smoking, aspirin/NSAID use, heartburn symptoms and TNM (OAC only) were imputed using multiple imputation by chained equations with the appropriate method selected based on the variable type (van Buuren & Groothuis-Oudshoorn, 2011).

The missing data were assumed to be missing completely at random, meaning that the probability of a value being missing is not related to other data. This assumption was based on the similar distribution observed for recorded and imputed data. Furthermore, baseline data were collected by numerous research staff, and based on our experience, we assumed that variations in the order of the CRF questions, completeness of each section and other factors may have impacted the quality and accuracy of the data collected. Therefore, we assumed that systematic exclusion of data was unlikely. The number of imputations (m) was set to the percent value of the variable with the highest amount of missing data in each dataset which was aspirin/NSAID use with approximately 50-60% missing data. The number of iterations (n) was set to 20 as typically recommended (Nguyen et al., 2017).

Survival analysis

Kaplan-Meier curves and the log-rank test were used to examine OAC phenotypes as a univariable predictor of overall survival and Cox proportional hazards regression models were used to obtain unadjusted and adjusted HRs and 95% CIs for this association. The proportional hazards (PH) assumption was tested by using Kaplan-Meier curves and an interaction term between covariates and follow-up time. There was no violation of the PH assumption. The minimal adjustment set was identical to Sawas *et al.* (2018) which included age, gender, TNM and Siewert Classification. The full adjustment set was further adjusted for BMI group, smoking status, aspirin/NSAID use and heartburn symptoms. Missing data were coded as indicator variable.

Sensitivity analyses were performed by excluding OAC cases with a history of participating in a BO surveillance programme or excluding cases with under one year of recorded follow-up time. In OCCAMS, overall survival time (years) was calculated from the date of diagnosis to the date of death, or the date patient was last seen in the clinic. Vital status was ascertained from all-cause mortality. Person-years was calculated by summing the total follow-up time for all cases. Crude mortality rates (per 100 person-years) were calculated using by dividing the number of deaths by the total number of person-years.

Non-parametric data, transformations and multiple hypothesis testing

Statistical comparisons between groups were performed using either the Kruskal-Wallis test or the Mann-Whitney U test, as indicated by the normality of the data distribution. When applicable, data were log-transformed to ensure normality. In cases where multiple comparisons were made, adjustment for false discovery rate using the Benjamini-Hochberg (BH) procedure was applied.

Computing environment

All analyses were performed using R Version 4.2.3 (R Foundation, Vienna, Austria) on macOS Ventura 13.3.1 with packages ‘rstatix’, ‘mice’, ‘survival’, ‘survminer’ and ‘coxme’.

Chapter 4

Results

4.1 Confirmation of the prognostic difference

First, we sought to confirm the survival advantage observed for cases with BO+ve OAC tumours compared to those with BO-ve tumours as previously described by Sawas *et al*. (2018). When examined according to OAC phenotype, the median overall survival time was 6.23 years among BO+ve OAC (95% CI: 5.21-6.96) compared to 4.19 years (95% CI: 3.54-5.12) among BO-ve OAC cases (log-rank p=0.00025; Figure 4.1). This observation is consistent with the findings reported in that by Sawas and colleagues in 2018. Although BO status was available for a total of 2,115 OAC cases, 15 cases had no vital status or follow-up time recorded, therefore, 2,100 cases were included in this analysis. Of note, the cohort of BO+ve OAC and BO-ve OAC cases used here is substantially in sample size (n=2,100) than the published Mayo and Cambridge cohorts combined (n=1,828). Additionally, the median survival for both phenotypes increased by approximately 2.5 years and nearly twice as many BO+ve OAC cases were annotated in this analysis (n=1,223) compared to the published Cambridge cohort (n=634) (Sawas et al., 2018). Having established that the prognostic difference has persisted over time and in a larger cohort, we next analysed the clinical and epidemiological characteristics of the cohort.

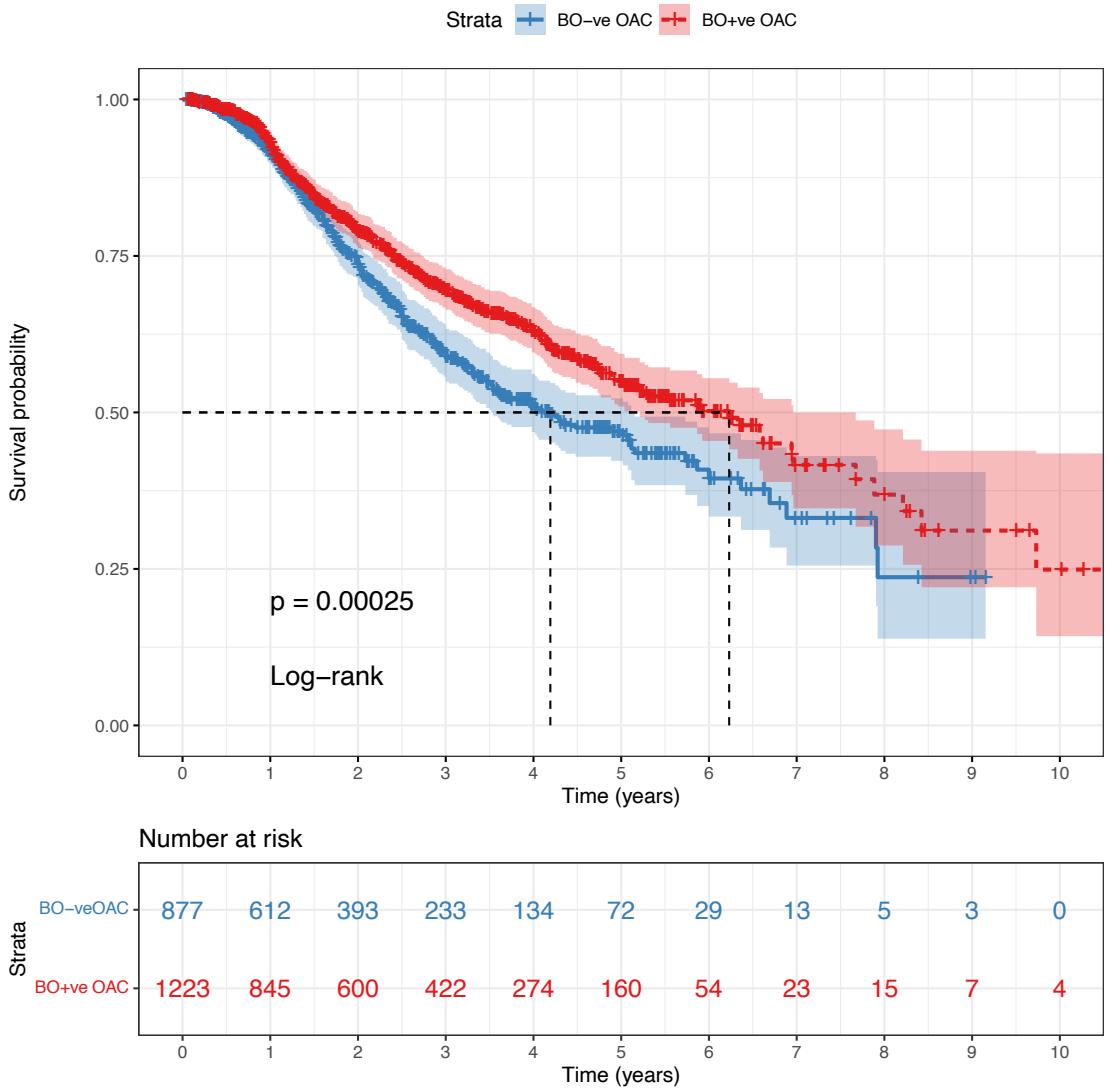

Figure 4.1 – Kaplan-Meier survival curve showing survival probability for BO+ve OAC and BO-ve OAC cases. Vertical ticks on the curve indicate an event (death) and shaded areas indicate the 95% confidence interval. Median survival time is marked with the dashed lines. The life table at the bottom shows the number of patients at risk at the beginning of each follow-up year.

4.2 Descriptive statistics

4.2.1 Baseline characteristics of oesophageal adenocarcinoma cases

A total of 3,100 cases of OAC were included in the cohort. Of these, tumour phenotype was ascertained for 2,115 (68.2%) cases while the phenotype was unascertainable for the remaining 985 (31.8%) cases. Among the tumours with an ascertained phenotype, 880 (41.6%) were classified as BO-ve OAC and 1,235 (58.4%) as BO+ve OAC cases. The distribution of baseline characteristics across the entire cohort and according to tumour phenotype is summarised in Tables 4.1-4.6.

Distribution of OAC cases per centre and region

The number of OAC cases included from across the OCCAMS study centres is shown in Table 4.1. Study centres were grouped based on geographic regions to provide a pooled estimate for the regional distribution of OAC phenotypes. This also helped to derive a more meaningful estimate for the distribution of OAC phenotypes as some centres had relatively smaller cases. Three regions (East of England, London and South East England) contributed more than half of all cases included in the thesis cohort. Across nine regions and 25 study centres, the proportion of BO+ve OAC and BO-ve OAC phenotypes was generally similar. In regions comprising 10% or more of the total cohort (n>310), the proportion of BO+ve OAC cases ranged from approximately 50% to 60% which is consistent with the literature. However, there was more variation in regions with a smaller share of cases. For example, in South West England, nearly 75% of tumours were classified as BO+ve OAC and only 25% were BO-ve OAC. Overall, there was a wide variation in the distribution of BO(?) OAC phenotype which ranges from 11.0% in Scotland to 57.3% in South West England.

Table 4.1 – Frequency of OAC cases per OCCAMS study centre and distribution of OAC phenotypes according to geographic regions. The regions and centres are arranged in descending order based on the total number of participants.

Region	Location	OCCAMS study centre	Total n (%)	Region total n (%)	BO+ve OAC n (%)	BO-ve OAC n (%)	BO(?) OAC n (%)
East of England	Cambridge	Addenbrooke's Hospital	471 (15.2)	597 (19.3)	285 (59.0)	198 (41.0)	114 (19.1)
	Norwich	Norfolk & Norwich University Hospital	126 (4.1)				
London	St Thomas	Guy's & St Thomas' Hospitals	356 (11.5)	521 (16.8)	160 (56.9)	121 (43.1)	240 (46.1)
	Imperial College London	St Mary's Hospital	80 (2.6)				
	University College London	University College Hospital	52 (1.7)				
	Romford	Queen's Hospital	33 (1.1)				
South East, England	Southampton	Southampton General Hospital	307 (9.9)	465 (15.0)	174 (50.9)	168 (49.1)	123 (26.5)
	Guildford	Royal Surrey County Hospital	156 (5.0)				
	Portsmouth	Queen Alexandra Hospital	2 (0.1)				
West Midlands, England	Birmingham	Queen Elizabeth Hospital	212 (6.8)	361 (11.6)	152 (60.8)	98 (39.2)	111 (30.7)
	Coventry	University Hospitals Coventry & Warwickshire	86 (2.8)				
	Stafford	Royal Stoke University Hospital	31 (1.0)				
	Bordesley Green	Heartlands Hospital	20 (0.6)				
	Worcester	Worcestershire Royal Hospital	12 (0.4)				
East Midlands, England	Nottingham	Nottingham City Hospital	324 (10.5)	324 (10.5)	116 (50.0)	116 (50.0)	92 (28.4)
Scotland	Edinburgh	Royal Infirmary of Edinburgh	296 (9.5)	308 (9.9)	178 (65.0)	96 (35.0)	34 (11.0)
	Dundee	Ninewells Hospital	10 (0.3)				
	Kilmarnock	University Hospital Crosshouse	2 (0.1)				
South West, England	Plymouth	Derriford Hospital	203 (6.5)	255 (8.2)	81 (74.3)	28 (25.7)	146 (57.3)
	Gloucester	Gloucestershire Royal Hospital	44 (1.4)				
	Bournemouth	Royal Bournemouth Hospital	8 (0.3)				
North West, England	Salford	Salford Royal Hospital	104 (3.4)	169 (5.5)	57 (58.8)	40 (41.2)	72 (42.6)
	Wythenshawe	Wythenshawe Hospital	59 (1.9)				
	Wigan	Wrightington Hospital	6 (0.2)				
Northern Ireland	Belfast	Belfast City Hospital	100 (3.2)	100 (3.2)	32 (68.1)	15 (31.9)	53 (53.0)
Total (%)	**25 (locations/centres)**		**3,100 (100.0)**		**1,235 (58.4)**	**880 (41.6)**	**985 (31.7)**

For Tables 4.2-4.6, the univariable odds ratio (OR) and 95% confidence interval (95% CI) were calculated using unconditional logistic regression for the association of baseline characteristics with BO-ve OAC cases as the outcome compared to the BO+ve OAC cases (primary comparison group). As mentioned in Methods (3.2.2, Table 3.1), variables showing statistical significance or deemed clinically important in this analysis were included in subsequent analyses. Additionally, data on BO cases and heartburn controls were harmonised according to the retained variables in this analysis.

Demographics of OAC cases

As expected, patients were predominately over 60 years old (74.5%, n=2,309) male (84.5%, n=2,618) with white British ethnicity (98.2%, n=2,902). Increasing age was associated with reduced risk of the BO-ve OAC phenotype (OR=0.99, 95% CI: 0.98-1.00, p=0.003). Of note, male cases were less frequent in the BO-ve OAC phenotype group compared to the BO+ve OAC phenotype (OR=0.69, 95% CI: 0.55-0.88, p=0.003). Ethnicity was not associated with either phenotype (Table 4.2).

Table 4.2 – Baseline demographics of OAC cases overall and according to tumour phenotype. The univariable odds ratios and 95% confidence intervals are for the association of characteristics with risk of BO-ve OAC phenotype compared to BO+ve OAC (primary comparison set).

Characteristic	BO+ve OAC (n=1,235)	BO-ve OAC (n=880)	BO(?) OAC (n=985)	Overall (n=3,100)	Univariable OR (95% CI, p) BO-ve OAC vs. BO+ve OAC
Age at diagnosis; *years*					
Mean [SD]	66.7 [9.2]	65.5 [9.8]	65.3 [10.2]	65.9 [9.7]	0.99 (0.98-1.00, p=0.003)
Median [Q1, Q3]	67.5 [61.4, 73.5]	66.7 [59.0, 72.6]	66.7 [58.6, 72.9]	67.0 [59.8, 73.0]	-
Age group at diagnosis, n (%)					
< 50 years old	67 (5.4)	59 (6.7)	75 (7.6)	201 (6.5)	1.00 (Referent)
50 - 59 years old	193 (15.6)	184 (20.9)	213 (21.6)	590 (19.0)	1.08 (0.72-1.62, p=0.700)
60 - 69 years old	497 (40.2)	317 (36.0)	333 (33.8)	1147 (37.0)	0.72 (0.50-1.06, p=0.094)
70+ years old	478 (38.7)	320 (36.4)	364 (37.0)	1162 (37.5)	0.76 (0.52-1.11, p=0.155)
Gender, n (%)					-
Female	163 (13.2)	158 (18.0)	161 (16.3)	482 (15.5)	1.00 (Referent)
Male	1072 (86.8)	722 (82.0)	824 (83.7)	2618 (84.5)	0.69 (0.55-0.88, p=0.003)
Ethnicity, n (%)					
Other	20 (1.71)	11 (1.32)	23 (2.42)	54 (1.83)	1.00 (Referent)
White British	1152 (98.3)	822 (98.7)	928 (97.6)	2902 (98.2)	1.30 (0.63-2.82, p=0.491)
Missing	63 (5.1)	47 (5.3)	34 (3.5)	144 (4.6)	1.36 (0.60-3.19, p=0.470)

Risk factor exposures among OAC cases

The distribution of risk factors is presented in Table 4.3. The mean BMI at baseline was 27.1 (standard deviation [SD]=4.9) kg/m^2 with 42.3% (n=1,046) and 26.5% (n=654) of patients in the overweight or obese BMI range, respectively. BMI five years prior to diagnosis

was slightly more elevated than the baseline with a mean of 29.2 (SD=5.7) kg/m^2 and a higher proportion of obese cases (37.0%, n=338). Furthermore, cases lost an average of 2.20 (SD=3.8) kg/m^2 of BMI from five years prior to OAC diagnosis. This BMI shift likely reflects cancer-associated dietary and metabolic changes leading to weight loss which tends to be profound in this disease due to oesophageal obstruction. Relative to BO+ve OAC cases, cases with BO-ve OAC tumours were more likely to have a lower BMI and while this association was statistically significant, it was not substantial (OR=0.96, 95% CI: 0.94-0.98, p<0.001). Similarly, when examined based on BMI groups and against normal BMI, BO-ve OAC cases were more likely to be underweight (OR=2.45, 95% CI: 1.12-5.76, p=0.029) and less likely to be obese at baseline (OR=0.56, 95% CI: 0.43-0.73, p<0.001). Neither BMI five years prior to diagnosis nor the difference in BMI was associated with the outcome of BO-ve OAC phenotype. This lack of association may be due to the reduced statistical power as both variables had a high proportion of missing data.

Self-reported cigarette smoking was prevalent in the cohort with 62.5% (n=1,759) of cases having ever smoked. Compared to BO+ve OAC cases, BO-ve OAC cases were only marginally more likely to report ever smoking (OR=1.21, 95% CI: 1.01-1.46, p=0.041). The mean number of cigarettes smoked per day was 18.1 (SD=11.5) which is nearly equivalent to a standard pack of cigarettes (20 cigarettes/pack). In addition, the mean length of time for cigarette smoking was 29.7 (SD=15.1) years and the mean number of pack-years was calculated to be 28.7 (SD=24.3). No association was observed for the number of cigarettes smoked per day, years of cigarette smoking and the number of pack-years and risk of either phenotype. This may be due to the relatively small number of cases with recorded pack-years of smoking.

Overall, current heavy drinking was self-reported by 14.2% (n=300) of cases. The mean units of any alcoholic beverage intake (beer, wine and/or spirits) per week was 20.4 (SD=30.1). However, the distribution of units of alcohol intake per week was left-skewed due to 119 cases with outlying values ($\geq$ 30 units/week), the median and interquartile range (IQR) are more robust measures (median=12.0, IQR: 5.0-24.0). Not unexpectedly, none of the alcohol intake measures were associated with risk of BO-ve OAC compared to BO+ve OAC.

Table 4.3 – Baseline risk factors of OAC cases overall and according to tumour phenotype. The univariable odds ratios and 95% confidence intervals are for the association of characteristics with risk of BO-ve OAC phenotype compared to BO+ve OAC (primary comparison set).

Characteristic	BO+ve OAC (n=1,235)	BO-ve OAC (n=880)	BO(?) OAC (n=985)	Overall (n=3,100)	Univariable OR (95% CI, p) BO-ve OAC vs. BO+ve OAC
BMI at baseline; *kg/m²*					
Mean [SD]	27.5 [4.9]	26.7 [4.9]	26.8 [5.0]	27.1 [4.9]	0.96 (0.94-0.98, p<0.001)
Median [Q1, Q3]	27.0 [24.0, 30.0]	26.0 [23.0, 29.0]	26.0 [24.0, 30.0]	27.0 [24.0, 30.0]	-
Missing n (%)	241 (19.5)	153 (17.4)	235 (23.9)	629 (20.3)	0.70 (0.53-0.91, p=0.009)
BMI group at baseline, n (%)					
Normal	259 (26.1)	235 (32.3)	217 (28.9)	711 (28.8)	1.00 (Referent)
Underweight	9 (0.9)	20 (2.8)	31 (4.1)	60 (2.43)	2.45 (1.12-5.76, p=0.029)
Overweight	422 (42.5)	317 (43.6)	307 (40.9)	1046 (42.3)	0.83 (0.66-1.04, p=0.106)
Obese	304 (30.6)	155 (21.3)	195 (26.0)	654 (26.5)	0.56 (0.43-0.73, p<0.001)
Missing	241 (19.5)	153 (17.4)	235 (23.9)	629 (20.3)	0.70 (0.53-0.91, p=0.009)
BMI five years prior to diagnosis; *kg/m²*					
Mean [SD]	29.4 [5.6]	29.2 [5.8]	28.8 [5.8]	29.2 [5.7]	0.99 (0.97-1.02, p=0.686)
Median [Q1, Q3]	28.5 [25.9, 32.1]	28.0 [25.2, 32.2]	28.0 [25.2, 31.2]	28.2 [25.5, 31.9]	-
Missing n (%)	874 (70.8)	593 (67.4)	704 (71.5)	2171 (70.0)	0.85 (0.71-1.03, p=0.104)
BMI group five years prior to diagnosis, n (%)					
Normal	66 (18.6)	60 (21.2)	62 (22.5)	188 (20.6)	1.00 (Referent)
Underweight	0 (0)	0 (0)	2 (0.7)	2 (0.2)	NE
Overweight	148 (41.8)	117 (41.3)	120 (43.5)	385 (42.2)	0.87 (0.57-1.33, p=0.520)
Obese	140 (39.5)	106 (37.5)	92 (33.3)	338 (37.0)	0.83 (0.54-1.28, p=0.406)
Missing	874 (70.8)	593 (67.4)	704 (71.5)	2171 (70.0)	0.75 (0.52-1.08, p=0.114)
BMI difference (five years prior-diagnosis to baseline); *kg/m²*					
Mean [SD]	-1.9 [4.0]	-2.4 [3.9]	-2.4 [3.4]	-2.2 [3.8]	0.97 (0.93-1.01, p=0.123)
Median [Q1, Q3]	-1.29 [-3.4, -0.2]	-1.66 [-3.9, -0.3]	-1.69 [-4.0, -0.6]	-1.54 [-3.8, -0.3]	-
Missing n (%)	875 (70.9)	594 (67.5)	705 (71.6)	2174 (70.1)	0.85 (0.71-1.04, p=0.104)

Table continued next page…

Table 4.3 – Baseline risk factors of OAC cases overall and according to tumour phenotype. The univariable odds ratios and 95% confidence intervals are for the association of characteristics with risk of BO-ve OAC phenotype compared to BO+ve OAC (primary comparison set).

Characteristic	BO+ve OAC (n=1,235)	BO-ve OAC (n=880)	BO(?) OAC (n=985)	Overall (n=3,100)	Univariable OR (95% CI, p) BO-ve OAC vs. BO+ve OAC
Table continued from last page…					
Cigarette smoking status, n (%)					
Never	452 (40.5)	298 (35.9)	304 (35.1)	1054 (37.5)	1.00 (Referent)
Ever	665 (59.5)	532 (64.1)	562 (64.9)	1759 (62.5)	1.21 (1.01-1.46, p=0.041)
Missing	118 (9.6)	50 (5.7)	119 (12.1)	287 (9.3)	0.64 (0.44-0.92, p=0.017)
Number of cigarettes smoked per day					
Mean [SD]	18.9 [11.7]	19.2 [12.3]	16.2 [10.2]	18.1 [11.5]	1.00 (0.99-1.01, p=0.746)
Median [Q1, Q3]	20.0 [10.0, 20.0]	20.0 [10.0, 20.0]	15.0 [10.0, 20.0]	20.0 [10.0, 20.0]	
Missing n (%)	198 (29.8)	174 (32.7)	208 (37.0)	580 (33.0)	1.10 (0.87-1.39, p=0.431)
Duration of smoking cigarettes; *years*					
Mean [SD]	30.4 [15.3]	29.2 [15.6]	29.3 [14.5]	29.7 [15.1]	1.00 (0.99-1.00, p=0.310)
Median [Q1, Q3]	30.0 [20.0, 41.0]	30.0 [18.0, 40.0]	30.0 [20.0, 40.0]	30.0 [20.0, 40.0]	-
Missing n (%)	244 (36.7)	207 (38.9)	239 (42.5)	690 (39.2)	1.06 (0.85-1.32, p=0.597)
Number of pack-years of smoking					
Mean [SD]	30.4 [25.6]	29.3 [24.5]	25.8 [22.0]	28.7 [24.3]	1.00 (0.99-1.00, p=0.582)
Median [Q1, Q3]	25.0 [12.0, 41.3]	25.0 [11.5, 40.0]	21.0 [10.0, 37.5]	24.7 [10.5, 40.0]	-
Missing n (%)	270 (40.6)	233 (43.8)	271 (48.2)	774 (44.0)	1.08 (0.88-1.33, p=0.480)
Heavy alcohol drinking status, n (%)					
No	738 (87.6)	542 (85.9)	535 (83.3)	1815 (85.8)	1.00 (Referent)
Yes	104 (12.4)	89 (14.1)	107 (16.7)	300 (14.2)	1.17 (0.86-1.58, p=0.324)
Missing	393 (31.8)	249 (28.3)	343 (34.8)	985 (31.8)	0.86 (0.71-1.05, p=0.135)
Units of alcohol intake per week					
Mean [SD]	20.6 [28.3]	20.9 [30.5]	19.6 [32.0]	20.4 [30.1]	1.00 (0.99-1.01, p=0.928)
Median [Q1, Q3]	13.0 [5.00, 22.8]	14.0 [5.00, 26.5]	11.0 [5.00, 24.0]	12.0 [5.00, 24.0]	-
Missing n (%)	921 (74.6)	672 (76.4)	737 (74.8)	2330 (75.2)	1.10 (0.90-1.36, p=0.357)

Abbreviations: NE, not estimable.

Reflux and acid-suppressing medication use among OAC cases

The variables related to reflux and acid-suppressing medication use are summarised in Table 4.4. The history of reflux symptoms was assessed using the frequency and duration of symptom occurrence. Of note, 23.9% (n=310) of cases reported a 'never' frequency for reflux symptoms. For these cases, the duration since reflux symptoms began was also set to 'never' if other values were recorded. Most cases (25.9%, n=335) experienced a sporadic/unknown pattern followed by sometimes (22.5%, n=292), daily (16.3%, n=211) and often (11.4%, n=147). The most common duration since heartburn symptom occurrence began was under 5 years (43.9%, n=569) followed by greater than 10 years (18.2%, n=236) and between 5-10 years (9.7%, n=125). The sporadic/unknown duration was only observed among 55 cases overall (4.3%). No difference was seen in the frequency of reflux symptoms when comparing BO-ve OAC to BO+ve OAC cases. However, reflux symptoms duration of greater than 10 years was less prevalent among BO-ve OAC than BO+ve OAC cases (OR=0.51, 95% CI: 0.33-0.78, p=0.002).

On the OCCAMS CRF, two questions were asked related to patterns of reflux symptoms prior to diagnosis. The first question asked if patients were using any acid-suppressant medication prior to their OAC diagnosis, which was then followed up by a subsequent question asking if they experienced any symptoms despite using acid suppressants during that time. Overall, most cases (58.0%, n=1,288) reported using any acid-suppressant medication prior to baseline and most (57.9%, n=711) also reported not having experienced symptoms when using such medications. BO-ve OAC cases were less likely than BO+ve OAC cases to report use of acid suppressant medications prior to diagnosis (OR=0.73, 95% CI: 0.59-0.89, p=0.002). There was no association for persistent reflux symptoms while using such medications.

Not unexpectedly, acid suppressant medication use at baseline was ubiquitous in the cohort. Use of PPI, OTC and H2RA medications was reported by 76.4% (n=1,568), 56.1% (n=670) and 15.1% (n=177), respectively. On average, cases used PPI medications for 3.6 (SD=5.9) years, OTC medications for 8.0 (SD=10.3) years and H2RA medications for 3.3 (SD=5.4) years prior to diagnosis. The median estimates for years of use of these medications were much lower than the mean, therefore, it is likely that most patients were prescribed the medication close to diagnosis. A slightly shorter duration of PPI usage was reported by BO-ve OAC cases compared to BO+ve OAC cases (OR=0.95, 95% CI: 0.92-0.99, p=0.008). Similarly, OTC acid suppressant medications were less frequently reported by BO-ve OAC cases (OR=0.53, 95% CI: 0.40-0.70, p<0.001). There was no association between PPI use, duration of OTC medication use, or H2RA use frequency or duration and BO-ve OAC.

As described in Methods (3.2.1, Figure 3.4), two variables were constructed based on combinations of reflux and acid suppressant medication use variables. These variables are included under the "derived variables" label in Table 4.4. The first variable was based on the use and duration of all acid suppressant medications and is a single measure for use of any acid suppressant medications. The second variable combined the formerly derived variable with variables related to reflux symptoms to construct a single measure to infer overall whether cases had experienced heartburn symptoms. Both variables were constructed to address missing data and limitations in medication or symptom reporting (e.g., patients not remembering medication names or types). Based on the first variable, 77.5% (n=1,861) of cases reported using any acid suppressant medications up to diagnosis or baseline. The derived heartburn symptom status was positive for 81.2% (n=1,960) of cases, suggesting that heartburn or acid-suppressant medication use is relevant for most of the patient population. As expected, the association of any acid suppressant medications use with BO-ve OAC was similar to that seen for OTC acid suppressant use (OR=0.63, 95% CI: 0.50-0.79, p<0.001). Similarly, BO-ve OAC cases were less likely to have a positive response of 'present' for the derived heartburn symptom measure than BO+ve OAC cases (OR=0.62, 95% CI: 0.49-0.80, p<0.001). This association was driven mostly due to the association of the ever-use of any acid suppressant medications.

To summarise, across all statistically significant univariable associations, BO-ve OAC cases were less likely than BO+ve OAC cases to report symptoms of reflux or use of acid suppressant medications. Moreover, the former group was also less likely to have reported longer usage durations of such medication.

Table 4.4 – Baseline reflux symptoms characteristics and acid-suppressant medication use of OAC cases overall and according to tumour phenotype. The univariable odds ratios and 95% confidence intervals are for the association of characteristics with risk of BO-ve OAC phenotype compared to BO+ve OAC (primary comparison set).

Characteristic	BO+ve OAC (n=1,235)	BO-ve OAC (n=880)	BO(?) OAC (n=985)	Overall (n=3,100)	Univariable OR (95% CI, p) BO-ve OAC vs. BO+ve OAC
Frequency of reflux symptoms, n (%)					
Never	114 (21.7)	90 (24.3)	106 (26.5)	310 (23.9)	1.00 (Referent)
Sometimes	121 (23.0)	81 (21.9)	90 (22.5)	292 (22.5)	0.85 (0.57-1.26, p=0.412)
Often	66 (12.6)	36 (9.73)	45 (11.3)	147 (11.4)	0.69 (0.42-1.12, p=0.140)
Daily	84 (16.0)	68 (18.4)	59 (14.8)	211 (16.3)	1.03 (0.67-1.56, p=0.907)
Unknown/sporadic	140 (26.7)	95 (25.7)	100 (25.0)	335 (25.9)	0.86 (0.59-1.26, p=0.435)
Missing	710 (57.5)	510 (58.0)	585 (59.4)	1805 (58.2)	0.90 (0.67-1.21, p=0.492)
Duration since reflux symptoms began, n (%)					
Never	114 (21.7)	90 (24.3)	106 (26.5)	310 (23.9)	1.00 (Referent)
< 5 years	206 (39.2)	188 (50.8)	175 (43.8)	569 (43.9)	1.16 (0.82-1.63, p=0.403)
5-10 years	58 (11.0)	31 (8.4)	36 (9.0)	125 (9.7)	0.68 (0.40-1.13, p=0.139)
> 10 years	124 (23.6)	50 (13.5)	62 (15.5)	236 (18.2)	0.51 (0.33-0.78, p=0.002)
Unknown/sporadic	23 (4.38)	11 (2.97)	21 (5.25)	55 (4.25)	0.61 (0.27-1.28, p=0.202)
Missing	710 (57.5)	510 (58.0)	585 (59.4)	1805 (58.2)	0.90 (0.67-1.22, p=0.490)
Pre-baseline acid suppressant medications use, n (%)					
No	345 (38.3)	300 (46.2)	289 (43.0)	934 (42.0)	1.00 (Referent)
Yes	555 (61.7)	350 (53.8)	383 (57.0)	1288 (58.0)	0.73 (0.59-0.89, p=0.002)
Missing	335 (27.1)	230 (26.1)	313 (31.8)	878 (28.3)	0.79 (0.63-0.99, p=0.042)
Pre-baseline symptoms of reflux even while on acid suppressant medications, n (%)					
No	273 (55.5)	207 (60.0)	231 (59.2)	711 (57.9)	1.00 (Referent)
Yes	219 (44.5)	138 (40.0)	159 (40.8)	516 (42.1)	0.83 (0.63-1.10, p=0.194)
Missing	743 (60.2)	535 (60.8)	595 (60.4)	1873 (60.4)	0.95 (0.77-1.17, p=0.633)
PPI medication use, n (%)					
Never	188 (22.1)	151 (25.7)	145 (23.5)	484 (23.6)	1.00 (Referent)
Ever	661 (77.9)	436 (74.3)	471 (76.5)	1568 (76.4)	0.82 (0.64-1.05, p=0.117)
Missing	386 (31.3)	293 (33.3)	369 (37.5)	1048 (33.8)	0.95 (0.73-1.23, p=0.673)
Duration of PPI medication use; *years*					
Mean [SD]	4.1 [6.6]	2.7 [4.4]	3.7 [5.7]	3.6 [5.9]	0.95 (0.92-0.99, p=0.008)
Median [Q1, Q3]	0.8 [0.2, 5.0]	0.4 [0.2, 3.0]	0.8 [0.2, 5.0]	0.6 [0.2, 5.0]	-
Missing n (%)	300 (45.4)	222 (50.9)	233 (49.5)	755 (48.2)	1.12 (0.91-1.39, p=0.286)

Table continued next page…

Table 4.4 – Baseline reflux symptoms characteristics and acid-suppressant medication use of OAC cases overall and according to tumour phenotype. The univariable odds ratios and 95% confidence intervals are for the association of characteristics with risk of BO-ve OAC phenotype compared to BO+ve OAC (primary comparison set).

Characteristic	BO+ve OAC (n=1,235)	BO-ve OAC (n=880)	BO(?) OAC (n=985)	Overall (n=3,100)	Univariable OR (95% CI, p) BO-ve OAC vs. BO+ve OAC
Table continued from last` page…					
OTC acid suppressant medication use, n (%)					
Never	176 (36.7)	170 (52.3)	178 (45.8)	524 (43.9)	1.00 (Referent)
Ever	304 (63.3)	155 (47.7)	211 (54.2)	670 (56.1)	0.53 (0.40-0.70, p<0.001)
Missing	755 (61.1)	555 (63.1)	596 (60.5)	1906 (61.5)	0.76 (0.60-0.97, p=0.024)
Duration of OTC medication use; *years*					
Mean [SD]	8.5 [11.4]	7.4 [10.3]	7.8 [9.03]	8.0 [10.3]	0.99 (0.96-1.02, p=0.481)
Median [Q1, Q3]	5.0 [1.0, 10.0]	3.0 [0.8, 10.0]	4.0 [1.0, 10.0]	5.0 [1.0, 10.0]	-
Missing n (%)	164 (53.9)	86 (55.5)	90 (42.7)	340 (50.7)	1.03 (0.74-1.42, p=0.862)
H2RA medication use, n (%)					
Never	390 (84.1)	284 (86.1)	318 (84.8)	992 (84.9)	1.00 (Referent)
Ever	74 (15.9)	46 (13.9)	57 (15.2)	177 (15.1)	0.85 (0.57-1.27, p=0.436)
Missing n (%)	771 (62.4)	550 (62.5)	610 (61.9)	1931 (62.3)	0.98 (0.81-1.18, p=0.830)
Duration of H2RA medication use; *years*					
Mean [SD]	3.5 [6.1]	2.9 [4.7]	3.2 [5.3]	3.3 [5.4]	0.98 (0.87-1.08, p=0.709)
Median [Q1, Q3]	1.0 [0.3, 4.9]	1.0 [0.2, 3.0]	1.00 [0.2, 3.0]	1.0 [0.3, 4.0]	-
Missing n (%)	40 (54.1)	23 (50.0)	32 (56.1)	95 (53.7)	0.93 (0.49-1.74, p=0.806)
Derived variables					
Derived any acid suppressant medications use, n (%)					
Never	180 (18.7)	187 (26.8)	173 (23.4)	540 (22.5)	1.00 (Referent)
Ever	783 (81.3)	512 (73.2)	566 (76.6)	1861 (77.5)	0.63 (0.50-0.79, p<0.001)
Missing	272 (22.0)	181 (20.6)	246 (25.0)	699 (22.5)	0.64 (0.48-0.85, p=0.002)
Derived heartburn symptom status, n (%)					
Absent	150 (15.5)	160 (22.8)	144 (19.4)	454 (18.8)	1.00 (Referent)
Present	818 (84.5)	543 (77.2)	599 (80.6)	1960 (81.2)	0.62 (0.49-0.80, p<0.001)
Missing	267 (21.6)	177 (20.1)	242 (24.6)	686 (22.1)	0.62 (0.46-0.83, p=0.001)

Abbreviations: PPI, proton pump inhibitor; OTC, over-the-counter; H2RA, histamine H-2 receptor antagonist, NE, not estimable.

Anti-inflammatory medication use among OAC cases

Table 4.5 shows the summary statistics for measures of aspirin and NSAID use. The information on use of aspirin and other NSAIDs was recorded separately and also combined into a single measure. Ever-use of aspirin was observed among 42.1% (n=561) of cases overall with a mean duration of 6.9 (SD=7.1) years of use. For non-aspirin NSAIDs (i.e. ibuprofen, naproxen and diclofenac), 37.0% reported ever-use with a mean of 5.3 (SD=7.7) years of use. When combined, 57.4% (n=834) of cases overall reported ever-use of aspirin or other NSAIDs (aspirin/NSAID use). While no association was observed for the measures of aspirin use, ever-use of other NSAIDs was more likely to be reported among BO-ve OAC than BO+ve OAC cases (OR=1.50, 95% CI: 1.11-2.02, p=0.008). This effect was attenuated when aspirin and NSAIDs were combined into a single measure and did not reach statistical significance (OR=1.18, 95% CI: 0.91-1.53, p=0.202). There was no difference in the duration of use of aspirin or NSAIDs between the phenotypes.

Table 4.5 – Baseline anti-inflammatory medication use of OAC cases overall and according to tumour phenotype. The univariable odds ratios and 95% confidence intervals are for the association of characteristics with risk of BO-ve OAC phenotype compared to BO+ve OAC (primary comparison set).

Characteristic	BO+ve OAC (n=1,235)	BO-ve OAC (n=880)	BO(?) OAC (n=985)	Overall (n=3,100)	Univariable OR (95% CI, p) BO-ve OAC vs. BO+ve OAC
Aspirin use, n (%)					
Never	306 (57.6)	209 (57.3)	256 (58.7)	771 (57.9)	1.00 (Referent)
Ever	225 (42.4)	156 (42.7)	180 (41.3)	561 (42.1)	1.02 (0.77-1.33, p=0.913)
Missing	704 (57.0)	515 (58.5)	549 (55.7)	1768 (57.0)	1.07 (0.87-1.32, p=0.521)
Duration of aspirin use; *years*					
Mean [SD]	7.8 [7.9]	6.0 [5.8]	6.60 [7.0]	6.94 [7.1]	0.96 (0.91-1.01, p=0.132)
Median [Q1, Q3]	5.0 [1.1, 11.3]	4.0 [1.4, 10.0]	4.00 [1.0, 10.0]	4.3 [1.00, 10.0]	
Missing n (%)	133 (59.1)	100 (64.1)	99 (55.0)	332 (59.2)	1.08 (0.78-1.51, p=0.632)
NSAID use, n (%)					
Never	305 (66.6)	177 (57.1)	239 (63.4)	721 (63.0)	1.00 (Referent)
Ever	153 (33.4)	133 (42.9)	138 (36.6)	424 (37.0)	1.50 (1.11-2.02, p=0.008)
Missing	777 (62.9)	570 (64.8)	608 (61.7)	1955 (63.1)	1.26 (1.02-1.57, p=0.032)
Duration of NSAID use; *years*					
Mean [SD]	6.4 [8.6]	3.8 [6.4]	5.56 [7.6]	5.3 [7.7]	0.95 (0.89-1.01, p=0.142)
Median [Q1, Q3]	2.0 [0.4, 9.5]	0.7 [0.4, 5.0]	2.00 [0.5, 7.3]	2.0 [0.2, 6.0]	-
Missing n (%)	107 (69.9)	94 (70.1)	88 (63.8)	289 (68.2)	1.01 (0.70-1.45, p=0.998)
Aspirin/NSAID use, n (%)					
Never	257 (43.9)	161 (39.9)	201 (43.3)	619 (42.6)	1.00 (Referent)
Ever	328 (56.1)	243 (60.1)	263 (56.7)	834 (57.4)	1.18 (0.91-1.53, p=0.202)
Missing	650 (52.6)	476 (54.1)	521 (52.9)	1647 (53.1)	1.17 (0.93-1.47, p=0.183)

Abbreviations: NSAID, non-steroidal anti-inflammatory drugs; NE, not estimable.

Clinical and pathological characteristics of OAC cases

The clinical and pathological characteristics of the OAC cases are presented in Table 4.6. Overall, the mean tumour length was 4.0 (SD=3.0) centimetres. Predominately, tumours were located in the gastro-oesophageal junction (GOJ) region (66.9%, n=796) with Siewert-Stein Type II tumours (straddling the junction) tumours being the most common (48.7%, n=524), followed by predominantly oesophageal Type I (36.8%, n=396) and predominantly proximal stomach Type III (14.5%, n=156). As expected due to the late stage at presentation for this disease (stage III or higher), and since these cases had to be deemed suitable for a curative pathway (stage III or lower, with some exceptions for stage IV) to be recruited into OCCAMS, most tumours were diagnosed with TNM stage III (52.2%, n=1352) followed by stage II (25.9%, n=671), stage I (19.2%, n=497) and stage IV (2.7%, n=70). Following treatment and as measured by the change in T-stage from clinical-stage to resection pathology stage, the majority of tumours (50.7%, n=1,431) had no change in size to resection pathology stage while 31.1% (n=879) of tumours shrank and 18.2% grew (n=515). Lastly, 16.5% (n=214) of all patients had a history of enrolment in a BO surveillance programme. The effect of including these patients is examined in a sensitivity analysis in subsequent results.

No association was observed between the length of tumours and risk of BO-ve OAC compared to BO+ve OAC phenotype. Relative to the GOJ, BO-ve OAC tumours were less frequently located in the oesophagus (OR=0.60, 95% CI: 0.43-0.81, p=0.001) and more frequently located in the gastric region although the CI was wide (OR=3.20, 95% CI: 1.63-6.63, p=0.001). In addition, relative to the Siewert Type I, BO-ve OAC tumours were more likely to be classified as Type II (OR=2.29, 95% CI: 1.67-3.14, p<0.001) and even more likely to be Type III (OR=3.83, 95% CI: 2.48-5.95, p<0.001).

The TNM stage was strongly and linearly associated with the risk of BO-ve OAC relative to the BO+ve OAC phenotype. Cases with the BO-ve OAC phenotype were 2.42, 3.14 and 3.38 times more likely to be diagnosed with TNM stage II, III, or IV, relative to stage I, respectively (p-trend <0.001, Cochran-Armitage test). In addition, BO-ve OAC tumours were less likely to shrink in size following neo-adjuvant chemotherapy as measured using the change from clinical to resection pathology T stage (OR=0.71, 95% CI: 0.58-0.86, p=0.001). As expected, a history of participation in a BO surveillance programme was unlikely among the BO-ve OAC cases compared to the BO+ve OAC cases (OR=0.17, 95% CI: 0.10-0.27, p<0.001).

Table 4.6 – Clinicopathological characteristics of OAC cases overall and according to tumour phenotype. The univariable odds ratios and 95% confidence intervals are for the association of characteristics with risk of BO-ve OAC phenotype compared to BO+ve OAC (primary comparison set).

Characteristic	BO+ve OAC (n=1,235)	BO-ve OAC (n=880)	BO(?) OAC (n=985)	Overall (n=3,100)	Univariable OR (95% CI, p) BO-ve OAC vs. BO+ve OAC
Tumour length; *cm*					
Mean [SD]	3.9 [2.7]	4.1 [2.4]	4.2 [3.9]	4.0 [3.0]	1.03 (0.99-1.07, p=0.199)
Median [Q1, Q3]	3.5 [2.5, 4.5]	3.50 [2.5, 5.0]	3.5 [2.5, 5.0]	3.5 [2.5, 5.0]	
Missing n (%)	390 (31.6)	272 (30.9)	440 (44.7)	1102 (35.5)	0.98 (0.82-1.17, p=0.807)
Tumour location (resection pathology), n (%)					
GOJ	363 (66.6)	265 (72.2)	168 (60.6)	796 (66.9)	1.00 (Referent)
Oesophageal	170 (31.2)	74 (20.2)	99 (35.7)	343 (28.8)	0.60 (0.43-0.81, p=0.001)
Gastric	12 (2.2)	28 (7.6)	10 (3.6)	50 (4.2)	3.20 (1.63-6.63, p=0.001)
Missing	690 (55.9)	513 (58.3)	708 (71.9)	1911 (61.6)	1.02 (0.84-1.24, p=0.855)
Siewert-Stein Classification, n (%)					
Type I	220 (46.7)	91 (25.3)	85 (34.6)	396 (36.8)	1.00 (Referent)
Type II	203 (43.1)	192 (53.5)	129 (52.4)	524 (48.7)	2.29 (1.67-3.14, p<0.001)
Type III	48 (10.2)	76 (21.2)	32 (13.0)	156 (14.5)	3.83 (2.48-5.95, p<0.001)
Missing	764 (61.9)	521 (59.2)	739 (75.0)	2024 (65.3)	1.65 (1.26-2.16, p<0.001)
TNM, n (%)					
I	294 (27.2)	87 (11.4)	116 (15.5)	497 (19.2)	1.00 (Referent)
II	279 (25.8)	200 (26.3)	192 (25.6)	671 (25.9)	2.42 (1.80-3.28, p<0.001)
III	481 (44.5)	447 (58.8)	424 (56.5)	1352 (52.2)	3.14 (2.40-4.14, p<0.001)
IV	26 (2.4)	26 (3.4)	18 (2.4)	70 (2.7)	3.38 (1.86-6.14, p<0.001)
Missing	155 (12.6)	120 (13.6)	235 (23.9)	510 (16.5)	2.62 (1.87-3.68, p<0.001)
Tumour Growth (T stage pre-operation to T stage post-operation), n (%)					
No change	555 (47.2)	460 (53.7)	416 (52.5)	1431 (50.7)	1.00 (Referent)
Shrink	412 (35.0)	241 (28.2)	226 (28.5)	879 (31.1)	0.71 (0.58-0.86, p=0.001)
Grow	209 (17.8)	155 (18.1)	151 (19.0)	515 (18.2)	0.89 (0.70-1.14, p=0.367)
Missing	59 (4.8)	24 (2.7)	192 (19.5)	275 (8.9)	0.49 (0.30-0.79, p=0.004)
History of undergoing BO surveillance, n (%)					
No	469 (76.4)	395 (95.0)	222 (82.2)	1086 (83.5)	1.00 (Referent)
Yes	145 (23.6)	21 (5.0)	48 (17.8)	214 (16.5)	0.17 (0.10-0.27, p<0.001)
Missing	621 (50.3)	464 (52.7)	715 (72.6)	1800 (58.1)	0.89 (0.74-1.06, p=0.192)

Distribution of selected baseline characteristics of OAC cases per region

Based on the observed univariable associations presented to this point, seven variables were selected using the previously outlined steps (3.2.2 and Table 3.1). These variables are included in Table 4.7 which shows the distribution of baseline characteristics across the geographic regions covered by OCCAMS study centres. Across all centres, the mean age at diagnosis and BMI at baseline were very similar. Similar to the overall descriptive statistics, the medians for age and BMI were close to their means (data not shown). Male cases accounted for greater than 80% of all cases within each region. There was a wider variation in the distribution of cigarette smoking status, aspirin/NSAID use and heartburn symptoms. The proportion of missing data for these factors also varied substantially between regions, and the London region had the highest share of missing data. The distribution of TNM was in line with expectations with TNM III tumours being most common in each region.

Table 4.7 – Distribution of selected baseline characteristics of OAC cases according to geographic region. The regions are arranged in descending order from left to right based on the total number of participants.

Characteristic	East of England (n=597)	London (n=521)	South East, England (n=465)	West Midlands, England (n=361)	East Midlands, England (n=324)	Scotland (n=308)	South West, England (n=255)	North West, England (n=169)	Northern Ireland (n=100)	Total (n=3,100)
BO+ve, n (%)	285 (59.0)	160 (56.9)	174 (50.9)	152 (60.8)	116 (50.0)	178 (65.0)	81 (74.3)	57 (58.8)	32 (68.1)	1,235 (58.4)
BO-ve, n (%)	198 (41.0)	121 (43.1)	168 (49.1)	98 (39.2)	116 (50.0)	96 (35.0)	28 (25.7)	40 (41.2)	15 (31.9)	880 (41.6)
BO(?), n (%)	114 (19.1)	240 (46.1)	123 (26.5)	111 (30.7)	92 (28.4)	34 (11.0)	146 (57.3)	72 (42.6)	53 (53.0)	985 (31.7)
Age at diagnosis; *years*										
Mean [SD]	66.6 [9.0]	65.5 [9.7]	65.8 [10.4]	65.6 [9.1]	67.0 [9.9]	65.3 [9.5]	65.9 [9.7]	65.8 [10.4]	64.0 [10.1]	65.9 [9.7]
Gender, n (%)										
Female	91 (15.2)	82 (15.7)	73 (15.5)	57 (15.8)	58 (18.2)	50 (16.2)	26 (10.2)	31 (18.3)	14 (14.0)	482 (15.5)
Male	506 (84.8)	439 (84.3)	398 (84.5)	304 (84.2)	260 (81.8)	258 (83.8)	229 (89.8)	138 (81.7)	86 (86.0)	2618 (84.5)
BMI at baseline; kg/m^2										
Mean [SD]	27.4 [4.6]	27.2 [5.1]	27.0 [4.9]	26.7 [5.0]	26.8 [5.2]	27.4 [5.2]	27.0 [4.7]	27.0 [5.0]	26.4 [5.2]	27.1 [4.9]
Missing n (%)	67 (11.2)	263 (50.5)	40 (8.5)	20 (5.5)	103 (32.4)	63 (20.5)	32 (12.5)	21 (12.4)	20 (20.0)	629 (20.3)
Cigarette smoking status, n (%)										
Never	168 (28.9)	118 (40.0)	152 (33.2)	140 (40.6)	152 (47.8)	146 (47.4)	101 (39.8)	53 (33.5)	24 (25.3)	1054 (37.5)
Ever	414 (71.1)	177 (60.0)	306 (66.8)	205 (59.4)	166 (52.2)	162 (52.6)	153 (60.2)	105 (66.5)	71 (74.7)	1759 (62.5)
Missing	15 (2.5)	226 (43.4)	13 (2.8)	16 (4.4)	0 (0)	0 (0)	1 (0.4)	11 (6.5)	5 (5.0)	287 (9.3)
Aspirin/NSAID use, n (%)										
Never	179 (44.3)	102 (65.0)	64 (32.2)	82 (42.7)	35 (41.7)	15 (14.7)	60 (44.1)	49 (52.1)	33 (38.8)	619 (42.6)
Ever	225 (55.7)	55 (35.0)	135 (67.8)	110 (57.3)	49 (58.3)	87 (85.3)	76 (55.9)	45 (47.9)	52 (61.2)	834 (57.4)
Missing	193 (32.3)	364 (69.9)	272 (57.7)	169 (46.8)	234 (73.6)	206 (66.9)	119 (46.7)	75 (44.4)	15 (15.0)	1647 (53.1)
Derived heartburn symptom status, n (%)										
Absent	99 (20.1)	92 (36.4)	52 (12.6)	56 (19.3)	60 (22.6)	28 (10.4)	32 (16.2)	27 (19.0)	8 (8.6)	454 (18.8)
Present	393 (79.9)	161 (63.6)	360 (87.4)	234 (80.7)	205 (77.4)	242 (89.6)	165 (83.8)	115 (81.0)	85 (91.4)	1960 (81.2)
Missing	105 (17.6)	268 (51.4)	59 (12.5)	71 (19.7)	53 (16.7)	38 (12.3)	58 (22.7)	27 (16.0)	7 (7.0)	686 (22.1)
TNM, n (%)										
I	105 (21.6)	82 (19.4)	85 (21.6)	40 (14.5)	59 (21.1)	46 (15.9)	43 (19.9)	28 (20.0)	9 (10.3)	497 (19.2)
II	112 (23.0)	104 (24.6)	120 (30.5)	70 (25.5)	72 (25.7)	76 (26.2)	55 (25.5)	42 (30.0)	20 (23.0)	671 (25.9)
III	256 (52.7)	227 (53.7)	166 (42.2)	159 (57.8)	143 (51.1)	167 (57.6)	113 (52.3)	66 (47.1)	55 (63.2)	1352 (52.2)
IV	13 (2.7)	10 (2.4)	22 (5.6)	6 (2.2)	6 (2.1)	1 (0.3)	5 (2.3)	4 (2.9)	3 (3.4)	70 (2.7)
Missing	111 (18.6)	98 (18.8)	78 (16.6)	86 (23.8)	38 (11.9)	18 (5.8)	39 (15.3)	29 (17.2)	13 (13.0)	510 (16.5)

Abbreviation: NSAID, non-steroidal anti-inflammatory drugs.

Distribution of selected baseline characteristics of OAC cases with a history of BO surveillance

An important finding was that 214 cases or 7% of the 3,100 total cases had a history of participating in a BO surveillance programme. Of these, BO phenotype for the tumour was ascertainable for 166 (75.6%) with 145 (87.3%) classified as BO+ve OAC and 21 (12.7%) classified as BO-ve OAC. BO phenotype was unascertainable for 48 of the 214 tumours (22.4%) which were classified as BO(?) OAC.

We separately examined the baseline characteristics of these BO-ve OAC cases given their relevance to our hypothesis (Table 4.8). Due to the relatively small size of this group, only the baseline characteristics noted above are shown for this group. Similar to the overall cohort, male cases were less likely to be among the BO-ve OAC group compared to the BO+ve OAC group (OR=0.30, 95% CI: 0.11-0.88, p=0.022). Importantly, OAC cases in this group were diagnosed at earlier TNM stages compared to the overall cohort.

Table 4.8 – Distribution of selected baseline characteristics for OAC cases with a history of undergoing BO surveillance. The univariable odds ratios and 95% confidence intervals are for the association of characteristics with risk of BO-ve OAC phenotype compared to BO+ve OAC (primary comparison set).

Characteristic	BO+ve OAC (n=145)	BO-ve OAC (n=21)	BO (?) OAC (n=48)	Overall (n=214)	Univariable OR (95% CI, p) BO-ve OAC vs. BO+ve OAC
Age at diagnosis; *years*					
Mean [SD]	66.3 [9.3]	66.9 [9.1]	63.9 [11.6]	65.9 [9.9]	0.99 (0.98-1.00, p=0.003)
Median [Q1, Q3]	67.5 [61.6, 72.3]	68.5 [60.7, 73.6]	64.8 [55.6, 73.5]	67.1 [59.1, 72.4]	-
Age groups at diagnosis, n (%)					
< 50 years old	11 (7.6)	0 (0)	5 (10.4)	16 (7.5)	NE
50 - 59 years old	19 (13.1)	5 (23.8)	16 (33.3)	40 (18.7)	1.00 (Referent)
60 - 69 years old	60 (41.4)	8 (38.1)	11 (22.9)	79 (36.9)	0.51 (0.15-1.85, p=0.279)
70+ years old	55 (37.9)	8 (38.1)	16 (33.3)	79 (36.9)	0.55 (0.16-2.02, p=0.346)
Gender, n (%)					-
Female	19 (13.1)	7 (33.3)	7 (14.6)	33 (15.4)	1.00 (Referent)
Male	126 (86.9)	14 (66.7)	41 (85.4)	181 (84.6)	0.30 (0.11-0.88, p=0.022)
BMI at baseline; *kg/m²*					
Mean [SD]	28.0 [4.0]	25.1 [3.8]	27.3 [4.29]	27.6 [4.10]	0.82 (0.70-0.94, p=0.006)
Median [Q1, Q3]	28.0 [25.0, 30.0]	26.0 [22.0, 28.0]	27.0 [25.3, 29.0]	27.5 [25.0, 30.0]	-
Missing n (%)	16 (11.0)	3 (14.3)	6 (12.5)	25 (11.6)	-
BMI group at baseline, n (%)					
Normal	23 (17.8)	6 (33.3)	5 (11.9)	34 (18.0)	1.00 (Referent)
Underweight	0 (0)	1 (5.56)	2 (4.76)	3 (1.59)	NE
Overweight	60 (46.5)	10 (55.6)	25 (59.5)	95 (50.3)	0.64 (0.21-2.06, p=0.433)
Obese	46 (35.7)	1 (5.56)	10 (23.8)	57 (30.2)	0.08 (0.00-0.53, p=0.025)
Missing	16 (11.0)	3 (14.3)	6 (12.5)	25 (11.6)	0.72 (0.14-3.16, p=0.671)
Cigarette smoking status, n (%)					
Never	54 (37.0)	7 (33.3)	14 (29.2)	75 (34.9)	1.00 (Referent)
Ever	92 (63.0)	14 (66.7)	34 (70.8)	140 (65.1)	1.19 (0.46-3.30, p=0.729)
Aspirin/NSAID use, n (%)					
Never	22 (30.1)	2 (20.0)	6 (24.0)	30 (27.8)	1.00 (Referent)
Ever	51 (69.9)	8 (80.0)	19 (76.0)	78 (72.2)	0.71 (0.11-13.97, p=0.759)
Missing	73 (50.0)	11 (52.4)	23 (47.9)	107 (49.8)	0.83 (0.03-24.90, p=0.906)
Derived heartburn symptom status, n (%)					
Absent	5 (3.6)	1 (5.0)	3 (6.4)	9 (4.4)	1.00 (Referent)
Present	134 (96.4)	19 (95.0)	44 (93.6)	197 (95.6)	1.73 (0.39-12.05, p=0.511)
Missing	6 (4.1)	1 (4.8)	1 (2.1)	8 (3.7)	1.68 (0.41-11.39, p=0.520)
TNM, n (%)					
I	62 (52.1)	7 (41.2)	17 (48.6)	86 (50.3)	1.00 (Referent)
II	34 (28.6)	3 (17.6)	8 (22.9)	45 (26.3)	0.78 (0.16-3.01, p=0.733)
III	22 (18.5)	7 (41.2)	10 (28.6)	39 (22.8)	2.82 (0.87-9.14, p=0.079)
IV	1 (0.8)	0 (0)	0 (0)	1 (0.6)	NE
Missing	26 (17.8)	4 (19.0)	13 (27.1)	43 (20.0)	1.36 (0.33-4.92, p=0.644)

Abbreviations: NSAID, non-steroidal anti-inflammatory drugs; NE, not estimable.

4.2.2 Baseline characteristics of Barrett's oesophagus cases and reflux controls

The BEST2 cohort included 1,595 patients comprised of 1,091 (68.4%) cases of BO and 504 (31.6%) controls with reflux symptoms. Tables 4.9-4.13 summarise the baseline characteristics across cases and controls. The baseline characteristics of BO cases and reflux controls were not directly compared as the scope of the hypothesis did not include evaluating differences between these two groups.

Distribution of BO cases and reflux controls per centre and region

The distribution of BO cases and reflux controls included from across 11 BEST2 study centres and regions is shown in Table 4.9. Close to half of all BO cases and reflux controls were from East of England and almost all of these were from Addenbrooke's Hospital in Cambridge. Within East of England, BO cases and reflux controls were nearly equally included with 436 (56.5%) BO cases and 336 (43.5%) reflux controls. In all other regions, the difference between the proportion of BO cases and reflux controls was greater.

Table 4.9 – Frequency of BO cases and reflux controls per BEST2 study centre and according to geographic regions. The regions and centres are arranged in descending order based on the total number of participants.

Region	Location	BEST2 study centre	Total n (%)	Region total n (%)	Barrett's Cases n (%)	Reflux Controls n (%)
East of England	Cambridge	Addenbrooke's Hospital	759 (47.6)	772 (48.4)	436 (56.5)	336 (43.5)
	Welwyn Garden City	Queen Elizabeth II Hospital	13 (0.8)			
London	University College London	University College Hospital	316 (19.8)	332 (20.8)	270 (81.3)	62 (18.7)
	Harrow	St Mark's Hospital	16 (1.0)			
North East, England	Newcastle	Royal Victoria Hospital	184 (11.5)	270 (16.9)	206 (76.3)	64 (23.7)
	Stockton-On-Tees	University Hospital of North Tees	24 (1.5)			
	North Shields	North Tyneside General Hospital	23 (1.4)			
	South Tyneside	South Tyneside District Hospital	21 (1.3)			
	County Durham & Darlington	University Hospital of North Durham	18 (1.1)			
East Midlands, England	Nottingham	Queen's Medical Centre	174 (10.9)	174 (10.9)	134 (77.0)	40 (23.0)
South East, England	Portsmouth	Queen Alexandra Hospital	47 (2.9)	47 (2.9)	45 (95.7)	2 (4.3)
Total (%)	**11 (locations/centres)**		**1,595 (100.0)**		**1,091 (68.4)**	**504 (31.6)**

Demographics of BO cases and reflux controls

BO cases were generally older and more likely to be male than reflux controls (Table 4.10). Additionally, the proportion of patients with white British ethnicity was slightly higher among BO cases compared to the reflux controls.

Table 4.10 – Baseline demographics of BO cases and reflux controls.

Characteristic	Barrett's Cases (n=1,091)	Reflux Controls (n=504)
Age at BO diagnosis or recruitment as control; *years*		
Mean [SD]	60.1 [12.1]	54.7 [14.8]
Median [Q1, Q3]	61.0 [53.0, 69.0]	56.0 [44.0, 66.0]
Age group at BO diagnosis or recruitment as control, n (%)		
< 50 years old	203 (18.6)	169 (33.5)
50 - 59 years old	281 (25.7)	123 (24.4)
60 - 69 years old	353 (32.3)	132 (26.2)
70+ years old	254 (23.3)	80 (15.9)
Gender, n (%)		
Female	217 (19.9)	275 (54.6)
Male	874 (80.1)	229 (45.4)
Ethnicity, n (%)		
Other	15 (1.4)	39 (7.7)
White British	1071 (98.6)	465 (92.3)
Missing	5 (0.5)	0 (0)

Risk factor exposures among BO cases and reflux controls

The distribution of baseline risk factors is shown in Table 4.11. As expected, overweight and obesity were more prevalent among BO cases (45.3% and 33.9%, respectively) compared to reflux controls (37.6% overweight and 26.8% obese).

Cigarette ever-smoking was reported by 63.6% (n=686) of BO cases compared to 48.7% of reflux controls (n=243). The mean number of pack-years of smoking was greater among BO cases (25.6, SD=24.8) than among reflux controls (14.0, SD=15.8).

Current or recent heavy drinking of alcoholic beverages was self-reported by 20.5% of BO cases and 11.6% (n=54) of reflux controls. The mean units of any alcoholic beverage intake (beer, wine and/or spirits) per week was 12.0 (SD=14.0) for BO cases and 10.1 (SD=15.3) for reflux controls.

Table 4.11 – Baseline risk factors of BO cases and reflux controls.

Characteristic	Barrett's Cases (n=1,091)	Reflux Controls (n=504)
BMI at baseline; *kg/m²*		
Mean [SD]	28.3 [4.9]	27.2 [5.5]
Median [Q1, Q3]	28.0 [25.0, 31.0]	26.0 [23.0, 30.0]
Missing n (%)	47 (4.3)	23 (4.6)
BMI group at baseline, n (%)		
Normal	213 (20.4)	160 (33.3)
Underweight	4 (0.4)	11 (2.29)
Overweight	473 (45.3)	181 (37.6)
Obese	354 (33.9)	129 (26.8)
Missing	47 (4.3)	23 (4.6)
Cigarette smoking status, n (%)		
Never	392 (36.4)	256 (51.3)
Ever	686 (63.6)	243 (48.7)
Missing	13 (1.2)	5 (1.0)
Number of cigarettes smoked per day		
Mean [SD]	18.1 [13.0]	12.0 [9.4]
Median [Q1, Q3]	20.0 [10.0, 20.0]	10.0 [5.0, 20.0]
Missing n (%)	483 (44.3)	278 (55.2)
Duration of smoking cigarettes; *years*		
Mean [SD]	24.2 [15.1]	19.2 [14.8]
Median [Q1, Q3]	23.0 [13.0, 36.0]	17.0 [8.00, 30.0]
Missing n (%)	538 (49.3)	321 (63.7)
Number of pack-years of smoking		
Mean [SD]	25.6 [24.8]	14.0 [15.8]
Median [Q1, Q3]	20.0 [7.00, 36.0]	8.40 [2.5, 21.0]
Missing n (%)	590 (54.1)	333 (66.1)
Heavy alcohol drinking status, n (%)		
Never	839 (79.5)	413 (88.4)
Ever	216 (20.5)	54 (11.6)
Missing	36 (3.3)	37 (7.3)
Units of alcohol intake per week		
Mean [SD]	12.0 [14.9]	10.1 [15.3]
Median [Q1, Q3]	8.0 [3.0, 15.0]	6.00 [2.0, 14.0]
Missing n (%)	346 (31.7)	190 (37.7)

Reflux and acid-suppressing medication use among BO cases and reflux controls

The distribution of reflux-related variables and acid-suppressant medication use is described in Table 4.12. As expected, most BO cases and reflux controls reported a history of reflux symptoms. The majority of BO cases (59.1%, n=634) reported a sporadic/unknown pattern

for the frequency of reflux symptoms and this pattern was also most frequent among the controls (33.9%, n=169). Among BO cases, other patterns of 'sometimes', 'often' and 'daily' were experienced by 31.2% (n=335), 6.2% (n=66) and 4.9% (n=52), respectively. Among reflux controls with a known pattern for frequency of reflux symptoms, most experienced a 'sometimes' pattern (30.9%, n=154) followed by daily (17.2%, n=86) and often (11.0%, n=55). The 'never' frequency of reflux symptoms was reported by a small proportion of BO cases (7.8%, n=84) and reflux controls (7.0%, n=35). Most BO cases experienced reflux for greater than 10 years since symptom occurrence (59.1%, n=634) followed by 5-10 years (17.0%, n=182) and fewer than 5 years (14.7%, n=158). Among reflux cases, most reported experiencing reflux symptoms for fewer than 5 years (55.5%, n=277) followed by greater than 10 years (22.6% n=113) and 5-10 years (13.2%, n=66) since symptom occurrence.

A majority of both BO cases (78.6%, n=857) and reflux controls (52.8%, n=266) reported using at least one type of acid suppressing medication at baseline. The average duration of PPI use was 4.9 (SD=3.9) years for BO cases and 2.6 (SD=1.5) years for reflux controls. Use of OTC and H2RA medications was much less frequent with 3.0% (n=33) and 0.2% (n=2) of BO cases reporting the use of these medications, respectively. A small proportion of reflux controls reported using OTC medications (2.6%, n=13) while none reported use of H2RA medications. The duration of use for OTC or H2RA medications was not estimable as none of the users reported a value for the number of years of use.

When the three measures of acid suppressant medication use were combined, a majority of BO cases (78.9%, n=861) and reflux controls (53.4%, n=269) reported using any type of these medications. This distribution nearly mirrors the distribution of PPI medication use as these medications were highly prevalent among both groups.

As before, to address limitations in the reporting of heartburn symptoms as well as missing data, the derived variable for any acid suppressant medication use was combined with the measures of reflux symptoms (frequency and duration of symptoms) to create a single measure for the presence of heartburn symptoms at baseline. Not unexpectedly, nearly all BO cases (96.6%, n=1,054) and reflux controls (93.3%, n=470) had likely experienced symptoms of heartburn at baseline.

Table 4.12 – Baseline reflux symptoms characteristics and acid-suppressant medication use of BO cases and reflux controls.

Characteristic	Barrett's Cases (n=1,091)	Reflux Controls (n=504)
Frequency of reflux symptoms, n (%)		
Never	84 (7.8)	35 (7.0)
Sometimes	335 (31.2)	154 (30.9)
Often	66 (6.2)	55 (11.0)
Daily	52 (4.9)	86 (17.2)
Unknown/sporadic	536 (50.0)	169 (33.9)
Missing	18 (1.6)	5 (1.0)
Duration since reflux symptoms began, n (%)		
Never	84 (7.8)	35 (7.0)
< 5 years	158 (14.7)	277 (55.5)
5-10 years	182 (17.0)	66 (13.2)
> 10 years	634 (59.1)	113 (22.6)
Unknown/sporadic	15 (1.4)	8 (1.6)
Missing	18 (1.6)	5 (1.0)
PPI medication use, n (%)		
Never	234 (21.4)	238 (47.2)
Ever	857 (78.6)	266 (52.8)
OTC acid suppressant medication use, n (%)		
Never	1058 (97.0)	491 (97.4)
Ever	33 (3.0)	13 (2.6)
H2RA medication use		
Never	1089 (99.8)	504 (100.0)
Ever	2 (0.232)	0 (0)
Derived variables		
Derived any acid suppressant medications use, n (%)		
Never	230 (21.1)	235 (46.6)
Ever	861 (78.9)	269 (53.4)
Derived heartburn symptom status, n (%)		
Absent	37 (3.4)	34 (6.7)
Present	1054 (96.6)	470 (93.3)

Abbreviations: PPI, proton pump inhibitor; OTC, over-the-counter; H2RA, histamine H-2 receptor antagonist.

Anti-inflammatory medication use among BO cases and reflux controls

Table 4.13 presents the summary statistics for aspirin and NSAID use among BO cases and reflux controls selected from BEST2. In BO cases, 22.8% (n=174) reported ever-use of aspirin, while 17.4% (n=124) reported ever-use of other NSAIDs. When both aspirin and other NSAID use were combined, 32.2% (n=279) reported ever-use of either medication. Among reflux controls, 14.9% (n=41) reported ever-use of aspirin, and 28.4% (n=93) reported

ever-use of other NSAIDs. When aspirin and other NSAID use were combined, 34.3% (n=122) reported ever-use of either medication.

Table 4.13 – Baseline anti-inflammatory medication use of BO cases and reflux controls.

Characteristic	Barrett's Cases (n=1,091)	Reflux Controls (n=504)
Aspirin use, n (%)		
Never	588 (77.2)	234 (85.1)
Ever	174 (22.8)	41 (14.9)
Missing	329 (30.2)	229 (45.4)
NSAID use, n (%)		
Never	588 (82.6)	234 (71.6)
Ever	124 (17.4)	93 (28.4)
Missing	379 (34.7)	177 (35.1)
Aspirin/NSAID use, n (%)		
Never	588 (67.8)	234 (65.7)
Ever	279 (32.2)	122 (34.3)
Missing	224 (20.5)	148 (29.4)

Abbreviation: NSAID, non-steroidal anti-inflammatory drugs.

Distribution of selected baseline characteristics of BO cases and reflux controls per region

The baseline variables selected for BO cases and reflux controls are the same as those selected after analysing the OAC phenotypes. These include age at diagnosis for BO cases or recruitment for reflux controls, gender, BMI at baseline, cigarette smoking status, aspirin/NSAID use and heartburn symptoms using the derived variable. TNM was not applicable to this patient population.

Table 4.14 presents the distribution of these variables across the geographic regions covered and according to participant type (BO case or reflux control). The mean age was generally higher among BO cases than reflux controls in each region. The proportion of male patients was higher among BO cases than reflux controls by at least 20% in all regions except for South East (England), however, this region only had two reflux controls and both were male. The mean BMI and self-reported cigarette smoking was also generally higher among BO cases than among reflux controls across regions. There was no distinct pattern for self-reported aspirin/NSAID use. While the proportion of those reporting ever-use was lower among BO cases than among reflux cases in East of England (24.5% vs. 36.7%) and East

Midland (44.7% vs 5.0%), it was nearly equal for the London region (28.3%) but higher for North East (38.5 vs. 22.0%). The presence of heartburn symptoms was derived for at least 94.5% of BO cases and at least 85.9% of reflux controls across regions, with 100% of both in the East Midlands region.

Table 4.14 – Distribution of selected baseline characteristics of BO cases and reflux controls according to geographic region. The regions are arranged in descending order from left to right based on the total number of participants.

Characteristic	East of England (n=772)		London (n=332)		North East, Eng. (n=270)		East Midlands, Eng. (n=174)		South East, Eng. (n=47)	
	BO ca. n=436 (56.5%)	**Reflux co.** n=336 (43.5%)	**BO ca.** n=270 (81.3%)	**Reflux co.** n=62 (18.7%)	**BO ca.** n=206 (76.3%)	**Reflux co.** n=64 (23.7%)	**BO ca.** n=134 (77.0%)	**Reflux co.** n=40 (23.0%)	**BO ca.** n=45 (95.7%)	**Reflux co.** n=2 (4.3%)
Age*; *years*										
Mean [SD]	61.9 [11.6]	57.0 [14.6]	59.5 [13.5]	48.2 [15.2]	58.0 [11.1]	52.3 [13.2]	61.6 [11.9]	53.0 [15.2]	60.5 [10.2]	55.0 [2.8]
Gender, n (%)										
Female	85 (19.5)	194 (57.7)	43 (15.9)	23 (37.1)	58 (28.2)	32 (50.0)	23 (17.2)	26 (65.0)	8 (17.8)	0 (0)
Male	351 (80.5)	142 (42.3)	227 (84.1)	39 (62.9)	148 (71.8)	32 (50.0)	111 (82.8)	14 (35.0)	37 (82.2)	2 (100.0)
BMI at baseline; *kg/m²*										
Mean [SD]	28.3 [4.7]	26.9 [5.3]	27.9 [4.9]	26.6 [4.5]	28.6 [5.2]	29.2 [7.2]	28.1 [3.8]	27.0 [4.9]	29.2 [6.9]	22.5 [2.1]
Missing n (%)	27 (6.2)	16 (4.8)	11 (4.1)	2 (3.2)	6 (2.9)	4 (6.2)	2 (1.5)	1 (2.5)	1 (2.2)	0 (0)
Cigarette smoking status, n (%)										
Never	158 (36.8)	177 (53.0)	101 (38.0)	31 (50.0)	92 (45.1)	29 (47.5)	31 (23.1)	17 (42.5)	10 (22.2)	2 (100.0)
Ever	271 (63.2)	157 (47.0)	165 (62.0)	31 (50.0)	112 (54.9)	32 (52.5)	103 (76.9)	23 (57.5)	35 (77.8)	0 (0)
Missing	7 (1.6)	2 (0.6)	4 (1.5)	0 (0)	2 (1.0)	3 (4.7)	0 (0)	0 (0)	0 (0)	0 (0)
Aspirin/NSAID use, n (%)										
Never	216 (75.5)	140 (63.3)	162 (71.7)	39 (70.9)	120 (61.5)	39 (78.0)	68 (55.3)	14 (50.0)	22 (59.5)	2 (100.0)
Ever	70 (24.5)	81 (36.7)	64 (28.3)	16 (29.1)	75 (38.5)	11 (22.0)	55 (44.7)	14 (50.0)	15 (40.5)	0 (0)
Missing	150 (34.4)	115 (34.2)	44 (16.3)	7 (11.3)	11 (5.3)	14 (21.9)	11 (8.2)	12 (30.0)	8 (17.8)	0 (0)
Derived heartburn symptom status, n (%)										
Absent	24 (5.5)	22 (6.5)	7 (2.6)	3 (4.8)	5 (2.4)	9 (14.1)	0 (0)	0 (0)	1 (2.2)	0 (0)
Present	412 (94.5)	314 (93.5)	263 (97.4)	59 (95.2)	201 (97.6)	55 (85.9)	134(100.0)	40 (100.0)	44 (97.8)	2 (100.0)

*Age at diagnosis for BO cases or at recruitment for reflux controls.
Abbreviations: Ca., cases; Co., controls; Eng., England; NSAID, non-steroidal anti-inflammatory drugs.

4.2.3 Summary

We first re-established the previously reported prognostic effect of OAC phenotypes by Sawas *et al.* (2018) in a larger and updated cohort used here. Next, examined the distribution of OAC cases by region and found that the overall distribution of cases, as well as the distribution by phenotype, was largely consistent across all regions covered by OCCAMS study centres.

Descriptive statistics were calculated for the entire cohort (n=3,100) and stratified by OAC phenotype: 58.4% with BO+ve OAC (n=1,235), 41.6% with BO-ve OAC (n=880) and 31.8% (n=985/3,100) with BO(?) OAC. The distribution of 34 baseline variables among the OAC cases was consistent with previous reports of this disease. Upon comparison of BO-ve OAC to BO+ve OAC cases, we observed that older age, male gender, higher BMI and reflux symptoms were less likely among BO-ve OAC cases, while self-reported ever-smoking and use of aspirin/NSAIDs were more likely. In addition, TNM stage among clinical variables and it was linearly associated with increased risk of BO-ve OAC compared to BO+ve OAC. Among all clinicopathological variables, TNM had the lowest missing data. The variables highlighted here comprise the set of covariates which will be used in subsequent analyses.

Among 214 OAC cases with a history of participating in BO surveillance, BO phenotype was ascertained for 166 tumours. Of these, the finding of BO-ve OAC for 21 (12.7%) patients supports the tumour overgrowth hypothesis. It is worth noting that the pathologists evaluating BO adjacent status for tumours were blind to the patient's history of participating in a BO surveillance programme, making it unlikely that these cases were subjected to more scrutiny when determining BO adjacent status. However, due to the small sample size, this finding should be interpreted with caution.

The regional distribution of variables with a significant univariable association was also examined. Similar to the distribution of cases and phenotypes, baseline characteristics were mostly consistent between regions but there was wide variation in missing data. Lastly, the prevalence of baseline characteristics among the BO(?) OAC group generally falls between those of BO-ve and BO+ve OAC phenotypes. This suggests that the group with unascertainable BO adjacent to OAC is likely a mixture of both phenotypes.

The baseline characteristics of BO cases and reflux controls were as expected for these conditions. The rate of missing data was lower for the BO cases and reflux controls compared to the OAC cases. The prevalence of heartburn symptoms was very high across the cohort (95.5%) which was expected as BO patients may have long-standing reflux and controls were indicated for endoscopy due to dyspepsia and/or reflux symptoms. However, lower rates of heartburn were observed among OAC cases (81.2%). These differences may be explained by distinctions in study design and CRF structure between BEST2 and OCCAMS. With this in mind, we compared OAC phenotypes to BO cases and reflux controls to understand whether

these OAC phenotypes are part of the same disease or a different aetiology related to risk factor exposures.

4.3 Association of clinical and epidemiological characteristics

4.3.1 Factors associated with prognostic phenotypes

As described, a collection of 34 variables collected in OCCAMS were screened for inclusion in the fully adjusted models (Methods, 3.2.2). From these variables, age at diagnosis, gender, BMI at baseline, smoking, aspirin/NSAID use, heartburn and TNM (for cancer only) were selected and are included in the primary comparison of BO-ve OAC vs. BO+ve OAC as well as other comparison sets. To provide a more interpretable comparison for age at diagnosis and BMI at baseline, these continuous measures were categorised into groups as described in 3.2.1. These categories were used in all other comparison sets.

Table 4.15 presents the association of baseline characteristics with OAC phenotypes in the primary comparison set that included BO-ve OAC as outcome vs. BO+ve OAC as reference. The OR and 95% CI estimates were derived using univariable (unadjusted/crude), minimally adjusted (age and gender as covariates) and fully adjusted (all covariates included) models. A fully adjusted model that excluded heartburn symptom status as a covariate was used to examine the effect of adjusting for heartburn.

Age group at diagnosis was not statistically significantly associated with risk of BO-ve OAC in any of the models. As reported in the results of descriptive analysis, the univariable association for gender showed that male cases were less prevalent among the BO-ve OAC group compared to the BO+ve OAC group (OR=0.69, 95% CI: 0.55-0.88, p=0.003). This association persisted in subsequent adjusted models with a fully adjusted OR (aOR) of 0.67 (95%CI: 0.52-0.86, p=0.002).

In the analysis of BMI groups, the underweight BMI group was merged with the normal BMI group due to its few observations (n=29). The overweight group was weakly associated with a decreased risk of BO-ve OAC compared to BO+ve OAC in the unadjusted model (OR=0.79, CI: 0.63-0.99, p=0.039). However, this association became null in the adjusted models. Compared to BO+ve cases, BO-ve OAC cases were consistently less likely to be obese at baseline with similar estimates in the univariable model (OR=0.54, 95% CI: 0.41-0.69, p<0.001) and in the fully adjusted model (aOR=0.57, 95% CI: 0.44-0.75, p<0.001). The self-reported cigarette smoking status slightly differed between BO+ve OAC and BO-ve OAC, with the BO-ve OAC group being more likely than the BO+ve OAC group to report ever smoking (aOR=1.26, 95% CI: 1.03-1.54, p=0.022).

The univariable association between aspirin/NSAID use and risk of BO-ve OAC was not significant. This association became weakly statistically significant in the fully adjusted

model (aOR=1.35, 95% CI: 1.03-1.76, p=0.031), suggesting that the BO-ve OAC cases were slightly more likely to have reported use of such medications compared to BO+ve OAC cases. However, this association was the only one that became null in the fully adjusted model with heartburn excluded as a covariate (aOR=1.28, CI: 0.98-1.67, p=0.072). Therefore, it is possible that the association of aspirin/NSAID and increased risk of BO-ve OAC reflects the confounding effects of the covariates in the fully adjusted model, specifically heartburn, which can affect the association between aspirin/NSAID use and the risk of BO-ve OAC.

Similar to the inverse association of male gender and obesity with BO-ve OAC cases, these cases were also less likely than BO+ve OAC cases to exhibit heartburn symptoms as measured using the derived variable (aOR=0.63, CI: 0.49-0.82, p=0.001). Lastly, there was a strong association between increasing TNM stage and BO-ve OAC as the outcome when compared to the reference BO+ve OAC phenotype, with an aOR of 2.42, 2.93, and 3.19 for stages II, III, and IV, respectively, relative to stage I.

As noted above, except for aspirin/NSAID use, the derived OR and 95% CI estimates for all other variables remained virtually unchanged between the fully adjusted model and the fully adjusted model that excluded heartburn as a covariate. A likelihood ratio test between these models resulted in a p-value of 0.856, indicating an equal model fit. There were no significant interactions between the variables included in the fully adjusted models.

Table 4.15 – Associations of baseline characteristics with risk of BO-ve OAC phenotype compared to BO+ve OAC cases (primary comparison set).

Characteristic	BO+ve OAC n (%)	BO-ve OAC n (%)	Univariable model OR (95% CI, p)	Minimally adjusted model[a] OR (95% CI, p)	Fully adjusted model[b] OR (95% CI, p)	Fully adjusted model[b] excluding heartburn OR (95% CI, p)
Age group at diagnosis						
< 50	67 (5.4)	59 (6.7)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
50 - 59	193 (15.6)	184 (20.9)	1.08 (0.72-1.62, p=0.700)	1.10 (0.73-1.65, p=0.645)	1.12 (0.74-1.71, p=0.595)	1.12 (0.73-1.70, p=0.610)
60 - 69	497 (40.2)	317 (36.0)	0.72 (0.50-1.06, p=0.094)	0.75 (0.51-1.09, p=0.129)	0.72 (0.49-1.07, p=0.108)	0.72 (0.49-1.07, p=0.106)
70+	478 (38.7)	320 (36.4)	0.76 (0.52-1.11, p=0.155)	0.77 (0.53-1.13, p=0.185)	0.72 (0.48-1.07, p=0.100)	0.74 (0.50-1.09, p=0.123)
Gender						
Female	163 (13.2)	158 (18.0)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Male	1072 (86.8)	722 (82.0)	0.69 (0.55-0.88, p=0.003)	0.71 (0.56-0.90, p=0.004)	0.67 (0.52-0.86, p=0.002)	0.68 (0.53-0.87, p=0.003)
BMI group at baseline						
Normal	268 (21.7)	255 (29.0)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Overweight	422 (34.2)	317 (36.0)	0.79 (0.63-0.99, p=0.039)	0.80 (0.64-1.00, p=0.055)	0.89 (0.70-1.12, p=0.309)	0.86 (0.68-1.09, p=0.212)
Obese	304 (24.6)	155 (17.6)	0.54 (0.41-0.69, p<0.001)	0.51 (0.39-0.66, p<0.001)	0.57 (0.44-0.75, p<0.001)	0.56 (0.43-0.73, p<0.001)
Missing	241 (19.5)	153 (17.4)	0.67 (0.51-0.87, p=0.003)	0.66 (0.50-0.86, p=0.002)	0.87 (0.63-1.20, p=0.385)	0.88 (0.64-1.20, p=0.417)
Cigarette smoking status						
Never	452 (36.6)	298 (33.9)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Ever	665 (53.8)	532 (60.5)	1.21 (1.01-1.46, p=0.041)	1.25 (1.04-1.51, p=0.019)	1.26 (1.03-1.54, p=0.022)	1.24 (1.02-1.51, p=0.033)
Missing	118 (9.6)	50 (5.7)	0.64 (0.44-0.92, p=0.017)	0.65 (0.45-0.93, p=0.020)	0.51 (0.32-0.80, p=0.004)	0.54 (0.35-0.83, p=0.005)
Aspirin/NSAID use						
Never	257 (20.8)	161 (18.3)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Ever	328 (26.6)	243 (27.6)	1.18 (0.91-1.53, p=0.202)	1.22 (0.94-1.58, p=0.139)	1.35 (1.03-1.76, p=0.031)	1.28 (0.98-1.67, p=0.072)
Missing	650 (52.6)	476 (54.1)	1.17 (0.93-1.47, p=0.183)	1.17 (0.93-1.48, p=0.180)	1.31 (1.02-1.69, p=0.038)	1.32 (1.03-1.69, p=0.027)
Heartburn symptoms status						
Absent	150 (12.1)	160 (18.2)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	-
Present	818 (66.2)	543 (61.7)	0.62 (0.49-0.80, p<0.001)	0.61 (0.48-0.78, p<0.001)	0.63 (0.49-0.82, p=0.001)	-
Missing	267 (21.6)	177 (20.1)	0.62 (0.46-0.83, p=0.001)	0.62 (0.46-0.83, p=0.001)	0.79 (0.56-1.13, p=0.200)	-
TNM						
I	294 (23.8)	87 (9.9)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
II	279 (22.6)	200 (22.7)	2.42 (1.80-3.28, p<0.001)	2.44 (1.81-3.31, p<0.001)	2.43 (1.79-3.31, p<0.001)	2.40 (1.78-3.27, p<0.001)
III	481 (38.9)	447 (50.8)	3.14 (2.40-4.14, p<0.001)	3.15 (2.41-4.16, p<0.001)	2.93 (2.23-3.88, p<0.001)	3.02 (2.30-3.99, p<0.001)
IV	26 (2.1)	26 (3.0)	3.38 (1.86-6.14, p<0.001)	3.29 (1.81-6.01, p<0.001)	3.19 (1.74-5.86, p<0.001)	3.19 (1.74-5.84, p<0.001)
Missing	155 (12.6)	120 (13.6)	2.62 (1.87-3.68, p<0.001)	2.66 (1.90-3.75, p<0.001)	2.64 (1.88-3.74, p<0.001)	2.67 (1.89-3.77, p<0.001)

[a]adjusted for age group at diagnosis and gender.
[b]adjusted for all covariates.

4.3.2 Sensitivity analyses

Three sensitivity analyses were conducted to evaluate the effects of missing data and the influence of the OAC cases with a history of BO surveillance on the point estimates obtained using the fully adjusted model. In the first analysis, missing data were imputed using multiple imputation (MI) as described in 3.4.2. In the second analysis, a complete case approach was taken to exclude any observation with missing data for the variables included in the fully adjusted model. In the third analysis, the OAC cases with a history of participation in BO surveillance programmes (n=214) were excluded from the fully adjusted model. The results of the sensitivity analyses were compared against results from the fully adjusted model reported in the section above.

Table 4.16 summarises the results of the sensitivity analyses conducted for the primary comparison set (BO-ve OAC as outcome vs. BO+ve OAC). Overall, results remained highly robust across all three sensitivity analyses. Using imputed data, the adjusted odds ratios for the associations of all variables but one remained statistically significant and changed in magnitude by less than 10%. Only the association of ever-smoking became non-significant (MI aOR=1.21, 95% CI: 0.99-1.47, p=0.060). In complete-case analysis, the aORs for the association of male gender, aspirin/NSAID ever-use and presence of heartburn symptoms remained statistically significant and did not change by more than 10%. The association of obese BMI was affected and became null (aOR=0.78, 95% CI: 0.52-1.16, p=0.222). Similarly, the observed higher likelihood of self-reported ever-smoking among BO-ve OAC compared to BO+ve OAC cases was no longer statistically significant (aOR=1.34, 95% CI: 0.97-1.86, p=0.082). Finally, no material deviation was observed in the fully adjusted estimates when OAC cases with a history of undergoing BO surveillance were excluded compared to the results with these patients included.

As the results obtained from the fully adjusted model were similar to the other adjusted models and remained robust in the sensitivity analyses, only the results from the fully adjusted model (aOR) with missing data as an indicator variable will be reported to ease the interpretation of the results.

Table 4.16 – Sensitivity analyses of the adjusted association of baseline characteristics with risk of BO-ve OAC compared to BO+ve OAC cases using multiple imputation, complete case analysis and exclusion of OAC cases with a history of undergoing BO surveillance.

	Multiple imputation	Complete case analysis			Excluding BO surveillance OAC cases (n=214)		
Characteristic	**Fully adjusted model[a] OR (95% CI, p)**	**BO+ve OAC n (%)**	**BO-ve OAC n (%)**	**Fully adjusted model[a] OR (95% CI, p)**	**BO+ve OAC n (%)**	**BO-ve OAC n (%)**	**Fully adjusted model[a] OR (95% CI, p)**
Age group at diagnosis							
< 50	1.00 (Referent)	28 (6.2)	24 (7.5)	1.00 (Referent)	56 (5.1)	59 (6.9)	1.00 (Referent)
50 - 59	1.13 (0.74-1.73, p=0.577)	65 (14.4)	58 (18.1)	1.20 (0.61-2.39, p=0.598)	174 (16.0)	179 (20.8)	1.02 (0.66-1.58, p=0.917)
60 - 69	0.72 (0.48-1.07, p=0.107)	168 (37.2)	130 (40.5)	1.02 (0.55-1.92, p=0.946)	437 (40.1)	309 (36.0)	0.68 (0.45-1.02, p=0.064)
70+	0.70 (0.47-1.04, p=0.077)	191 (42.3)	109 (34.0)	0.69 (0.37-1.31, p=0.253)	423 (38.8)	312 (36.3)	0.68 (0.45-1.02, p=0.064)
Gender							
Female	1.00 (Referent)	56 (12.4)	58 (18.1)	1.00 (Referent)	144 (13.2)	151 (17.6)	1.00 (Referent)
Male	0.67 (0.52-0.87, p=0.002)	396 (87.6)	263 (81.9)	0.61 (0.40-0.93, p=0.022)	946 (86.8)	708 (82.4)	0.68 (0.53-0.88, p=0.004)
BMI group at baseline							
Normal	1.00 (Referent)	126 (27.9)	99 (30.8)	1.00 (Referent)	245 (22.5)	248 (28.9)	1.00 (Referent)
Overweight	0.89 (0.70-1.13, p=0.359)	191 (42.3)	143 (44.5)	1.10 (0.77-1.57, p=0.612)	362 (33.2)	307 (35.7)	0.91 (0.72-1.16, p=0.445)
Obese	0.57 (0.44-0.75, p<0.001)	135 (29.9)	79 (24.6)	0.78 (0.52-1.16, p=0.222)	258 (23.7)	154 (17.9)	0.63 (0.48-0.83, p=0.001)
Missing	-	-	-	-	225 (20.6)	150 (17.5)	0.93 (0.67-1.29, p=0.662)
Cigarette smoking status							
Never	1.00 (Referent)	155 (34.3)	89 (27.7)	1.00 (Referent)	398 (36.5)	291 (33.9)	1.00 (Referent)
Ever	1.21 (0.99-1.47, p=0.060)	297 (65.7)	232 (72.3)	1.34 (0.97-1.86, p=0.082)	574 (52.7)	518 (60.3)	1.29 (1.05-1.58, p=0.016)
Missing	-	-	-	-	118 (10.8)	50 (5.8)	0.48 (0.30-0.76, p=0.002)
Aspirin/NSAID use							
Never	1.00 (Referent)	208 (46.0)	129 (40.2)	1.00 (Referent)	235 (21.6)	159 (18.5)	1.00 (Referent)
Ever	1.36 (1.04-1.77, p=0.023)	244 (54.0)	192 (59.8)	1.45 (1.08-2.00, p=0.015)	277 (25.4)	235 (27.4)	1.40 (1.06-1.85, p=0.017)
Missing	-	-	-	-	578 (53.0)	465 (54.1)	1.39 (1.07-1.81, p=0.014)
Heartburn symptoms status							
Absent	1.00 (Referent)	70 (15.5)	73 (22.7)	1.00 (Referent)	145 (13.3)	159 (18.5)	1.00 (Referent)
Present	0.62 (0.47-0.81, p=0.001)	382 (84.5)	248 (77.3)	0.56 (0.38-0.83, p=0.004)	684 (62.8)	524 (61.0)	0.69 (0.53-0.89, p=0.005)
Missing	-	-	-	-	261 (23.9)	176 (20.5)	0.79 (0.55-1.12, p=0.182)
TNM							
I	1.00 (Referent)	132 (29.2)	44 (13.7)	1.00 (Referent)	232 (21.3)	80 (9.3)	1.00 (Referent)
II	2.40 (1.78-3.25, p<0.001)	110 (24.3)	85 (26.5)	2.33 (1.48-3.69, p<0.001)	245 (22.5)	197 (22.9)	2.31 (1.68-3.20, p<0.001)
III	2.92 (2.22-3.84, p<0.001)	200 (44.2)	186 (57.9)	2.68 (1.80-4.06, p<0.001)	459 (42.1)	440 (51.2)	2.61 (1.96-3.50, p<0.001)
IV	3.16 (1.73-5.78, p<0.001)	10 (2.2)	6 (1.9)	1.92 (0.60-5.69, p=0.250)	25 (2.3)	26 (3.0)	2.84 (1.53-5.28, p=0.001)
Missing	-	-	-	-	129 (11.8)	116 (13.5)	2.63 (1.83-3.80, p<0.001)

[a]adjusted for all covariates.

4.3.3 Similarity of prognostic phenotypes with unascertainable phenotype

It is likely the unascertainable BO(?) OAC group is a mixture of both tumour phenotypes that with an excess of BO-ve OAC. To confirm this, we compared the baseline characteristics of BO+ve OAC and BO-ve OAC to the BO(?) OAC cases. We expected that the associations observed in the primary comparison group would weaken when BO+ve was compared to BO(?) OAC cases while the associations seen for BO-ve OAC would disappear. The results for the association of baseline characteristics comparing the BO+ve OAC and BO-ve OAC to BO(?) OAC cases are presented in Table 4.17 and Table 4.18, respectively.

Indeed, all aORs previously noted when comparing BO-ve OAC to BO+OAC cases were attenuated when the later phenotype was compared to BO(?) OAC. For example, male cases were less likely to be in the BO-ve OAC phenotype group than the BO+ve OAC group (aOR=0.67, 95% CI: 0.52-0.86, p=0.002; Table 4.15). When the BO+ve OAC phenotype group was compared to the BO(?) OAC group, this association became less prominent, indicating a nearly equal likelihood of male cases in either group (aOR=1.32, 95% CI: 1.03-1.70, p=0.026; Table 4.17).

When the BO-ve OAC phenotype was compared against the BO(?) OAC group, nearly all statistically significant associations seen in the primary comparison set disappeared. Similarly, symptoms of heartburn were less likely to be 'present' in the BO-ve OAC phenotype group compared to the BO+ve OAC group (aOR=0.63, 95% CI: 0.49-0.82, p=0.001; Table 4.15). However, when comparing BO-ve OAC to BO(?) OAC, this association was no longer statistically significant (aOR=0.80, 95% CI: 0.61-1.03, p=0.085; Table 4.18). Similar attenuations occurred for the association of BMI group and cigarette smoking previously observed in the primary comparison set. Only TNM III versus I remained correlated with BO-ve OAC cases, but this was a weak effect (TNM III vs. I OR=1.41, 95% CI: 1.03-1.94, p=0.031). Taken together, it is likely that the BO(?) OAC phenotype is comprised of an excess number of BO-ve OAC than BO+ve OAC cases.

The same three sensitivity analyses conducted for the primary comparison set (BO-ve OAC vs. BO+ve OAC) were also performed for the comparison set described here. The results of these analyses were not substantially different from the adjusted associations described above (Tables A.1 and A.2).

Table 4.17 – Associations of baseline characteristics with risk of BO+ve OAC phenotype compared to BO(?) OAC cases.

Characteristic	BO(?) OAC n (%)	BO+ve OAC n (%)	Univariable model OR (95% CI, p)	Minimally adjusted model[a] OR (95% CI, p)	Fully adjusted model[b] OR (95% CI, p)	Fully adjusted model excluding heartburn OR (95% CI, p)
Age group at diagnosis						
< 50	75 (7.6)	67 (5.4)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
50 - 59	213 (21.6)	193 (15.6)	1.01 (0.69-1.49, p=0.942)	0.99 (0.68-1.46, p=0.965)	0.98 (0.66-1.46, p=0.929)	0.99 (0.67-1.47, p=0.969)
60 - 69	333 (33.8)	497 (40.2)	1.67 (1.17-2.39, p=0.005)	1.63 (1.14-2.33, p=0.008)	1.64 (1.13-2.38, p=0.009)	1.64 (1.14-2.38, p=0.008)
70+	364 (37.0)	478 (38.7)	1.47 (1.03-2.10, p=0.034)	1.44 (1.01-2.06, p=0.045)	1.46 (1.01-2.11, p=0.047)	1.45 (1.00-2.10, p=0.049)
Gender						
Female	161 (16.3)	163 (13.2)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Male	824 (83.7)	1072 (86.8)	1.29 (1.01-1.63, p=0.037)	1.25 (0.99-1.59, p=0.063)	1.32 (1.03-1.70, p=0.026)	1.30 (1.02-1.67, p=0.036)
BMI group at baseline						
Normal	248 (25.2)	268 (21.7)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Overweight	307 (31.2)	422 (34.2)	1.27 (1.01-1.60, p=0.038)	1.25 (1.00-1.58, p=0.052)	1.14 (0.90-1.44, p=0.282)	1.16 (0.91-1.46, p=0.227)
Obese	195 (19.8)	304 (24.6)	1.44 (1.12-1.85, p=0.004)	1.49 (1.16-1.92, p=0.002)	1.34 (1.03-1.74, p=0.028)	1.36 (1.05-1.76, p=0.021)
Missing	235 (23.9)	241 (19.5)	0.95 (0.74-1.22, p=0.681)	0.96 (0.75-1.24, p=0.763)	0.90 (0.66-1.24, p=0.522)	0.89 (0.65-1.22, p=0.469)
Cigarette smoking status						
Never	304 (30.9)	452 (36.6)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Ever	562 (57.1)	665 (53.8)	0.80 (0.66-0.96, p=0.015)	0.79 (0.65-0.95, p=0.011)	0.78 (0.64-0.95, p=0.014)	0.79 (0.65-0.96, p=0.019)
Missing	119 (12.1)	118 (9.6)	0.67 (0.50-0.89, p=0.007)	0.67 (0.50-0.90, p=0.007)	0.88 (0.59-1.32, p=0.531)	0.82 (0.57-1.20, p=0.310)
Aspirin/NSAID use						
Never	201 (20.4)	257 (20.8)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Ever	263 (26.7)	328 (26.6)	0.98 (0.76-1.25, p=0.842)	0.94 (0.73-1.20, p=0.613)	0.92 (0.71-1.19, p=0.521)	0.94 (0.73-1.21, p=0.635)
Missing	521 (52.9)	650 (52.6)	0.98 (0.78-1.21, p=0.825)	0.96 (0.77-1.20, p=0.716)	1.03 (0.80-1.31, p=0.835)	1.00 (0.79-1.27, p=0.976)
Heartburn symptoms status						
Absent	144 (14.6)	150 (12.1)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	-
Present	599 (60.8)	818 (66.2)	1.31 (1.02-1.69, p=0.035)	1.34 (1.04-1.72, p=0.025)	1.29 (0.99-1.67, p=0.060)	-
Missing	242 (24.6)	267 (21.6)	1.06 (0.79-1.41, p=0.695)	1.07 (0.80-1.43, p=0.644)	1.08 (0.76-1.54, p=0.670)	-
TNM						
I	116 (11.8)	294 (23.8)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
II	192 (19.5)	279 (22.6)	0.57 (0.43-0.76, p<0.001)	0.56 (0.42-0.75, p<0.001)	0.57 (0.43-0.76, p<0.001)	0.58 (0.43-0.77, p<0.001)
III	424 (43.0)	481 (38.9)	0.45 (0.35-0.57, p<0.001)	0.45 (0.35-0.58, p<0.001)	0.48 (0.37-0.62, p<0.001)	0.47 (0.37-0.61, p<0.001)
IV	18 (1.8)	26 (2.1)	0.57 (0.30-1.09, p=0.084)	0.57 (0.30-1.09, p=0.083)	0.58 (0.31-1.12, p=0.101)	0.57 (0.30-1.10, p=0.090)
Missing	235 (23.9)	155 (12.6)	0.26 (0.19-0.35, p<0.001)	0.26 (0.19-0.34, p<0.001)	0.27 (0.20-0.36, p<0.001)	0.26 (0.20-0.36, p<0.001)

[a]adjusted for age group at diagnosis and gender.
[b]adjusted for all covariates.

Table 4.18 – Associations of baseline characteristics with risk of BO-ve OAC phenotype compared to BO(?) OAC cases.

Characteristic	BO(?) OAC n (%)	BO-ve OAC n (%)	Univariable model OR (95% CI, p)	Minimally adjusted model[a] OR (95% CI, p)	Fully adjusted model[b] OR (95% CI, p)	Fully adjusted model excluding heartburn OR (95% CI, p)
Age group at diagnosis						
< 50	75 (7.6)	59 (6.7)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
50 - 59	213 (21.6)	184 (20.9)	1.10 (0.74-1.63, p=0.642)	1.11 (0.75-1.65, p=0.612)	1.15 (0.77-1.73, p=0.494)	1.14 (0.76-1.71, p=0.529)
60 - 69	333 (33.8)	317 (36.0)	1.21 (0.83-1.76, p=0.318)	1.22 (0.84-1.78, p=0.295)	1.24 (0.84-1.82, p=0.279)	1.23 (0.84-1.81, p=0.296)
70+	364 (37.0)	320 (36.4)	1.12 (0.77-1.63, p=0.559)	1.13 (0.78-1.64, p=0.530)	1.14 (0.78-1.68, p=0.497)	1.14 (0.78-1.67, p=0.507)
Gender						
Female	161 (16.3)	158 (18.0)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Male	824 (83.7)	722 (82.0)	0.89 (0.70-1.14, p=0.357)	0.89 (0.70-1.13, p=0.332)	0.87 (0.68-1.12, p=0.277)	0.88 (0.69-1.13, p=0.322)
BMI group at baseline						
Normal	248 (25.2)	255 (29.0)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Overweight	307 (31.2)	317 (36.0)	1.00 (0.79-1.27, p=0.972)	1.01 (0.80-1.28, p=0.928)	1.00 (0.79-1.27, p=0.995)	1.00 (0.78-1.27, p=0.970)
Obese	195 (19.8)	155 (17.6)	0.77 (0.59-1.02, p=0.066)	0.78 (0.59-1.03, p=0.075)	0.79 (0.60-1.05, p=0.101)	0.79 (0.60-1.04, p=0.098)
Missing	235 (23.9)	153 (17.4)	0.63 (0.48-0.83, p=0.001)	0.63 (0.48-0.83, p=0.001)	0.78 (0.56-1.09, p=0.143)	0.79 (0.57-1.09, p=0.153)
Cigarette smoking status						
Never	304 (30.9)	298 (33.9)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Ever	562 (57.1)	532 (60.5)	0.97 (0.79-1.18, p=0.731)	0.97 (0.79-1.18, p=0.739)	0.98 (0.79-1.21, p=0.839)	0.97 (0.79-1.20, p=0.790)
Missing	119 (12.1)	50 (5.7)	0.43 (0.30-0.62, p<0.001)	0.43 (0.29-0.61, p<0.001)	0.44 (0.27-0.69, p=0.001)	0.43 (0.28-0.66, p<0.001)
Aspirin/NSAID use						
Never	201 (20.4)	161 (18.3)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Ever	263 (26.7)	243 (27.6)	1.15 (0.88-1.51, p=0.301)	1.15 (0.88-1.51, p=0.307)	1.18 (0.89-1.56, p=0.247)	1.14 (0.86-1.50, p=0.354)
Missing	521 (52.9)	476 (54.1)	1.14 (0.90-1.45, p=0.286)	1.13 (0.89-1.45, p=0.309)	1.33 (1.02-1.74, p=0.036)	1.31 (1.02-1.70, p=0.037)
Heartburn symptoms status						
Absent	144 (14.6)	160 (18.2)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	-
Present	599 (60.8)	543 (61.7)	0.82 (0.63-1.05, p=0.115)	0.81 (0.63-1.04, p=0.101)	0.80 (0.61-1.03, p=0.085)	-
Missing	242 (24.6)	177 (20.1)	0.66 (0.49-0.89, p=0.006)	0.65 (0.48-0.88, p=0.005)	0.83 (0.58-1.19, p=0.322)	-
TNM						
I	116 (11.8)	87 (9.9)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
II	192 (19.5)	200 (22.7)	1.39 (0.99-1.96, p=0.059)	1.40 (0.99-1.97, p=0.056)	1.39 (0.98-1.96, p=0.065)	1.37 (0.97-1.95, p=0.071)
III	424 (43.0)	447 (50.8)	1.41 (1.03-1.92, p=0.030)	1.42 (1.04-1.94, p=0.026)	1.42 (1.03-1.94, p=0.030)	1.41 (1.03-1.94, p=0.031)
IV	18 (1.8)	26 (3.0)	1.93 (1.00-3.78, p=0.052)	1.95 (1.01-3.84, p=0.048)	1.94 (1.00-3.86, p=0.054)	1.94 (1.00-3.85, p=0.054)
Missing	235 (23.9)	120 (13.6)	0.68 (0.48-0.97, p=0.034)	0.69 (0.48-0.98, p=0.038)	0.67 (0.47-0.96, p=0.029)	0.67 (0.47-0.96, p=0.027)

[a]adjusted for age group at diagnosis and gender.
[b]adjusted for all covariates.

4.3.4 Comparison of characteristics between prognostic phenotypes and Barrett's oesophagus

We next compared the characteristics of OAC phenotypes to BO cases to determine if certain factors associate more strongly with one phenotype than the other. We rationalised that if the BO-ve OAC phenotype does not involve BO during its progression, then the association of risk factors for this phenotype compared to BO cases may be more distinct than associations for BO+ve OAC against BO cases. Table 4.19 and Table 4.20 present the association of baseline characteristics comparing BO+ve OAC and BO-ve OAC cases to BO cases. The sensitivity analysis results excluding OAC cases with a history of undergoing BO surveillance are also presented in these tables to ease interpretation.

As anticipated, older age groups were found to be associated with an increased risk of both OAC phenotypes when compared to BO cases, and the strength of these associations was similar for both OAC phenotypes. BO+ve OAC cases were more likely to be male than BO controls (aOR=1.51, 95% CI: 1.16-1.97, p=0.003; Table 4.19), but the proportion of male cases was equal among BO-ve OAC cases and BO controls (aOR=1.05, 95% CI: 0.79-1.40, p=0.737; Table 4.20). This is in line with earlier results showing that male cases were less frequent in the BO-ve OAC phenotype group compared to the BO+ve OAC phenotype.

Compared to BO cases, no association was observed between BMI groups and risk of BO+ve OAC. This lack of association may be explained by BO-ve OAC cases having a higher TNM stage and therefore being frailer, however, TNM was not included in the model as it does not apply to BO cases. By excluding OAC cases with a history of undergoing BO surveillance, who were more likely to be diagnosed at an earlier stage, the association of overweight and obese moved to favour BO cases more than BO+ve OAC but results did not reach statistical significance (Table 4.19). In contrast, BO-ve OAC was less likely among overweight (aOR=0.64, 95% CI: 0.49-0.84, p=0.001; Table 4.20) and obese groups cases (aOR=0.39, 95% CI: 0.29-0.53, p<0.001; Table 4.20). Taken together, it is likely that compared to BO cases, both OAC phenotypes have a lower likelihood of including overweight or obese cases. The lack of an association between BMI groups and BO+ve OAC cases compared to BO cases is likely attributed to early tumour-stage patients who did not experience significant weight loss, thus masking the association.

Similar to BMI, smoking is also a risk factor for both BO and OAC. Compared to BO cases, smoking was not associated with risk of either BO+ve OAC or BO-ve OAC phenotype across adjusted models. The associations of aspirin/NSAID use and reflux symptoms with OAC phenotypes were generally similar in magnitude.

The results of the other two sensitivity analyses (MI and complete case) and for the fully adjusted model with heartburn symptoms excluded as a covariate are presented in Tables A.3

and A.4 for BO+ve OAC and BO-ve OAC compared to BO cases, respectively. The results did not differ substantially from the adjusted estimates presented here.

Furthermore, we compared the BO(?) OAC phenotype to BO cases (Table A.5) and, as expected, saw generally similar point estimates obtained from comparing BO-ve OAC cases to BO cases. This further confirms the excess number of BO-ve OAC among the BO(?) OAC group for which BO phenotype was unascertainable. Results remained robust in sensitivity analyses (Table A.6).

Table 4.19 – Associations of baseline characteristics with risk of BO+ve OAC phenotype compared to BO cases.

Characteristic	BO cases n (%)	BO+ve OAC n (%)	Univariable model OR (95% CI, p)	Minimally adjusted model[a] OR (95% CI, p)	Fully adjusted model[b] OR (95% CI, p)	Fully adjusted model[b] excl. BO surveillance OACs OR (95% CI, p)
Age group at diagnosis						
< 50	203 (18.6)	67 (5.4)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
50 - 59	281 (25.8)	193 (15.6)	2.08 (1.50-2.91, p<0.001)	2.10 (1.51-2.93, p<0.001)	1.95 (1.35-2.86, p<0.001)	2.12 (1.43-3.19, p<0.001)
60 - 69	353 (32.4)	497 (40.2)	4.27 (3.15-5.84, p<0.001)	4.24 (3.13-5.81, p<0.001)	3.60 (2.56-5.13, p<0.001)	3.73 (2.59-5.47, p<0.001)
70+	254 (23.3)	478 (38.7)	5.70 (4.18-7.86, p<0.001)	5.72 (4.19-7.89, p<0.001)	4.61 (3.24-6.62, p<0.001)	4.68 (3.22-6.91, p<0.001)
Gender						
Female	217 (19.9)	163 (13.2)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Male	874 (80.1)	1072 (86.8)	1.63 (1.31-2.04, p<0.001)	1.62 (1.28-2.04, p<0.001)	1.51 (1.16-1.97, p=0.003)	1.47 (1.11-1.94, p=0.007)
BMI group at baseline						
Normal	217 (19.9)	268 (21.7)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Overweight	473 (43.4)	422 (34.2)	0.72 (0.58-0.90, p=0.004)	0.75 (0.60-0.95, p=0.016)	0.83 (0.64-1.07, p=0.148)	0.79 (0.61-1.02, p=0.073)
Obese	354 (32.4)	304 (24.6)	0.70 (0.55-0.88, p=0.003)	0.84 (0.66-1.08, p=0.178)	0.85 (0.65-1.11, p=0.230)	0.78 (0.59-1.04, p=0.086)
Missing	47 (4.3)	241 (19.5)	4.15 (2.92-6.00, p<0.001)	4.56 (3.17-6.68, p<0.001)	2.05 (1.29-3.30, p=0.003)	1.88 (1.16-3.06, p=0.010)
Cigarette smoking status						
Never	392 (35.9)	452 (36.6)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Ever	686 (62.9)	665 (53.8)	0.84 (0.71-1.00, p=0.048)	0.78 (0.65-0.93, p=0.006)	0.92 (0.75-1.12, p=0.408)	0.92 (0.74-1.14, p=0.441)
Missing	13 (1.2)	118 (9.6)	7.87 (4.54-14.86, p<0.001)	7.04 (4.01-13.40, p<0.001)	0.02 (0.00-0.07, p<0.001)	0.02 (0.00-0.08, p<0.001)
Aspirin/NSAID use						
Never	588 (53.9)	257 (20.8)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Ever	279 (25.6)	328 (26.6)	2.69 (2.17-3.34, p<0.001)	2.46 (1.97-3.09, p<0.001)	2.60 (2.06-3.28, p<0.001)	2.39 (1.88-3.06, p<0.001)
Missing	224 (20.5)	650 (52.6)	6.64 (5.38-8.21, p<0.001)	6.35 (5.11-7.91, p<0.001)	4.08 (3.23-5.18, p<0.001)	3.65 (2.86-4.68, p<0.001)
Heartburn symptoms status						
Absent	37 (3.4)	150 (12.1)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Present	1054 (96.6)	818 (66.2)	0.19 (0.13-0.27, p<0.001)	0.21 (0.14-0.30, p<0.001)	0.16 (0.10-0.24, p<0.001)	0.14 (0.09-0.22, p<0.001)
Missing	0 (0)	267 (21.6)	NE	NE	NE	NE

[a]adjusted for age groups and gender.
[b]adjusted for all covariates.
Abbreviation: NE, not estimable.

Table 4.20 – Associations of baseline characteristics with risk of BO-ve OAC phenotype compared to BO cases.

Characteristic	BO cases n (%)	BO-ve OAC n (%)	Univariable model OR (95% CI, p)	Minimally adjusted model[a] OR (95% CI, p)	Fully adjusted model[b] OR (95% CI, p)	Fully adjusted model[b] excl. BO surveillance OACs OR (95% CI, p)
Age group at diagnosis						
< 50	203 (18.6)	59 (6.7)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
50 - 59	281 (25.8)	184 (20.9)	2.25 (1.60-3.20, p<0.001)	2.26 (1.61-3.21, p<0.001)	2.11 (1.40-3.22, p<0.001)	2.03 (1.35-3.11, p=0.001)
60 - 69	353 (32.4)	317 (36.0)	3.09 (2.24-4.31, p<0.001)	3.10 (2.25-4.33, p<0.001)	2.85 (1.94-4.27, p<0.001)	2.78 (1.89-4.16, p<0.001)
70+	254 (23.3)	320 (36.4)	4.33 (3.12-6.09, p<0.001)	4.34 (3.13-6.10, p<0.001)	3.03 (2.04-4.57, p<0.001)	2.94 (1.98-4.44, p<0.001)
Gender						
Female	217 (19.9)	158 (18.0)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Male	874 (80.1)	722 (82.0)	1.13 (0.90-1.43, p=0.277)	1.14 (0.91-1.44, p=0.265)	1.05 (0.79-1.40, p=0.737)	1.08 (0.81-1.45, p=0.580)
BMI group at baseline						
Normal	217 (19.9)	255 (29.0)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Overweight	473 (43.4)	317 (36.0)	0.57 (0.45-0.72, p<0.001)	0.59 (0.47-0.75, p<0.001)	0.64 (0.49-0.84, p=0.001)	0.64 (0.49-0.84, p=0.001)
Obese	354 (32.4)	155 (17.6)	0.37 (0.29-0.48, p<0.001)	0.41 (0.31-0.53, p<0.001)	0.39 (0.29-0.53, p<0.001)	0.40 (0.29-0.55, p<0.001)
Missing	47 (4.3)	153 (17.4)	2.77 (1.92-4.06, p<0.001)	2.84 (1.95-4.19, p<0.001)	1.46 (0.88-2.43, p=0.147)	1.45 (0.87-2.44, p=0.152)
Cigarette smoking status						
Never	392 (35.9)	298 (33.9)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Ever	686 (62.9)	532 (60.5)	1.02 (0.85-1.23, p=0.836)	0.95 (0.78-1.16, p=0.622)	1.20 (0.95-1.51, p=0.138)	1.18 (0.93-1.50, p=0.165)
Missing	13 (1.2)	50 (5.7)	5.06 (2.78-9.87, p<0.001)	4.98 (2.70-9.82, p<0.001)	0.00 (0.00-0.67, p=0.966)	0.00 (0.00-0.07, p=0.966)
Aspirin/NSAID use						
Never	588 (53.9)	161 (18.3)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Ever	279 (25.6)	243 (27.6)	3.18 (2.49-4.07, p<0.001)	3.03 (2.37-3.90, p<0.001)	3.49 (2.66-4.59, p<0.001)	3.42 (2.61-4.51, p<0.001)
Missing	224 (20.5)	476 (54.1)	7.76 (6.14-9.85, p<0.001)	7.45 (5.88-9.50, p<0.001)	5.14 (3.92-6.77, p<0.001)	5.02 (3.82-6.63, p<0.001)
Heartburn symptoms status						
Absent	37 (3.4)	160 (18.2)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Present	1054 (96.6)	543 (61.7)	0.12 (0.08-0.17, p<0.001)	0.12 (0.08-0.18, p<0.001)	0.08 (0.05-0.12, p<0.001)	0.08 (0.05-0.12, p<0.001)
Missing	0 (0)	177 (20.1)	NE	NE	NE	NE

[a]adjusted for age groups and gender.
[b]adjusted for all covariates.
Abbreviation: NE, not estimable.

4.3.5 Comparison of characteristics between prognostic phenotypes and reflux controls

There is substantial overlap between the risk factors for BO and OAC as reviewed earlier. The case-case analyses presented above may be limited in disentangling significant differences in the association of baseline factors for the OAC phenotypes. Therefore, we compared all three OAC phenotypes against reflux controls without BO/OAC to further evaluate the strength of the observed associations for these phenotypes.

When compared against reflux controls, both BO+ve OAC and BO-ve OAC phenotypes were more likely to include cases that were older, male, reported ever-use of aspirin/NSAIDs and were inferred to have heartburn symptoms using the derived variable. As expected, these associations were strengthened in magnitude compared to previous results and the aORs were largely similar for BO+ve OAC and BO-ve OAC cases. Only the association of BMI groups and cigarette smoking status differed between the phenotypes. Compared to reflux controls, the BO+ve OAC group was more likely to include obese cases (aOR=2.10, 95% CI:1.45-3.05, p<0.001; Table A.7). However, similar to the comparison against BO cases, this association was weakened when OAC cases with a history of undergoing BO surveillance were excluded (aOR=1.90, 95% CI: 1.30-2.80, p=0.001; Table A.8). The BO-ve OAC group was more likely than reflux controls to report ever-smoking (aOR=1.85, 95% CI: 1.38-2.48, p<0.001; Table A.9), but there was considerable variation for this association in sensitivity analyses (Table A.10). Finally, as expected, the observed associations were attenuated when comparing the BO(?) OAC to reflux controls (Table A.11) with sensitivity analyses demonstrating some variations in results (Table A.12).

4.3.6 Summary

To summarise the work to this point, after selecting the most significant and well-recorded variables to include in a fully adjusted model, we first compared the cancer phenotypes to each other to determine the degree of overlap between the epidemiological characteristic of BO+ve OAC and BO-ve OAC. We observed similar age group distribution for these and a higher likelihood of self-reported cigarette smoking and aspirin/NSAID use among the BO-ve OAC cases compared to BO+ve OAC cases. Additionally, male cases, overweight or obese BMI, and inferred reflux symptoms were more common among BO-ve OAC cases than BO+ve OAC cases. The association of these epidemiological factors was generally weak or modest and was likely explained by other factors (e.g., BMI and tumour stage) or was not robust in sensitivity analyses, such as the association of smoking with BO-ve OAC using MI. However, the strongest and most significant association was between higher TNM

and increased risk of BO-ve OAC, demonstrating a linear trend. Thus, it could be that the tumour overgrew the extant BO in these cases. Furthermore, by comparing the characteristics of BO+ve and BO-ve to the BO(?) OAC, we confirmed that this group includes an excess of BO-ve OAC tumours.

We then moved to compare the OAC phenotypes with BO cases to better understand the degree of shared characteristics between them. Similar to before, male gender was more strongly associated with the BO+ve OAC than the BO-ve OAC phenotype. The cause of this observation is unclear, but study design and selection bias should be considered as the BO cases were sourced from a different study (BEST2) than the OAC cases (OCCAMS). OAC phenotypes were also compared against reflux controls who were otherwise healthy. In this analysis, we observed a similar magnitude of the aOR for most baseline risk factors in relation to BO+ve OAC or BO-ve OAC. Only cigarette smoking was more likely to be self-reported among BO-ve OAC, but not BO+ve OAC, compared to reflux controls. Taken together, there is considerable overlap in the association of the characteristics between the OAC phenotypes.

4.4 Predictors of survival in prognostic phenotypes

We first re-established the persistence of the favourable prognosis related to BO+ve OAC patients compare to BO-ve OAC patients. This association was similar to that observed in the study by Sawas *et al*. (2018) to further evaluate our hypothesis and address the aims, we conducted additional analyses to interrogate whether the observed prognostic difference is explained by the factors which were associated with the OAC phenotypes as described in previous chapters.

4.4.1 Survival trends among oesophageal adenocarcinoma cases

The survival metrics for OAC cases overall and according to tumour phenotype are presented in Table 25. Of 3,100 OAC cases included in this study from the OCCAMS cohort, vital status and follow-up data were complete for 2,566 cases, resulting in an 82.8% follow-up rate as of June 4th, 2022. No vital status was recorded for 510 cases (16.4%), and these were right censored. Vital status and follow-up duration were not recorded for 24 cases (0.8%) and therefore were excluded from survival analyses. Among the 2,566 cases with complete survival data, a total of 862 deaths due to all causes were documented during the follow-up period: 335 (38.9%) of deaths occurred in the BO+ve OAC group, 283 (32.8%) in the BO-ve OAC group and 244 (28.3%) in the BO(?) OAC group. A total of 6,885.1 person-years of follow-up was collected with BO+ve OAC, BO-ve OAC and BO(?) OAC cases contributing 2,974.7, 1,907.6 and 2,002.8 person-years, respectively.

Table 4.21 – Survival statistics of OAC cases, overall and according to OAC phenotype.

Metric	BO+ve OAC n=1,235	BO-ve OAC n=880	BO(?) OAC n=985	Overall n=3,100
Follow-up rate†, n (%)	1053 (85.2)	773 (87.8)	740 (75.1)	2566 (82.8)
Right censored, n (%)	170 (13.7)	104 (11.8)	236 (23.9)	510 (16.4)
Excluded*, n (%)	12 (0.9)	3 (0.3)	9 (0.9)	24 (0.8)
Deaths, n (%)	335 (27.1)	283 (32.1)	244 (24.7)	862 (27.8)
Person-years	2974.7	1907.6	2002.8	6885.1
Crude mortality rate#	11.2	14.8	12.2	12.5
Years of follow-up, median (min-max)	1.9 (0.1-12.0)	1.7 (0.1-9.2)	1.5 (0.1-10.9)	1.7 (0.1-12.0)

†Cases with complete vital status and follow-up duration.
*Cases with missing vital status and follow-up duration.
#Per 100 person-years

Tables 4.22-4.29 present summary survival statistics according to the selected baseline characteristics for all OAC cases and by OAC phenotypes. Although Siewert Classification

was not included among these, it was analysed in relation to survival because it was one of the variables originally used by Sawas *et al*. (2018). We collected all-cause mortality for OAC cases, therefore, survival refers to overall survival (OS).

Demographic factors

Overall, a similar follow-up rate and proportion of deaths were observed across the age groups (Table 4.22). Cases in the 60-69 age group contributed the highest number of person-years (2665.5) followed by the 70+ group (2452.7), the 50-59 group (1341.8) and the <50 group (425.1). A similar crude mortality rate (CMR) was observed across the three youngest age groups, however, a slightly higher rate was seen among those in the 70+ age group. When examined according to OAC phenotype, a lower follow-up rate was seen for all age groups among BO(?) OAC tumours.

The survival metrics overall and by OAC phenotypes according to patient gender are presented in Table 4.23. Overall, these metrics were similar between female and male cases. Consistent with the results for age group, BO-ve OAC tumours had an elevated CMR. However, the crude mortality rate for female BO-ve OAC cases was slightly higher than for male cases.

Table 4.22 – Survival statistics of OAC cases by age group at diagnosis, overall and according to OAC phenotype.

Phenotype	BO+ve OAC				BO-ve OAC				BO(?) OAC				Overall			
Age group at diagnosis	**< 50**	**50 - 59**	**60 - 69**	**70+**	**< 50**	**50 - 59**	**60 - 69**	**70+**	**< 50**	**50 - 59**	**60 - 69**	**70+**	**< 50**	**50 - 59**	**60 - 69**	**70+**
Frequency, n	66	190	492	475	59	183	316	319	75	212	332	357	200	585	1140	1151
Follow-up rate[†], n (%)	57 (86.4)	166 (87.4)	424 (86.2)	406 (85.5)	48 (81.4)	167 (91.3)	273 (86.4)	285 (89.3)	55 (73.3)	163 (76.9)	262 (78.9)	260 (72.8)	160 (80.0)	496 (84.8)	959 (84.1)	951 (82.6)
Right censored, n (%)	9 (13.6)	24 (12.6)	68 (13.8)	69 (14.5)	11 (18.6)	16 (8.7)	43 (13.6)	34 (10.7)	20 (26.7)	49 (23.1)	70 (21.1)	97 (27.2)	40 (20.0)	89 (15.2)	181 (15.9)	200 (17.4)
Deaths, n (%)	20 (30.3)	49 (25.8)	124 (25.2)	142 (29.9)	15 (25.4)	56 (30.6)	104 (32.9)	108 (33.9)	16 (21.3)	54 (25.5)	91 (27.4)	83 (23.2)	51 (25.5)	159 (27.2)	319 (28.0)	333 (28.9)
Person-years	163.4	472.1	1207.6	1131.6	133.9	424.1	722.5	627.1	127.8	445.6	735.5	693.9	425.1	1341.8	2665.5	2452.7
CMR[#]	12.2	10.4	10.3	12.5	11.2	13.2	14.4	17.2	12.5	12.1	12.4	12.0	12.0	11.8	12.0	13.6
Years of follow-up, median (min-max)	2.4 (0.1-6.5)	2.0 (0.1-7.7)	1.9 (0.1-12.0)	1.9 (0.1-9.7)	1.6 (0.3-8.4)	1.8 (0.2-9.2)	1.8 (0.1-9.0)	1.6 (0.0-7.9)	1.2 (0.2-7.1)	1.4 (0.1-8.5)	1.6 (0.1-9.8)	1.4 (0.1-10.9)	1.8 (0.1-8.4)	1.7 (0.1-9.2)	1.8 (0.1-12.0)	1.6 (0.0-10.9)

†Cases with complete vital status and follow-up duration.
[#]Per 100 person-years.
Abbreviation: CMR, crude mortality rate.

Table 4.23 – Survival statistics of OAC cases by gender, overall and according to tumour phenotype.

Phenotype	BO+ve OAC		BO-ve OAC		BO(?) OAC		Overall	
Gender	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**
Frequency, n	163	1060	158	719	161	815	482	2594
Follow-up rate[†], n (%)	136 (83.4)	917 (86.5)	133 (84.2)	640 (89.0)	129 (80.1)	611 (75.0)	398 (82.6)	2168 (83.6)
Right censored, n (%)	27 (16.6)	143 (13.5)	25 (15.8)	79 (11.0)	32 (19.9)	204 (25.0)	84 (17.4)	426 (16.4)
Deaths, n (%)	39 (23.9)	296 (27.9)	52 (32.9)	231 (32.1)	38 (23.6)	206 (25.3)	129 (26.8)	733 (28.3)
Person-years	410	2564.7	326.2	1581.3	334.3	1668.5	1070.6	5814.5
CMR[#]	9.5	11.5	15.9	14.6	11.4	12.3	12.0	12.6
Years of follow-up, median (min-max)	2.0 (0.1-10.3)	1.9 (0.1-12.0)	1.5 (0.2-9.0)	1.7 (0.0-9.2)	1.3 (0.1-10.9)	1.5 (0.1-9.8)	1.6 (0.1-10.9)	1.7 (0.0-12.0)

†Cases with complete vital status and follow-up duration.
[#]Per 100 person-years.
Abbreviation: CMR, crude mortality rate.

Risk factors

Table 4.24 shows the survival statistics for all OAC cases based on the OAC phenotype and according to BMI group at baseline. Overall, a similar follow-up rate was achieved for across BMI groups, however, those missing BMI had the lowest follow-up rate (73.6%). Overweight cases provided the greatest number of person-years (2388.4) followed by cases with normal (1659.1) and obese (1517.5) BMI. As expected, the CMR was highest among cases with a normal BMI (14.8) followed by overweight (11.7) and obese (9.7) cases. This trend is likely explained by the severe weight loss experienced by patients with more advanced tumours at baseline when BMI was measured. Indeed, among patients with early-stage tumours (I/II) 20.3% had a normal BMI while this prevalence was 27.6% among patients with late-stage tumours (III/IV). Conversely, 24.3% of early-stage patients were obese relative to 18.2% of late-stage patients. The CMR was elevated across all BMI groups among the BO-ve OAC phenotype compared to the other phenotypes.

Table 4.24 – Survival statistics of OAC cases by BMI group at baseline, overall and according to tumour phenotype.

Phenotype	BO+ve OAC				BO-ve OAC				BO(?) OAC				Overall			
BMI group at diagnosis	**Normal**	**Overweight**	**Obese**	**Missing**	**Normal**	**Overweight**	**Obese**	**Missing**	**Normal**	**Overweight**	**Obese**	**Missing**	**Normal**	**Overweight**	**Obese**	**Missing**
Frequency, n	266	418	300	239	255	315	155	152	245	305	193	233	766	1038	648	624
Follow-up rate[†], n (%)	236 (88.7)	364 (87.1)	271 (90.3)	182 (76.2)	226 (88.6)	287 (91.1)	136 (87.7)	124 (81.6)	208 (84.9)	239 (78.4)	140 (72.5)	153 (65.7)	670 (87.5)	890 (85.7)	547 (84.4)	459 (73.6)
Right censored, n (%)	30 (11.3)	54 (12.9)	29 (9.7)	57 (23.8)	29 (11.4)	28 (8.9)	19 (12.3)	28 (18.4)	37 (15.1)	66 (21.6)	53 (27.5)	80 (34.3)	96 (12.5)	148 (14.3)	101 (15.6)	165 (26.4)
Deaths, n (%)	86 (32.3)	111 (26.6)	62 (20.7)	76 (31.8)	87 (34.1)	96 (30.5)	43 (27.7)	57 (37.5)	72 (29.4)	73 (23.9)	42 (21.8)	57 (24.5)	245 (32.0)	280 (27.0)	147 (22.7)	190 (30.4)
Person-years	601	1025.6	781.2	566.9	552.7	709.8	348.5	296.6	505.4	653.1	387.8	456.6	1659.1	2388.4	1517.5	1320.1
CMR[#]	14.3	10.8	7.9	13.4	15.7	13.5	12.3	19.2	14.2	11.2	10.8	12.5	14.8	11.7	9.7	14.4
Years of follow-up, median (min-max)	1.7 (0.1-7.3)	2.0 (0.1-10.0)	2.2 (0.1-8.6)	1.8 (0.1-12.0)	1.6 (0.0-9.2)	1.8 (0.1-9.0)	1.9 (0.3-7.6)	1.6 (0.1-8.4)	1.5 (0.1-10.9)	1.5 (0.1-9.0)	1.4 (0.1-7.1)	1.2 (0.1-8.3)	1.7 (0.0-10.9)	1.8 (0.1-10.0)	1.8 (0.1-8.6)	1.5 (0.1-12.0)

[†]Cases with complete vital status and follow-up duration.
[#]Per 100 person-years.
Abbreviation: CMR, crude mortality rate.

Table 4.25 summarises the survival metrics for the cohort according to cigarette smoking status. Overall, these metrics were similar between those reporting ever- and never-smoking. Similar to other characteristics examined above, the CMR was higher among both never- and ever-smokers in the BO-ve OAC group compared to BO+ve OAC and BO(?) OAC.

Table 4.25 – Survival statistics of OAC cases by cigarette smoking group, overall and according to tumour phenotype.

Phenotype	BO+ve OAC			BO-ve OAC			BO(?) OAC			Overall		
Smoking status	**Never**	**Ever**	**Missing**	**Never**	**Ever**	**Missing**	**Never**	**Ever**	**Missing**	**Never**	**Ever**	**Missing**
Frequency, n	448	658	117	297	531	49	301	557	118	1046	1746	284
Follow-up rate†, n (%)	398 (88.8)	588 (89.4)	67 (57.3)	265 (89.2)	475 (89.5)	33 (67.3)	241 (80.1)	441 (79.2)	58 (49.2)	904 (86.4)	1504 (86.1)	158 (55.6)
Right censored, n (%)	50 (11.2)	70 (10.6)	50 (42.7)	32 (10.8)	56 (10.5)	16 (32.7)	60 (19.9)	116 (20.8)	60 (50.8)	142 (13.6)	242 (13.9)	126 (44.4)
Deaths, n (%)	117 (26.1)	189 (28.7)	29 (24.8)	103 (34.7)	163 (30.7)	17 (34.7)	78 (25.9)	138 (24.8)	28 (23.7)	298 (28.5)	490 (28.1)	74 (26.1)
Person-years	1141.4	1668.7	164.6	657.4	1181.6	68.6	645.4	1196.1	161.4	2444.2	4046.4	394.6
CMR#	10.3	11.3	17.6	15.7	13.8	24.8	12.1	11.5	17.3	12.2	12.1	18.8
Years of follow-up, median (min-max)	2.1 (0.1-12.0)	2.1 (0.1-10.0)	0.7 (0.1-6.2)	1.8 (0.1-9.0)	1.7 (0.0-9.2)	1.1 (0.2-4.2)	1.5 (0.1-10.9)	1.6 (0.1-9.8)	0.6 (0.1-5.5)	1.8 (0.1-12.0)	1.8 (0.0-10.0)	0.7 (0.1-6.2)

†Cases with complete vital status and follow-up duration.
#Per 100 person-years.
Abbreviation: CMR, crude mortality rate.

Table 4.26 provides descriptive survival statistics, which are presented both overall and by OAC phenotypes, according to aspirin/NSAID use. Overall, the follow-up rate was slightly higher for ever users than never users. Additionally, ever-users contributed more Person-Years (2014.4) than never-users (1430.4) and the CMR was slightly higher among ever-users (10.8) compared to never-users (9.3). Among BO-ve OAC tumours, the CMR was found to be greater for both never- and ever-users was higher than the other tumour groups and the CMR was also greater in ever-users than never-users in this tumour phenotype group.

The survival statistics overall and by tumour phenotype are summarised according to heartburn status in Table 4.27. Overall, little difference was observed in these statistics between those with and without heartburn symptoms. As expected, the CMR was again elevated among BO-ve OAC tumours compared to other tumour groups.

Table 4.26 – Survival statistics of OAC cases by aspirin/NSAID use status, overall and according to tumour phenotype.

Phenotype	BO+ve OAC			BO-ve OAC			BO(?) OAC			Overall		
Aspirin/NSAID use	**Never**	**Ever**	**Missing**	**Never**	**Ever**	**Missing**	**Never**	**Ever**	**Missing**	**Never**	**Ever**	**Missing**
Frequency, n	256	323	644	160	243	474	198	259	519	614	825	1637
Follow-up rate†, n (%)	221 (86.3)	294 (91.0)	538 (83.5)	134 (83.8)	221 (90.9)	418 (88.2)	148 (74.7)	211 (81.5)	381 (73.4)	503 (81.9)	726 (88.0)	1337 (81.7)
Right censored, n (%)	35 (13.7)	29 (9.0)	106 (16.5)	26 (16.2)	22 (9.1)	56 (11.8)	50 (25.3)	48 (18.5)	138 (26.6)	111 (18.1)	99 (12.0)	300 (18.3)
Deaths, n (%)	60 (23.4)	82 (25.4)	193 (30.0)	35 (21.9)	80 (32.9)	168 (35.4)	38 (19.2)	55 (21.2)	151 (29.1)	133 (21.7)	217 (26.3)	512 (31.3)
Person-years	662	874.3	1438.3	354.9	571.2	981.5	413.4	568.9	1020.5	1430.4	2014.4	3440.3
CMR#	9.1	9.4	13.4	9.9	14.0	17.1	9.2	9.7	14.8	9.3	10.8	14.9
Years of follow-up, median (min-max)	2.3 (0.1-8.2)	2.3 (0.1-9.7)	1.7 (0.1-12.0)	2.0 (0.1-6.9)	1.8 (0.1-9.0)	1.6 (0.0-9.2)	1.5 (0.1-7.1)	1.7 (0.1-9.8)	1.3 (0.1-10.9)	1.9 (0.1-8.2)	1.9 (0.1-9.8)	1.5 (0.0-12.0)

†Cases with complete vital status and follow-up duration.
#Per 100 person-years.
Abbreviation: CMR, crude mortality rate.

Table 4.27 – Survival statistics of OAC cases by derived heartburn symptoms status, overall and according to tumour phenotype.

Phenotype	BO+ve OAC			BO-ve OAC			BO(?) OAC			Overall		
Heartburn symptoms	**Absent**	**Present**	**Missing**	**Absent**	**Present**	**Missing**	**Absent**	**Present**	**Missing**	**Absent**	**Present**	**Missing**
Frequency, n	149	810	264	159	543	175	142	593	241	450	1946	680
Follow-up rate†, n (%)	127 (85.2)	734 (90.6)	192 (72.7)	136 (85.5)	492 (90.6)	145 (82.9)	109 (76.8)	480 (80.9)	151 (62.7)	372 (82.7)	1706 (87.7)	488 (71.8)
Right censored, n (%)	22 (14.8)	76 (9.4)	72 (27.3)	23 (14.5)	51 (9.4)	30 (17.1)	33 (23.2)	113 (19.1)	90 (37.3)	78 (17.3)	240 (12.3)	192 (28.2)
Deaths, n (%)	40 (26.8)	210 (25.9)	85 (32.2)	46 (28.9)	163 (30.0)	74 (42.3)	32 (22.5)	147 (24.8)	65 (27.0)	118 (26.2)	520 (26.7)	224 (32.9)
Person-years	349.7	2094	531	359.9	1202.4	345.3	287.2	1313.3	402.3	996.8	4609.7	1278.7
CMR#	11.4	10.0	16.0	12.8	13.6	21.4	11.1	11.2	16.2	11.8	11.3	17.5
Years of follow-up, median (min-max)	2.2 (0.1-8.6)	2.2 (0.1-11.2)	1.2 (0.1-12.0)	1.9 (0.1-9.2)	1.7 (0.0-9.0)	1.7 (0.2-8.4)	1.5 (0.1-10.9)	1.6 (0.1-9.8)	0.8 (0.1-8.3)	1.8 (0.1-10.9)	1.8 (0.0-11.2)	1.3 (0.1-12.0)

†Cases with complete vital status and follow-up duration.
#Per 100 person-years.
Abbreviation: CMR, crude mortality rate.

Clinical factors

Table 4.28 shows the descriptive survival statistics, overall and by tumour phenotype, according to the TNM stage. Overall, these statistics were largely consistent across TNM stages. The CMR showed an upward trend from stage I to IV, in line with expectations, and this trend was also observed among the tumour phenotypes. As observed for other baseline factors examined above, the CMR was generally higher among BO-ve OAC tumours compared to other tumour groups. Lastly, the survival metrics were generally similar according to Siewert Classification (Table 4.29).

Table 4.28 – Survival statistics of OAC cases by TNM, overall and according to tumour phenotype.

Phenotype	BO+ve OAC					BO-ve OAC					BO(?) OAC					Overall				
TNM	**I**	**II**	**III**	**IV**	**Mis.**	**I**	**II**	**III**	**IV**	**Mis.**	**I**	**II**	**III**	**IV**	**Mis.**	**I**	**II**	**III**	**IV**	**Mis.**
Frequency, n	293	278	475	26	151	86	200	445	26	120	115	189	424	18	230	494	667	1344	70	501
Follow-up rate†, n (%)	244 (83.3)	239 (86.0)	424 (89.3)	18 (69.2)	128 (84.8)	77 (89.5)	181 (90.5)	396 (89.0)	22 (84.6)	97 (80.8)	82 (71.3)	138 (73.0)	326 (76.9)	12 (66.7)	182 (79.1)	403 (81.6)	558 (83.7)	1146 (85.3)	52 (74.3)	407 (81.2)
Right censored, n (%)	49 (16.7)	39 (14.0)	51 (10.7)	8 (30.8)	23 (15.2)	9 (10.5)	19 (9.5)	49 (11.0)	4 (15.4)	23 (19.2)	33 (28.7)	51 (27.0)	98 (23.1)	6 (33.3)	48 (20.9)	91 (18.4)	109 (16.3)	198 (14.7)	18 (25.7)	94 (18.8)
Deaths, n (%)	43 (14.7)	65 (23.4)	196 (41.3)	5 (19.2)	26 (17.2)	16 (18.6)	42 (21.0)	185 (41.6)	15 (57.7)	25 (20.8)	9 (7.8)	31 (16.4)	129 (30.4)	7 (38.9)	68 (29.6)	68 (13.8)	138 (20.7)	510 (37.9)	27 (38.6)	119 (23.8)
Person-years	814.5	716.5	985.6	41.8	416.2	251.2	467.6	868.4	34.2	286.2	285.3	454	809.9	28.6	425	1351	1638.1	2663.9	104.6	1127.5
CMR#	5.3	9.1	19.9	12.0	6.2	6.4	9.0	21.3	43.9	8.7	3.2	6.8	15.9	24.5	16	5.0	8.4	19.1	25.8	10.6
Years of follow-up, median (min-max)	2.8 (0.1-10.3)	2.3 (0.1-8.6)	1.6 (0.1-12.0)	1.1 (0.2-5.7)	2.4 (0.1-11.2)	2.8 (0.1-9.0)	2.0 0.0-9.0)	1.6 (0.1-8.4)	1.0 (0.2-6.4)	1.8 (0.3-9.2)	2.1 (0.1-7.7)	1.8 (0.1-9.8)	1.4 (0.1-9.3)	0.9 (0.4-6.9)	1.1 (0.1-10.9)	2.5 (0.1-10.3)	2.1 (0.0-9.8)	1.5 (0.1-12.0)	1.0 (0.2-6.9)	1.6 (0.1-11.2)

†Cases with complete vital status and follow-up duration.
#Per 100 person-years.
Abbreviations: CMR, crude mortality rate; Mis., missing.

Table 4.29 – Survival statistics of OAC cases by Siewert classification, overall and according to tumour phenotype.

Phenotype	BO+ve OAC				BO-ve OAC				BO(?) OAC				Overall			
Siewert classification Type	**I**	**II**	**III**	**Mis.**	**I**	**II**	**III**	**Mis.**	**I**	**II**	**III**	**Mis.**	**I**	**II**	**III**	**Mis.**
Frequency, n	220	201	46	756	91	192	76	518	83	129	32	732	394	522	154	2006
Follow-up rate†, n (%)	205 (93.2)	175 (87.1)	45 (97.8)	628 (83.1)	84 (92.3)	183 (95.3)	73 (96.1)	433 (83.6)	74 (89.2)	103 (79.8)	26 (81.2)	537 (73.4)	363 (92.1)	461 (88.3)	144 (93.5)	1598 (79.7)
Right censored, n (%)	15 (6.8)	26 (12.9)	1 (2.2)	128 (16.9)	7 (7.7)	9 (4.7)	3 (3.9)	85 (16.4)	9 (10.8)	26 (20.2)	6 (18.8)	195 (26.6)	31 (7.9)	61 (11.7)	10 (6.5)	408 (20.3)
Deaths, n (%)	112 (50.9)	91 (45.3)	20 (43.5)	112 (14.8)	49 (53.8)	103 (53.6)	45 (59.2)	86 (16.6)	44 (53.0)	54 (41.9)	14 (43.8)	132 (18.0)	205 (52.0)	248 (47.5)	79 (51.3)	330 (16.5)
Person-years	593.3	524.3	142.1	1715	268.1	488.3	193.8	957.4	231.7	344.8	83.6	1342.7	1093.1	1357.4	419.6	4015.1
CMR#	18.9	17.4	14.1	6.5	18.3	21.1	23.2	9.0	19.0	15.7	16.7	9.8	18.8	18.3	18.8	8.2
Years of follow-up, median (min-max)	2.2 (0.1-8.6)	1.9 (0.1-11.2)	2.6 (0.2-9.5)	1.8 (0.1-12.0)	2.4 (0.4-9.0)	2.0 (0.1-9.2)	1.9 (0.3-9.0)	1.4 (0.0-7.3)	1.9 (0.2-10.9)	2.5 (0.2-9.3)	1.9 (0.2-7.7)	1.2 (0.1-7.8)	2.2 (0.1-10.9)	2.0 (0.1-11.2)	2.0 (0.2-9.5)	1.5 (0.0-12.0)

†Cases with complete vital status and follow-up duration.
#Per 100 person-years.
Abbreviations: CMR, crude mortality rate; Mis., missing.

Overall survival

The median overall survival for all OAC cases in this cohort was 5.52 years (95% CI: 5.02-6.13) as shown on the Kaplan-Meier survival curve in Figure 4.2. It should be noted that patients recruited into OCCAMS are on a curative pathway, therefore, the survival for OAC cases included here is longer than the overall survival seen for all OAC cases (approximately <20% at 5 years).

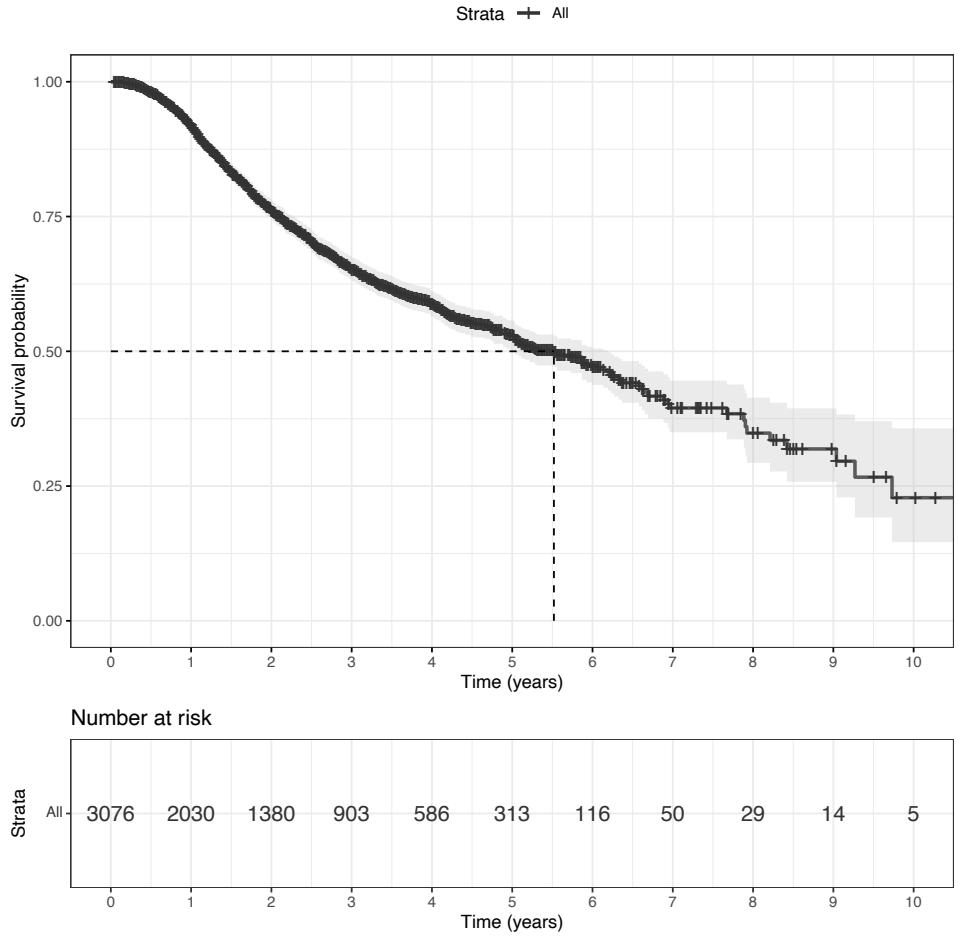

Figure 4.2 – Kaplan-Meier survival curve showing survival probability for all OAC cases. Vertical ticks on the curve indicate an event (death) and shaded areas indicate the 95% confidence interval. Median survival time is marked with the dashed lines. The life table at the bottom shows the number of patients at risk at the beginning of each follow-up year.

The median overall survival for BO+ve OAC or BO-ve OAC cases was similar to BO(?) OAC cases, as expected (Figure 4.3). The median overall survival among BO(?) OAC cases was 5.87 years (95% CI: 5.04-not estimable) which was more similar to the observed survival among BO+ve OAC tumours.

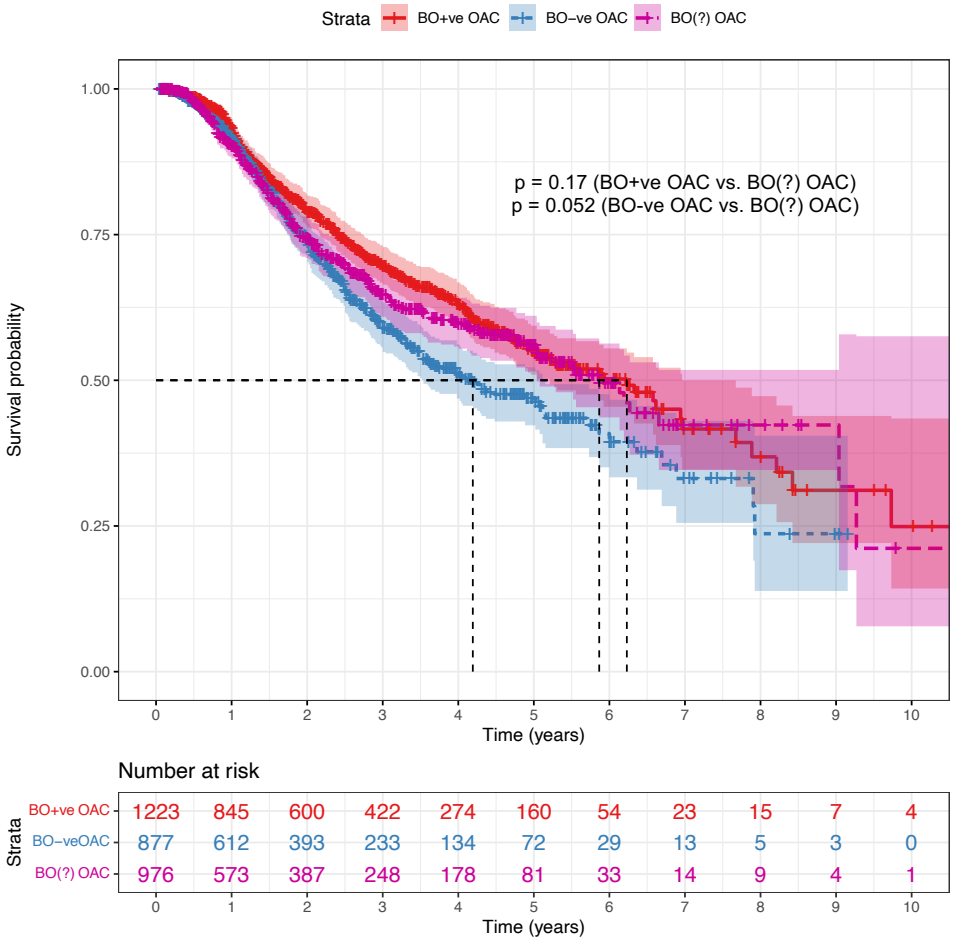

Figure 4.3 – Kaplan-Meier survival curves showing survival probability for all three tumour phenotypes. Vertical ticks on the curve indicate an event (death) and shaded areas indicate the 95% confidence interval. Median survival time is marked with the dashed lines. The life table at the bottom shows the number of patients at risk at the beginning of each follow-up year.

4.4.2 Association of characteristics with survival in prognostic phenotypes

After identifying the association of the selected baseline characteristics with the OAC phenotypes and individually reviewing these factors in relation to survival, we performed follow-up survival analyses to evaluate if other factors explain the prognostic effect of the OAC phenotypes. Table 4.30 presents the characteristics of the cases included in the survival analysis and the unadjusted and adjusted hazard ratios for the association of the BO+ve OAC phenotype with overall survival compared to the BO-ve OAC phenotype. Previously, BO-ve OAC were compared to BO+ve OAC cases to characterise the association of baseline factors. This comparison is reversed here to align with the study by Sawas *et al.* (2018) and ease the interpretation of results.

In unadjusted analysis, BO+ve OAC was associated with a significantly reduced risk of overall mortality compared to the BO-ve OAC cases (HR=0.75, 95% CI: 0.64-0.87, p<0.001). This association was attenuated but persisted in the model adjusted for age group at diagnosis, gender, TNM and Siewert classification (HR=0.85, 95% CI: 0.76-0.94, p=0.001). Further adjustment for BMI group, smoking status, aspirin/NSAID use and heartburn symptoms dampened the hazard ratio slightly more, however, the association of BO+ve OAC tumours and reduced risk of mortality was significant (HR=0.88, 95% CI: 0.77-0.95, p=0.015).

Among the baseline characteristics, TNM was strongly correlated with an increased risk of mortality in a stepwise manner in univariable and adjusted models. In unadjusted analysis, improved survival was observed for overweight (HR=0.79, 95% CI: 0.64-0.96, p=0.020) and obese (HR=0.61, 95% CI: 0.48-0.78, p<0.001) cases compared to those with a normal BMI. This is likely reflecting that cases with low BMI at diagnosis were at an advanced cancer stage. While the association of overweight BMI attenuated and did not reach significance in adjusted models, obese BMI was significantly associated with reduced risk of mortality in the minimally adjusted model (HR=0.76, 95% CI:0.60-0.97, p=0.030) and the fully adjusted model (HR=0.75, 95% CI:0.59-0.97, p=0.025). No significant association was observed between other baseline characteristics and risk of mortality.

4.4.3 Sensitivity analyses

A sensitivity analysis was performed which excluded the OAC cases with a history of undergoing BO surveillance (n=211). As described previously, a majority of these cases were BO+ve OAC (67.8%, n=145), but BO-ve OAC was not completely absent (9.8%, n=21) and BO(?) OAC was frequent (22.4%, n=48). Initially, a total of 214 cases reported a history of undergoing BO surveillance but no vital status or follow-up data was recorded for 3 cases

Table 4.30 – Association of OAC phenotype and baseline characteristics with overall survival.

Variable	Alive n (%)	Died n (%)	Univariable model HR (95% CI, p)	Minimally adjusted model[a] HR (95% CI, p)	Fully adjusted model[b] HR (95% CI, p)
OAC phenotype					
BO-ve OAC	594 (67.7)	283 (32.3)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
BO+ve OAC	888 (72.6)	335 (27.4)	0.75 (0.64-0.87, p<0.001)	0.85 (0.76-0.94, p=0.001)	0.88 (0.77-0.95, p=0.015)
Age group at diagnosis					
< 50	90 (72.0)	35 (28.0)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
50 - 59	268 (71.8)	105 (28.2)	1.00 (0.68-1.46, p=0.987)	1.21 (0.82-1.78, p=0.332)	1.14 (0.77-1.68, p=0.510)
60 - 69	580 (71.8)	228 (28.2)	1.00 (0.70-1.42, p=0.980)	1.22 (0.85-1.74, p=0.282)	1.15 (0.80-1.65, p=0.442)
70+	544 (68.5)	250 (31.5)	1.21 (0.85-1.72, p=0.298)	1.51 (1.06-2.16, p=0.023)	1.46 (1.02-2.10, p=0.039)
Gender					
Female	230 (71.7)	91 (28.3)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Male	1252 (70.4)	527 (29.6)	1.02 (0.81-1.27, p=0.881)	1.04 (0.83-1.30, p=0.748)	1.05 (0.84-1.32, p=0.661)
TNM					
I	320 (84.4)	59 (15.6)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
II	371 (77.6)	107 (22.4)	1.68 (1.22-2.32, p=0.001)	1.63 (1.18-2.24, p=0.003)	1.59 (1.15-2.19, p=0.005)
III	539 (58.6)	381 (41.4)	4.00 (3.03-5.27, p<0.001)	3.72 (2.81-4.92, p<0.001)	3.64 (2.74-4.82, p<0.001)
IV	32 (61.5)	20 (38.5)	5.89 (3.54-9.80, p<0.001)	5.22 (3.13-8.72, p<0.001)	5.30 (3.15-8.93, p<0.001)
Missing	220 (81.2)	51 (18.8)	1.34 (0.92-1.94, p=0.130)	1.31 (0.90-1.90, p=0.165)	1.28 (0.88-1.87, p=0.199)
Siewert Type					
Type I	150 (48.2)	161 (51.8)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Type II	199 (50.6)	194 (49.4)	1.04 (0.84-1.28, p=0.744)	0.97 (0.78-1.20, p=0.781)	0.94 (0.75-1.16, p=0.546)
Type III	57 (46.7)	65 (53.3)	1.05 (0.79-1.40, p=0.731)	0.87 (0.65-1.16, p=0.333)	0.90 (0.67-1.21, p=0.479)
Missing	1076 (84.5)	198 (15.5)	0.40 (0.33-0.50, p<0.001)	0.41 (0.34-0.51, p<0.001)	0.42 (0.34-0.53, p<0.001)
BMI group at baseline					
Normal	348 (66.8)	173 (33.2)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Overweight	526 (71.8)	207 (28.2)	0.79 (0.64-0.96, p=0.020)	0.87 (0.71-1.07, p=0.181)	0.86 (0.70-1.06, p=0.153)
Obese	350 (76.9)	105 (23.1)	0.61 (0.48-0.78, p<0.001)	0.76 (0.60-0.97, p=0.030)	0.75 (0.59-0.97, p=0.025)
Missing	258 (66.0)	133 (34.0)	1.02 (0.81-1.28, p=0.860)	0.98 (0.78-1.23, p=0.863)	0.78 (0.60-1.02, p=0.065)
Cigarette smoking status					
Never	525 (70.5)	220 (29.5)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Ever	837 (70.4)	352 (29.6)	1.02 (0.86-1.21, p=0.815)	1.07 (0.90-1.27, p=0.441)	1.11 (0.93-1.33, p=0.250)
Missing	120 (72.3)	46 (27.7)	1.86 (1.35-2.55, p<0.001)	1.86 (1.35-2.57, p<0.001)	1.68 (1.15-2.46, p=0.007)
Aspirin/NSAID use					
Never	321 (77.2)	95 (22.8)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Ever	404 (71.4)	162 (28.6)	1.18 (0.92-1.52, p=0.193)	1.05 (0.81-1.36, p=0.698)	1.04 (0.80-1.35, p=0.761)
Missing	757 (67.7)	361 (32.3)	1.61 (1.28-2.01, p<0.001)	1.29 (1.02-1.63, p=0.030)	1.20 (0.93-1.53, p=0.157)
Heartburn symptoms status					
Absent	222 (72.1)	86 (27.9)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Present	980 (72.4)	373 (27.6)	0.93 (0.73-1.17, p=0.520)	1.23 (0.97-1.56, p=0.093)	1.25 (0.98-1.59, p=0.067)
Missing	280 (63.8)	159 (36.2)	1.53 (1.17-1.99, p=0.002)	1.60 (1.22-2.08, p=0.001)	1.44 (1.06-1.94, p=0.018)

[a]adjsuted for age group at diagnosis, gender, TNM and Siewert Classification.
[b]adjusted for same covariates as [a] and further adjusted for BMI, smoking status, aspirin/NSAID use and derived heartburn symptoms.

(1.4%, all BO+ve OAC) which were excluded from the analysis, therefore resulting in 211 cases with a history of undergoing BO surveillance.

After excluding cases undergoing BO surveillance, the median overall survival of the cohort was 5.12 years (95% CI: 4.77-5.92; Figure 4.4) which was not significantly different from the overall median survival of 5.52 years (95% CI: 5.02-6.13) obtained when these cases were included (p=0.455, log-rank test). Initially, the median overall survival time was 6.23 years among BO+ve OAC (95% CI: 5.21-6.96) compared to 4.19 years (95% CI: 3.54-5.12) among BO-ve OAC cases (log-rank p=0.00025; Figure 4.1). After excluding the BO surveillance cases, the difference in median overall survival between BO+ve OAC and BO-ve OAC cases became smaller but remained statistically significant. The median survival was 5.32 years (95% CI:4.75-6.62) among BO+ve OAC and 4.25 years (95% CI: 3.54-5.73) among BO-ve OAC cases (Figure 4.5).

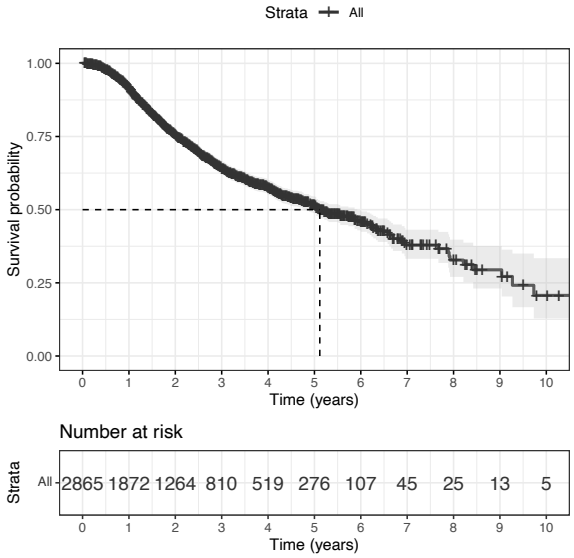

Figure 4.4 – Kaplan-Meier survival curve showing survival probability among all OAC cases after excluding cases with a history of undergoing BO surveillance (n=211). Vertical ticks on the curve indicate an event (death) and shaded areas indicate the 95% confidence interval. Median survival time is marked with the dashed lines. The life table at the bottom shows the number of patients at risk at the beginning of each follow-up year.

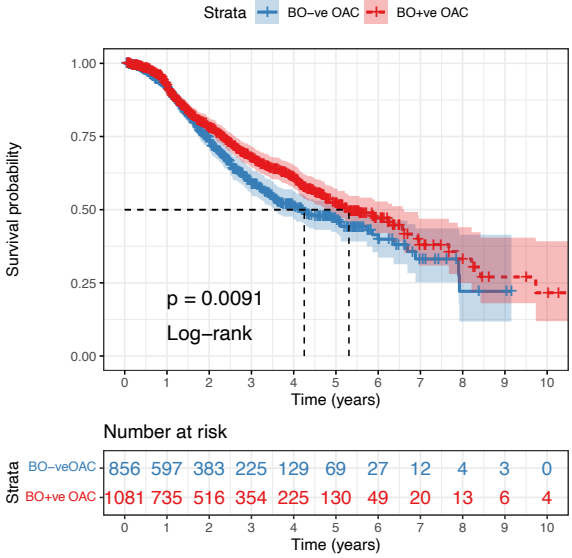

Figure 4.5 – Kaplan-Meier survival curve showing survival probability for BO+ve OAC and BO-ve OAC cases after excluding cases with a history of undergoing BO surveillance (n=211). Vertical ticks on the curve indicate an event (death) and shaded areas indicate the 95% confidence interval. Median survival time is marked with the dashed lines. The life table at the bottom shows the number of patients at risk at the beginning of each follow-up year.

The results of the Cox regression survival analysis excluding OAC cases with a history of undergoing BO surveillance are shown in Table 4.31. After excluding OAC cases with a history of undergoing BO surveillance, the association of the BO+ve OAC phenotype

with survival relative to the BO-ve OAC phenotype became attenuated as evidenced by the unadjusted and adjusted HRs. Relative to BO-ve OAC cases, the unadjusted HR for the association of the BO+ve OAC phenotype demonstrated a 20% reduced risk of mortality (HR=0.80, 95% CI: 0.68-0.95, p=0.009; Table 4.31). This association was weakened compared to the association seen for the BO+ve OAC cases before excluding cases with a history of undergoing surveillance (HR=0.75, 95% CI: 0.64-0.87, p<0.001; Table 4.30). The fully adjusted association for the BO+ve OAC phenotype and risk of mortality compared to the BO-ve phenotypes was borderline statistically significant (HR=0.92, 95% CI: 0.82-1.01, p=0.052; Table 4.31).

Table 4.31 – Association of OAC phenotype and baseline characteristics with overall survival in sensitivity analysis excluding cases (n=211) with a history of undergoing BO surveillance.

Variable	Alive n (%)	Died n (%)	Univariable model HR (95% CI, p)	Minimally adjusted model[a] HR (95% CI, p)	Fully adjusted model[b] HR (95% CI, p)
OAC phenotype					
BO-ve OAC	583 (68.1)	273 (31.9)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
BO+ve OAC	772 (71.4)	309 (28.6)	0.80 (0.68-0.95, p=0.009)	0.89 (0.79-0.97, p=0.023)	0.92 (0.82-1.01, p=0.055)
Age group at diagnosis					
< 50	81 (70.4)	34 (29.6)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
50 - 59	249 (71.3)	100 (28.7)	0.97 (0.66-1.43, p=0.872)	1.16 (0.78-1.72, p=0.456)	1.10 (0.74-1.63, p=0.647)
60 - 69	525 (70.8)	217 (29.2)	0.97 (0.68-1.40, p=0.887)	1.18 (0.82-1.70, p=0.367)	1.11 (0.77-1.61, p=0.573)
70+	500 (68.4)	231 (31.6)	1.13 (0.79-1.62, p=0.498)	1.42 (0.99-2.05, p=0.056)	1.36 (0.94-1.97, p=0.098)
Gender					
Female	215 (72.9)	80 (27.1)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Male	1140 (69.4)	502 (30.6)	1.12 (0.89-1.42, p=0.329)	1.10 (0.87-1.39, p=0.431)	1.12 (0.88-1.43, p=0.358)
TNM					
I	259 (83.5)	51 (16.5)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
II	342 (77.6)	99 (22.4)	1.58 (1.13-2.22, p=0.008)	1.54 (1.10-2.16, p=0.013)	1.48 (1.05-2.08, p=0.024)
III	528 (59.2)	364 (40.8)	3.63 (2.71-4.88, p<0.001)	3.41 (2.53-4.59, p<0.001)	3.31 (2.45-4.47, p<0.001)
IV	31 (60.8)	20 (39.2)	5.40 (3.21-9.07, p<0.001)	4.89 (2.90-8.25, p<0.001)	4.88 (2.87-8.31, p<0.001)
Missing	195 (80.2)	48 (19.8)	1.33 (0.90-1.97, p=0.156)	1.27 (0.85-1.89, p=0.239)	1.23 (0.83-1.83, p=0.306)
Siewert Type					
Type I	125 (45.3)	151 (54.7)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Type II	189 (50.4)	186 (49.6)	0.98 (0.79-1.21, p=0.824)	0.96 (0.77-1.20, p=0.723)	0.93 (0.74-1.16, p=0.498)
Type III	56 (47.1)	63 (52.9)	0.96 (0.71-1.28, p=0.763)	0.84 (0.63-1.13, p=0.261)	0.87 (0.65-1.18, p=0.372)
Missing	985 (84.4)	182 (15.6)	0.38 (0.31-0.48, p<0.001)	0.40 (0.32-0.50, p<0.001)	0.41 (0.33-0.52, p<0.001)
BMI group at baseline					
Normal	326 (66.4)	165 (33.6)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Overweight	470 (70.8)	194 (29.2)	0.81 (0.66-1.00, p=0.050)	0.88 (0.71-1.09, p=0.234)	0.87 (0.71-1.07, p=0.199)
Obese	312 (76.1)	98 (23.9)	0.63 (0.49-0.80, p<0.001)	0.75 (0.58-0.97, p=0.027)	0.74 (0.58-0.96, p=0.023)
Missing	247 (66.4)	125 (33.6)	0.99 (0.78-1.25, p=0.900)	0.97 (0.76-1.23, p=0.776)	0.76 (0.57-1.00, p=0.050)
Cigarette smoking status					
Never	474 (69.3)	210 (30.7)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Ever	761 (70)	326 (30)	1.02 (0.86-1.21, p=0.825)	1.06 (0.89-1.27, p=0.512)	1.09 (0.91-1.31, p=0.356)
Missing	120 (72.3)	46 (27.7)	1.72 (1.25-2.37, p=0.001)	1.78 (1.28-2.46, p=0.001)	1.65 (1.13-2.43, p=0.010)
Aspirin/NSAID use					
Never	302 (77)	90 (23)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Ever	356 (69.9)	153 (30.1)	1.25 (0.97-1.63, p=0.089)	1.10 (0.84-1.43, p=0.484)	1.10 (0.84-1.43, p=0.498)
Missing	697 (67.3)	339 (32.7)	1.68 (1.33-2.12, p<0.001)	1.34 (1.06-1.70, p=0.015)	1.26 (0.97-1.62, p=0.078)
Heartburn symptoms status					
Absent	216 (71.5)	86 (28.5)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Present	862 (71.7)	341 (28.3)	0.96 (0.75-1.21, p=0.705)	1.18 (0.93-1.50, p=0.171)	1.21 (0.95-1.54, p=0.128)
Missing	277 (64.1)	155 (35.9)	1.48 (1.13-1.92, p=0.004)	1.51 (1.16-1.97, p=0.002)	1.35 (1.00-1.83, p=0.052)

[a]adjsuted for age group at diagnosis, gender, TNM and Siewert Classification.
[b]adjusted for same covariates as [a] and further adjusted for BMI, smoking status, aspirin/NSAID use and derived heartburn symptoms.

Lastly, we also performed a sensitivity analysis which excluded cases that were followed up for less than one year (n=1,046). This excluded 378 of 1,223 (31.0%) BO+ve OAC, 265 of 877 (30.2%) BO-ve OAC and 403 of 976 (41.3%) BO(?) OAC tumours. After excluding these cases, the obtained median overall survival estimates remained very similar to those obtained when all cases were included. For example, the median overall survival among all BO+ve OAC cases was 6.23 years (95% CI:5.21-6.96) and this was slightly increased to 6.58 (95% CI: 5.87-8.21) after removing cases with less than one year of follow-up. The results of the sensitivity analysis are provided in Table 4.32.

Table 4.32 – Frequency distribution and median overall survival among all OAC cases and by phenotype before and after excluding cases (n=1,046) with less than one year of follow-up.

Statistic	BO+ve OAC	BO-ve OAC	BO(?) OAC	Overall
All cases, n (%)	1,223 (100.0)	877 (100.0)	976 (100.0)	3,076 (100.0)
Excluded cases, n (%)	378 (31.0)	265 (30.2)	403 (41.3)	1,046 (34.0)
OS among all cases, median (95% CI)	6.23 (5.21-6.96)	4.19 (3.54-5.12)	5.87 (5.04-6.62)	5.52 (5.02-6.13)
OS after case exclusion, median (95% CI)	6.58 (5.87-8.21)	5.08 (4.06-6.37)	6.27 (5.87-NE)	6.19 (5.73-6.88)

Abbreviations: OS, overall survival; NE, not estimable.

As shown in Figure 4.6, the favourable prognosis persisted for BO+ve OAC versus BO-ve OAC (log-rank p=0.00084). While no significant difference in prognosis was observed in comparing BO+ve OAC to BO(?) OAC tumours (log-rank p=0.85), there was a statistically significant difference between BO-ve OAC and BO(?) OAC tumours (log-rank p=0.0057).

The results of the survival analysis using the unadjusted and adjusted Cox PH models for BO+ve OAC and BO-ve OAC are shown in Table 4.33. These results essentially mirrored those observed in the analysis that included all cases, indicating that the results were not significantly influenced by cases with a short duration of follow-up.

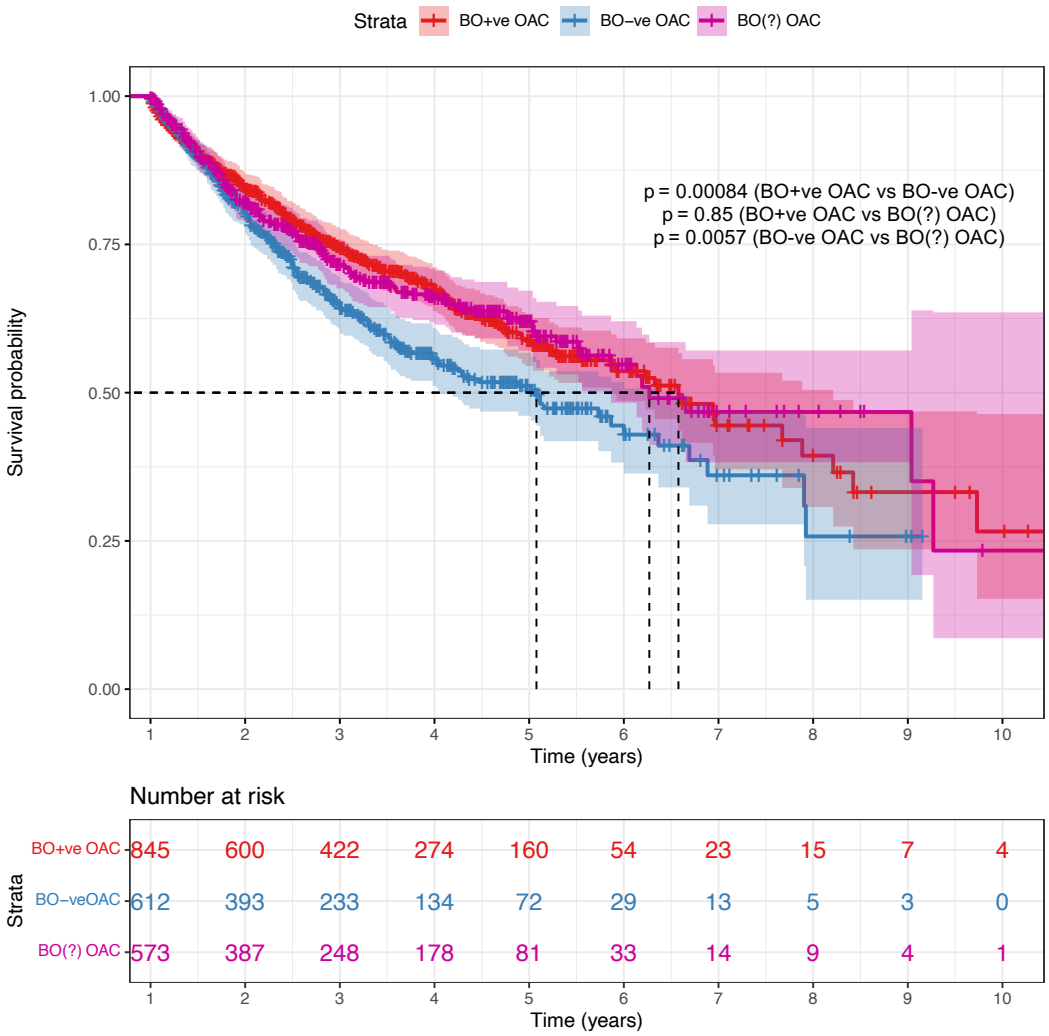

Figure 4.6 – Kaplan-Meier survival curves showing survival probability for all three tumour phenotypes in a sensitivity analysis excluding cases with less than one year of follow-up (n=1,046). Vertical ticks on the curve indicate an event (death) and shaded areas indicate the 95% confidence interval. Median survival time is marked with the dashed lines. The life table at the bottom shows the number of patients at risk at the beginning of each follow-up year.

Table 4.33 – Association of OAC phenotype and baseline characteristics with overall survival in sensitivity analysis excluding cases (n=1,046) with less than one year of follow-up time.

Variable	Alive n (%)	Died n (%)	Univariable model HR (95% CI, p)	Minimally adjusted model[a] HR (95% CI, p)	Fully adjusted model[b] HR (95% CI, p)
OAC phenotype					
BO-ve OAC	389 (63.6)	223 (36.4)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
BO+ve OAC	573 (67.8)	272 (32.2)	0.74 (0.62-0.88, p=0.001)	0.83 (0.74-0.92, p=0.001)	0.86 (0.76-0.93, p=0.012)
Age group at diagnosis					
< 50	55 (63.2)	32 (36.8)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
50 - 59	190 (67.9)	90 (32.1)	0.95 (0.63-1.42, p=0.802)	1.17 (0.78-1.76, p=0.443)	1.09 (0.73-1.65, p=0.668)
60 - 69	370 (66.5)	186 (33.5)	0.90 (0.62-1.30, p=0.566)	1.10 (0.75-1.60, p=0.633)	1.03 (0.70-1.51, p=0.882)
70+	347 (65.0)	187 (35.0)	1.01 (0.69-1.47, p=0.954)	1.28 (0.87-1.86, p=0.206)	1.23 (0.84-1.81, p=0.286)
Gender					
Female	143 (65.9)	74 (34.1)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Male	819 (66.0)	421 (34.0)	1.00 (0.78-1.29, p=0.973)	1.01 (0.79-1.30, p=0.913)	1.01 (0.78-1.30, p=0.938)
TNM					
I	215 (80.5)	52 (19.5)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
II	255 (73.5)	92 (26.5)	1.70 (1.21-2.39, p=0.002)	1.64 (1.16-2.30, p=0.005)	1.60 (1.13-2.25, p=0.008)
III	334 (53)	296 (47)	3.86 (2.87-5.20, p<0.001)	3.58 (2.65-4.84, p<0.001)	3.52 (2.59-4.77, p<0.001)
IV	13 (48.1)	14 (51.9)	5.62 (3.11-10.16, p<0.001)	5.03 (2.77-9.12, p<0.001)	5.57 (3.03-10.25, p<0.001)
Missing	145 (78.0)	41 (22.0)	1.24 (0.82-1.87, p=0.305)	1.19 (0.79-1.80, p=0.397)	1.17 (0.77-1.77, p=0.455)
Siewert Type					
Type I	108 (44.1)	137 (55.9)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Type II	147 (48.7)	155 (51.3)	0.99 (0.79-1.25, p=0.927)	0.93 (0.73-1.17, p=0.515)	0.88 (0.69-1.11, p=0.275)
Type III	47 (48.5)	50 (51.5)	0.96 (0.70-1.33, p=0.819)	0.80 (0.57-1.10, p=0.172)	0.84 (0.61-1.17, p=0.314)
Missing	660 (81.2)	153 (18.8)	0.38 (0.30-0.48, p<0.001)	0.39 (0.31-0.49, p<0.001)	0.39 (0.31-0.49, p<0.001)
BMI group at baseline					
Normal	223 (61.9)	137 (38.1)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Overweight	353 (68.3)	164 (31.7)	0.77 (0.62-0.97, p=0.026)	0.87 (0.69-1.09, p=0.226)	0.86 (0.68-1.08, p=0.194)
Obese	243 (73.4)	88 (26.6)	0.63 (0.48-0.82, p=0.001)	0.77 (0.58-1.01, p=0.056)	0.77 (0.58-1.01, p=0.058)
Missing	143 (57.4)	106 (42.6)	1.00 (0.77-1.29, p=0.992)	0.96 (0.74-1.24, p=0.734)	0.74 (0.55-1.00, p=0.051)
Cigarette smoking status					
Never	355 (67.9)	168 (32.1)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Ever	565 (66.2)	289 (33.8)	1.10 (0.91-1.34, p=0.307)	1.18 (0.97-1.43, p=0.106)	1.23 (1.00-1.50, p=0.049)
Missing	42 (52.5)	38 (47.5)	2.17 (1.52-3.09, p<0.001)	2.23 (1.56-3.19, p<0.001)	2.05 (1.34-3.13, p=0.001)
Aspirin/NSAID use					
Never	215 (71.7)	85 (28.3)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Ever	296 (70.1)	126 (29.9)	1.03 (0.79-1.36, p=0.812)	0.91 (0.69-1.21, p=0.520)	0.90 (0.68-1.19, p=0.445)
Missing	451 (61.4)	284 (38.6)	1.45 (1.14-1.85, p=0.003)	1.14 (0.89-1.47, p=0.295)	1.05 (0.80-1.37, p=0.744)
Heartburn symptoms status					
Absent	139 (66.8)	69 (33.2)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Present	678 (69.4)	299 (30.6)	0.92 (0.71-1.20, p=0.559)	1.22 (0.93-1.59, p=0.151)	1.25 (0.96-1.64, p=0.099)
Missing	145 (53.3)	127 (46.7)	1.55 (1.16-2.08, p=0.003)	1.61 (1.20-2.17, p=0.002)	1.51 (1.08-2.12, p=0.017)

[a]adjsuted for age group at diagnosis, gender, TNM and Siewert Classification.
[b]adjusted for same covariates as [a] and further adjusted for BMI, smoking status, aspirin/NSAID use and derived heartburn symptoms.

4.4.4 Survival among oesophageal adenocarcinoma cases undergoing surveillance

To further evaluate the association of OAC phenotypes with survival, we examined the group of cases with a history of participating in a BO surveillance programme. The aim of this analysis was to examine if the association of OAC phenotypes with survival would persist, even among a group of OAC cases with a history of BO. Table 4.34 presents the survival statistics of these OAC cases.

Table 4.34 – Survival statistics of OAC cases with a history of undergoing BO surveillance, overall and according to tumour phenotype.

Metric	BO+ve OAC	BO-ve OAC	BO(?) OAC	Overall
History of BO surveillance; n (row %)	145 (67.8)	21 (9.8)	48 (22.4)	214 (100.0)
Follow-up rate[†]; n (col. %)	130 (92.4)	20 (95.2)	42 (87.5)	194 (91.0)
Right censored; n (col. %)	12 (8.3)	1 (4.8)	6 (12.5)	19 (8.8)
Excluded[*]; n (col. %)	3 (2.1)	0 (0)	0 (0)	3 (1.4)
Deaths; n (col. %)	26 (17.9)	10 (50.0)	10 (20.8)	46 (21.5)
Person-years	418.7	54.8	119.0	592.5
Crude mortality rate[#]	6.3	18.2	8.4	7.8
Years of follow-up, median (min-max)	2.8 (0.1-9.7)	1.8 (0.3-9.0)	1.8 (0.2-8.5)	2.5 (0.1-9.7)

[†]Cases with complete vital status and follow-up duration.
*Cases with missing vital status and follow-up duration.
[#]Per 100 person-years.

Overall survival was prolonged among cases undergoing BO surveillance and the median overall survival was not reached within the follow-up period (75^{th} percentile survival=3.31 years, 95% CI: 2.15-5.87; Figure 4.7).

Next, we examined the overall survival among OAC cases with a reported BO surveillance history against all other cases in the cohort. The overall survival was significantly longer among cases undergoing BO surveillance compared to all other cases (Figure 4.8). Additionally, the prognostic benefit for BO+ve OAC tumours was also observed among the cases with a history of undergoing BO surveillance, although the sample size was small (Figure 4.9).

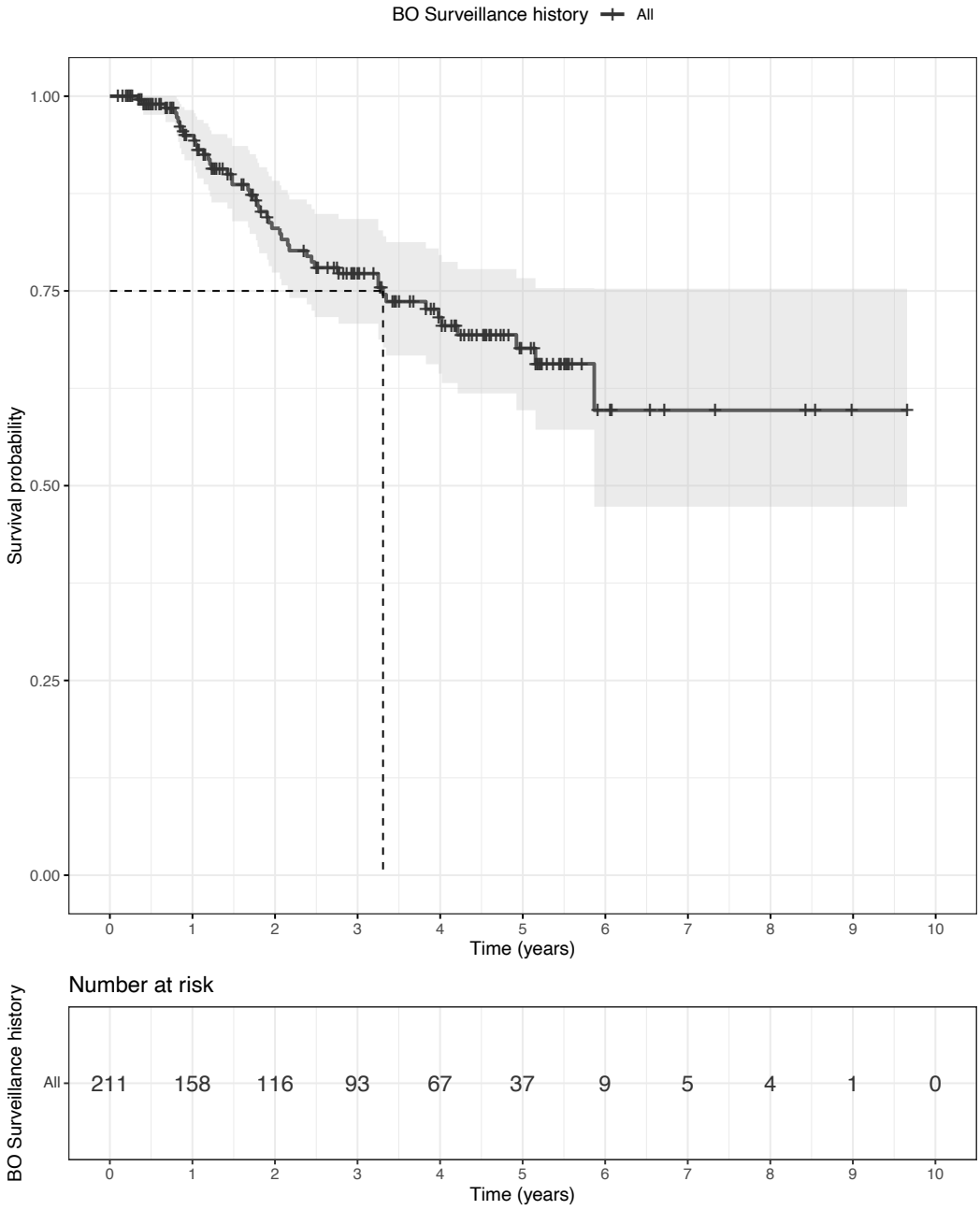

Figure 4.7 – Kaplan-Meier survival curves showing survival probability for OAC cases with a history of undergoing BO surveillance (n=211). Vertical ticks on the curve indicate an event (death) and shaded areas indicate the 95% confidence interval. 75th percentile survival time is marked with the dashed lines. The life table at the bottom shows the number of patients at risk at the beginning of each follow-up year.

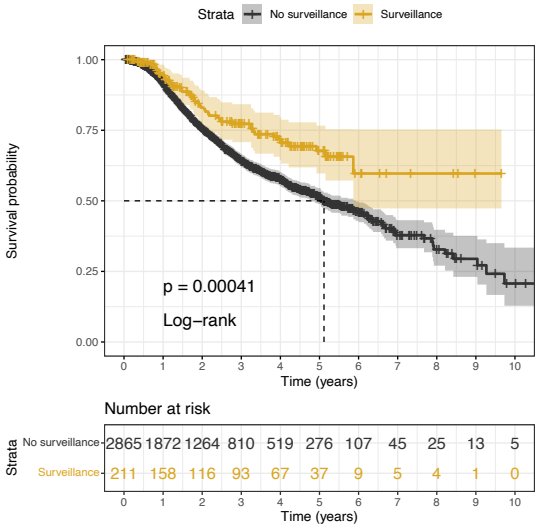

Figure 4.8 – Kaplan-Meier survival curve showing survival probability among all OAC cases and cases with a history of undergoing BO surveillance (n=211). Vertical ticks on the curve indicate an event (death) and shaded areas indicate the 95% confidence interval. Median survival time is marked with the dashed lines. The life table at the bottom shows the number of patients at risk at the beginning of each follow-up year.

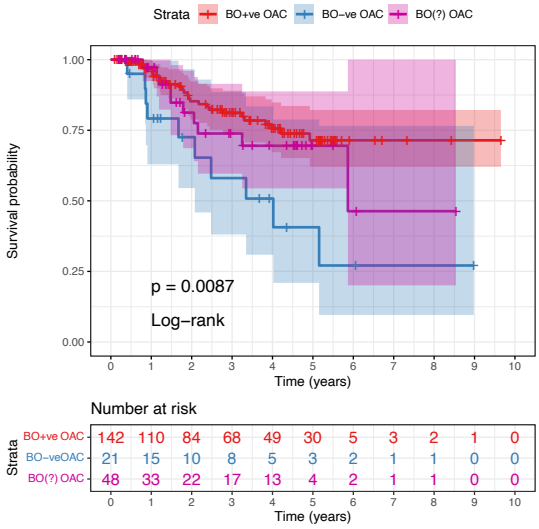

Figure 4.9 – Kaplan-Meier survival curve showing survival probability according to OAC phenotype among all cases with a history of undergoing BO surveillance (n=211). Vertical ticks on the curve indicate an event (death) and shaded areas indicate the 95% confidence interval. The life table at the bottom shows the number of patients at risk at the beginning of each follow-up year.

4.4.5 Summary

The results of the survival analyses largely show a persistent association between BO+ve OAC and improved survival. In the overall analysis, adjusting for baseline factors in addition to the adjustment set used in the initial analysis by Sawas *et al.* (2018) did not appreciably change the results. However, excluding cases with a history of undergoing BO surveillance attenuated the relation between BO+ve OAC status and improved survival compared to BO-ve OAC cases, indicating that the observed association was partly driven by this group of cases. Notably, among these cases with a history of BO surveillance, BO+ve OAC was associated with better survival compared to BO-ve OAC phenotype. It is likely that the effect of OAC phenotype on survival is independent of other factors.

4.5 Genomic features of prognostic phenotypes

Attribution

Dr Sarah Killcoyne performed copy number calls using GISTIC2.0 and generated WGD, ploidy and purity estimates using the PCAWG-11 pipeline. Mutational signatures were extracted by Dr Maria Secrier and Dr Sujath Abbas. Dr Alvin Ng provided the data for amplicon events. All this data was generated using the whole-genome sequencing cases included in this thesis. I processed all the data for mutation load and cohort characteristics and conducted all analyses.

4.5.1 Whole-genome cohort characteristics

Among all OAC cases with WGS data (n=950), the distribution of baseline characteristics was generally similar to the overall study cohort of 3,100 OAC cases, although the amount of missing data was generally lower in the WGS cohort. Table 4.35 provides a description of the clinico-demographic characteristics of the WGS cohort. This WGS cohort was used in the mutation load analysis described below and a subset comprising 710 cases was used for analyses beyond mutation load. The baseline characteristics and OAC phenotype distribution of this subset were similar larger WGS cohort (Table A.13). One tumour sample was included for each patient.

In the cohort of 950 cases with WGS, the OAC phenotype was ascertained for 571 tumours with 332 (58%) of cases classified as BO+ve OAC and 239 (42%) classified as BO-ve OAC. The OAC phenotype was unascertainable for the remaining 379 (40% of n=950) which were classified as BO (?) OAC case. Although were small differences in baseline characteristics such as in gender and cigarette smoking between the OAC phenotypes in the overall cohort, these differences were not seen ($p>0.05$) except for TNM. The BO+ve OAC group contained more stage I/II tumours, while the BO-ve OAC group had more stage III/IV tumours ($p=8.9\times10^{-6}$, chi-squared test).

Table 4.35 – Baseline characteristics of the OAC cases with WGS (n=950), overall and according to tumour phenotype.

Characteristic	BO+ve OAC (n=332)	BO-ve OAC (n=239)	BO(?) OAC (n=379)	Overall (n=950)
Age at diagnosis; *years*				
Mean [SD]	67.2 [9.3]	65.3 [9.6]	67.0 [10.4]	66.6 [9.8]
Median [Q1, Q3]	67.8 [61.9, 73.9]	66.2 [58.5, 72.5]	68.0 [59.3, 75.5]	67.4 [60.1, 73.8]
Age group at diagnosis, n (%)				
< 50 years old	14 (4.2)	13 (5.4)	21 (5.5)	48 (5.1)
50 - 59 years old	54 (16.3)	56 (23.4)	78 (20.6)	188 (19.8)
60 - 69 years old	124 (37.3)	91 (38.1)	117 (30.9)	332 (34.9)
70+ years old	140 (42.2)	79 (33.1)	163 (43.0)	382 (40.2)
Gender, n (%)				
Female	47 (14.2)	38 (15.9)	59 (15.6)	144 (15.2)
Male	285 (85.8)	201 (84.1)	320 (84.4)	806 (84.8)
Ethnicity, n (%)				
White	318 (95.8)	224 (99.6)	363 (98.6)	905 (99.3)
Other	0 (0)	1 (0.4)	5 (1.4)	6 (0.7)
Missing	14 (4.2)	14 (5.9)	11 (2.9)	39 (4.1)
BMI group at diagnosis, n (%)				
Underweight	1 (0.4)	5 (2.6)	12 (4.3)	18 (2.4)
Normal	77 (28.7)	58 (29.7)	88 (31.8)	223 (30.1)
Overweight	97 (36.2)	85 (43.6)	107 (38.6)	289 (39.1)
Obese	93 (34.7)	47 (24.1)	70 (25.3)	210 (28.4)
Missing	64 (19.3)	44 (18.4)	102 (26.9)	210 (22.1)
Cigarette smoking status, n (%)				
Never	106 (34.8)	77 (34.7)	112 (33.0)	295 (34.1)
Ever	199 (65.2)	145 (65.3)	227 (67.0)	571 (65.9)
Missing	27 (8.1)	17 (7.1)	40 (10.6)	84 (8.8)
Aspirin/NSAID use, n (%)				
Never	75 (45.5)	46 (45.1)	59 (42.1)	180 (44.2)
Ever	90 (54.5)	56 (54.9)	81 (57.9)	227 (55.8)
Missing	167 (50.3)	137 (57.3)	239 (63.1)	543 (57.2)
Heartburn symptoms status, n (%)				
Absent	50 (19.0)	39 (21.7)	60 (21.6)	149 (20.7)
Present	213 (81.0)	141 (78.3)	218 (78.4)	572 (79.3)
Missing	69 (20.8)	59 (24.7)	101 (26.6)	229 (24.1)
Total alcohol intake/week; *units*				
Mean [SD]	25.6 [34.8]	22.0 [17.0]	25.5 [42.5]	24.5 [34.1]
Median [Q1, Q3]	16.0 [6.0, 28.0]	19.0 [8.3, 31.0]	16.0 [7.0, 31.0]	18.0 [7.0, 30.0]
Missing	267 (80.4)	181 (75.7)	304 (80.2)	752 (79.2)
TNM, n (%)				
I	77 (26.1)	26 (12.3)	19 (10.8)	122 (17.9)
II	75 (25.4)	48 (22.7)	36 (20.5)	159 (23.3)
III	137 (46.4)	126 (59.7)	116 (65.9)	379 (55.6)
IV	6 (2.0)	11 (5.2)	5 (2.8)	22 (3.2)
Missing	37 (11.1)	28 (11.7)	203 (53.6)	268 (28.2)

4.5.2 Mutation load

Using the cohort of 950 WGS samples, we began by examining the distribution of mutation load of single nucleotide variants (SNV) and small insertions or deletions (IND) across the cohort.

Among all tumours (n=950), the median mutational load was 24,801 (IQR: 16,830-37,851) across the genome and 7.9 mutations per megabase (Mut/Mb, IQR: 5.1-12.0) which is consistent with published studies. The mutation load of each sample according to OAC phenotype is shown in Figure 4.10a. The median mutation load among the 332 cases with BO+ve OAC was 26,425 (IQR: 17,523-36,102), among the 239 with BO-ve OAC was 21,495 (IQR: 13,685-35,913) and among the 379 cases with BO(?) OAC was 25,537 (IQR: 16,501-40,467). The average proportion of mutations per genome attributed to IND was 9.9%, which was comparable across phenotypes with 10.1%, 9.3%, and 10.0% observed in BO+ve, BO-ve, and BO(?) tumours, respectively.

The proportion of malignant tissue in samples undergoing WGS can affect the downstream variant calling process. If the proportion of cancer cells is too low, too few variants may be detectable. In this cohort, there was no correlation between the estimated fraction of tumour cells and total mutations (Figure 4.10b). Cancer cell fractions were estimated using the PCAWG-11 consensus purity pipeline for ICGC/Mutographs samples or Strelka for ESCALATE/GEL samples (Methods).

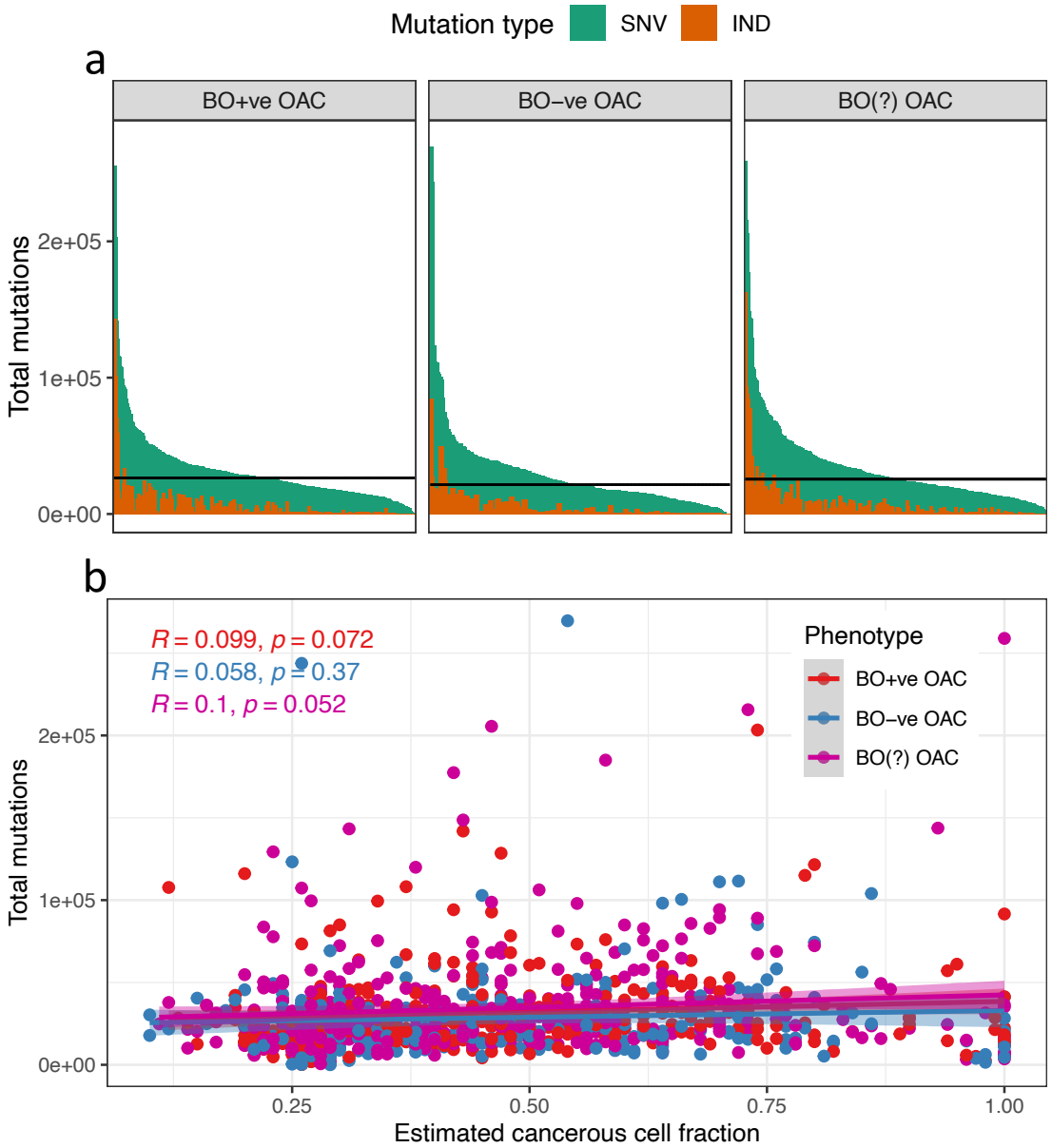

Figure 4.10 – Total mutation burden across the tumour phenotypes. a, waterfallplot showing the frequency of mutations per sample and by SNV/IND type according to OAC phenotype. The horizontal line indicates the median SNV/IND count across the tumour phenotype group.
b, bivariate plot of mutation load and estimated cancer cell fraction of the sample (GISTIC2.0/Strelka). *R*, Pearson's r. Samples: total 950 including 332 BO+ve OAC, 239 BO-ve OAC and 379 BO(?) OAC.

As shown in Figure 4.11, the median Mut/Mb was 8.4 (IQR: 5.6-11.5) for BO+ve tumours which was significantly higher than the median of 6.9 (IQR: 4.4-11.4) among BO-ve tumours.

The median Mut/Mb among BO(?) tumours was 8.1 (IQR: 5.3-12.9) which was significantly higher than the BO-ve tumours, but similar to BO+ve tumours. This may suggest a higher prevalence of BO+ve tumours among the pool of tumours with an unascertainable BO/IM status.

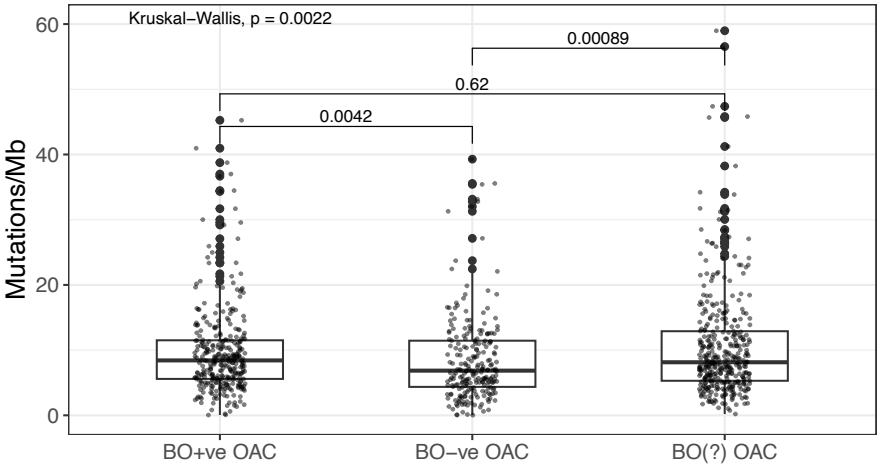

Figure 4.11 – Mutational load compared between OAC phenotypes. P-values calculated using the Mann–Whitney *U* test and adjusted using the BH procedure for multiple testing. Samples: total 950 including 332 BO+ve OAC, 239 BO-ve OAC and 379 BO(?) OAC.

In the analysis of baseline characteristics using the overall cohort (n=3,100) cases, the mean age of BO+ve OAC cases was slightly higher than BO-ve OAC. Furthermore, cigarette smoking was more strongly correlated with the BO-ve OAC than the BO+ve OAC phenotype. Age and smoking status were similar between the phenotypes similar in this WGS cohort (n=950), however, ageing and smoking have been associated with a higher mutational burden in several cancer types. Thus, it might affect the observed difference here. Furthermore, the BO+ve OAC phenotype showed a higher prevalence of early-stage tumours, thereby necessitating a stratified analysis to adjust for potential confounders. It is important to note that a stratified analysis could have affected the statistical power to detect significant differences between the OAC phenotypes in some strata. Therefore, logistic regression was also used to assess the impact of age, smoking and TNM.

To stratify by age. three age groups of '< 60 years old' (25%, n=236), '60-69 years old' (35%, n=332) and '70+ years old' (40%, n=382) were created. Figure 4.12 illustrates the distribution of Mut/Mb among OAC phenotypes across different age groups, revealing statistically significant differences only in the 60-69 years old age group. The median

Mut/Mb of the BO+ve phenotype (8.6, IQR: 5.8-11.2) was significantly higher than that of the BO-ve phenotype (6.2, IQR: 3.8-10.9). The median Mut/Mb of BO(?) OAC (8.7, IQR: 5.9-13.3) was comparable to that of BO+ve tumours but significantly higher than that of BO-ve OAC tumours. Therefore, the association between the OAC phenotypes observed in the overall analysis was partly driven by the 60-69 years old age group.

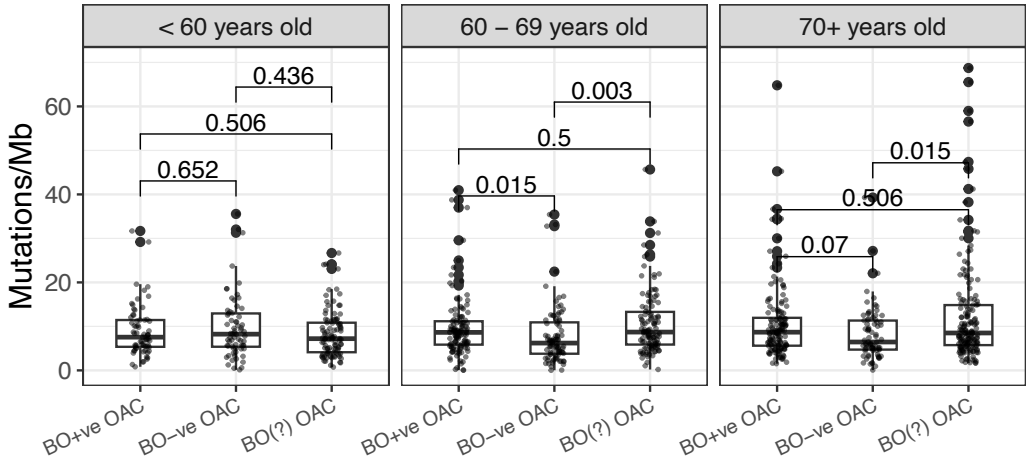

Figure 4.12 – Mutational load of OAC phenotypes according to age strata. P-values calculated using the Mann–Whitney *U* test and adjusted using the BH procedure for multiple testing. Samples: total 950 including 332 BO+ve OAC, 239 BO-ve OAC and 379 BO(?) OAC.

When stratified by smoking status (Figure 4.13), no statistically significant difference was seen in median Mut/Mb between the phenotypes according to smoking status.

Cases were also combined into TNM stage I/II (early) or stage III/IV (late) strata (Figure 4.14). After correcting for multiple hypothesis testing, the only statistically significant difference was observed among stage III/IV cases where the median Mut/Mb for BO(?) OAC tumours (8.6, IQR: 6.0-12.0) was higher than BO-ve OAC tumours (6.1, IQR: 4.3-10.6). In the same stratum, BO+ve OAC appeared to have a higher median Mut/Mb compared to BO-ve OAC tumours but this did not reach statistical significance. It may be that TNM stage is at least partly driving the higher mutational burden seen among BO+ve and BO-ve relative to BO(?) tumours.

Finally, two separate logistic regression models were used to obtain a single adjusted effect estimate between OAC phenotypes and Mut/Mb using age, smoking and TNM as covariates. As the distribution of Mut/Mb tended to be right-skewed, these values were

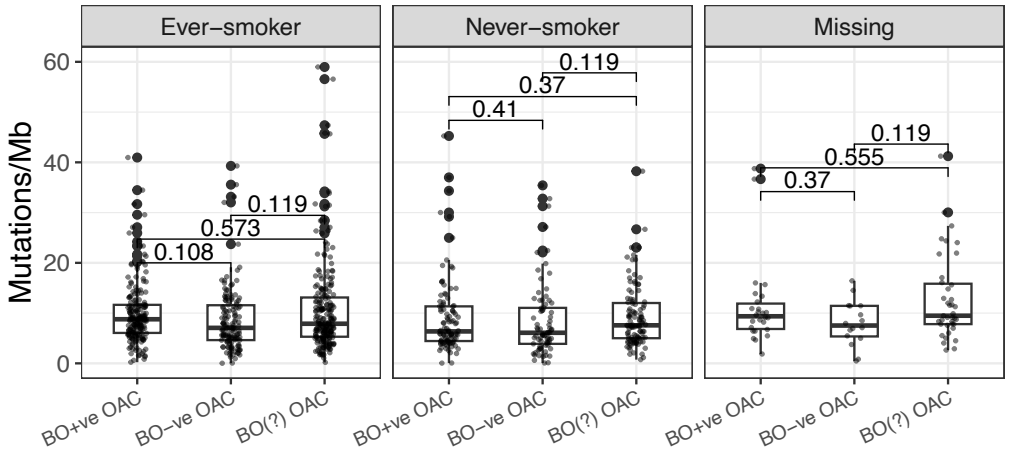

Figure 4.13 – Mutational load of OAC phenotypes according to smoking strata. P-values calculated using the Mann–Whitney *U* test and adjusted using the BH procedure for multiple testing. Samples: total 950 including 332 BO+ve OAC, 239 BO-ve OAC and 379 BO(?) OAC.

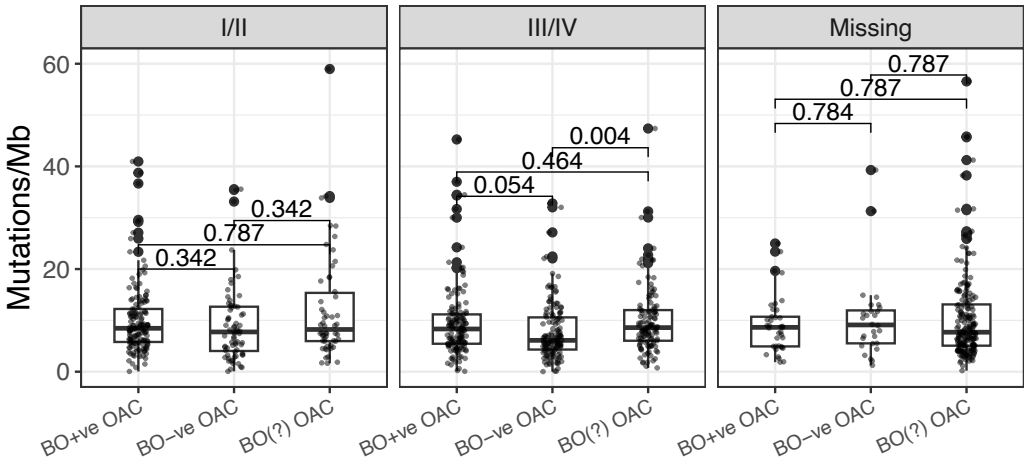

Figure 4.14 – Mutational load of OAC phenotypes according to TNM strata. P-values calculated using the Mann–Whitney *U* test and corrected using the BH procedure for multiple testing. Samples: total 950 including 332 BO+ve OAC, 239 BO-ve OAC and 379 BO(?) OAC.

$\log_{10}$-transformed. The BO-ve OAC phenotype was used as the reference group for BO+ve or BO-ve OAC phenotypes.

Table 4-36 presents the unadjusted and adjusted association of log-transformed Mut/Mb with BO+ve OAC in reference to the BO-ve OAC phenotype. In the unadjusted model, each unit increase in Mut/Mb was associated with a 2.44-fold increased risk of BO+ve OAC

(95% CI: 1.33-4.25, p=0.004). In the adjusted model, this association was attenuated but remained statistically significant (aOR=2.22, 95% CI: 1.19-4.18, p=0.013). Therefore, the higher mutation load among BO+ve OAC compared to BO-ve OAC tumours is likely to be in part related to age, smoking status and TNM. Although there is a large overlap between the confidence intervals of both unadjusted and adjusted models, the observed attenuation could be due to random variability.

Table 4-36 – Association of mutation load and risk of BO+ve OAC compared to BO-ve OAC in unadjusted and adjusted models.

Variable	BO-ve OAC (n=239)	BO+ve OAC (n=332)	Univariable model OR (95% CI, p)	Adjusted model[a] OR (95% CI, p)
Mut/Mb; *log$_{10}$*				
Mean (SD)	0.9 (0.3)	1.0 (0.3)	2.44 (1.33-4.52, p=0.004)	2.22 (1.19-4.18, p=0.013)
Age at diagnosis; *years*				
Mean (SD)	65.3 (9.6)	67.2 (9.3)	1.02 (1.01-1.04, p=0.011)	1.02 (1.00-1.04, p=0.016)
Cigarette smoking status, n (%)				
Never	77 (32.2)	106 (32.0)	1.00 (referent)	1.00 (referent)
Ever	145 (61.0)	199 (60.0)	1.05 (0.73-1.52, p=0.782)	0.95 (0.65-1.38, p=0.778)
Missing	17 (7.1)	27 (8.0)	1.21 (0.61-2.46, p=0.580)	1.17 (0.58-2.42, p=0.656)
TNM, n (%)				
I/II	74 (30.1)	152 (45.8)	1.00 (referent)	1.00 (referent)
III/IV	137 (57.3)	143 (43.1)	0.50 (0.35-0.72, p<0.001)	0.52 (0.36-0.75, p=0.001)
Missing	28 (11.6)	37 (11.1)	0.63 (0.36-1.11, p=0.110)	0.65 (0.36-1.15, p=0.134)

[a]adjusted for age at diagnosis, cigarette smoking status and TNM.

The adjusted and unadjusted association of Mut/Mb with the BO(?) OAC compared to the BO-ve OAC phenotype is shown in Table 4-37. There was a statistically significant association between increasing Mut/Mb and odds of the BO(?) OAC phenotype in the unadjusted model (OR=2.94, 95% CI: 1.65-5.34, p<0.001) which was also attenuated in the adjusted model (aOR=2.70, 95% CI: 1.42-5.23, p=0.003). Taken together with the results of the BO+ve and BO-ve phenotype comparison, it is likely that age, smoking and TNM at least partially explain the higher Mut/Mb among BO+ve and BO(?) phenotypes compared to BO-ve OAC.

Table 4-37 – Association of mutation load and risk of BO(?) OAC compared to BO-ve OAC in unadjusted and adjusted models.

Variable	BO-ve OAC (n=239)	BO(?) OAC (n=379)	Univariable model OR (95% CI, p)	Adjusted model[a] OR (95% CI, p)
Mut/Mb; *log$_{10}$*				
Mean (SD)	0.9 (0.3)	1.0 (0.3)	2.44 (1.33-4.52, p=0.004)	2.22 (1.19-4.18, p=0.013)
Age at diagnosis; *years*				
Mean (SD)	65.3 (9.6)	67.0 (10.4)	1.02 (1.01-1.04, p=0.011)	1.02 (1.00-1.04, p=0.016)
Cigarette smoking status, n (%)				
Never	77 (32.2)	112 (29.6)	1.00 (referent)	1.00 (referent)
Ever	145 (61.0)	227 (60.0)	1.05 (0.73-1.52, p=0.782)	0.95 (0.65-1.38, p=0.778)
Missing	17 (7.1)	40 (10.4)	1.21 (0.61-2.46, p=0.580)	1.17 (0.58-2.42, p=0.656)
TNM, n (%)				
I/II	74 (30.1)	55 (14.5)	1.00 (referent)	1.00 (referent)
III/IV	137 (57.3)	121 (31.9)	0.50 (0.35-0.72, p<0.001)	0.52 (0.36-0.75, p=0.001)
Missing	28 (11.6)	203 (53.6)	0.63 (0.36-1.11, p=0.110)	0.65 (0.36-1.15, p=0.134)

[a]adjusted for age at diagnosis, cigarette smoking status and TNM.

4.5.3 Mutational signatures

Mutational signatures were extracted on a subset of the WGS cohort comprised of 710 tumours with 252 (58%) BO+ve OAC tumours and 183 (42%) BO-ve OAC. BO phenotype was unascertainable for 275 (39% of 710) tumours. We explored mutational signatures to better understand the patterns of mutations in the cohort and identify potential differences in signature prevalence according to OAC phenotype. Compared to the total mutation load, mutational signatures add context to each mutation by considering the flanking base pairs (3' and 5') thus enabling inference of specific endogenous or exogenous mutagenic processes. As previously described, mutational signatures were identified across the cohort using non-negative matrix factorisation (NMF) via the SigProfiler method (Alexandrov et al., 2013). In total, 14 mutational signatures were reconstructed and compared against the database of known mutational signatures (COSMIC) to determine their similarities (Tate et al., 2019).

Ageing-associated signatures (SBS1, 5 and 40) were found in more than 80% of tumours regardless of phenotype. SBS2 (APOBEC-activity) and SBS18 (ROS) were also present in approximately 80% of the cohort. The prevalence of SBS3 (13.5%) and SBS8 (69.3%) which have been associated with DNA damage repair (DDR) impairment was consistent with previous estimates. SBS17a/b which are considered hallmark signatures in BO/OAC were also found in over 80% of cases. It has been shown that the mutational contribution of these signatures is preserved across the BO to OAC continuum (i.e., from NDBO to dysplasia, to OAC) as well as in tumour and extant/adjacent BO samples (Katz-Summercorn et al.,

2022; Ross-Innes et al., 2015). Here, we observe an equal number of OAC cases harbouring SBS17a/b mutations regardless of phenotype ($\geq$90% of cases in each phenotype). SBS28, 30, 35, 41 and 44 are not fully characterised and, except for SBS41, were present in <75% of cases. The aetiology of most of these signatures remains unknown, however, SBS30 has been associated with mismatch repair (MMR) deficiency and SBS35 is related to platinum treatment (Fluorouracil). No statistically significant difference was found in the proportion of cases with at least one mutation attributed to each signature based on the OAC phenotype (all p>0.05 (omitted), Mann–Whitney *U* test with BH adjustment; Figure 4.15).

Figure 4.16 shows the distribution of the fraction of mutations attributed to each signature according to each OAC phenotype. As SBS3, 8, 28, 30, 35, 41 and 44 had low signature proportions and some currently have an unclear aetiology, these were combined into "SBS Other".

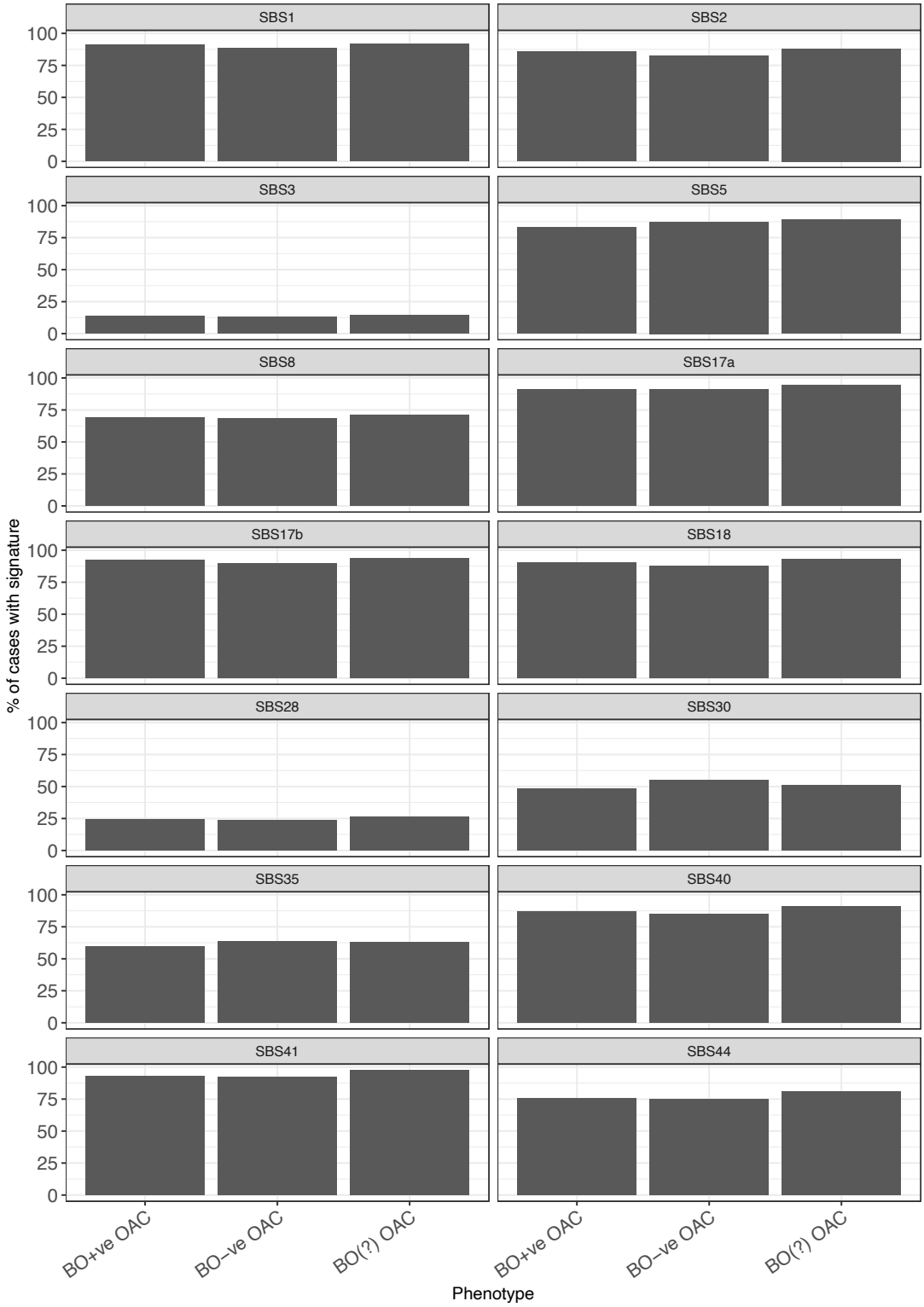

Figure 4.15 – Proportion of OAC cases harbouring mutational signatures according to phenotype.
Samples: total 710 including 252 BO+ve OAC, 183 BO-ve OAC and 275 BO(?) OAC.

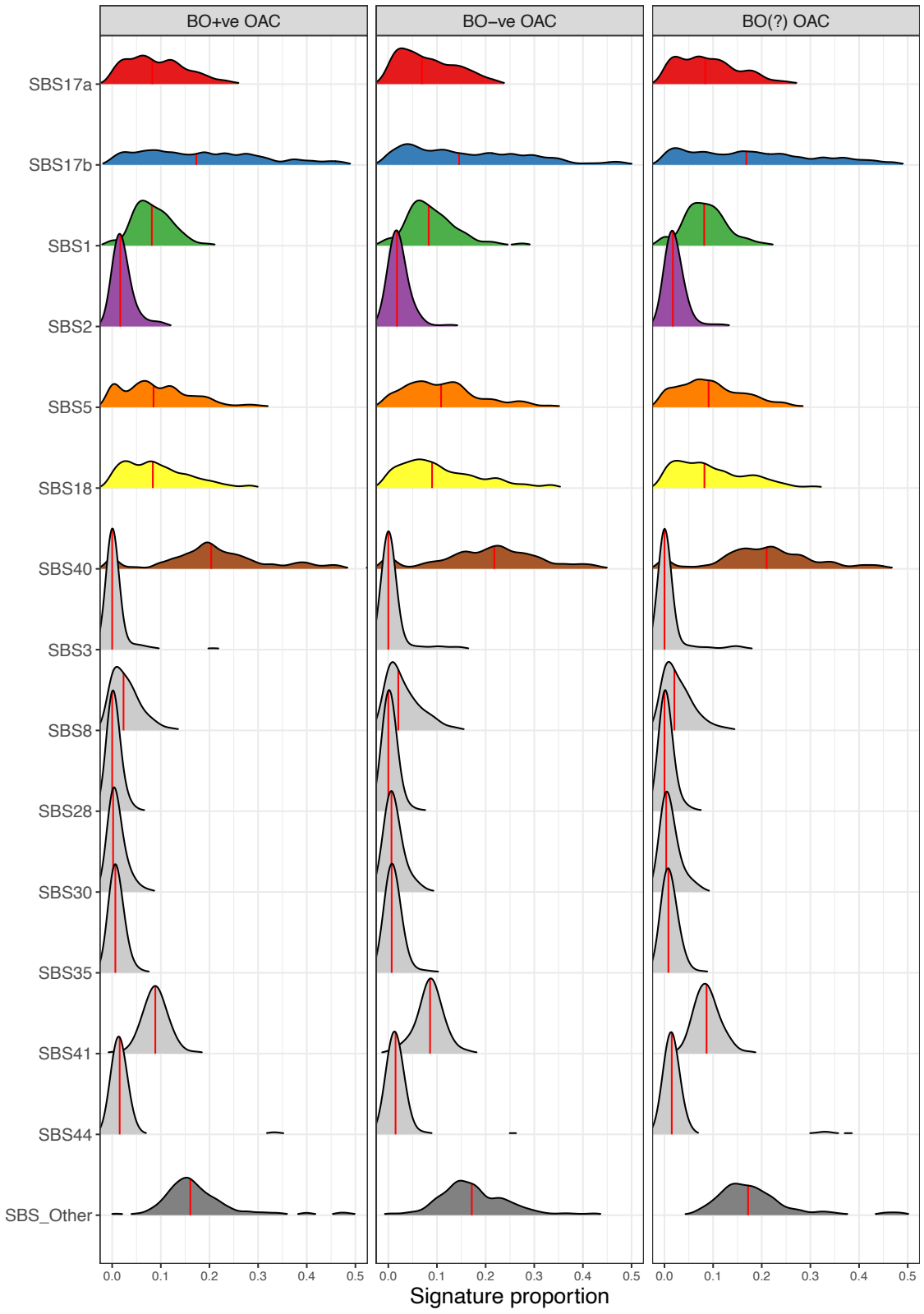

Figure 4.16 – Distribution of signature contributions in each OAC phenotype group. Signatures with light grey plots (SBS3, 8, 28, 30, 35, 41 and 44) were combined to create 'SBS Other' shown in dark grey.
Samples: total 710 including 252 BO+ve OAC, 183 BO-ve OAC and 275 BO(?) OAC.

The number of mutations per case attributed to each signature is illustrated in Figure 4.17a. Little to no difference was observed in the proportion of each signature between the OAC phenotypes (Figure 4.17b). Borderline significant differences were observed for SBS17a and SBS5 after correcting for multiple hypothesis testing (BH procedure). The median proportion of SBS17a mutations was slightly higher at 8.3% (IQR: 4.6-13.2) in BO+ve OAC compared to 6.9% (IQR: 3.1-12.8) in BO-ve OAC tumours, however, this difference was not statistically significant (p=0.068, Mann-Whitney *U* test, BH adjusted). It is known that SBS17a mutations correlate with total mutation load which was seen in these samples (Figure 4.17c). There was a weak yet statistically significant difference in the median proportion of SBS5 mutations in BO+ve OAC tumours (8.5%, IQR: 4.4-15.0), compared to BO-ve OAC tumours (11.0%, IQR: 5.7-16.0; p=0.042, Mann-Whitney *U* test, BH adjusted).

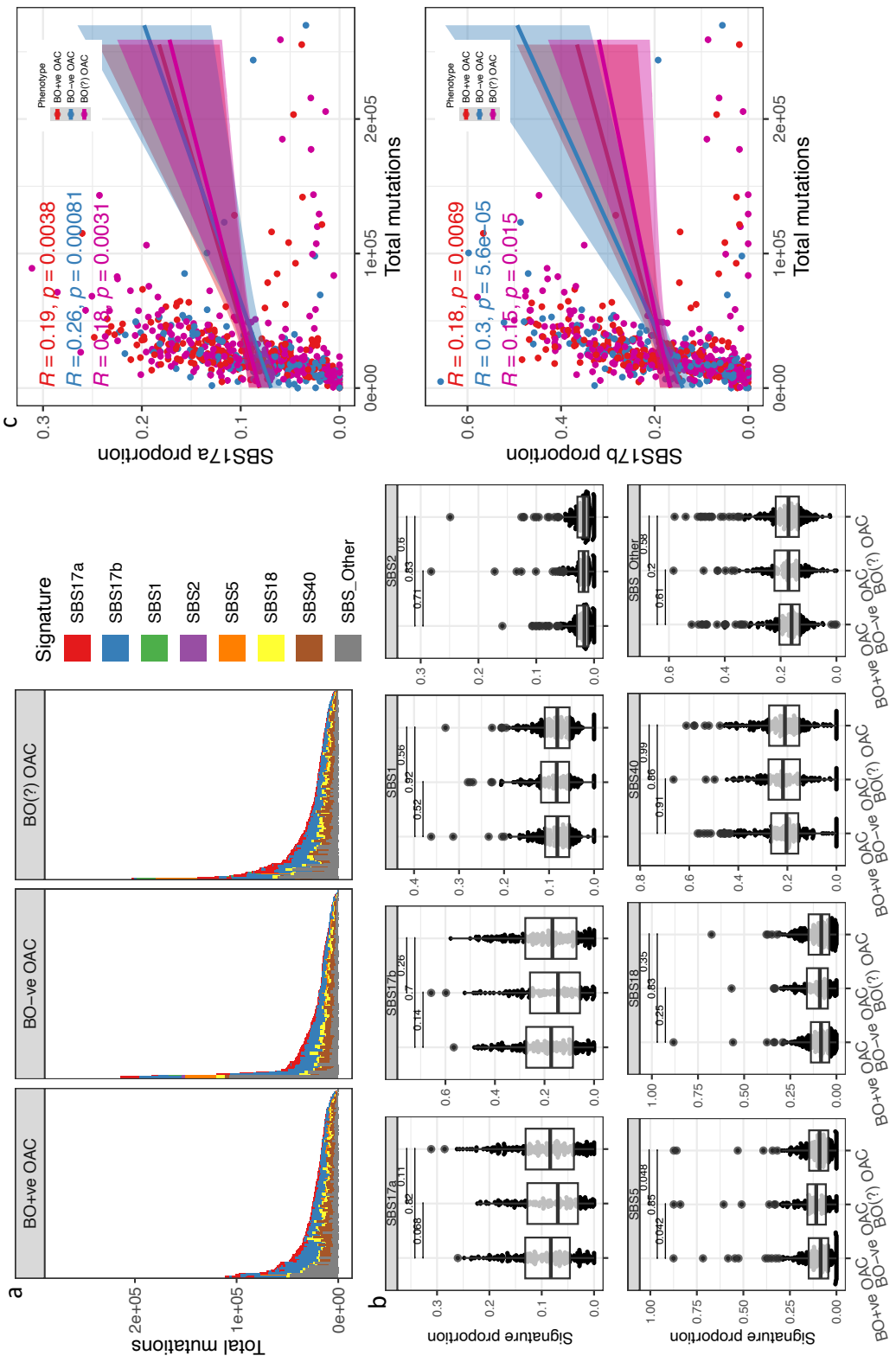

Figure 4.17 – The landscape of mutational signatures across tumour phenotypes. a, waterfallplot showing the total number of mutations per sample with colours corresponding to mutations of each signature. b, boxplots comparing the median contribution of mutational signatures across the three OAC phenotypes. P-values were calculated using the Mann–Whitney *U* test and adjusted using the BH procedure for multiple testing. c, bivariate plots of mutation load and proportion of SBS17a/b. *R*, Pearson's r. Samples: total 710 including 252 BO+ve OAC, 183 BO-ve OAC and 275 BO(?) OAC.

Finally, we analysed baseline characteristics (per Table 4.35) to identify potential exogenous factors associated with changes in signature mutations. This correlational analysis should not be considered to link the aetiology of signatures with environmental exposure.

As the distribution of the total number of mutations for each signature was right-skewed with some samples having zero mutations, one was added to this value and then log10-transformed. Univariable generalised linear models were used to assess the correlation between variables of interest (e.g., cigarette smoking status) and the transformed value for mutation number per each signature. To ease the interpretation of the results, statistically significant coefficients were transformed using $e^{(\text{coefficient})\text{-}1} \times 100$ to derive the percent change (increase or decrease) in the number of mutations per one unit change in the variable of interest. Correction for multiple hypothesis testing using the BH procedure was also applied.

The correlations between the baseline factors and mutational signatures are shown for all OAC cases (Figure 4.18) and separately among OAC phenotypes (Figure 4.19). Compared to never-smokers, cases with a self-reported history of cigarette smoking had a 23.6% higher SBS17a 18.7% higher SBS17b mutations. This was also seen among BO+ve OAC cases with those reporting ever-smoking having 25.4% higher SBS17a and 25.0% higher SBS17b mutations than never-smokers. This association was most evident among BO-ve OAC cases with smoking correlated with 51.2% and 42.9% increase in SBS17a and 17b mutations, respectively, compared to never-smokers.

Self-reported ever-aspirin/NSAID use was correlated with a 22.1% lower SBS5 mutations relative to never-use. Similarly, among BO+ve OAC tumours, aspirin/NSAID use was correlated with a 40.2% lower number of SBS5 mutations. Among BO (?) OAC tumours, aspirin/NSAID use was associated with a 32.8% reduction in SBS40 mutations. Among all cases, TNM stage III/IV was related to 15.1% lower SBS17a mutations. Additionally, TNM stage III/IV tumours had 22.0% lower SBS17a mutations as compared to TNM stage I/II tumours. Similarly, TNM stage III/IV tumours correlated with a 41.7% lower number of SBS5 mutations.

Figure 4.18 – Associations of baseline factors with changes in mutational signatures. Blue shades indicate a decrease and red an increase in the number of mutations contributed to a signature. Only statistically significant changes are illustrated with %. The reference for each variable is indicated in parentheses. Samples: total 710 including 252 BO+ve OAC, 183 BO-ve OAC and 275 BO(?) OAC.

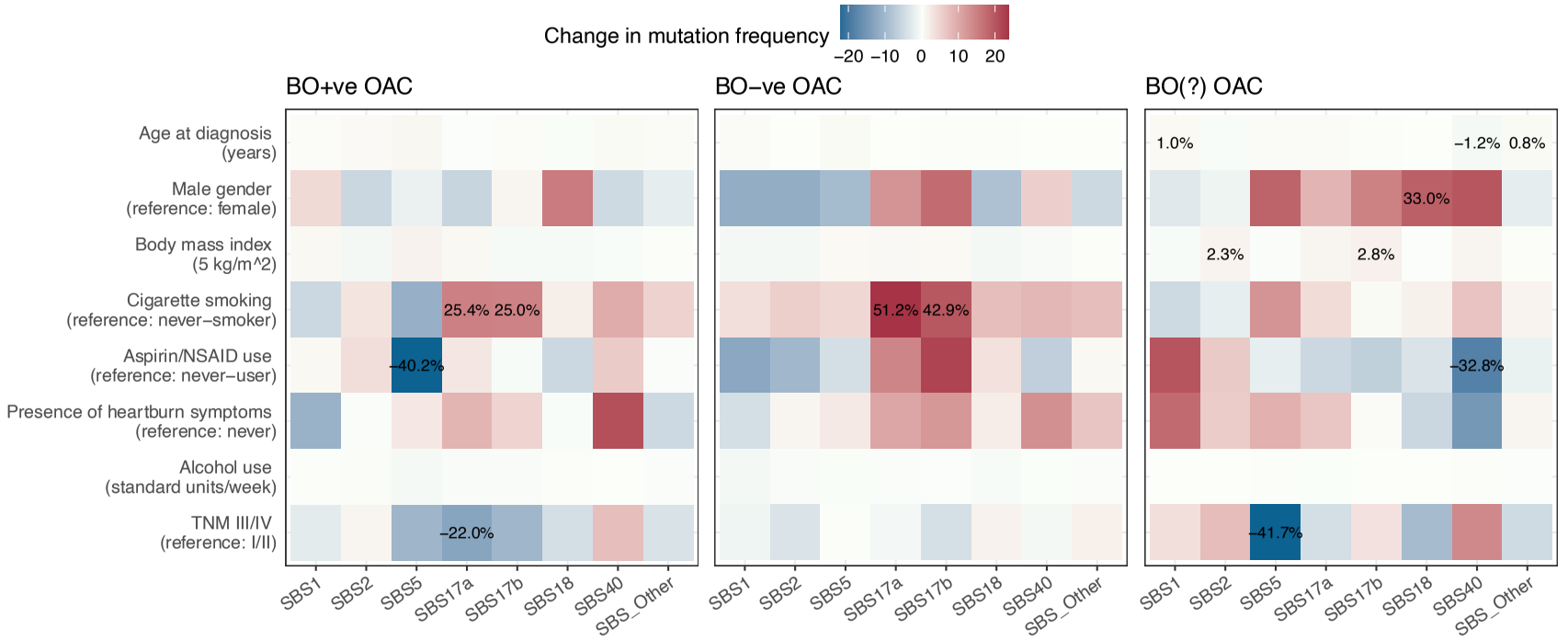

Figure 4.19 – Associations of baseline factors with changes in mutational signatures in each OAC phenotype group. Blue shades indicate a decrease and red an increase in the number of mutations contributed to a signature. Only statistically significant changes are illustrated with %. The reference for each variable is indicated in parentheses. Samples: total 710 including 252 BO+ve OAC, 183 BO-ve OAC and 275 BO(?) OAC

4.5.4 Driver gene alterations

We analysed alterations in specific genes that have been identified as OAC driver genes to investigate their potential association with OAC phenotypes. As driver gene analysis excludes passenger (synonymous) mutations, this provides a more targeted approach to examining the molecular history of each OAC genome, especially given the importance of such genes in the BO-OAC continuum. To date, 77 recurrently altered genes have been found in OAC and these were included in this analysis. This collection of genes is comprised of both oncogenes and tumour suppressor genes. As mentioned, there is significant heterogeneity in driver gene alterations between tumours, therefore we considered both single nucleotide variants (SNVs) copy number alterations (CNAs) together as detailed in Methods. The results described here are restricted to the driver genes which were found in >10% of the WGS subcohort (n=710).

First, we examined driver gene alterations in the cohort (n=710). The median number of driver alterations was 5 (IQR: 3-8). Only 6 cases (<1%) had no identifiable driver mutations. As expected, *TP53* was the most recurrently altered gene (85%) in the cohort followed by *CDKN2A* (22%). Consistent with published data, CNA gains predominated in *GATA6*, and CNA losses were frequent in *CDKN2A*. Figure 4.20 depicts the landscape of altered driver genes among all OAC cases (n=706).

Next, we examined the driver gene alterations in BO+ve OAC, BO-ve OAC and BO (?) OAC phenotypes (Figure 4.21). Overall, the driver mutation landscapes of OAC phenotypes were remarkably similar. The median number of driver alterations was not different between the phenotypes with a median of 5 (IQR: 3-8) for BO+ve OAC, 5 (IQR: 3-7) for BO-ve OAC and 5 (IQR: 3-7) for BO (?) OAC cases (p=0.542, Kruskal-Wallis test). As in the overall cohort, *TP53* and *CDKN2A* alterations were predominant across all three phenotypes. Besides these two genes, seven additional genes – *SMAD4*, *KRAS*, *LRRK2*, *MUC6*, *PCDH17*, *KCNQ3* and *ARID1A* – were identified as recurrently mutated in over 10% of the entire cohort as well as across all three phenotypes. No significant differences were observed in the frequency of cases harbouring mutations in these according to OAC phenotypes (Figure 4.22, all p>0.05 (omitted), Mann-Whitney *U* test with BH adjustment).

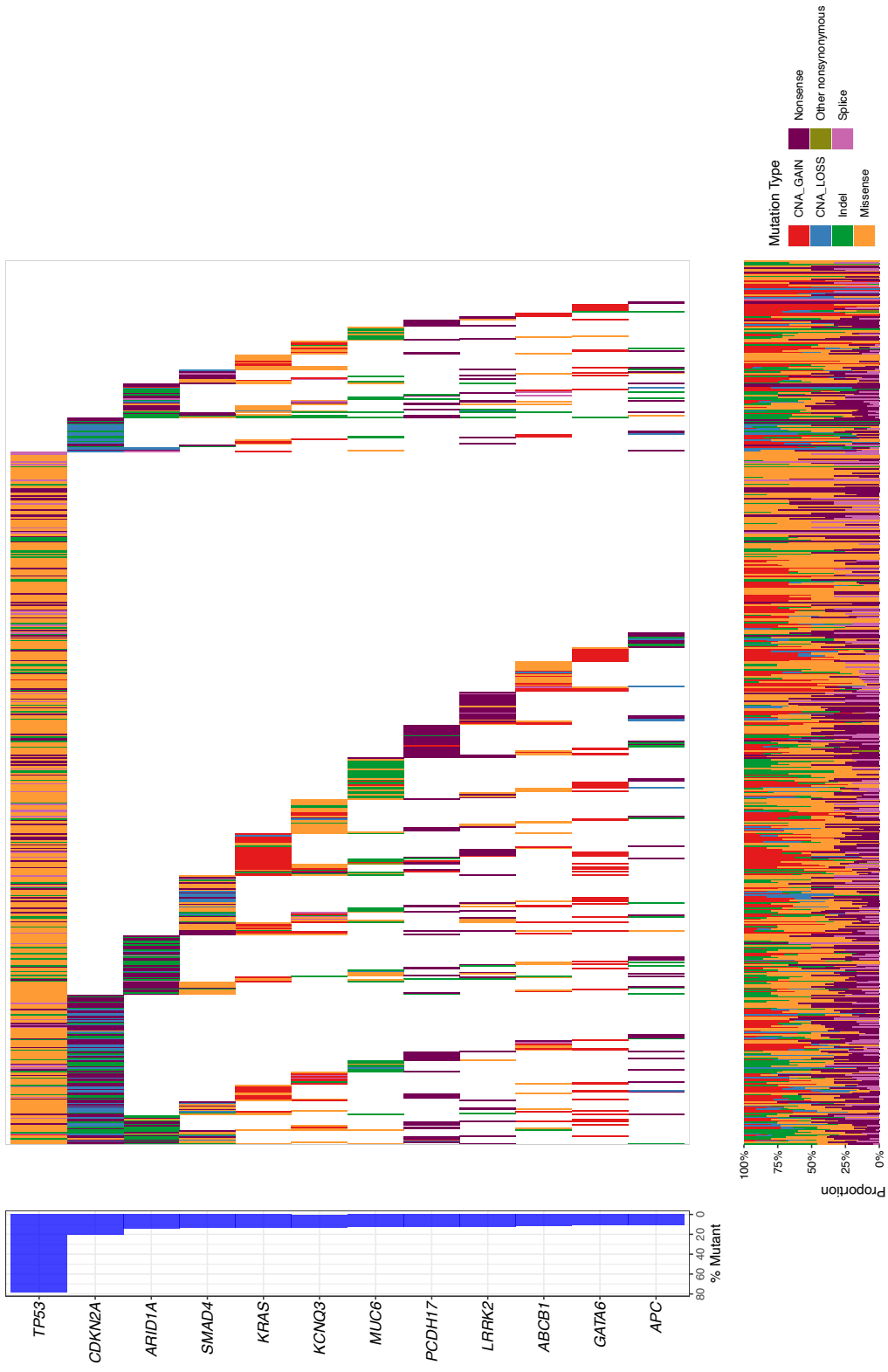

Figure 4.20 – Oncoplot of the driver gene alteration frequency in the cohort. Each column is a sample, and each row is a driver gene in OAC, ordered by frequency and coloured according to the mutation type. The bottom plot shows the proportion of mutation types per sample. The proportion of mutant samples for each gene is plotted on the left. Abbreviations: CNA, copy number alteration. Samples: total 710 including 252 BO+ve OAC, 183 BO-ve OAC and 275 BO(?) OAC.

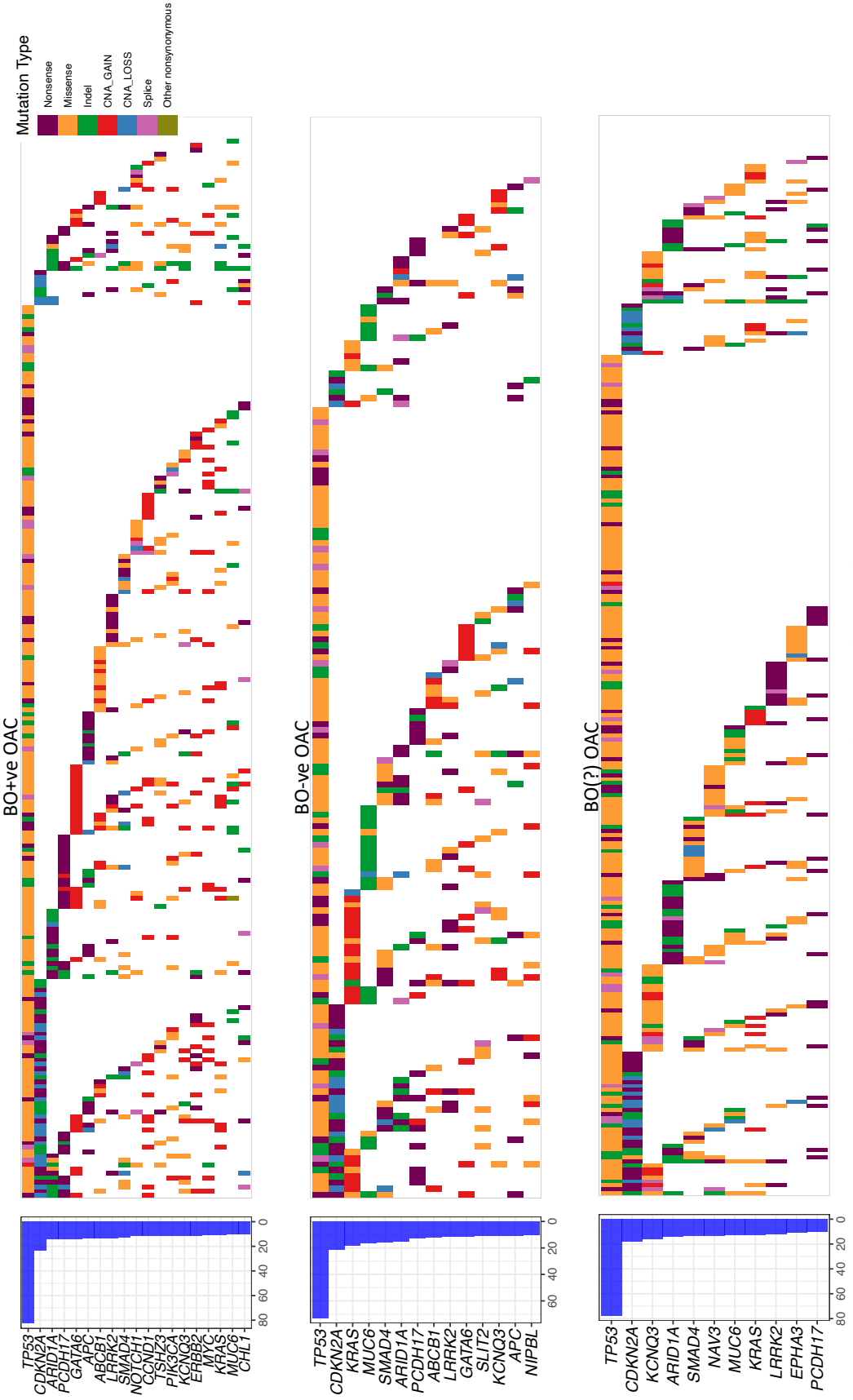

Figure 4.21 – Oncoplot of the driver gene alteration frequency according to OAC phenotype. Each column is a sample, and each row is a driver gene in OAC, ordered by frequency and coloured according to the mutation type. The proportion of mutant samples for each gene is plotted on the left. Abbreviations: CNA, copy number alteration. Samples: total 710 including 252 BO+ve OAC, 183 BO-ve OAC and 275 BO(?) OAC.

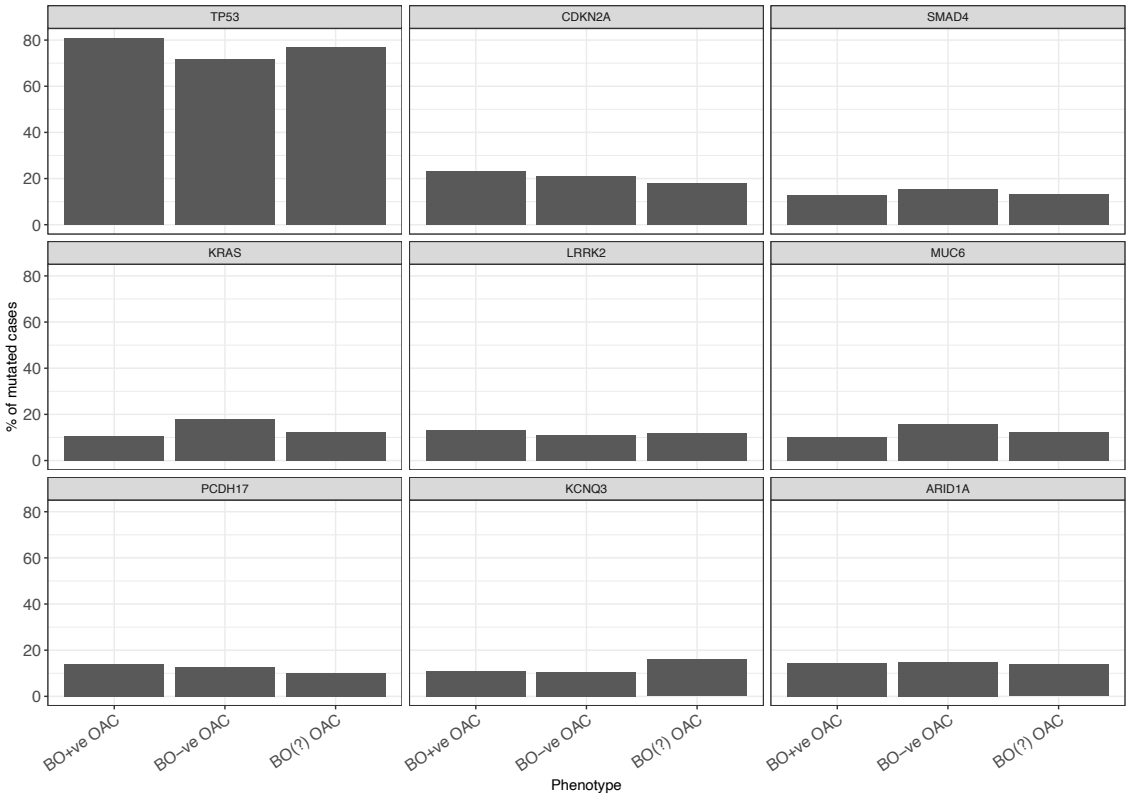

Figure 4.22 – Proportion of OAC cases mutant for the set of genes with greater than 10% recurrence among each phenotype group. The plotted proportions indicate the number of samples with one or more alterations in each driver gene according to OAC phenotype. P-values (omitted) were calculated using the Mann–Whitney *U* test and adjusted using the BH procedure. Samples: total 710 including 252 BO+ve OAC, 183 BO-ve OAC and 275 BO(?) OAC.

4.5.5 Large-scale and catastrophic genomic events

In addition to a high burden of point mutations and copy number alterations, OAC is characterised by whole-genome duplication (WGD), increased aneuploidy and markers of genome catastrophes such as circular extrachromosomal DNA (ecDNA) events. Early mutations of *TP53* and *CDKN2A* IN BO are typically followed by WGD, seen in approximately 65% of tumours, and high aneuploidy. Furthermore, catastrophic alterations such as breakage-fusion-bridges (BFB) cycles and ecDNA are present in about 25-55% of tumours and have been associated with poor prognosis. Based on this evidence, a model of catastrophe-driven OAC development with rapid progression from BO has been proposed. Therefore, examining these events in the context of OAC phenotypes is important for evaluating the questions of whether alternative progression pathways exist and what may explain the difference in prognosis.

Overall, among the 710 tumours included, WGD was seen for 75.0% of tumours and for 73.8%, 77.0% and 72.7% of BO+ve OAC, BO-ve OAC and BO(?) OAC tumours. The overall median ploidy was 2.9 (IQR: 2.2-3.6) and was 2.9 (IQR: 2.1-3.7), 2.9 (IQR: 2.4-3.6) and 3.0 (IQR: 2.1-3.6) among BO+ve, BO-ve and BO(?) OAC tumours. ecDNA events were identified in 44% of tumours (n=317) and BFB events in 39% (n=280) of OAC cases with similar proportions according to OAC phenotype. There was no difference in the distribution of WGD, aneuploidy, ecDNA or BFB events when examined according to OAC phenotype (Figure 4.23a-d, all p>0.05 (omitted), chi-squared test or Kruskal-Wallis test as appropriate).

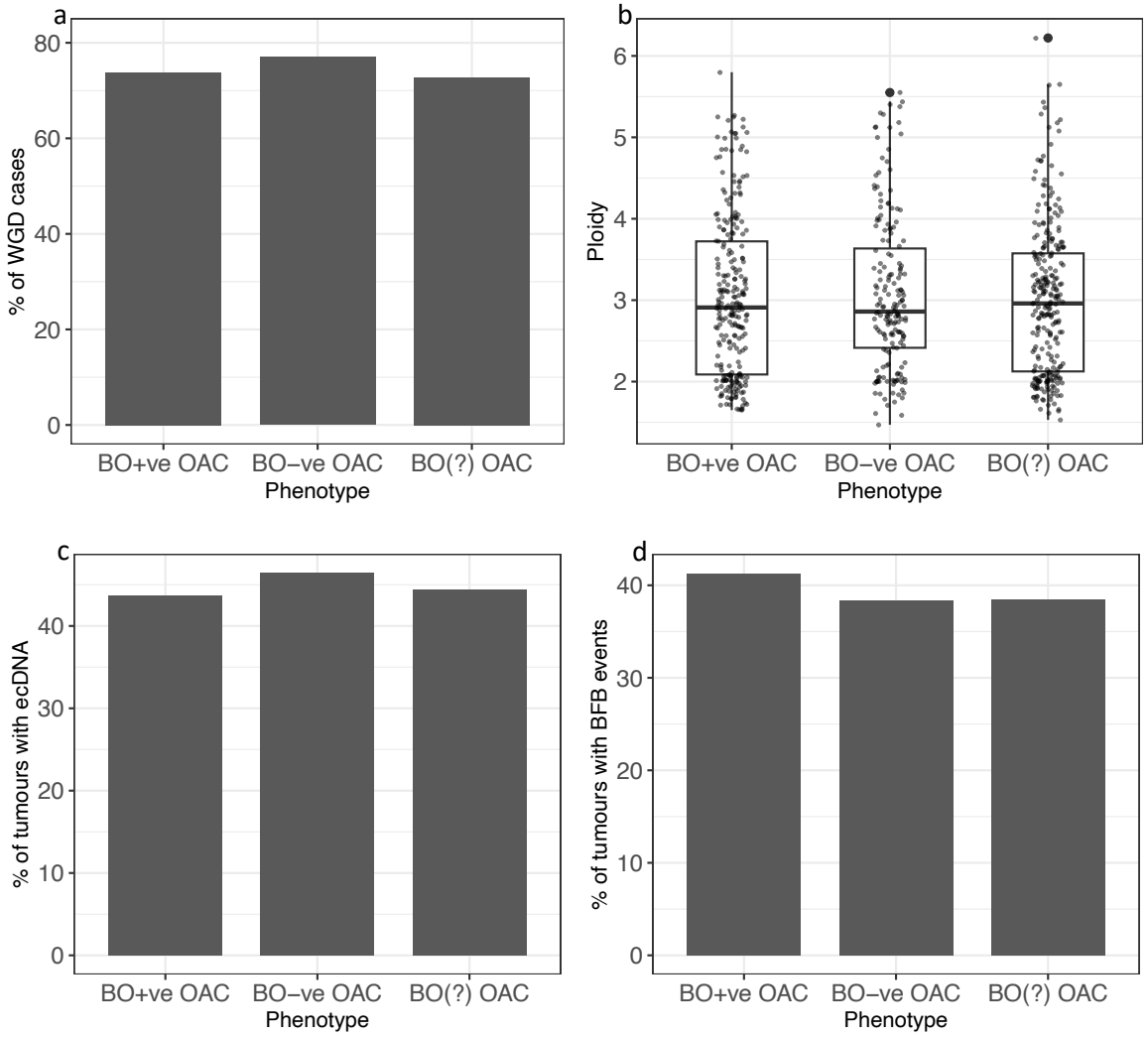

Figure 4.23 – Distribution of large-scale and catastrophic events across OAC phenotypes. a, proportion of samples with whole-genome duplication. b, distribution of ploidy estimated using the PCAWG-11 pipeline. c, proportion of samples harbouring extrachromosomal DNA. d, proportion of samples with Breakage-Fusion-Bridges events. Samples: total 710 including 252 BO+ve OAC, 183 BO-ve OAC and 275 BO(?) OAC.

4.5.6 Summary

In summary, mutation load was slightly higher in BO+ve OAC tumours compared to BO-ve OAC tumours and adjusting for age and cigarette smoking factors only partially explained this variation. Signature SBS17a/b were equally prevalent in both phenotypes and contributed a similar number of mutations. Likewise, for each tumour phenotype, a similar proportion of tumours was mutant for the selected driver genes and *TP53* and *CDKN2A* were the dominant driver genes. Lastly, there were no differences in the frequency or distribution of WGD, aneuploidy, ecDNA or BFB events by tumour phenotype. Overall, the trends of genomic features analysed significantly overlapped between the OAC phenotypes.

Chapter 5

Summary and discussion

It is currently debated whether all oesophageal adenocarcinoma (OAC) arise from Barrett's oesophagus (BO). There has been a lack of consolidated data and investigations to address this question. The work in this thesis triangulated clinical, epidemiological and genomic factors to comprehensively assess the prognostic phenotypes of oesophageal adenocarcinoma. To evaluate the hypothesis of whether all oesophageal adenocarcinoma arise from Barrett's oesophagus or whether there are additional pathogenic pathways, we first assembled a large UK-wide cohort comprised of OAC cases, BO cases and reflux controls.

5.1 Epidemiological characterisation of prognostic phenotypes

We re-established the prognostic difference between OAC cases with BO tissue present adjacent to the tumour (BO+ve OAC) and OAC cases without apparent BO tissue next to the tumour (BO-ve OAC) in the cohort since it was first described in 2018. The size of our cohort had increased substantially since then, giving us more statistical power to examine this difference and evaluate the hypothesis. Next, we sought to elucidate the association of epidemiological factors with the OAC phenotypes. In adjusted analyses, we observed weak associations for male gender, high BMI and chronic heartburn symptoms which were more strongly associated with reduced risk of BO-ve OAC phenotype compared to BO+ve OAC phenotype. Conversely, cigarette smoking was and more strongly associated with increased risk of BO-ve tumours. In addition, aspirin/NSAID use was more strongly associated with BO-ve OAC compared to BO+ve OAC, however, the effect size was small and not observed consistently. Finally, higher TNM stage was consistently and more strongly associated with the BO-ve OAC phenotype than the BO+ve OAC phenotype. These results remained highly

robust in sensitivity analyses and in comparisons of the OAC phenotypes against BO cases or reflux controls.

The described associations should be interpreted in the context of OAC and our study design. The association of BMI likely reflects the significant cancer-associated weight loss and cachexia which tends to be profound in OAC. While BMI was objectively measured at baseline, the finding that a higher BMI at baseline was less likely among BO-ve OAC cases may be attributed to more advanced tumours in this group. The association of aspirin/NSAID use may be confounded if a condition or an indication for use of such medicines is also related to the outcome of BO-ve OAC. It may also be a spurious association. Although we adjusted for several factors, including demographics and tumour stage, residual confounding due to unmeasured factors cannot be ruled out. Furthermore, as aspirin/NSAID was missing at a high rate, this association might be spurious and related to the missing data patterns. The finding that male cases were more likely than female cases to have BO+OAC than BO-ve OAC requires further evaluation, especially as evidence suggests poorer survival for female individuals (Codipilly et al., 2021).

The high degree of overlap of the baseline epidemiological characteristics between the phenotypes as well as with BO cases points to a shared aetiology for these phenotypes, thus a non-BO progression pathway is unlikely. However, the increased risk of BO-ve OAC associated with higher TNM suggests that extant BO tissue may have been overgrown by the tumour at the time of diagnosis. This is in congruence with a recent meta-analysis demonstrating that studies with only early-stage OAC had a 91.3% (95% CI: 82.4%-97.6%) prevalence of concurrent BO compared to the 39.7% (95% CI: 33.7%-45.9%) in studies with less than half early OAC (Tan et al., 2020). However, in the original study of the prognostic phenotypes, and the data presented here, the prevalence of phenotypes was similar within TNM stages.

It is plausible that extant BO was overgrown by the tumour, therefore, limiting macroscopic or microscopic identification of BO at diagnosis or resection. Furthermore, short-segment BO might be even more readily overgrown by tumours, even in early-stage cancer. As we lack longitudinally tracked cases that developed BO-ve OAC, examining this plausibility is impossible. Regardless, any scenario where BO is overgrown still represents a BO pathogenic pathway which is consistent with the current model of OAC pathogenesis, but perhaps with a more aggressive tumour behaviour as has been suggested for BO-ve OAC tumours.

We observed that chronic heartburn, a primary risk factor for BO/OAC, was less frequent among BO-ve OAC cases than BO+ve OAC cases and given a more advanced TNM stage at diagnosis, the BO-ve OAC might involve a compressed natural history with rapid progression

to late-stage disease among individuals without chronic reflux. Moreover, experimental evidence suggests cigarette smoke condensate may promote cellular proliferation, invasion and metastasis in BO and OAC cell lines (Xi et al., 2023). Here, we observed an enrichment of ever-smokers in the BO-ve OAC phenotype group, albeit the difference was small. The theory that the BO-ve OAC phenotype may be more aggressive and rapidly progressive may also be supported by the finding that 9.8% of BO surveillance participants (n=214) were classified as BO-ve OAC at diagnosis or resection pathology. Such cases could be interval cancers that developed between surveillance endoscopies. In breast tumours, interval cancers tend to be larger, faster growing and have worse survival rates (Holm et al., 2015). This observation should be interpreted with caution due to the small number of cases.

5.2 Evaluation of predictors of survival

We also examined if the prognostic difference was due to epidemiological factors. When adjusted for factors such as smoking and BMI, the hazard ratio for the association of OAC phenotype and risk of death did not change significantly. This confirms that while epidemiological factors are differentially associated with the OAC phenotypes, these do not explain the prognostic difference. However, we also found evidence for lead time bias which may have contributed to the difference in survival. Specifically, 214 OAC cases had a history of enrolment in BO surveillance programmes. Of these, a majority were BO+ve OAC (67.8%, n=145), but BO-ve OAC was not completely absent (9.8%, n=21) and BO(?) OAC was frequent (22.4%, n=48). When BO surveillance cases were removed from the survival analysis, the median overall survival among BO+ve OAC tumours decreased by nearly a year (from 6.2 to 5.3 years), but the favourable prognosis persisted in the unadjusted analysis (HR=0.80, 95% CI: 0.68-0.95, p=0.009). Adjusting for age at diagnosis, gender, TNM and Siewert Classification attenuated this association (HR=0.89, 95% CI: 0.79-0.97, p=0.023) and further adjustment for BMI, smoking status, aspirin/NSAID use and derived heartburn symptoms resulted in an association with borderline statistical significance (HR=0.92, 95% CI: 0.82-1.01, p=0.055). Therefore, there was some influence from cases under BO surveillance on the prognostic difference.

The cases with a history of undergoing BO surveillance are a unique group to understand whether all OAC, regardless of phenotype, progress through BO or if there are alternative pathogenic pathways. As described, the BO-ve OAC phenotype was seen for approximately 10% of patients in this group. Furthermore, the survival benefit related to BO+ve OAC persisted in this group, although the confidence intervals were wide due to the small sample size. Nevertheless, this adds evidence to the hypothesis that all OAC arise from BO, regardless

of OAC phenotype at diagnosis or resection. It may also be possible that among the 10% of BO patients with BO-ve OAC, the tumour had overgrown the extant BO. It is important to note that the history of BO surveillance was ascertained using medical record review or self-reported by patients. Therefore, the potential influence of information bias or recall bias should be considered.

5.3 Genomic landscape of prognostic phenotypes

We further examined the genomic landscape of the OAC phenotypes and found a remarkable similarity between them. Among 950 OAC cases with whole-genome sequencing (WGS), the mutational load was higher among BO+ve OAC than BO-ve OAC cases. This difference persisted when adjusted for the effect of ageing and smoking, which are known to increase the mutational load. A possible explanation for the higher mutation load among BO+ve cases could be the higher subclonal diversity in early-stage tumours prior to clonal sweeps in late-stage tumours (internal data). However, adjusting for TNM stage did not change this association. Future investigations are needed to better understand what may be driving this difference.

Subsequently, the relationship between mutational signatures and OAC phenotypes was investigated in 710 WGS cases from the initial 950 cases. This drop in sample size was due to time constraints and data availability. The distribution of all signatures was found to be consistent across phenotypes. Notably, hallmark SBS17a/b mutations are present during early pathogenesis even in non-dysplastic BO and remain proportionally preserved across progression grades to OAC. In our analysis, no significant difference in the proportion of SBS17a/b was observed between phenotypes, implying that the molecular history of both phenotypes involves premalignant BO. We also characterised the correlation of signatures with the epidemiological factors collected in the cohort and found consistent associations between cigarette smoking and increased SBS17a/b mutations. However, this association has not been previously described and smoking is instead related to SBS4 which was not present in among our tumours. Therefore, robust investigations are needed to evaluate this relationship. Increasing BMI was weakly associated with SBS17a/b among all tumours and BO(?) OAC only. It has been shown that a high BMI increases the risk of gastro-oesophageal reflux disease likely due to excess abdominal adipose tissue that can mechanistically promote acid reflux. Given the speculation that SBS17 mutations are related to acid reflux, this observation warrants further investigation. A decrease in SBS5 (ageing signature) was seen among aspirin/NSAID users which was especially evident among BO+ve OAC cases. Chemopreventive use of NSAID has been associated with lower mutations and risk of

OAC (Galipeau et al., 2018). Lastly, as the molecular effect of alcohol consumption was of particular interest, we examined the effect of total units of alcohol consumption per week. Consistent with epidemiological studies, no association was observed between alcohol consumption and any of the signatures.

Mutation load is a crude measurement of genomic instability as it includes the 'passenger' mutations which are unlikely to drive tumorigenesis and it does not consider copy number alterations (CNA) which are important driver events in OAC. Therefore, to derive richer information about the molecular history of tumours, we examined the landscape of known OAC driver genes was examined in relation to the phenotypes. Both single nucleotide variants and copy number alterations in driver genes were examined. Consistent with published data, *TP53* and *CDKN2A* were recurrently mutated in and dominated the landscape of mutations in each phenotype. No significant differences were elicited in the proportion of mutant cases for nine driver genes that had a recurrence of 10% or greater. Such results demonstrate a high similarity in mutated driver genes between the phenotypes and again point to a shared aetiology. However, more data is needed to compare the driver landscapes given the high heterogeneity of driver alterations in OAC and relatively smaller sample sizes per phenotype. Furthermore, the frequency of mutant cases by phenotype provides only a superficial driver event analysis, therefore future work should focus on mutation type, diversity and timing to provide a more in-depth comparison. Such analysis likely requires additional samples which are now available to our research group through the recently sequenced cases by Genomic England (n=250).

It has been hypothesised that the BO-ve OAC phenotype may be the outcome of rapid progression of BO driven by catastrophic events. To explore this, we examined the prevalence of select catastrophic events in OAC phenotypes. We would expect to see a higher prevalence of catastrophic events in the BO-ve OAC. The scope of this analysis was limited since other major catastrophic events in OAC such as chromothripsis, kataegis and chromoplexy were not examined due to data availability and time constraints. In addition to expanding the scope of this analysis, future work should consider the combinatorial effect of catastrophic alterations as multiple events co-exist in each genome and contribute to other features such as high-level copy number amplifications.

5.4 Strengths and limitations

The large size, detailed data collection and curation process is the primary strength of the cohort used in this thesis. The OCCAMS Consortium enabled the inclusion of a large number of the OAC cases and the BEST2 study provided the BO cases and reflux controls in

order to assemble the cohort. For each study, baseline data were extensively and carefully processed to ensure data quality. The cases included in this cohort were all prospectively recruited from across the UK as both the OCCAMS and BEST2 were multi-centre studies. Furthermore, each OAC case was evaluated by multiple expert pathologists to ascertain its phenotype which was not known by the study staff who recorded baseline data. Therefore, the risks of misclassification or differential biases were minimised. It is possible that sampling error would result in the extant small focus of BO/IM being missed which would lead to a misclassification bias that cannot be completely ruled out. Vital status and follow-up data collection spanned over a decade for OAC cases, yielding over 6,800 person-years of follow-up. Lastly, epidemiological and clinical data was well-recorded for cases with whole-genome sequencing, which is uncommon in the majority of genomic cohorts. This enabled orthogonal evaluations of our hypothesis.

This work was not without limitations. Missing data was high for some variables, such as pack-years of smoking or BMI five years prior to diagnosis, therefore limiting our assessment of these risk factors. Nevertheless, data on smoking status and BMI at baseline were well-recorded and included in the analysis. Furthermore, all efforts were made to recover missing data by combining and binarising variables or recoding values based on related variables. Additionally, we performed sensitivity analyses using a complete case or multiple imputation approach and the obtained estimates did not show large deviations, thus it is unlikely that missing data significantly altered our results. In some instances, information collected on the CRF was open-ended and not specifically defined. For example, in the question about smoking history, "former smoking" was left to the patient's or data collector's interpretations and was not defined based on a specific time period (e.g., 5 years) since smoking cessation. However, we binarised smoking status to overcome this limitation. In addition, we have also been collecting detailed lifestyle and epidemiological information using a separate questionnaire which was improved compared to the CRF. More details about this questionnaire instrument and the resulting dataset are presented in the future works section. Whole-genome sequencing was performed using tissue collected from formalin-fixed paraffin-embedded (FFPE) samples. DNA from FFPE has a low yield and is prone to quality degradation over time. However, FFPE biopsies are used in clinical practice and data generated from such samples would be better extrapolated into a clinical setting. Finally, the potential for residual confounding by unmeasured or unrecognized factors is inherent due to the nature of the observational data included here.

5.5 Conclusion

In conclusion, the orthogonal lines of evidence presented here do not support major pathogenic pathways in oesophageal adenocarcinoma that are independent of Barrett's oesophagus. Tumours without histologically apparent BO tissue may represent a more aggressive oesophageal adenocarcinoma, necessitating further investigation. Our findings emphasise the importance of Barrett's oesophagus screening for the secondary prevention of oesophageal adenocarcinoma. Screening strategies should focus on identifying and risk-stratifying prevalent cases of Barrett's oesophagus which could in turn reduce the public health burden of oesophageal adenocarcinoma.

Chapter 6

Ongoing and future work

The cohort and data generated in this project can be further utilised to examine questions related to the OAC phenotypes and beyond. An additional 250 OAC cases were recently whole-genome sequenced and while most of these were included in the mutation load analysis, they were not included in other genomic analyses due to time constraints and data availability. The inclusion of these cases would be very ideal, especially for the comparison of mutational signatures and driver genes between the phenotypes. In the future, a more comprehensive analysis of large-scale and catastrophic genomic events in relation to the OAC phenotypes should include additional alterations such as structural variants, chromothripsis and kataegis (Li et al., 2020; Ng et al., 2022). This would enable a deeper evaluation of the hypothesis that BO-ve OAC tumour pathogenesis may be driven rapidly and non-linearly by major genomic catastrophes. It would also be important to interrogate epigenomics as methylation could also correlate with the phenotypes. Methylation profiling has been performed on a subset of tumours (n=285) included in this cohort and could provide an extra layer of information. Previous work from our group has demonstrated that methylation profiles are related to subtypes of OAC (Jammula et al., 2020). One identified subtype showed a poor prognosis and was associated with a higher macrophage and neutrophil infiltration. It would be interesting to determine if this subtype is enriched with BO-ve OAC and examine the immunogenomics of the OAC phenotypes.

Starting in 2018, we employed a comprehensive questionnaire instrument to gather pre-diagnostic epidemiological and lifestyle data from OAC patients enrolled in the OCCAMS Consortium. To date, a total of 423 patients across 20 OCCAMS study sites have completed this questionnaire. Patients returned paper questionnaires to the Cambridge study site where data was entered on digitised forms. As part of the work for this project, I developed a processing pipeline to generate an analysis-ready dataset from these forms. We recently conducted a thorough quality control to ensure data integrity and accuracy, resulting in

a rich dataset for examining novel risk factors in relation to OAC phenotypes and other research questions. In addition, WGS has been performed on 104 tumours comprised of 41 BO+ve, 24 BO-ve and 37 BO(?) OAC tumours. Future work utilising this dataset should aim to 1) confirm the association of epidemiological factors identified here using more detailed measurement variables such as pack-years of cigarette smoking, 2) examine the association of novel factors such as physical activity, sedentary behaviours and dietary factors as well as co-morbid conditions in relation to OAC phenotypes and survival, and 3) conduct integrated molecular epidemiology analyses using questionnaire and WGS data to explore associations between risk factors and genomic features, such as mutational signatures. Efforts are underway to gather additional questionnaires, and it is hoped that this dataset will also serve as a resource for future studies examining patient-reported factors in OAC. Finally, work is ongoing to link mortality data (vital status, date and cause of death) for OAC cases in the OCCAMS Consortium using data from the Office of National Statistics. This would provide more complete, accurate and updated survival data for all OAC cases included here.

References

Abbas, S., Pich, O., Devonshire, G., Zamani, S. A., Katz-Summercorn, A., Killcoyne, S., Cheah, C., Nutzinger, B., Lopez-Bigas, N., the Oesophageal Cancer Clinical and Molecular Stratification (OCCAMS) Consortium, Secrier, M., and Fitzgerald, R. C. (2023). Mutational processes unveil bottlenecks that shape the evolution of oesophageal adenocarcinoma. Manuscript in preparation.

Abnet, C. C., Arnold, M., and Wei, W.-Q. (2018). Epidemiology of esophageal squamous cell carcinoma. *Gastroenterology*, 154(2):360–373.

Abnet, C. C., Freedman, N. D., Hollenbeck, A. R., Fraumeni, J. F., Leitzmann, M., and Schatzkin, A. (2008). A prospective study of BMI and risk of oesophageal and gastric adenocarcinoma. *European Journal of Cancer*, 44(3):465–471.

Abrams, J. A., Fields, S., Lightdale, C. J., and Neugut, A. I. (2008). Racial and ethnic disparities in the prevalence of Barrett's esophagus among patients who undergo upper endoscopy. *Clinical Gastroenterology and Hepatology*, 6(1):30–34.

Akdemir, K. C., Le, V. T., Kim, J. M., Killcoyne, S., King, D. A., Lin, Y.-P., Tian, Y., Inoue, A., Amin, S. B., Robinson, F. S., Nimmakayalu, M., Herrera, R. E., Lynn, E. J., Chan, K., Seth, S., Klimczak, L. J., Gerstung, M., Gordenin, D. A., O'Brien, J., Li, L., Deribe, Y. L., Verhaak, R. G., Campbell, P. J., Fitzgerald, R., Morrison, A. J., Dixon, J. R., and Futreal, P. A. (2020). Somatic mutation distributions in cancer genomes vary with three-dimensional chromatin structure. *Nature Genetics*, 52(11):1178–1188.

Alexandrov, L. B., Kim, J., Haradhvala, N. J., Huang, M. N., Ng, A. W., Wu, Y., Boot, A., Covington, K. R., Gordenin, D. A., Bergstrom, E. N., Islam, S. M. A., Lopez-Bigas, N., Klimczak, L. J., McPherson, J. R., Morganella, S., Sabarinathan, R., Wheeler, D. A., Mustonen, V., Getz, G., Rozen, S. G., and Stratton, M. R. (2020). The repertoire of mutational signatures in human cancer. *Nature*, 578(7793):94–101.

Alexandrov, L. B., Nik-Zainal, S., Wedge, D. C., Aparicio, S. A. J. R., Behjati, S., Biankin, A. V., Bignell, G. R., Bolli, N., Borg, A., Børresen-Dale, A.-L., Boyault, S., Burkhardt, B., Butler, A. P., Caldas, C., Davies, H. R., Desmedt, C., Eils, R., Eyfjörd, J. E., Foekens, J. A., Greaves, M., Hosoda, F., Hutter, B., Ilicic, T., Imbeaud, S., Imielinski, M., Jäger, N., Jones, D. T. W., Jones, D., Knappskog, S., Kool, M., Lakhani, S. R., López-Otín, C., Martin, S., Munshi, N. C., Nakamura, H., Northcott, P. A., Pajic, M., Papaemmanuil, E., Paradiso, A., Pearson, J. V., Puente, X. S., Raine, K., Ramakrishna, M., Richardson, A. L., Richter, J., Rosenstiel, P., Schlesner, M., Schumacher, T. N., Span, P. N., Teague, J. W., Totoki, Y., Tutt, A. N. J., Valdés-Mas, R., van Buuren, M. M., van 't Veer, L., Vincent-Salomon, A., Waddell, N., Yates, L. R., Zucman-Rossi, J., Futreal, P. A., McDermott, U., Lichter, P., Meyerson, M., Grimmond, S. M., Siebert, R., Campo, E., Shibata, T., Pfister, S. M., Campbell, P. J., and Stratton, M. R. (2013). Signatures of mutational processes in human cancer. *Nature*, 500(7463):415–421.

Andrici, J. and Eslick, G. D. (2015). Hot food and beverage consumption and the risk of esophageal cancer. *American Journal of Preventive Medicine*, 49(6):952–960.

Armstrong, B. K. (2018). Cancer epidemiology and prevention. *International Journal of Epidemiology*, 47(6):2097–2098.

Arnold, M., Ferlay, J., van Berge Henegouwen, M. I., and Soerjomataram, I. (2020). Global burden of oesophageal and gastric cancer by histology and subsite in 2018. *Gut*, 69(9):1564–1571.

Arnold, M., Laversanne, M., Brown, L. M., Devesa, S. S., and Bray, F. (2017). Predicting the future burden of esophageal cancer by histological subtype: International trends in incidence up to 2030. *American Journal of Gastroenterology*, 112(8):1247–1255.

Barrett, N. R. (1950). Chronic peptic ulcerz of the œophagus and 'œsophagitis'. *British Journal of Surgery*, 38(150):175–182.

Bhat, S., Coleman, H. G., Yousef, F., Johnston, B. T., McManus, D. T., Gavin, A. T., and Murray, L. J. (2011). Risk of malignant progression in Barrett's esophagus patients: Results from a large population-based study. *Journal of the National Cancer Institute*, 103(13):1049–1057.

Boffetta, P., Hashibe, M., Vecchia, C. L., Zatonski, W., and Rehm, J. (2006). The burden of cancer attributable to alcohol drinking. *International Journal of Cancer*, 119(4):884–887.

Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., and Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians*, 68(6):394–424.

Cameron, A. J., Zinsmeister, A. R., Ballard, D. J., and Carney, J. (1990). Prevalence of columnar-lined (Barrett's) esophagus. *Gastroenterology*, 99(4):918–922.

Campbell, P. J., Getz, G., Stuart, J. M., Korbel, J. O., and Stein, L. D. (2020). Pan-cancer analysis of whole genomes. *Nature*, 578(7793):82–93.

Chak, A., Ochs-Balcom, H., Falk, G., Grady, W. M., Kinnard, M., Willis, J. E., Elston, R., and Eng, C. (2006). Familiality in Barrett's esophagus, adenocarcinoma of the esophagus, and adenocarcinoma of the gastroesophageal junction. *Cancer Epidemiology, Biomarkers & Prevention*, 15(9):1668–1673.

Codipilly, D. C., Sawas, T., Dhaliwal, L., Johnson, M. L., Lansing, R., Wang, K. K., Leggett, C. L., Katzka, D. A., and Iyer, P. G. (2021). Epidemiology and outcomes of young-onset esophageal adenocarcinoma: An analysis from a population-based database. *Cancer Epidemiology, Biomarkers & Prevention*, 30(1):142–149.

Coleman, H. G., Murray, L. J., Hicks, B., Bhat, S. K., Kubo, A., Corley, D. A., Cardwell, C. R., and Cantwell, M. M. (2013). Dietary fiber and the risk of precancerous lesions and cancer of the esophagus: A systematic review and meta-analysis. *Nutrition Reviews*, 71(7):474–482.

Coleman, H. G., Xie, S.-H., and Lagergren, J. (2018). The epidemiology of esophageal adenocarcinoma. *Gastroenterology*, 154(2):390–405.

Cook, M. B., Chow, W.-H., and Devesa, S. S. (2009). Oesophageal cancer incidence in the United States by race, sex, and histologic type, 1977–2005. *British Journal of Cancer*, 101(5):855–859.

Cook, M. B., Coburn, S. B., Lam, J. R., Taylor, P. R., Schneider, J. L., and Corley, D. A. (2017). Cancer incidence and mortality risks in a large US Barrett's oesophagus cohort. *Gut*, 67(3):418–529.

Cook, M. B., Corley, D. A., Murray, L. J., Liao, L. M., Kamangar, F., Ye, W., Gammon, M. D., Risch, H. A., Casson, A. G., Freedman, N. D., Chow, W.-H., Wu, A. H., Bernstein, L., Nyrén, O., Pandeya, N., Whiteman, D. C., and Vaughan, T. L. (2014). Gastroesophageal reflux in relation to adenocarcinomas of the esophagus: A pooled analysis from the Barrett's and esophageal adenocarcinoma consortium (BEACON). *PLoS ONE*, 9(7):e103508.

Cook, M. B., Wild, C. P., and Forman, D. (2005). A systematic review and meta-analysis of the sex ratio for Barrett's esophagus, erosive reflux disease, and nonerosive reflux disease. *American Journal of Epidemiology*, 162(11):1050–1061.

Cunningham, F., Allen, J. E., Allen, J., Alvarez-Jarreta, J., Amode, M. R., Armean, I. M., Austine-Orimoloye, O., Azov, A. G., Barnes, I., Bennett, R., Berry, A., Bhai, J., Bignell, A., Billis, K., Boddu, S., Brooks, L., Charkhchi, M., Cummins, C., Fioretto, L. D. R., Davidson, C., Dodiya, K., Donaldson, S., Houdaigui, B. E., Naboulsi, T. E., Fatima, R., Giron, C. G., Genez, T., Martinez, J. G., Guijarro-Clarke, C., Gymer, A., Hardy, M., Hollis, Z., Hourlier, T., Hunt, T., Juettemann, T., Kaikala, V., Kay, M., Lavidas, I., Le, T., Lemos, D., Marugán, J. C., Mohanan, S., Mushtaq, A., Naven, M., Ogeh, D. N., Parker, A., Parton,

A., Perry, M., Piližota, I., Prosovetskaia, I., Sakthivel, M. P., Salam, A. I. A., Schmitt, B. M., Schuilenburg, H., Sheppard, D., Pérez-Silva, J. G., Stark, W., Steed, E., Sutinen, K., Sukumaran, R., Sumathipala, D., Suner, M.-M., Szpak, M., Thormann, A., Tricomi, F. F., Urbina-Gómez, D., Veidenberg, A., Walsh, T. A., Walts, B., Willhoft, N., Winterbottom, A., Wass, E., Chakiachvili, M., Flint, B., Frankish, A., Giorgetti, S., Haggerty, L., Hunt, S. E., IIsley, G. R., Loveland, J. E., Martin, F. J., Moore, B., Mudge, J. M., Muffato, M., Perry, E., Ruffier, M., Tate, J., Thybert, D., Trevanion, S. J., Dyer, S., Harrison, P. W., Howe, K. L., Yates, A. D., Zerbino, D. R., and Flicek, P. (2021). Ensembl 2022. *Nucleic Acids Research*, 50(D1):D988–D995.

Dentro, S. C., Leshchiner, I., Haase, K., Tarabichi, M., Wintersinger, J., Deshwar, A. G., Yu, K., Rubanova, Y., Macintyre, G., Demeulemeester, J., Vázquez-García, I., Kleinheinz, K., Livitz, D. G., Malikic, S., Donmez, N., Sengupta, S., Anur, P., Jolly, C., Cmero, M., Rosebrock, D., Schumacher, S., Fan, Y., Fittall, M., Drews, R. M., Yao, X., Lee, J., Schlesner, M., Zhu, H., Adams, D. J., Getz, G., Boutros, P. C., Imielinski, M., Beroukhim, R., Sahinalp, S. C., Ji, Y., Peifer, M., Martincorena, I., Markowetz, F., Mustonen, V., Yuan, K., Gerstung, M., Spellman, P. T., Wang, W., Morris, Q. D., Wedge, D. C., and Van Loo, P. (2021). Characterizing genetic intra-tumor heterogeneity across 2,658 human cancer genomes. *Cell*, 184(8):2239–2254.

Desai, T. K., Krishnan, K., Samala, N., Singh, J., Cluley, J., Perla, S., and Howden, C. W. (2011). The incidence of oesophageal adenocarcinoma in non-dysplastic Barrett's oesophagus: A meta-analysis. *Gut*, 61(7):970–976.

Deshpande, V., Luebeck, J., Nguyen, N.-P. D., Bakhtiari, M., Turner, K. M., Schwab, R., Carter, H., Mischel, P. S., and Bafna, V. (2019). Exploring the landscape of focal amplifications in cancer using AmpliconArchitect. *Nature Communications*, 10. Article number: 392.

Duits, L. C., Phoa, K. N., Curvers, W. L., ten Kate, F. J. W., Meijer, G. A., Seldenrijk, C. A., Offerhaus, G. J., Visser, M., Meijer, S. L., Krishnadath, K. K., Tijssen, J. G. P., Mallant-Hent, R. C., and Bergman, J. J. G. H. M. (2014). Barrett's oesophagus patients

with low-grade dysplasia can be accurately risk-stratified after histological review by an expert pathology panel. *Gut*, 64(5):700–706.

Dulak, A. M., Stojanov, P., Peng, S., Lawrence, M. S., Fox, C., Stewart, C., Bandla, S., Imamura, Y., Schumacher, S. E., Shefler, E., McKenna, A., Carter, S. L., Cibulskis, K., Sivachenko, A., Saksena, G., Voet, D., Ramos, A. H., Auclair, D., Thompson, K., Sougnez, C., Onofrio, R. C., Guiducci, C., Beroukhim, R., Zhou, Z., Lin, L., Lin, J., Reddy, R., Chang, A., Landrenau, R., Pennathur, A., Ogino, S., Luketich, J. D., Golub, T. R., Gabriel, S. B., Lander, E. S., Beer, D. G., Godfrey, T. E., Getz, G., and Bass, A. J. (2013). Exome and whole-genome sequencing of esophageal adenocarcinoma identifies recurrent driver events and mutational complexity. *Nature Genetics*, 45(5):478–486.

Edge, S. B. and Compton, C. C. (2010). The American Joint Committee on Cancer: The 7th edition of the AJCC cancer staging manual and the future of TNM. *Annals of Surgical Oncology*, 17(6):1471–1474.

Edgren, G., Adami, H.-O., Weiderpass, E., and Nyrén, O. (2012). A global assessment of the oesophageal adenocarcinoma epidemic. *Gut*, 62(10):1406–1414.

Falk, G. W. (2009). Risk factors for esophageal cancer development. *Surgical Oncology Clinics of North America*, 18(3):469–485.

Farrow, D. C., Vaughan, T. L., Sweeney, C., Gammon, M. D., Chow, W.-H., Risch, H. A., Stanford, J. L., Hansten, P. D., Mayne, S. T., Schoenberg, J. B., Rotterdam, H., Ahsan, H., West, A. B., Dubrow, R., Fraumeni, J. F., and Blot, W. J. (2000). Gastroesophageal reflux disease, use of H2 receptor antagonists, and risk of esophageal and gastric cancer. *Cancer Causes and Control*, 11(3):231–238.

Fitzgerald, R. C., di Pietro, M., Ragunath, K., Ang, Y., Kang, J.-Y., Watson, P., Trudgill, N., Patel, P., Kaye, P. V., Sanders, S., O'Donovan, M., Bird-Lieberman, E., Bhandari, P., Jankowski, J. A., Attwood, S., Parsons, S. L., Loft, D., Lagergren, J., Moayyedi, P., Lyratzopoulos, G., and de Caestecker, J. (2013). British Society of Gastroenterology guidelines on the diagnosis and management of Barrett's oesophagus. *Gut*, 63(1):7–42.

Fitzgerald, R. C., Lascar, R., and Triadafilopoulos, G. (2001). Barrett's oesophagus, dysplasia and pharmacologic acid suppression. *Alimentary Pharmacology & Therapeutics*, 15(3):269–276.

Frankell, A. M., Jammula, S., Li, X., Contino, G., Killcoyne, S., Abbas, S., Perner, J., Bower, L., Devonshire, G., Ococks, E., Grehan, N., Mok, J., O'Donovan, M., MacRae, S., Eldridge, M. D., Tavaré, S., and Fitzgerald, R. C. (2019). The landscape of selection in 551 esophageal adenocarcinomas defines genomic biomarkers for the clinic. *Nature Genetics*, 51(3):506–516.

Freedman, N. D., Murray, L. J., Kamangar, F., Abnet, C. C., Cook, M. B., Nyren, O., Ye, W., Wu, A. H., Bernstein, L., Brown, L. M., Ward, M. H., Pandeya, N., Green, A. C., Casson, A. G., Giffen, C., Risch, H. A., Gammon, M. D., Chow, W.-H., Vaughan, T. L., Corley, D. A., and Whiteman, D. C. (2011). Alcohol intake and risk of oesophageal adenocarcinoma: A pooled analysis from the BEACON consortium. *Gut*, 60(8):1029–1037.

Gajperia, C., Barbiere, J. M., Greenberg, D., Wright, K., and Lyratzopoulos, G. (2009). Recent incidence trends and sociodemographic features of oesophageal and gastric cancer types in an English region. *Alimentary Pharmacology & Therapeutics*, 30(8):873–880.

Galipeau, P. C., Oman, K. M., Paulson, T. G., Sanchez, C. A., Zhang, Q., Marty, J. A., Delrow, J. J., Kuhner, M. K., Vaughan, T. L., Reid, B. J., and Li, X. (2018). NSAID use and somatic exomic mutations in Barrett's esophagus. *Genome Medicine*, 10. Article number: 17.

Gavin, A., Francisci, S., Foschi, R., Donnelly, D., Lemmens, V., Brenner, H., and Anderson, L. (2012). Oesophageal cancer survival in Europe: A EUROCARE-4 study. *Cancer Epidemiology*, 36(6):505–512.

Gharahkhani, P., Fitzgerald, R. C., Vaughan, T. L., Palles, C., Gockel, I., Tomlinson, I., Buas, M. F., May, A., Gerges, C., Anders, M., Becker, J., Kreuser, N., Noder, T., Venerito, M., Veits, L., Schmidt, T., Manner, H., Schmidt, C., Hess, T., Böhmer, A. C., Izbicki, J. R.,

Hölscher, A. H., Lang, H., Lorenz, D., Schumacher, B., Hackelsberger, A., Mayershofer, R., Pech, O., Vashist, Y., Ott, K., Vieth, M., Weismüller, J., Nöthen, M. M., Attwood, S., Barr, H., Chegwidden, L., de Caestecker, J., Harrison, R., Love, S. B., MacDonald, D., Moayyedi, P., Prenen, H., Watson, R. G. P., Iyer, P. G., Anderson, L. A., Bernstein, L., Chow, W.-H., Hardie, L. J., Lagergren, J., Liu, G., Risch, H. A., Wu, A. H., Ye, W., Bird, N. C., Shaheen, N. J., Gammon, M. D., Corley, D. A., Caldas, C., Moebus, S., Knapp, M., Peters, W. H. M., Neuhaus, H., Rösch, T., Ell, C., MacGregor, S., Pharoah, P., Whiteman, D. C., Jankowski, J., and Schumacher, J. (2016). Genome-wide association studies in oesophageal adenocarcinoma and Barrett's oesophagus: A large-scale meta-analysis. *The Lancet Oncology*, 17(10):1363–1373.

Groves, C., Jankowski, J., Barker, F., and Holdstock, G. (2005). A family history of Barrett's oesophagus: Another risk factor? *Scandinavian Journal of Gastroenterology*, 40(9):1127–1128.

Hadi, K., Yao, X., Behr, J. M., Deshpande, A., Xanthopoulakis, C., Tian, H., Kudman, S., Rosiene, J., Darmofal, M., DeRose, J., Mortensen, R., Adney, E. M., Shaiber, A., Gajic, Z., Sigouros, M., Eng, K., Wala, J. A., Wrzeszczyński, K. O., Arora, K., Shah, M., Emde, A.-K., Felice, V., Frank, M. O., Darnell, R. B., Ghandi, M., Huang, F., Dewhurst, S., Maciejowski, J., de Lange, T., Setton, J., Riaz, N., Reis-Filho, J. S., Powell, S., Knowles, D. A., Reznik, E., Mishra, B., Beroukhim, R., Zody, M. C., Robine, N., Oman, K. M., Sanchez, C. A., Kuhner, M. K., Smith, L. P., Galipeau, P. C., Paulson, T. G., Reid, B. J., Li, X., Wilkes, D., Sboner, A., Mosquera, J. M., Elemento, O., and Imielinski, M. (2020). Distinct classes of complex structural variation uncovered across thousands of cancer genome graphs. *Cell*, 183(1):197–210.

Haidry, R. J., Dunn, J. M., Butt, M. A., Burnell, M. G., Gupta, A., Green, S., Miah, H., Smart, H. L., Bhandari, P., Smith, L. A., Willert, R., Fullarton, G., Morris, J., Pietro, M. D., Gordon, C., Penman, I., Barr, H., Patel, P., Boger, P., Kapoor, N., Mahon, B., Hoare, J., Narayanasamy, R., O'Toole, D., Cheong, E., Direkze, N. C., Ang, Y., Novelli, M., Banks, M. R., and Lovat, L. B. (2013). Radiofrequency ablation and endoscopic mucosal resection

for dysplastic Barrett's esophagus and early esophageal adenocarcinoma: Outcomes of the UK National HALO RFA registry. *Gastroenterology*, 145(1):87–95.

Hanahan, D. and Weinberg, R. A. (2011). Hallmarks of cancer: The next generation. *Cell*, 144(5):646–674.

Hidayat, K., Du, X., and Shi, B.-M. (2018). Body fatness at a young age and risks of eight types of cancer: Systematic review and meta-analysis of observational studies. *Obesity Reviews*, 19(10):1385–1394.

Holm, J., Humphreys, K., Li, J., Ploner, A., Cheddad, A., Eriksson, M., Törnberg, S., Hall, P., and Czene, K. (2015). Risk factors and tumor characteristics of interval cancers by mammographic density. *Journal of Clinical Oncology*, 33(9):1030–1037.

Hoyo, C., Cook, M. B., Kamangar, F., Freedman, N. D., Whiteman, D. C., Bernstein, L., Brown, L. M., Risch, H. A., Ye, W., Sharp, L., Wu, A. H., Ward, M. H., Casson, A. G., Murray, L. J., Corley, D. A., Nyrén, O., Pandeya, N., Vaughan, T. L., Chow, W.-H., and Gammon, M. D. (2012). Body mass index in relation to oesophageal and oesophagogastric junction adenocarcinomas: A pooled analysis from the international BEACON consortium. *International Journal of Epidemiology*, 41(6):1706–1718.

Huang, W., Han, Y., Xu, J., Zhu, W., and Li, Z. (2012). Red and processed meat intake and risk of esophageal adenocarcinoma: A meta-analysis of observational studies. *Cancer Causes & Control*, 24(1):193–201.

Hvid-Jensen, F., Pedersen, L., Drewes, A. M., Sørensen, H. T., and Funch-Jensen, P. (2011). Incidence of adenocarcinoma among patients with Barrett's esophagus. *New England Journal of Medicine*, 365(15):1375–1383.

Jammula, S., Katz-Summercorn, A. C., Li, X., Linossi, C., Smyth, E., Killcoyne, S., Biasci, D., Subash, V. V., Abbas, S., Blasko, A., Devonshire, G., Grantham, A., Wronowski, F., O'Donovan, M., Grehan, N., Eldridge, M. D., Tavaré, S., the Oesophageal Cancer Clinical and Molecular Stratification (OCCAMS) Consortium, and Fitzgerald, R. C.

(2020). Identification of subtypes of Barrett's esophagus and esophageal adenocarcinoma based on DNA methylation profiles and integration of transcriptome and genome data. *Gastroenterology*, 158(6):1682–1697.

Jankowski, J., de Caestecker, J., Love, S., Reilly, G., Watson, P., Sanders, S., Ang, Y., Morris, D., Bhandari, P., Brooks, C., Attwood, S., Harrison, R., Barr, H., Moayyedi, P., and the AspECT Trial Team (2018). Esomeprazole and aspirin in Barrett's oesophagus (AspECT): A randomised factorial trial. *The Lancet*, 392(10145):400–408.

Katz-Summercorn, A. C., Jammula, S., Frangou, A., Peneva, I., O'Donovan, M., Tripathi, M., Malhotra, S., di Pietro, M., Abbas, S., Devonshire, G., Januszewicz, W., Blasko, A., Nowicki-Osuch, K., MacRae, S., Northrop, A., Redmond, A. M., Wedge, D. C., and Fitzgerald, R. C. (2022). Multi-omic cross-sectional cohort study of pre-malignant Barrett's esophagus reveals early structural variation and retrotransposon activity. *Nature Communications*, 13. Article number: 1407.

Kim, S., Scheffler, K., Halpern, A. L., Bekritsky, M. A., Noh, E., Källberg, M., Chen, X., Kim, Y., Beyter, D., Krusche, P., and Saunders, C. T. (2018). Strelka2: Fast and accurate calling of germline and somatic variants. *Nature Methods*, 15(8):591–594.

Kubo, A., Cook, M. B., Shaheen, N. J., Vaughan, T. L., Whiteman, D. C., Murray, L., and Corley, D. A. (2013). Sex-specific associations between body mass index, waist circumference and the risk of Barrett's oesophagus: A pooled analysis from the international BEACON consortium. *Gut*, 62(12):1684–1691.

Kubo, A. and Corley, D. A. (2007). Meta-analysis of antioxidant intake and the risk of esophageal and gastric cardia adenocarcinoma. *The American Journal of Gastroenterology*, 102(10):2323–2330.

Lagergren, J. (2011). Influence of obesity on the risk of esophageal disorders. *Nature Reviews Gastroenterology & Hepatology*, 8(6):340–347.

Lagergren, J., Andersson, G., Talbäck, M., Drefahl, S., Bihagen, E., Härkönen, J., Feychting, M., and Ljung, R. (2015). Marital status, education, and income in relation to the risk of esophageal and gastric cancer by histological type and site. *Cancer*, 122(2):207–212.

Lagergren, J., Bergström, R., Lindgren, A., and Nyrén, O. (1999). Symptomatic gastroesophageal reflux as a risk factor for esophageal adenocarcinoma. *New England Journal of Medicine*, 340(11):825–831.

Li, H. (2013). Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv pre-print:1303.3997.

Li, Y., Roberts, N. D., Wala, J. A., Shapira, O., Schumacher, S. E., Khurana, E., Korbel, J. O., Haber, J. E., Imielinski, M., the PCAWG Structural Variation Working Group, Weischenfeldt, J., Beroukhim, R., Campbell, P. J., and the PCAWG Consortium (2020). Patterns of somatic structural variation in human cancer genomes. *Nature*, 578(7793):112–121.

Liao, L. M., Vaughan, T. L., Corley, D. A., Cook, M. B., Casson, A. G., Kamangar, F., Abnet, C. C., Risch, H. A., Giffen, C., Freedman, N. D., Chow, W.-H., Sadeghi, S., Pandeya, N., Whiteman, D. C., Murray, L. J., Bernstein, L., Gammon, M. D., and Wu, A. H. (2012). Nonsteroidal anti-inflammatory drug use reduces risk of adenocarcinomas of the esophagus and esophagogastric junction in a pooled analysis. *Gastroenterology*, 142(3):442–452.

Luebeck, J., Ng, A. W. T., Galipeau, P. C., Li, X., Sanchez, C. A., Katz-Summercorn, A. C., Kim, H., Jammula, S., He, Y., Lippman, S. M., Verhaak, R. G. W., Maley, C. C., Alexandrov, L. B., Reid, B. J., Fitzgerald, R. C., Paulson, T. G., Chang, H. Y., Wu, S., Bafna, V., and Mischel, P. S. (2023). Extrachromosomal DNA in the cancerous transformation of Barrett's oesophagus. *Nature*, 616(7958):798–805.

Martínez-Jiménez, F., Muiños, F., Sentís, I., Deu-Pons, J., Reyes-Salazar, I., Arnedo-Pac, C., Mularoni, L., Pich, O., Bonet, J., Kranas, H., Gonzalez-Perez, A., and Lopez-Bigas, N. (2020). A compendium of mutational cancer driver genes. *Nature Reviews Cancer*, 20(10):555–572.

Masclee, G. M. C., Coloma, P. M., de Wilde, M., Kuipers, E. J., and Sturkenboom, M. C. J. M. (2014). The incidence of Barrett's oesophagus and oesophageal adenocarcinoma in the United Kingdom and The Netherlands is levelling off. *Alimentary Pharmacology & Therapeutics*, 39(11):1321–1330.

McLaren, W., Gil, L., Hunt, S. E., Riat, H. S., Ritchie, G. R. S., Thormann, A., Flicek, P., and Cunningham, F. (2016). The Ensembl Variant Effect Predictor. *Genome Biology*, 17. Article number: 122.

Mermel, C. H., Schumacher, S. E., Hill, B., Meyerson, M. L., Beroukhim, R., and Getz, G. (2011). GISTIC2.0 facilitates sensitive and confident localization of the targets of focal somatic copy-number alteration in human cancers. *Genome Biology*, 12. Article number: R41.

Murnane, J. P. (2012). Telomere dysfunction and chromosome instability. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, 730(1-2):28–36.

Newell, F., Patel, K., Gartside, M., Krause, L., Brosda, S., Aoude, L. G., Loffler, K. A., Bonazzi, V. F., Patch, A.-M., Kazakoff, S. H., Holmes, O., Xu, Q., Wood, S., Leonard, C., Lampe, G., Lord, R. V., Whiteman, D. C., Pearson, J. V., Nones, K., Waddell, N., and Barbour, A. P. (2019). Complex structural rearrangements are present in high-grade dysplastic Barrett's oesophagus samples. *BMC Medical Genomics*, 12. Article number: 31.

Ng, A. W. T., Contino, G., Killcoyne, S., Devonshire, G., Hsu, R., Abbas, S., Su, J., Redmond, A. M., Weaver, J. M. J., Eldridge, M. D., Tavaré, S., the Oesophageal Cancer Clinical and Molecular Stratification (OCCAMS) Consortium, Edwards, P. A. W., and Fitzgerald, R. C. (2022). Rearrangement processes and structural variations show evidence of selection in oesophageal adenocarcinomas. *Communications Biology*, 5. Article number: 335.

Nguyen, C. D., Carlin, J. B., and Lee, K. J. (2017). Model checking in multiple imputation: An overview and case study. *Emerging Themes in Epidemiology*, 14.

Nones, K., Waddell, N., Wayte, N., Patch, A.-M., Bailey, P., Newell, F., Holmes, O., Fink, J. L., Quinn, M. C. J., Tang, Y. H., Lampe, G., Quek, K., Loffler, K. A., Manning, S., Idrisoglu, S., Miller, D., Xu, Q., Waddell, N., Wilson, P. J., Bruxner, T. J. C., Christ, A. N., Harliwong, I., Nourse, C., Nourbakhsh, E., Anderson, M., Kazakoff, S., Leonard, C., Wood, S., Simpson, P. T., Reid, L. E., Krause, L., Hussey, D. J., Watson, D. I., Lord, R. V., Nancarrow, D., Phillips, W. A., Gotley, D., Smithers, B. M., Whiteman, D. C., Hayward, N. K., Campbell, P. J., Pearson, J. V., Grimmond, S. M., and Barbour, A. P. (2014). Genomic catastrophes frequently arise in esophageal adenocarcinoma and drive tumorigenesis. *Nature Communications*, 5. Article number: 31.

Noorani, A., Bornschein, J., Lynch, A. G., Secrier, M., Achilleos, A., Eldridge, M., Bower, L., Weaver, J. M., Crawte, J., Ong, C.-A., Shannon, N., MacRae, S., Grehan, N., Nutzinger, B., O'Donovan, M., Hardwick, R., Tavaré, S., the Oesophageal Cancer Clinical and Molecular Stratification (OCCAMS) Consortium, and Fitzgerald, R. C. (2017). A comparative analysis of whole genome sequencing of esophageal adenocarcinoma pre- and post-chemotherapy. *Genome Research*, 27(6):902–912.

Nowicki-Osuch, K., Zhuang, L., Jammula, S., Bleaney, C. W., Mahbubani, K. T., Devonshire, G., Katz-Summercorn, A., Eling, N., Wilbrey-Clark, A., Madissoon, E., Gamble, J., Pietro, M. D., O'Donovan, M., Meyer, K. B., Saeb-Parsy, K., Sharrocks, A. D., Teichmann, S. A., Marioni, J. C., and Fitzgerald, R. C. (2021). Molecular phenotyping reveals the identity of Barrett's esophagus and its malignant transition. *Science*, 373(6556):760–767.

Offman, J., Pesola, F., and Sasieni, P. (2018). Trends and projections in adenocarcinoma and squamous cell carcinoma of the oesophagus in England from 1971 to 2037. *British Journal of Cancer*, 118(10):1391–1398.

Paulson, T. G., Galipeau, P. C., Oman, K. M., Sanchez, C. A., Kuhner, M. K., Smith, L. P., Hadi, K., Shah, M., Arora, K., Shelton, J., Johnson, M., Corvelo, A., Maley, C. C., Yao, X., Sanghvi, R., Venturini, E., Emde, A.-K., Hubert, B., Imielinski, M., Robine, N., Reid, B. J., and Li, X. (2022). Somatic whole genome dynamics of precancer in Barrett's esophagus

reveals features associated with disease progression. *Nature Communications*, 13. Article number: 2300.

Pennathur, A., Gibson, M. K., Jobe, B. A., and Luketich, J. D. (2013). Oesophageal carcinoma. *The Lancet*, 381(9864):400–412.

Peters, Y., Al-Kaabi, A., Shaheen, N. J., Chak, A., Blum, A., Souza, R. F., Pietro, M. D., Iyer, P. G., Pech, O., Fitzgerald, R. C., and Siersema, P. D. (2019). Barrett oesophagus. *Nature Reviews Disease Primers*, 5(1).

Phoa, K. N., van Vilsteren, F. G. I., Weusten, B. L. A. M., Bisschops, R., Schoon, E. J., Ragunath, K., Fullarton, G., Pietro, M. D., Ravi, N., Visser, M., Offerhaus, G. J., Seldenrijk, C. A., Meijer, S. L., ten Kate, F. J. W., Tijssen, J. G. P., and Bergman, J. J. G. H. M. (2014). Radiofrequency ablation vs endoscopic surveillance for patients with Barrett esophagus and low-grade dysplasia. *JAMA*, 311(12):1209.

Pich, O., Muiños, F., Lolkema, M. P., Steeghs, N., Gonzalez-Perez, A., and Lopez-Bigas, N. (2019). The mutational footprints of cancer therapies. *Nature Genetics*, 51(12):1732–1740.

Qumseya, B. J., Bukannan, A., Gendy, S., Ahemd, Y., Sultan, S., Bain, P., Gross, S. A., Iyer, P., and Wani, S. (2019). Systematic review and meta-analysis of prevalence and risk factors for Barrett's esophagus. *Gastrointestinal Endoscopy*, 90(5):707–717.

Rastogi, A., Puli, S., El-Serag, H. B., Bansal, A., Wani, S., and Sharma, P. (2008). Incidence of esophageal adenocarcinoma in patients with Barrett's esophagus and high-grade dysplasia: A meta-analysis. *Gastrointestinal Endoscopy*, 67(3):394–398.

Rice, T. W., Patil, D. T., and Blackstone, E. H. (2017). 8th edition AJCC/UICC staging of cancers of the esophagus and esophagogastric junction: Application to clinical practice. *Annals of Cardiothoracic Surgery*, 6(2):119–130.

Ronkainen, J., Aro, P., Storskrubb, T., Johansson, S.-E., Lind, T., Bolling-Sternevald, E., Vieth, M., Stolte, M., Talley, N. J., and Agréus, L. (2005). Prevalence of Barrett's

esophagus in the general population: An endoscopic study. *Gastroenterology*, 129(6):1825–1831.

Rosenthal, R., McGranahan, N., Herrero, J., Taylor, B. S., and Swanton, C. (2016). deconstructSigs: Delineating mutational processes in single tumors distinguishes DNA repair deficiencies and patterns of carcinoma evolution. *Genome Biology*, 17. Article number: 31.

Ross-Innes, C. S., Becq, J., Warren, A., Cheetham, R. K., Northen, H., O'Donovan, M., Malhotra, S., di Pietro, M., Ivakhno, S., He, M., Weaver, J. M. J., Lynch, A. G., Kingsbury, Z., Ross, M., Humphray, S., Bentley, D., the Oesophageal Cancer Clinical and Molecular Stratification (OCCAMS) Consortium, and Fitzgerald, R. C. (2015). Whole-genome sequencing provides new insights into the clonal architecture of Barrett's esophagus and esophageal adenocarcinoma. *Nature Genetics*, 47(9):1038–1046.

Ross-Innes, C. S., Chettouh, H., Achilleos, A., Galeano-Dalmau, N., Debiram-Beecham, I., MacRae, S., Fessas, P., Walker, E., Varghese, S., Evan, T., Lao-Sirieix, P. S., O'Donovan, M., Malhotra, S., Novelli, M., Disep, B., Kaye, P. V., Lovat, L. B., Haidry, R., Griffin, M., Ragunath, K., Bhandari, P., Haycock, A., Morris, D., Attwood, S., Dhar, A., Rees, C., Rutter, M. D., Ostler, R., Aigret, B., Sasieni, P. D., and Fitzgerald, R. C. (2017). Risk stratification of Barrett's oesophagus using a non-endoscopic sampling method coupled with a biomarker panel: A cohort study. *The Lancet Gastroenterology & Hepatology*, 2(1):23–31.

Rubenstein, J. H. and Taylor, J. B. (2010). Meta-analysis: The association of oesophageal adenocarcinoma with symptoms of gastro-oesophageal reflux. *Alimentary Pharmacology & Therapeutics*, 32(10):1222–1227.

Runge, T. M., Abrams, J. A., and Shaheen, N. J. (2015). Epidemiology of Barrett's esophagus and esophageal adenocarcinoma. *Gastroenterology Clinics of North America*, 44(2):203–231.

Rutegård, M., Shore, R., Lu, Y., Lagergren, P., and Lindblad, M. (2010). Sex differences in the incidence of gastrointestinal adenocarcinoma in Sweden 1970–2006. *European Journal of Cancer*, 46(6):1093–1100.

Salehi, M., Moradi-Lakeh, M., Salehi, M. H., Nojomi, M., and Kolahdooz, F. (2013). Meat, fish, and esophageal cancer risk: A systematic review and dose-response meta-analysis. *Nutrition Reviews*, 71(5):257–267.

Sawas, T., Killcoyne, S., Iyer, P. G., Wang, K. K., Smyrk, T. C., Kisiel, J. B., Qin, Y., Ahlquist, D. A., Rustgi, A. K., Costa, R. J., Gerstung, M., the Oesophageal Cancer Clinical and Molecular Stratification (OCCAMS) Consortium, Fitzgerald, R. C., and Katzka, D. A. (2018). Identification of prognostic phenotypes of esophageal adenocarcinoma in 2 independent cohorts. *Gastroenterology*, 155(6):1720–1728.

Sawas, T., Zamani, S. A., Killcoyne, S., Dullea, A., Wang, K. K., Iyer, P. G., Fitzgerald, R. C., and Katzka, D. A. (2022). Limitations of heartburn and other societies' criteria in Barrett's screening for detecting de novo esophageal adenocarcinoma. *Clinical Gastroenterology and Hepatology*, 20(8):1709–1718.

Secrier, M., Li, X., de Silva, N., Eldridge, M. D., Contino, G., Bornschein, J., MacRae, S., Grehan, N., O'Donovan, M., Miremadi, A., Yang, T.-P., Bower, L., Chettouh, H., Crawte, J., Galeano-Dalmau, N., Grabowska, A., Saunders, J., Underwood, T., Waddell, N., Barbour, A. P., Nutzinger, B., Achilleos, A., Edwards, P. A. W., Lynch, A. G., Tavaré, S., and Fitzgerald, R. C. (2016). Mutational signatures in esophageal adenocarcinoma define etiologically distinct subgroups with therapeutic relevance. *Nature Genetics*, 48(10):1131–1141.

Siewert, J. R. and Stein, H. J. (1998). Classification of adenocarcinoma of the oesophagogastric junction. *British Journal of Surgery*, 85(11):1457–1459.

Singh, S., Devanna, S., Varayil, J. E., Murad, M. H., and Iyer, P. G. (2014). Physical activity is associated with reduced risk of esophageal cancer, particularly esophageal

adenocarcinoma: A systematic review and meta-analysis. *BMC Gastroenterology*, 14. Article number: 101.

Singh, S., Sharma, A. N., Murad, M. H., Buttar, N. S., El-Serag, H. B., Katzka, D. A., and Iyer, P. G. (2013a). Central adiposity is associated with increased risk of esophageal inflammation, metaplasia, and adenocarcinoma: A systematic review and meta-analysis. *Clinical Gastroenterology and Hepatology*, 11(11):1399–1412.

Singh, S., Garg, S. K., Singh, P. P., Iyer, P. G., and El-Serag, H. B. (2013b). Acid-suppressive medications and risk of oesophageal adenocarcinoma in patients with Barrett's oesophagus: A systematic review and meta-analysis. *Gut*, 63(8):1229–1237.

Smith, K. J., O'Brien, S. M., Smithers, B. M., Gotley, D. C., Webb, P. M., Green, A. C., and Whiteman, D. C. (2005). Interactions among smoking, obesity, and symptoms of acid reflux in Barrett's esophagus. *Cancer Epidemiology, Biomarkers & Prevention*, 14(11):2481–2486.

Smyth, E. C., Lagergren, J., Fitzgerald, R. C., Lordick, F., Shah, M. A., Lagergren, P., and Cunningham, D. (2017). Oesophageal cancer. *Nature Reviews Disease Primers*, 3(1).

Spechler, S. J. (2002). Barrett's esophagus. *New England Journal of Medicine*, 346(11):836–842.

Stachler, M. D., Camarda, N. D., Deitrick, C., Kim, A., Agoston, A. T., Odze, R. D., Hornick, J. L., Nag, A., Thorner, A. R., Ducar, M., Noffsinger, A., Lash, R. H., Redston, M., Carter, S. L., Davison, J. M., and Bass, A. J. (2018). Detection of mutations in Barrett's esophagus before progression to high-grade dysplasia or adenocarcinoma. *Gastroenterology*, 155(1):156–167.

Stachler, M. D., Taylor-Weiner, A., Peng, S., McKenna, A., Agoston, A. T., Odze, R. D., Davison, J. M., Nason, K. S., Loda, M., Leshchiner, I., Stewart, C., Stojanov, P., Seepo, S., Lawrence, M. S., Ferrer-Torres, D., Lin, J., Chang, A. C., Gabriel, S. B., Lander, E. S., Beer, D. G., Getz, G., Carter, S. L., and Bass, A. J. (2015). Paired exome analysis of Barrett's esophagus and adenocarcinoma. *Nature Genetics*, 47(9):1047–1055.

Steffen, A., Huerta, J.-M., Weiderpass, E., de Mesquita, H. B., May, A. M., Siersema, P. D., Kaaks, R., Neamat-Allah, J., Pala, V., Panico, S., Saieva, C., Tumino, R., Naccarati, A., Dorronsoro, M., Sánchez-Cantalejo, E., Ardanaz, E., Quirós, J. R., Ohlsson, B., Johansson, M., Wallner, B., Overvad, K., Halkjaer, J., Tjønneland, A., Fagherazzi, G., Racine, A., Clavel-Chapelon, F., Key, T. J., Khaw, K.-T., Wareham, N., Lagiou, P., Bamia, C., Trichopoulou, A., Ferrari, P., Freisling, H., Lu, Y., Riboli, E., Cross, A. J., Gonzalez, C. A., and Boeing, H. (2015). General and abdominal obesity and risk of esophageal and gastric adenocarcinoma in the European Prospective Investigation into Cancer and Nutrition. *International Journal of Cancer*, 137(3):646–657.

Stratton, M. R., Campbell, P. J., and Futreal, P. A. (2009). The cancer genome. *Nature*, 458(7239):719–724.

Talevich, E., Shain, A. H., Botton, T., and Bastian, B. C. (2016). CNVkit: Genome-wide copy number detection and visualization from targeted DNA sequencing. *PLOS Computational Biology*, 12(4):e1004873.

Tan, M. C., Mansour, N., White, D. L., Sisson, A., El-Serag, H. B., and Thrift, A. P. (2020). Systematic review with meta-analysis: Prevalence of prior and concurrent Barrett's oesophagus in oesophageal adenocarcinoma patients. *Alimentary Pharmacology & Therapeutics*, 52(1):20–36.

Tate, J. G., Bamford, S., Jubb, H. C., Sondka, Z., Beare, D. M., Bindal, N., Boutselakis, H., Cole, C. G., Creatore, C., Dawson, E., Fish, P., Harsha, B., Hathaway, C., Jupe, S. C., Kok, C. Y., Noble, K., Ponting, L., Ramshaw, C. C., Rye, C. E., Speedy, H. E., Stefancsik, R., Thompson, S. L., Wang, S., Ward, S., Campbell, P. J., and Forbes, S. A. (2018). COSMIC: The catalogue of somatic mutations in cancer. *Nucleic Acids Research*, 47(D1):D941–D947.

Taylor, J. B. and Rubenstein, J. H. (2010). Meta-analyses of the effect of symptoms of gastroesophageal reflux on the risk of Barrett's esophagus. *American Journal of Gastroenterology*, 105(8):1730–1737.

Thrift, A. P. (2018). Barrett's esophagus and esophageal adenocarcinoma: How common are they really? *Digestive Diseases and Sciences*, 63(8):1988–1996.

Thrift, A. P. (2021). Global burden and epidemiology of Barrett oesophagus and oesophageal cancer. *Nature Reviews Gastroenterology & Hepatology*, 18(6):432–443.

Thrift, A. P., Anderson, L. A., Murray, L. J., Cook, M. B., Shaheen, N. J., Rubenstein, J. H., El-Serag, H. B., Vaughan, T. L., Schneider, J. L., Whiteman, D. C., and Corley, D. A. (2016). Nonsteroidal anti-inflammatory drug use is not associated with reduced risk of Barrett's esophagus. *American Journal of Gastroenterology*, 111(11):1528–1535.

Thrift, A. P., Cook, M. B., Vaughan, T. L., Anderson, L. A., Murray, L. J., Whiteman, D. C., Shaheen, N. J., and Corley, D. A. (2014). Alcohol and the risk of Barrett's esophagus: A pooled analysis from the international BEACON consortium. *American Journal of Gastroenterology*, 109(10):1586–1594.

Turati, F., Galeone, C., Vecchia, C. L., Garavello, W., and Tavani, A. (2011). Coffee and cancers of the upper digestive and respiratory tracts: Meta-analyses of observational studies. *Annals of Oncology*, 22(3):536–544.

Turati, F., Tramacere, I., Vecchia, C. L., and Negri, E. (2013). A meta-analysis of body mass index and esophageal and gastric cardia adenocarcinoma. *Annals of Oncology*, 24(3):609–617.

Turner, K. M., Deshpande, V., Beyter, D., Koga, T., Rusert, J., Lee, C., Li, B., Arden, K., Ren, B., Nathanson, D. A., Kornblum, H. I., Taylor, M. D., Kaushal, S., Cavenee, W. K., Wechsler-Reya, R., Furnari, F. B., Vandenberg, S. R., Rao, P. N., Wahl, G. M., Bafna, V., and Mischel, P. S. (2017). Extrachromosomal oncogene amplification drives tumour evolution and genetic heterogeneity. *Nature*, 543(7643):122–125.

Van Buuren, S. and Groothuis-Oudshoorn, K. (2011). mice: Multivariate imputation by chained equations. *Journal of Statistical Software*, 45(3):122–125.

Van Loo, P., Nordgard, S. H., Lingjærde, O. C., Russnes, H. G., Rye, I. H., Sun, W., Weigman, V. J., Marynen, P., Zetterberg, A., Naume, B., Perou, C. M., Børresen-Dale, A.-L., and Kristensen, V. N. (2010). Allele-specific copy number analysis of tumors. *Proceedings of the National Academy of Sciences*, 107(39):16910–16915.

Vaughan, T. L. and Fitzgerald, R. C. (2015). Precision prevention of oesophageal adenocarcinoma. *Nature Reviews Gastroenterology & Hepatology*, 12(4):243–248.

Verbeek, R. E., Spittuler, L. F., Peute, A., van Oijen, M. G., ten Kate, F. J., Vermeijden, J. R., Oberndorff, A., van Baal, J. W., and Siersema, P. D. (2014). Familial clustering of Barrett's esophagus and esophageal adenocarcinoma in a European cohort. *Clinical Gastroenterology and Hepatology*, 12(10):1656–1663.e1.

Vingeliene, S., Chan, D. S. M., Aune, D., Vieira, A. R., Polemiti, E., Stevens, C., Abar, L., Rosenblatt, D. N., Greenwood, D. C., and Norat, T. (2016). An update of the WCRF/AICR systematic literature review on esophageal and gastric cancers and citrus fruits intake. *Cancer Causes & Control*, 27(7):837–851.

Vingeliene, S., Chan, D. S. M., Vieira, A., Polemiti, E., Stevens, C., Abar, L., Rosenblatt, D. N., Greenwood, D., and Norat, T. (2017). An update of the WCRF/AICR systematic literature review and meta-analysis on dietary and anthropometric factors and esophageal cancer risk. *Annals of Oncology*, 28(10):2409–2419.

Wang, S., Li, H., Song, M., Tao, Z., Wu, T., He, Z., Zhao, X., Wu, K., and Liu, X.-S. (2021). Copy number signature analysis tool and its application in prostate cancer reveals distinct mutational processes and clinical outcomes. *PLOS Genetics*, 17(5):e1009557.

Weaver, J. M. J., Ross-Innes, C. S., Shannon, N., Lynch, A. G., Forshew, T., Barbera, M., Murtaza, M., Ong, C.-A. J., Lao-Sirieix, P., Dunning, M. J., Smith, L., Smith, M. L., Anderson, C. L., Carvalho, B., O'Donovan, M., Underwood, T. J., May, A. P., Grehan, N., Hardwick, R., Davies, J., Oloumi, A., Aparicio, S., Caldas, C., Eldridge, M. D., Edwards, P. A. W., Rosenfeld, N., Tavaré, S., and Fitzgerald, R. C. (2014). Ordering of mutations in preinvasive disease stages of esophageal carcinogenesis. *Nature Genetics*, 46(8):837–843.

Westhoff, B., Brotze, S., Weston, A., McElhinney, C., Cherian, R., Mayo, M. S., Smith, H. J., and Sharma, P. (2005). The frequency of Barrett's esophagus in high-risk patients with chronic GERD. *Gastrointestinal Endoscopy*, 61(2):226–231.

Whiteman, D. C. and Wilson, L. F. (2016). The fractions of cancer attributable to modifiable factors: A global review. *Cancer Epidemiology*, 44:203–221.

Wu, S., Bafna, V., Chang, H. Y., and Mischel, P. S. (2022). Extrachromosomal DNA: An emerging hallmark in human cancer. *Annual Review of Pathology: Mechanisms of Disease*, 17(1):367–386.

Wu, S., Bafna, V., and Mischel, P. S. (2021). Extrachromosomal DNA (ecDNA) in cancer pathogenesis. *Current Opinion in Genetics & Development*, 66:78–82.

Xi, S., Oyetunji, S., Wang, H., Azoury, S., Liu, Y., Hsiao, S.-H., Zhang, M., Carr, S. R., Hoang, C. D., Chen, H., and Schrump, D. S. (2023). Cigarette smoke enhances the malignant phenotype of esophageal adenocarcinoma cells by disrupting a repressive regulatory interaction between miR-145 and LOXL2. *Laboratory Investigation*, 103(4):100014.

Xie, S.-H. and Lagergren, J. (2016). The male predominance in esophageal adenocarcinoma. *Clinical Gastroenterology and Hepatology*, 14(3):338–347.e1.

Zagari, R. M., Fuccio, L., Wallander, M.-A., Johansson, S., Fiocca, R., Casanova, S., Farahmand, B. Y., Winchester, C. C., Roda, E., and Bazzoli, F. (2008). Gastro-oesophageal reflux symptoms, oesophagitis and Barrett's oesophagus in the general population: The Loiano-Monghidoro study. *Gut*, 57(10):1354–1359.

Zhao, Z., Pu, Z., Yin, Z., Yu, P., Hao, Y., Wang, Q., Guo, M., and Zhao, Q. (2016). Dietary fruit, vegetable, fat and red and processed meat intakes and Barrett's esophagus risk: A systematic review and meta-analysis. *Scientific Reports*, 6. Article number: 27334.

Zhu, H.-C., Yang, X., Xu, L.-P., Zhao, L.-J., Tao, G.-Z., Zhang, C., Qin, Q., Cai, J., Ma, J.-X., Mao, W.-D., Zhang, X.-Z., Cheng, H.-Y., and Sun, X.-C. (2014). Meat consumption is

associated with esophageal cancer risk in a meat- and cancer-histological-type dependent manner. *Digestive Diseases and Sciences*, 59(3):664–673.

Appendix A

Additional tables

Table A.1 – Sensitivity analyses of the adjusted association of baseline characteristics with risk of BO+ve OAC compared to BO(?) OAC cases using multiple imputation, complete case analysis and exclusion of OAC cases with a history of undergoing BO surveillance.

	Multiple imputation	Complete case analysis			Excluding BO surveillance OAC cases (n=214)		
Characteristic	**Fully adjusted model[a] OR (95% CI, p)**	**BO(?) OAC n (%)**	**BO+ve OAC n (%)**	**Fully adjusted model[a] OR (95% CI, p)**	**BO(?) OAC n (%)**	**BO+ve OAC n (%)**	**Fully adjusted model[a] OR (95% CI, p)**
Age group at diagnosis							
< 50	1.00 (Referent)	15 (4.8)	28 (6.2)	1.00 (Referent)	70 (7.5)	56 (5.1)	1.00 (Referent)
50 - 59	0.96 (0.65-1.43, p=0.854)	70 (22.3)	65 (14.4)	0.52 (0.25-1.08, p=0.083)	197 (21.0)	174 (16.0)	1.06 (0.70-1.62, p=0.779)
60 - 69	1.60 (1.11-2.31, p=0.013)	104 (33.1)	168 (37.2)	0.90 (0.44-1.78, p=0.770)	322 (34.4)	437 (40.1)	1.63 (1.10-2.42, p=0.015)
70+	1.42 (0.98-2.05, p=0.064)	125 (39.8)	191 (42.3)	0.86 (0.42-1.69, p=0.668)	348 (37.1)	423 (38.8)	1.49 (1.01-2.21, p=0.045)
Gender							
Female	1.00 (Referent)	38 (12.1)	56 (12.4)	1.00 (Referent)	154 (16.4)	144 (13.2)	1.00 (Referent)
Male	1.33 (1.04-1.70, p=0.025)	276 (87.9)	396 (87.6)	1.02 (0.64-1.60, p=0.946)	783 (83.6)	946 (86.8)	1.32 (1.02-1.71, p=0.035)
BMI group at baseline							
Normal	1.00 (Referent)	103 (32.8)	126 (27.9)	1.00 (Referent)	241 (25.7)	245 (22.5)	1.00 (Referent)
Overweight	1.16 (0.92-1.45, p=0.216)	137 (43.6)	191 (42.3)	1.08 (0.76-1.54, p=0.649)	282 (30.1)	362 (33.2)	1.16 (0.91-1.48, p=0.240)
Obese	1.32 (1.03-1.70, p=0.031)	74 (23.6)	135 (29.9)	1.37 (0.92-2.04, p=0.123)	185 (19.7)	258 (23.7)	1.27 (0.97-1.66, p=0.087)
Missing	-	-	-	-	229 (24.4)	225 (20.6)	0.87 (0.63-1.22, p=0.418)
Cigarette smoking status							
Never	1.00 (Referent)	87 (27.7)	155 (34.3)	1.00 (Referent)	290 (30.9)	398 (36.5)	1.00 (Referent)
Ever	0.80 (0.66-0.96, p=0.019)	227 (72.3)	297 (65.7)	0.78 (0.56-1.08, p=0.138)	528 (56.4)	574 (52.7)	0.77 (0.63-0.95, p=0.013)
Missing	-	-	-	-	119 (12.7)	118 (10.8)	0.92 (0.61-1.40, p=0.709)
Aspirin/NSAID use							
Never	1.00 (Referent)	140 (44.6)	208 (46.0)	1.00 (Referent)	195 (20.8)	235 (21.6)	1.00 (Referent)
Ever	0.95 (0.70-1.28, p=0.718)	174 (55.4)	244 (54.0)	0.91 (0.67-1.23, p=0.534)	244 (26.0)	277 (25.4)	0.90 (0.69-1.17, p=0.437)
Missing	-	-	-	-	498 (53.1)	578 (53.0)	1.00 (0.77-1.29, p=0.989)
Heartburn symptoms status							
Absent	1.00 (Referent)	54 (17.2)	70 (15.5)	1.00 (Referent)	141 (15.0)	145 (13.3)	1.00 (Referent)
Present	1.28 (0.99-1.66, p=0.064)	260 (82.8)	382 (84.5)	1.13 (0.75-1.69, p=0.559)	555 (59.2)	684 (62.8)	1.19 (0.91-1.56, p=0.204)
Missing	-	-	-	-	241 (25.7)	261 (23.9)	1.06 (0.73-1.52, p=0.769)
TNM							
I	1.00 (Referent)	58 (18.5)	132 (29.2)	1.00 (Referent)	99 (10.6)	232 (21.3)	1.00 (Referent)
II	0.57 (0.43-0.76, p<0.001)	80 (25.5)	110 (24.3)	0.62 (0.40-0.94, p=0.027)	184 (19.6)	245 (22.5)	0.57 (0.42-0.77, p<0.001)
III	0.48 (0.37-0.62, p<0.001)	169 (53.8)	200 (44.2)	0.56 (0.38-0.81, p=0.003)	414 (44.2)	459 (42.1)	0.50 (0.38-0.66, p<0.001)
IV	0.56 (0.30-1.08, p=0.082)	7 (2.2)	10 (2.2)	0.61 (0.22-1.78, p=0.348)	18 (1.9)	25 (2.3)	0.60 (0.31-1.17, p=0.128)
Missing	-	-	-	-	222 (23.7)	129 (11.8)	0.25 (0.18-0.35, p<0.001)

[a]adjusted for all covariates.

Table A.2 – Sensitivity analyses of the adjusted association of baseline characteristics with risk of BO-ve OAC compared to BO(?) OAC cases using multiple imputation, complete case analysis and exclusion of OAC cases with a history of undergoing BO surveillance.

	Multiple imputation	Complete case analysis			Excluding BO surveillance OAC cases (n=214)		
Characteristic	**Fully adjusted model[a] OR (95% CI, p)**	**BO(?) OAC n (%)**	**BO-ve OAC n (%)**	**Fully adjusted model[a] OR (95% CI, p)**	**BO(?) OAC n (%)**	**BO-ve OAC n (%)**	**Fully adjusted model[a] OR (95% CI, p)**
Age group at diagnosis							
< 50	1.00 (Referent)	15 (4.8)	24 (7.5)	1.00 (Referent)	70 (7.5)	59 (6.9)	1.00 (Referent)
50 - 59	1.15 (0.77-1.72, p=0.494)	70 (22.3)	58 (18.1)	0.58 (0.27-1.21, p=0.147)	197 (21.0)	179 (20.8)	1.11 (0.74-1.68, p=0.621)
60 - 69	1.24 (0.84-1.81, p=0.278)	104 (33.1)	130 (40.5)	0.87 (0.42-1.76, p=0.708)	322 (34.4)	309 (36.0)	1.14 (0.77-1.69, p=0.510)
70+	1.11 (0.76-1.63, p=0.580)	125 (39.8)	109 (34.0)	0.61 (0.29-1.24, p=0.177)	348 (37.1)	312 (36.3)	1.07 (0.73-1.59, p=0.716)
Gender							
Female	1.00 (Referent)	38 (12.1)	58 (18.1)	1.00 (Referent)	154 (16.4)	151 (17.6)	1.00 (Referent)
Male	0.87 (0.68-1.11, p=0.263)	276 (87.9)	263 (81.9)	0.62 (0.39-0.97, p=0.039)	783 (83.6)	708 (82.4)	0.90 (0.70-1.16, p=0.403)
BMI group at baseline							
Normal	1.00 (Referent)	103 (32.8)	99 (30.8)	1.00 (Referent)	241 (25.7)	248 (28.9)	1.00 (Referent)
Overweight	1.03 (0.81-1.30, p=0.822)	137 (43.6)	143 (44.5)	1.15 (0.80-1.68, p=0.449)	282 (30.1)	307 (35.7)	1.04 (0.81-1.33, p=0.751)
Obese	0.78 (0.59-1.03, p=0.078)	74 (23.6)	79 (24.6)	1.17 (0.76-1.80, p=0.488)	185 (19.7)	154 (17.9)	0.82 (0.62-1.09, p=0.178)
Missing	-	-	-	-	229 (24.4)	150 (17.5)	0.81 (0.58-1.13, p=0.206)
Cigarette smoking status							
Never	1.00 (Referent)	87 (27.7)	89 (27.7)	1.00 (Referent)	290 (30.9)	291 (33.9)	1.00 (Referent)
Ever	0.97 (0.79-1.18, p=0.736)	227 (72.3)	232 (72.3)	1.02 (0.71-1.45, p=0.930)	528 (56.4)	518 (60.3)	0.99 (0.80-1.22, p=0.920)
Missing	-	-	-		119 (12.7)	50 (5.8)	0.44 (0.27-0.69, p=0.001)
Aspirin/NSAID use					-		
Never	1.00 (Referent)	140 (44.6)	129 (40.2)	1.00 (Referent)	195 (20.8)	159 (18.5)	1.00 (Referent)
Ever	1.25 (0.95-1.65, p=0.106)	174 (55.4)	192 (59.8)	1.33 (0.95-1.85, p=0.097)	244 (26.0)	235 (27.4)	1.20 (0.90-1.59, p=0.208)
Missing	-	-	-	-	498 (53.1)	465 (54.1)	1.37 (1.04-1.79, p=0.024)
Heartburn symptoms status							
Absent	1.00 (Referent)	54 (17.2)	73 (22.7)	1.00 (Referent)	141 (15.0)	159 (18.5)	1.00 (Referent)
Present	0.77 (0.58-1.02, p=0.070)	260 (82.8)	248 (77.3)	0.61 (0.40-0.92, p=0.020)	555 (59.2)	524 (61.0)	0.81 (0.62-1.06, p=0.121)
Missing	-	-	-	1.40 (0.84-2.34, p=0.196)	241 (25.7)	176 (20.5)	0.82 (0.57-1.18, p=0.286)
TNM							
I	1.00 (Referent)	58 (18.5)	44 (13.7)	1.00 (Referent)	99 (10.6)	80 (9.3)	1.00 (Referent)
II	1.34 (0.95-1.90, p=0.098)	80 (25.5)	85 (26.5)	1.40 (0.84-2.34, p=0.196)	184 (19.6)	197 (22.9)	1.30 (0.91-1.87, p=0.153)
III	1.36 (0.99-1.87, p=0.054)	169 (53.8)	186 (57.9)	1.42 (0.90-2.26, p=0.129)	414 (44.2)	440 (51.2)	1.31 (0.94-1.82, p=0.111)
IV	1.85 (0.94-3.62, p=0.074)	7 (2.2)	6 (1.9)	1.04 (0.31-3.44, p=0.949)	18 (1.9)	26 (3.0)	1.77 (0.90-3.55, p=0.100)
Missing	-	-	-	-	222 (23.7)	116 (13.5)	0.63 (0.43-0.92, p=0.016)

[a]adjusted for all covariates.

Table A.3 – Sensitivity analyses of the adjusted association of baseline characteristics with risk of BO+ve OAC compared to BO cases using multiple imputation and complete case analysis, and the analysis excluding heartburn as a covariate in the fully adjusted model.

	Multiple imputation	Complete case analysis			Excluding heartburn as a covariate		
Characteristic	**Fully adjusted model[a] OR (95% CI, p)**	**BO Cases n (%)**	**BO+ve OAC n (%)**	**Fully adjusted model[a] OR (95% CI, p)**	**BO Cases n (%)**	**BO+ve OAC n (%)**	**Fully adjusted model[a] excluding heartburn OR (95% CI, p)**
Age group at diagnosis							
< 50	1.00 (Referent)	155 (18.4)	31 (5.9)	1.00 (Referent)	203 (18.6)	67 (5.4)	1.00 (Referent)
50 - 59	2.10 (1.48-3.00, p<0.001)	223 (26.5)	79 (15.0)	1.84 (1.14-3.05, p=0.015)	281 (25.8)	193 (15.6)	2.12 (1.48-3.05, p<0.001)
60 - 69	4.03 (2.90-5.60, p<0.001)	283 (33.6)	190 (36.1)	3.26 (2.09-5.22, p<0.001)	353 (32.4)	497 (40.2)	3.95 (2.84-5.55, p<0.001)
70+	5.09 (3.63-7.13, p<0.001)	182 (21.6)	226 (43.0)	5.58 (3.55-9.00, p<0.001)	254 (23.3)	478 (38.7)	5.40 (3.85-7.65, p<0.001)
Gender							
Female	1.00 (Referent)	174 (20.6)	63 (12.0)	1.00 (Referent)	217 (19.9)	163 (13.2)	1.00 (Referent)
Male	1.49 (1.17-1.91, p=0.001)	669 (79.4)	463 (88.0)	1.76 (1.25-2.50, p=0.001)	874 (80.1)	1072 (86.8)	1.61 (1.25-2.09, p<0.001)
BMI group at baseline							
Normal	1.00 (Referent)	174 (20.6)	145 (27.6)	1.00 (Referent)	217 (19.9)	268 (21.7)	1.00 (Referent)
Overweight	0.82 (0.64-1.04, p=0.101)	384 (45.6)	228 (43.3)	0.83 (0.61-1.13, p=0.233)	473 (43.4)	422 (34.2)	0.75 (0.59-0.96, p=0.023)
Obese	0.86 (0.67-1.11, p=0.251)	285 (33.8)	153 (29.1)	0.83 (0.60-1.16, p=0.282)	354 (32.4)	304 (24.6)	0.79 (0.61-1.03, p=0.080)
Missing	-	-	-	-	47 (4.3)	241 (19.5)	2.49 (1.61-3.90, p<0.001)
Cigarette smoking status							
Never	1.00 (Referent)	304 (36.1)	176 (33.5)	1.00 (Referent)	392 (35.9)	452 (36.6)	1.00 (Referent)
Ever	0.86 (0.72-1.01, p=0.075)	539 (63.9)	350 (66.5)	0.99 (0.77-1.28, p=0.937)	686 (62.9)	665 (53.8)	0.85 (0.70-1.03, p=0.100)
Missing	-	-	-	-	13 (1.2)	118 (9.6)	1.31 (0.66-2.72, p=0.451)
Aspirin/NSAID use							
Never	1.00 (Referent)	569 (67.5)	238 (45.2)	1.00 (Referent)	588 (53.9)	257 (20.8)	1.00 (Referent)
Ever	2.62 (2.06-3.33, p<0.001)	274 (32.5)	288 (54.8)	2.54 (2.00-3.25, p<0.001)	279 (25.6)	328 (26.6)	2.50 (1.99-3.14, p<0.001)
Missing	-	-	-	-	224 (20.5)	650 (52.6)	5.18 (4.14-6.50, p<0.001)
Heartburn symptoms status							
Absent	1.00 (Referent)	15 (1.8)	81 (15.4)	1.00 (Referent)	37 (3.4)	150 (12.1)	-
Present	0.19 (0.13-0.28, p<0.001)	828 (98.2)	445 (84.6)	0.09 (0.05-0.16, p<0.001)	1054 (96.6)	818 (66.2)	-
Missing	-	-	-	-	0 (0)	267 (21.6)	-

[a]adjusted for all covariates.

Table A.4 – Sensitivity analyses of the adjusted association of baseline characteristics with risk of BO-ve OAC compared to BO cases using multiple imputation and complete case analysis, and the analysis excluding heartburn as a covariate in the fully adjusted model.

	Multiple imputation	Complete case analysis			Excluding heartburn as a covariate		
Characteristic	**Fully adjusted model[a] OR (95% CI, p)**	**BO Cases n (%)**	**BO-ve OAC n (%)**	**Fully adjusted model[a] OR (95% CI, p)**	**BO Cases n (%)**	**BO-ve OAC n (%)**	**Fully adjusted model[a] excluding heartburn OR (95% CI, p)**
Age group at diagnosis							
< 50	1.00 (Referent)	155 (18.4)	29 (7.9)	1.00 (Referent)	203 (18.6)	59 (6.7)	1.00 (Referent)
50 - 59	2.11 (1.43-3.11, p<0.001)	223 (26.5)	68 (18.6)	1.51 (0.90-2.58, p=0.127)	281 (25.8)	184 (20.9)	2.13 (1.47-3.13, p<0.001)
60 - 69	2.68 (1.86-3.86, p<0.001)	283 (33.6)	145 (39.6)	2.37 (1.47-3.91, p=0.001)	353 (32.4)	317 (36.0)	2.90 (2.03-4.17, p<0.001)
70+	3.36 (2.30-4.91, p<0.001)	182 (21.6)	124 (33.9)	2.76 (1.68-4.64, p<0.001)	254 (23.3)	320 (36.4)	3.47 (2.42-5.03, p<0.001)
Gender							
Female	1.00 (Referent)	174 (20.6)	60 (16.4)	1.00 (Referent)	217 (19.9)	158 (18.0)	1.00 (Referent)
Male	1.01 (0.77-1.32, p=0.943)	669 (79.4)	306 (83.6)	1.12 (0.78-1.62, p=0.546)	874 (80.1)	722 (82.0)	1.11 (0.86-1.45, p=0.418)
BMI group at baseline							
Normal	1.00 (Referent)	174 (20.6)	116 (31.7)	1.00 (Referent)	217 (19.9)	255 (29.0)	1.00 (Referent)
Overweight	0.62 (0.48-0.81, p<0.001)	384 (45.6)	165 (45.1)	0.71 (0.51-1.00, p=0.048)	473 (43.4)	317 (36.0)	0.62 (0.48-0.80, p<0.001)
Obese	0.38 (0.28-0.52, p<0.001)	285 (33.8)	85 (23.2)	0.46 (0.32-0.68, p<0.001)	354 (32.4)	155 (17.6)	0.41 (0.30-0.54, p<0.001)
Missing	-	-	-	-	47 (4.3)	153 (17.4)	1.95 (1.25-3.08, p=0.004)
Cigarette smoking status							
Never	1.00 (Referent)	304 (36.1)	97 (26.5)	1.00 (Referent)	392 (35.9)	298 (33.9)	1.00 (Referent)
Ever	0.94 (0.75-1.17, p=0.574)	539 (63.9)	269 (73.5)	1.36 (1.01-1.86, p=0.047)	686 (62.9)	532 (60.5)	1.09 (0.87-1.35, p=0.461)
Missing	-	-	-		13 (1.2)	50 (5.7)	0.86 (0.42-1.86, p=0.686)
Aspirin/NSAID use							
Never	1.00 (Referent)	569 (67.5)	154 (42.1)	1.00 (Referent)	588 (53.9)	161 (18.3)	1.00 (Referent)
Ever	3.55 (2.69-4.68, p<0.001)	274 (32.5)	212 (57.9)	3.34 (2.52-4.44, p<0.001)	279 (25.6)	243 (27.6)	3.08 (2.39-3.98, p<0.001)
Missing	-	-	-	-	224 (20.5)	476 (54.1)	6.56 (5.12-8.44, p<0.001)
Heartburn symptoms status							
Absent	1.00 (Referent)	15 (1.8)	79 (21.6)	1.00 (Referent)	37 (3.4)	160 (18.2)	-
Present	0.10 (0.07-0.15, p<0.001)	828 (98.2)	287 (78.4)	0.05 (0.03-0.09, p<0.001)	1054 (96.6)	543 (61.7)	-
Missing	-	-	-	-	0 (0)	177 (20.1)	-

[a]adjusted for all covariates.

Table A.5 – Associations of baseline characteristics with risk of BO(?) OAC phenotype compared to BO cases.

Characteristic	BO Cases n (%)	BO(?) OAC n (%)	Univariable model OR (95% CI, p)	Minimally adjusted model[a] OR (95% CI, p)	Fully adjusted model[b] OR (95% CI, p)	Fully adjusted model[b] excl. BO surveillance OAC cases (n=214) OR (95% CI, p)
Age group at diagnosis						
< 50	203 (18.6)	75 (7.6)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
50 - 59	281 (25.8)	213 (21.6)	2.05 (1.50-2.83, p<0.001)	2.05 (1.49-2.83, p<0.001)	2.34 (1.59-3.50, p<0.001)	2.27 (1.52-3.44, p<0.001)
60 - 69	353 (32.4)	333 (33.8)	2.55 (1.89-3.48, p<0.001)	2.55 (1.89-3.47, p<0.001)	2.59 (1.79-3.81, p<0.001)	2.72 (1.86-4.04, p<0.001)
70+	254 (23.3)	364 (37.0)	3.88 (2.86-5.31, p<0.001)	3.87 (2.85-5.30, p<0.001)	3.61 (2.48-5.31, p<0.001)	3.68 (2.50-5.49, p<0.001)
Gender						
Female	217 (19.9)	161 (16.3)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Male	874 (80.1)	824 (83.7)	1.27 (1.02-1.59, p=0.037)	1.26 (1.00-1.59, p=0.048)	1.24 (0.94-1.65, p=0.129)	1.24 (0.93-1.66, p=0.139)
BMI group at baseline						
Normal	217 (19.9)	248 (25.2)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Overweight	473 (43.4)	307 (31.2)	0.57 (0.45-0.72, p<0.001)	0.58 (0.46-0.73, p<0.001)	0.60 (0.46-0.79, p<0.001)	0.57 (0.43-0.74, p<0.001)
Obese	354 (32.4)	195 (19.8)	0.48 (0.37-0.62, p<0.001)	0.52 (0.40-0.67, p<0.001)	0.50 (0.37-0.66, p<0.001)	0.49 (0.36-0.65, p<0.001)
Missing	47 (4.3)	235 (23.9)	4.37 (3.07-6.34, p<0.001)	4.60 (3.20-6.71, p<0.001)	1.64 (1.01-2.68, p=0.048)	1.52 (0.93-2.51, p=0.099)
Cigarette smoking status						
Never	392 (35.9)	304 (30.9)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Ever	686 (62.9)	562 (57.1)	1.06 (0.88-1.27, p=0.565)	1.01 (0.83-1.22, p=0.935)	1.22 (0.98-1.53, p=0.082)	1.22 (0.97-1.54, p=0.090)
Missing	13 (1.2)	119 (12.1)	11.80 (6.78-22.34, $p<10^{-3}$)	11.82 (6.74-22.50, $p<10^{-3}$)	0.00 (0.00-1.06, p=0.967)	0.00 (0.00-0.15, p=0.967)
Aspirin/NSAID use						
Never	588 (53.9)	201 (20.4)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Ever	279 (25.6)	263 (26.7)	2.76 (2.19-3.48, p<0.001)	2.60 (2.05-3.30, p<0.001)	2.87 (2.23-3.71, p<0.001)	2.72 (2.11-3.53, p<0.001)
Missing	224 (20.5)	521 (52.9)	6.80 (5.45-8.53, p<0.001)	6.73 (5.36-8.47, p<0.001)	3.84 (2.96-4.98, p<0.001)	3.59 (2.76-4.68, p<0.001)
Heartburn symptoms status						
Absent	37 (3.4)	144 (14.6)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Present	1054 (96.6)	599 (60.8)	0.15 (0.10-0.21, p<0.001)	0.15 (0.10-0.22, p<0.001)	0.10 (0.06-0.16, p<0.001)	0.10 (0.06-0.15, p<0.001)
Missing	0 (0)	242 (24.6)	NE	NE	NE	NE

[a]adjusted for age groups and gender.
[b]adjusted for all covariates.
Abbreviation: NE, not estimable.

Table A.6 – Sensitivity analyses of the adjusted association of baseline characteristics with risk of BO(?) OAC compared to BO cases using multiple imputation and complete case analysis, and the analysis excluding heartburn as a covariate in the fully adjusted model.

	Multiple imputation	Complete case analysis			Excluding heartburn as a covariate		
Characteristic	**Fully adjusted model[a] OR (95% CI, p)**	**BO Cases n (%)**	**BO(?) OAC n (%)**	**Fully adjusted model[a] OR (95% CI, p)**	**BO Cases n (%)**	**BO(?) OAC n (%)**	**Fully adjusted model[a] excluding heartburn OR (95% CI, p)**
Age group at diagnosis							
< 50	1.00 (Referent)	155 (18.4)	27 (6.5)	1.00 (Referent)	203 (18.6)	75 (7.6)	1.00 (Referent)
50 - 59	2.06 (1.45-2.91, p<0.001)	223 (26.5)	91 (21.8)	2.24 (1.36-3.78, p=0.002)	281 (25.8)	213 (21.6)	2.12 (1.49-3.03, p<0.001)
60 - 69	2.35 (1.68-3.28, p<0.001)	283 (33.6)	134 (32.1)	2.35 (1.46-3.89, p=0.001)	353 (32.4)	333 (33.8)	2.49 (1.78-3.51, p<0.001)
70+	3.41 (2.43-4.80, p<0.001)	182 (21.6)	166 (39.7)	4.28 (2.65-7.11, p<0.001)	254 (23.3)	364 (37.0)	3.61 (2.57-5.11, p<0.001)
Gender							
Female	1.00 (Referent)	174 (20.6)	53 (12.7)	1.00 (Referent)	217 (19.9)	161 (16.3)	1.00 (Referent)
Male	1.06 (0.82-1.36, p=0.669)	669 (79.4)	365 (87.3)	1.48 (1.03-2.16, p=0.035)	874 (80.1)	824 (83.7)	1.30 (1.00-1.69, p=0.049)
BMI group at baseline							
Normal	1.00 (Referent)	174 (20.6)	138 (33.0)	1.00 (Referent)	217 (19.9)	248 (25.2)	1.00 (Referent)
Overweight	0.57 (0.44-0.74, p<0.001)	384 (45.6)	176 (42.1)	0.59 (0.43-0.81, p=0.001)	473 (43.4)	307 (31.2)	0.57 (0.44-0.73, p<0.001)
Obese	0.49 (0.37-0.65, p<0.001)	285 (33.8)	104 (24.9)	0.48 (0.34-0.68, p<0.001)	354 (32.4)	195 (19.8)	0.49 (0.38-0.65, p<0.001)
Missing	-	-	-	-	47 (4.3)	235 (23.9)	2.34 (1.52-3.69, p<0.001)
Cigarette smoking status							
Never	1.00 (Referent)	304 (36.1)	123 (29.4)	1.00 (Referent)	392 (35.9)	304 (30.9)	1.00 (Referent)
Ever	0.96 (0.78-1.19, p=0.733)	539 (63.9)	295 (70.6)	1.21 (0.91-1.61, p=0.189)	686 (62.9)	562 (57.1)	1.10 (0.89-1.36, p=0.364)
Missing	-	-	-	-	13 (1.2)	119 (12.1)	1.77 (0.89-3.71, p=0.115)
Aspirin/NSAID use							
Never	1.00 (Referent)	569 (67.5)	186 (44.5)	1.00 (Referent)	588 (53.9)	201 (20.4)	1.00 (Referent)
Ever	2.93 (2.26-3.80, p<0.001)	274 (32.5)	232 (55.5)	2.79 (2.14-3.64, p<0.001)	279 (25.6)	263 (26.7)	2.65 (2.08-3.38, p<0.001)
Missing	-	-	-	-	224 (20.5)	521 (52.9)	5.08 (4.00-6.48, p<0.001)
Heartburn symptoms status							
Absent	1.00 (Referent)	15 (1.8)	70 (16.7)	1.00 (Referent)	37 (3.4)	144 (14.6)	-
Present	0.13 (0.08-0.19, p<0.001)	828 (98.2)	348 (83.3)	0.08 (0.04-0.14, p<0.001)	1054 (96.6)	599 (60.8)	-
Missing	-	-	-	-	0 (0)	242 (24.6)	-

[a]adjusted for all covariates.

Table A.7 – Associations of baseline characteristics with risk of BO+ve OAC phenotype compared to reflux controls.

Characteristic	Reflux Controls n (%)	BO+ve OAC n (%)	Univariable model OR (95% CI, p)	Minimally adjusted model[a] OR (95% CI, p)	Fully adjusted model[b] OR (95% CI, p)	Fully adjusted model[b] excluding heartburn OR (95% CI, p)
Age group at diagnosis						
< 50	169 (33.5)	67 (5.4)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
50 - 59	123 (24.4)	193 (15.6)	3.96 (2.77-5.71, p<0.001)	4.54 (3.06-6.81, p<0.001)	3.96 (2.57-6.15, p<0.001)	4.32 (2.83-6.65, p<0.001)
60 - 69	132 (26.2)	497 (40.2)	9.50 (6.78-13.44, p<0.001)	10.08 (6.96-14.75, p<10^{-3})	8.89 (5.94-13.48, p<0.001)	9.57 (6.45-14.37, p<0.001)
70+	80 (15.9)	478 (38.7)	15.07 (10.48-21.94, p<10^{-3})	17.49 (11.71-26.45, p<10^{-3})	16.39 (10.60-25.74, p<10^{-3})	18.26 (11.92-28.39, p<10^{-3})
Gender						
Female	275 (54.6)	163 (13.2)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Male	229 (45.4)	1072 (86.8)	7.90 (6.22-10.07, p<0.001)	8.54 (6.54-11.19, p<0.001)	8.59 (6.38-11.66, p<0.001)	8.93 (6.69-11.99, p<0.001)
BMI group at baseline						
Normal	171 (33.9)	268 (21.7)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Overweight	181 (35.9)	422 (34.2)	1.49 (1.15-1.93, p=0.003)	1.28 (0.93-1.76, p=0.129)	1.28 (0.91-1.78, p=0.151)	1.23 (0.89-1.70, p=0.209)
Obese	129 (25.6)	304 (24.6)	1.50 (1.14-1.99, p=0.005)	2.15 (1.51-3.08, p<0.001)	2.10 (1.45-3.05, p<0.001)	1.99 (1.39-2.87, p<0.001)
Missing	23 (4.6)	241 (19.5)	6.69 (4.26-10.93, p<0.001)	8.04 (4.75-14.15, p<0.001)	3.89 (2.09-7.50, p<0.001)	5.15 (2.81-9.87, p<0.001)
Cigarette smoking status						
Never	256 (50.8)	452 (36.6)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Ever	243 (48.2)	665 (53.8)	1.55 (1.25-1.92, p<0.001)	1.11 (0.86-1.44, p=0.430)	1.25 (0.95-1.65, p=0.114)	1.13 (0.86-1.48, p=0.393)
Missing	5 (1.0)	118 (9.6)	13.37 (5.97-38.15, p<10^{-3})	9.33 (3.98-27.43, p<0.001)	0.11 (0.02-0.57, p=0.009)	2.04 (0.71-6.99, p=0.215)
Aspirin/NSAID use						
Never	233 (46.2)	257 (20.8)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Ever	123 (24.4)	328 (26.6)	2.42 (1.84-3.18, p<0.001)	1.99 (1.43-2.77, p<0.001)	2.04 (1.45-2.87, p<0.001)	1.95 (1.39-2.74, p<0.001)
Missing	148 (29.4)	650 (52.6)	3.98 (3.10-5.13, p<0.001)	3.83 (2.84-5.21, p<0.001)	2.53 (1.84-3.50, p<0.001)	3.05 (2.24-4.19, p<0.001)
Heartburn symptoms status						
Absent	34 (6.7)	150 (12.1)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	-
Present	470 (93.3)	818 (66.2)	0.39 (0.26-0.58, p<0.001)	0.41 (0.25-0.64, p<0.001)	0.31 (0.19-0.50, p<0.001)	-
Missing	0 (0.0)	267 (21.6)	NE	NE	NE	-

[a]adjusted for age group at diagnosis and gender.
[b]adjusted for all covariates.
Abbreviation: NE, not estimable.

Table A.8 – Sensitivity analyses of the adjusted association of baseline characteristics with risk of BO+ve OAC compared to reflux controls using multiple imputation, complete case analysis and exclusion of OAC cases with a history of undergoing BO surveillance.

	Multiple imputation	Complete case analysis			Excluding BO surveillance OAC cases (n=214)		
Characteristic	**Fully adjusted model[a] OR (95% CI, p)**	**Reflux Controls n (%)**	**BO+ve OAC n (%)**	**Fully adjusted model[a] OR (95% CI, p)**	**Reflux Controls n (%)**	**BO+ve OAC n (%)**	**Fully adjusted model[a] OR (95% CI, p)**
Age group at diagnosis							
< 50	1.00 (Referent)	113 (33.3)	31 (5.9)	1.00 (Referent)	169 (33.5)	56 (5.1)	1.00 (Referent)
50 - 59	4.34 (2.86-6.60, p<0.001)	75 (22.1)	79 (15.0)	4.49 (2.54-8.09, p<0.001)	123 (24.4)	174 (16.0)	4.34 (2.77-6.90, p<0.001)
60 - 69	9.78 (6.59-14.50, p<0.001)	95 (28.0)	190 (36.1)	7.90 (4.69-13.63, p<0.001)	132 (26.2)	437 (40.1)	9.38 (6.14-14.57, p<0.001)
70+	16.86 (10.99-25.86, $p<10^{-3}$)	56 (16.5)	226 (43.0)	16.51 (9.45-29.64, $p<10^{-3}$)	80 (15.9)	423 (38.8)	16.44 (10.44-26.37, $p<10^{-3}$)
Gender							
Female	1.00 (Referent)	189 (55.8)	63 (12.0)	1.00 (Referent)	275 (54.6)	144 (13.2)	1.00 (Referent)
Male	8.92 (6.71-11.87, p<0.001)	150 (44.2)	463 (88.0)	9.91 (6.72-14.83, p<0.001)	229 (45.4)	946 (86.8)	8.40 (6.18-11.51, p<0.001)
BMI group at baseline							
Normal	1.00 (Referent)	125 (36.9)	145 (27.6)	1.00 (Referent)	171 (33.9)	245 (22.5)	1.00 (Referent)
Overweight	1.27 (0.92-1.77, p=0.148)	124 (36.6)	228 (43.3)	1.25 (0.83-1.90, p=0.283)	181 (35.9)	362 (33.2)	1.21 (0.86-1.71, p=0.266)
Obese	2.14 (1.49-3.09, p<0.001)	90 (26.5)	153 (29.1)	2.13 (1.34-3.41, p=0.002)	129 (25.6)	258 (23.7)	1.90 (1.30-2.80, p=0.001)
Missing	-	-	-	-	23 (4.6)	225 (20.6)	3.51 (1.86-6.86, p<0.001)
Cigarette smoking status							
Never	1.00 (Referent)	176 (51.9)	176 (33.5)	1.00 (Referent)	256 (50.8)	398 (36.5)	1.00 (Referent)
Ever	1.08 (0.82-1.42, p=0.587)	163 (48.1)	350 (66.5)	1.35 (0.95-1.93, p=0.097)	243 (48.2)	574 (52.7)	1.26 (0.95-1.67, p=0.115)
Missing	-	-	-	-	5 (1.0)	118 (10.8)	0.13 (0.02-0.68, p=0.015)
Aspirin/NSAID use							
Never	1.00 (Referent)	218 (64.3)	238 (45.2)	1.00 (Referent)	233 (46.2)	235 (21.6)	1.00 (Referent)
Ever	2.02 (1.44-2.82, p<0.001)	121 (35.7)	288 (54.8)	1.90 (1.34-2.70, p<0.001)	123 (24.4)	277 (25.4)	1.95 (1.38-2.78, p<0.001)
Missing	-	-	-	-	148 (29.4)	578 (53.0)	2.29 (1.65-3.20, p<0.001)
Heartburn symptoms status							
Absent	1.00 (Referent)	26 (7.7)	81 (15.4)	1.00 (Referent)	34 (6.7)	145 (13.3)	1.00 (Referent)
Present	0.35 (0.22-0.56, p<0.001)	313 (92.3)	445 (84.6)	0.34 (0.19-0.61, p<0.001)	470 (93.3)	684 (62.8)	0.28 (0.17-0.46, p<0.001)
Missing	-	-	-	-	0 (0.0)	261 (23.9)	NE

[a]adjusted for all covariates.
Abbreviation: NE, not estimable.

Table A.9 – Associations of baseline characteristics with risk of BO-ve OAC phenotype compared to reflux controls.

Characteristic	Reflux Controls n (%)	BO-ve OAC n (%)	Univariable model OR (95% CI, p)	Minimally adjusted model[a] OR (95% CI, p)	Fully adjusted model[b] OR (95% CI, p)	Fully adjusted model[b] excluding heartburn OR (95% CI, p)
Age group at diagnosis						
< 50	169 (33.5)	59 (6.7)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
50 - 59	123 (24.4)	184 (20.9)	4.28 (2.96-6.26, p<0.001)	5.03 (3.38-7.57, p<0.001)	4.70 (2.98-7.53, p<0.001)	4.60 (3.00-7.13, p<0.001)
60 - 69	132 (26.2)	317 (36.0)	6.88 (4.83-9.91, p<0.001)	7.94 (5.42-11.76, p<0.001)	7.56 (4.88-11.88, p<0.001)	7.49 (4.98-11.41, p<0.001)
70+	80 (15.9)	320 (36.4)	11.46 (7.85-16.95, $p<10^{-3}$)	13.10 (8.71-19.96, $p<10^{-3}$)	10.28 (6.48-16.59, $p<10^{-3}$)	11.14 (7.23-17.41, $p<10^{-3}$)
Gender						
Female	275 (54.6)	158 (18.0)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Male	229 (45.4)	722 (82.0)	5.49 (4.30-7.03, p<0.001)	6.07 (4.65-7.96, p<0.001)	5.99 (4.42-8.17, p<0.001)	6.07 (4.56-8.13, p<0.001)
BMI group at baseline						
Normal	171 (33.9)	255 (29.0)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Overweight	181 (35.9)	317 (36.0)	1.17 (0.90-1.53, p=0.236)	0.97 (0.71-1.32, p=0.834)	0.97 (0.69-1.36, p=0.865)	0.94 (0.68-1.31, p=0.728)
Obese	129 (25.6)	155 (17.6)	0.81 (0.59-1.09, p=0.163)	0.81 (0.57-1.16, p=0.255)	0.76 (0.51-1.12, p=0.166)	0.77 (0.53-1.12, p=0.175)
Missing	23 (4.6)	153 (17.4)	4.46 (2.81-7.36, p<0.001)	4.20 (2.49-7.36, p<0.001)	2.13 (1.12-4.21, p=0.025)	3.00 (1.65-5.66, p<0.001)
Cigarette smoking status						
Never	256 (50.8)	298 (33.9)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Ever	243 (48.2)	532 (60.5)	1.88 (1.50-2.36, p<0.001)	1.46 (1.12-1.90, p=0.005)	1.85 (1.38-2.48, p<0.001)	1.64 (1.24-2.16, p=0.001)
Missing	5 (1.0)	50 (5.7)	8.59 (3.71-25.00, p<0.001)	9.00 (3.50-28.51, p<0.001)	0.00 (0.00-0.00, p=0.980)	2.25 (0.74-8.01, p=0.174)
Aspirin/NSAID use						
Never	233 (46.2)	161 (18.3)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Ever	123 (24.4)	243 (27.6)	2.86 (2.13-3.85, p<0.001)	2.64 (1.87-3.73, p<0.001)	2.96 (2.06-4.27, p<0.001)	2.64 (1.86-3.77, p<0.001)
Missing	148 (29.4)	476 (54.1)	4.65 (3.55-6.13, p<0.001)	4.63 (3.38-6.39, p<0.001)	3.44 (2.44-4.88, p<0.001)	4.20 (3.04-5.83, p<0.001)
Heartburn symptoms status						
Absent	34 (6.7)	160 (18.2)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	-
Present	470 (93.3)	543 (61.7)	0.25 (0.16-0.36, p<0.001)	0.25 (0.16-0.38, p<0.001)	0.18 (0.11-0.29, p<0.001)	-
Missing	0 (0.0)	177 (20.1)	NE	NE	NE	-

[a]adjusted for age group at diagnosis and gender.
[b]adjusted for all covariates.
Abbreviation: NE, not estimable.

Table A.10 – Sensitivity analyses of the adjusted association of baseline characteristics with risk of BO-ve OAC compared to reflux controls using multiple imputation, complete case analysis and exclusion of OAC cases with a history of undergoing BO surveillance.

Characteristic	Multiple imputation	Complete case analysis			Excluding BO surveillance OAC cases (n=214)		
	Fully adjusted model[a] OR (95% CI, p)	Reflux Controls n (%)	BO-ve OAC n (%)	Fully adjusted model[a] OR (95% CI, p)	Reflux Controls n (%)	BO-ve OAC n (%)	Fully adjusted model[a] OR (95% CI, p)
Age group at diagnosis							
< 50	1.00 (Referent)	113 (33.3)	29 (7.9)	1.00 (Referent)	169 (33.5)	59 (6.9)	1.00 (Referent)
50 - 59	4.74 (3.06-7.35, p<0.001)	75 (22.1)	68 (18.6)	3.71 (2.06-6.81, p<0.001)	123 (24.4)	179 (20.8)	4.56 (2.88-7.31, p<0.001)
60 - 69	7.11 (4.69-10.79, p<0.001)	95 (28.0)	145 (39.6)	5.90 (3.44-10.37, p<0.001)	132 (26.2)	309 (36.0)	7.38 (4.77-11.63, p<0.001)
70+	10.14 (6.50-15.81, p<10^{-3})	56 (16.5)	124 (33.9)	7.14 (4.02-13.00, p<0.001)	80 (15.9)	312 (36.3)	9.84 (6.20-15.90, p<0.001)
Gender							
Female	1.00 (Referent)	189 (55.8)	60 (16.4)	1.00 (Referent)	275 (54.6)	151 (17.6)	1.00 (Referent)
Male	6.27 (4.64-8.47, p<0.001)	150 (44.2)	306 (83.6)	6.54 (4.41-9.84, p<0.001)	229 (45.4)	708 (82.4)	6.19 (4.55-8.48, p<0.001)
BMI group at baseline							
Normal	1.00 (Referent)	125 (36.9)	116 (31.7)	1.00 (Referent)	171 (33.9)	248 (28.9)	1.00 (Referent)
Overweight	0.91 (0.65-1.27, p=0.573)	124 (36.6)	165 (45.1)	1.06 (0.69-1.63, p=0.777)	181 (35.9)	307 (35.7)	0.99 (0.70-1.39, p=0.942)
Obese	0.71 (0.48-1.05, p=0.083)	90 (26.5)	85 (23.2)	0.96 (0.59-1.58, p=0.886)	129 (25.6)	154 (17.9)	0.78 (0.52-1.17, p=0.230)
Missing	-	-	-	-	23 (4.6)	150 (17.5)	2.17 (1.13-4.31, p=0.023)
Cigarette smoking status							
Never	1.00 (Referent)	176 (51.9)	97 (26.5)	1.00 (Referent)	256 (50.8)	291 (33.9)	1.00 (Referent)
Ever	1.52 (1.14-2.02, p=0.004)	163 (48.1)	269 (73.5)	2.24 (1.54-3.27, p<0.001)	243 (48.2)	518 (60.3)	1.84 (1.37-2.48, p<0.001)
Missing	-	-		-	5 (1.0)	50 (5.8)	0.00 (0.00-0.00, p=0.980)
Aspirin/NSAID use							
Never	1.00 (Referent)	218 (64.3)	154 (42.1)	1.00 (Referent)	233 (46.2)	159 (18.5)	1.00 (Referent)
Ever	2.97 (2.04-4.34, p<0.001)	121 (35.7)	212 (57.9)	2.68 (1.85-3.92, p<0.001)	123 (24.4)	235 (27.4)	2.92 (2.03-4.24, p<0.001)
Missing	-	-	-	-	148 (29.4)	465 (54.1)	3.39 (2.40-4.82, p<0.001)
Heartburn symptoms status							
Absent	1.00 (Referent)	26 (7.7)	79 (21.6)	1.00 (Referent)	34 (6.7)	159 (18.5)	1.00 (Referent)
Present	0.20 (0.13-0.33, p<0.001)	313 (92.3)	287 (78.4)	0.23 (0.13-0.41, p<0.001)	470 (93.3)	524 (61.0)	0.18 (0.11-0.28, p<0.001)
Missing	-	-	-	-	0 (0.0)	176 (20.5)	NE

[a]adjusted for all covariates.
Abbreviation: NE, not estimable.

Table A.11 – Associations of baseline characteristics with risk of BO(?) OAC phenotype compared to reflux controls.

Characteristic	Reflux Controls n (%)	BO(?) OAC n (%)	Univariable model OR (95% CI, p)	Minimally adjusted model[a] OR (95% CI, p)	Fully adjusted model[b] OR (95% CI, p)	Fully adjusted model[b] excluding heartburn OR (95% CI, p)
Age group at diagnosis						
< 50	169 (33.5)	75 (7.6)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
50 - 59	123 (24.4)	213 (21.6)	3.90 (2.76-5.57, p<0.001)	4.15 (2.85-6.11, p<0.001)	4.93 (3.18-7.75, p<0.001)	4.17 (2.77-6.34, p<0.001)
60 - 69	132 (26.2)	333 (33.8)	5.68 (4.07-8.01, p<0.001)	6.23 (4.33-9.05, p<0.001)	6.75 (4.41-10.49, p<0.001)	6.18 (4.16-9.27, p<0.001)
70+	80 (15.9)	364 (37.0)	10.25 (7.16-14.84, $p<10^{-3}$)	11.08 (7.51-16.55, $p<10^{-3}$)	11.25 (7.20-17.87, $p<10^{-3}$)	10.89 (7.17-16.75, $p<10^{-3}$)
Gender						
Female	275 (54.6)	161 (16.3)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Male	229 (45.4)	824 (83.7)	6.15 (4.83-7.85, p<0.001)	6.51 (5.02-8.49, p<0.001)	6.88 (5.08-9.39, p<0.001)	6.85 (5.14-9.19, p<0.001)
BMI group at baseline						
Normal	171 (33.9)	248 (25.2)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Overweight	181 (35.9)	307 (31.2)	1.17 (0.89-1.53, p=0.252)	0.89 (0.65-1.23, p=0.486)	0.87 (0.61-1.22, p=0.412)	0.83 (0.60-1.15, p=0.259)
Obese	129 (25.6)	195 (19.8)	1.04 (0.78-1.40, p=0.784)	1.02 (0.72-1.45, p=0.915)	0.94 (0.65-1.38, p=0.770)	0.93 (0.65-1.34, p=0.709)
Missing	23 (4.6)	235 (23.9)	7.05 (4.48-11.54, p<0.001)	7.51 (4.51-13.00, p<0.001)	2.51 (1.34-4.86, p=0.005)	3.98 (2.22-7.44, p<0.001)
Cigarette smoking status						
Never	256 (50.8)	304 (30.9)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Ever	243 (48.2)	562 (57.1)	1.95 (1.56-2.44, p<0.001)	1.60 (1.23-2.07, p<0.001)	1.94 (1.46-2.59, p<0.001)	1.70 (1.29-2.23, p<0.001)
Missing	5 (1.0)	119 (12.1)	20.04 (8.93-57.29, $p<10^{-3}$)	20.15 (8.45-60.18, $p<10^{-3}$)	NE	3.86 (1.37-12.86, p=0.016)
Aspirin/NSAID use						
Never	233 (46.2)	201 (20.4)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Ever	123 (24.4)	263 (26.7)	2.48 (1.87-3.30, p<0.001)	2.07 (1.48-2.89, p<0.001)	2.20 (1.55-3.13, p<0.001)	2.08 (1.48-2.93, p<0.001)
Missing	148 (29.4)	521 (52.9)	4.08 (3.14-5.32, p<0.001)	4.30 (3.17-5.86, p<0.001)	2.53 (1.81-3.55, p<0.001)	3.24 (2.36-4.47, p<0.001)
Heartburn symptoms status						
Absent	34 (6.7)	144 (14.6)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	-
Present	470 (93.3)	599 (60.8)	0.30 (0.20-0.44, p<0.001)	0.33 (0.21-0.50, p<0.001)	0.24 (0.15-0.39, p<0.001)	-
Missing	0 (0.0)	242 (24.6)	NE	NE	NE	-

[a]adjusted for age group at diagnosis and gender.
[b]adjusted for all covariates.
Abbreviation: NE, not estimable.

Table A.12 – Sensitivity analyses of the adjusted association of baseline characteristics with risk of BO(?) OAC compared to reflux controls using multiple imputation, complete case analysis and exclusion of OAC cases with a history of undergoing BO surveillance.

Characteristic	Multiple imputation	Complete case analysis			Excluding BO surveillance OAC cases (n=214)		
	Fully adjusted model[a] OR (95% CI, p)	Reflux Controls n (%)	BO(?) OAC n (%)	Fully adjusted model[a] OR (95% CI, p)	Reflux Controls n (%)	BO(?) OAC n (%)	Fully adjusted model[a] OR (95% CI, p)
Age group at diagnosis							
< 50	1.00 (Referent)	113 (33.3)	27 (6.5)	1.00 (Referent)	169 (33.5)	59 (6.9)	1.00 (Referent)
50 - 59	4.11 (2.74-6.15, p<0.001)	75 (22.1)	91 (21.8)	5.43 (3.06-9.86, p<0.001)	123 (24.4)	179 (20.8)	4.85 (3.09-7.73, p<0.001)
60 - 69	5.84 (3.96-8.63, p<0.001)	95 (28.0)	134 (32.1)	6.27 (3.62-11.15, p<0.001)	132 (26.2)	309 (36.0)	7.15 (4.63-11.24, p<0.001)
70+	9.79 (6.47-14.82, p<0.001)	56 (16.5)	166 (39.7)	10.81 (6.13-19.57, $p<10^{-3}$)	80 (15.9)	312 (36.3)	11.38 (7.22-18.27, $p<10^{-3}$)
Gender							
Female	1.00 (Referent)	189 (55.8)	53 (12.7)	1.00 (Referent)	275 (54.6)	151 (17.6)	1.00 (Referent)
Male	6.25 (4.71-8.28, p<0.001)	150 (44.2)	365 (87.3)	8.40 (5.66-12.67, p<0.001)	229 (45.4)	708 (82.4)	6.86 (5.04-9.41, p<0.001)
BMI group at baseline							
Normal	1.00 (Referent)	125 (36.9)	138 (33.0)	1.00 (Referent)	171 (33.9)	248 (28.9)	1.00 (Referent)
Overweight	0.80 (0.58-1.12, p=0.197)	124 (36.6)	176 (42.1)	0.83 (0.54-1.27, p=0.390)	181 (35.9)	307 (35.7)	0.81 (0.57-1.14, p=0.235)
Obese	0.95 (0.66-1.37, p=0.796)	90 (26.5)	104 (24.9)	0.96 (0.60-1.54, p=0.860)	129 (25.6)	154 (17.9)	0.91 (0.62-1.34, p=0.627)
Missing	-	-	-	-	23 (4.6)	150 (17.5)	2.22 (1.18-4.33, p=0.016)
Cigarette smoking status							
Never	1.00 (Referent)	176 (51.9)	123 (29.4)	1.00 (Referent)	256 (50.8)	291 (33.9)	1.00 (Referent)
Ever	1.63 (1.23-2.15, p=0.001)	163 (48.1)	295 (70.6)	1.93 (1.34-2.79, p<0.001)	243 (48.2)	518 (60.3)	1.94 (1.45-2.61, p<0.001)
Missing	-	-	-	-	5 (1.0)	50 (5.8)	0.00 (0.00-0.00, p=0.981)
Aspirin/NSAID use							
Never	1.00 (Referent)	218 (64.3)	186 (44.5)	1.00 (Referent)	233 (46.2)	159 (18.5)	1.00 (Referent)
Ever	2.18 (1.54-3.07, p<0.001)	121 (35.7)	232 (55.5)	1.96 (1.37-2.84, p<0.001)	123 (24.4)	235 (27.4)	2.09 (1.47-3.00, p<0.001)
Missing	-	-	-	-	148 (29.4)	465 (54.1)	2.39 (1.71-3.37, p<0.001)
Heartburn symptoms status							
Absent	1.00 (Referent)	26 (7.7)	70 (16.7)	1.00 (Referent)	34 (6.7)	159 (18.5)	1.00 (Referent)
Present	0.28 (0.18-0.45, p<0.001)	313 (92.3)	348 (83.3)	0.35 (0.19-0.61, p<0.001)	470 (93.3)	524 (61.0)	0.24 (0.14-0.38, p<0.001)
Missing	-	-	-	-	0 (0.0)	176 (20.5)	NE

[a]adjusted for all covariates.
Abbreviation: NE, not estimable.

Table A.13 – Baseline characteristics of the OAC cases with WGS (n=710), overall and according to phenotype.

Characteristic	BO+ve OAC (n=252)	BO-ve OAC (n=183)	BO(?) OAC (n=275)	Overall (n=710)
Age at diagnosis; *years*				
Mean [SD]	67.2 [9.2]	65.6 [9.5]	67.4 [10.0]	66.9 [9.6]
Median [Q1, Q3]	67.7 [61.4, 74.5]	66.9 [59.3, 72.7]	68.0 [60.4, 75.4]	67.6 [60.4, 74.0]
Age group at diagnosis, n (%)				
< 50 years old	10 (4.0)	10 (5.5)	14 (5.1)	34 (4.8)
50 - 59 years old	44 (17.5)	39 (21.3)	53 (19.3)	136 (19.2)
60 - 69 years old	94 (37.3)	69 (37.7)	86 (31.3)	249 (35.1)
70+ years old	104 (41.3)	65 (35.5)	122 (44.3)	288 (40.6)
Gender, n (%)				
Female	33 (13.1)	27 (14.8)	47 (17.1)	107 (15.0)
Male	219 (86.9)	156 (85.2)	228 (82.9)	603 (84.9)
Ethnicity, n (%)				
White	241 (100)	175 (99.4)	266 (99.3)	682 (99.6)
Other	0 (0)	1 (0.6)	2 (0.7)	3 (0.4)
Missing	11 (4.4)	7 (3.8)	7 (2.5)	25 (3.5)
BMI group at diagnosis, n (%)				
Underweight	0 (0)	2 (1.27)	10 (4.88)	12 (2.11)
Normal	59 (28.6)	47 (29.7)	59 (28.8)	165 (29.0)
Overweight	77 (37.4)	70 (44.3)	81 (39.5)	228 (40.1)
Obese	70 (34.0)	39 (24.7)	55 (26.8)	164 (28.8)
Missing	46 (18.3)	25 (13.7)	70 (25.5)	141 (19.9)
Cigarette smoking status, n (%)				
Never	88 (36.8)	60 (33.9)	86 (33.7)	234 (34.9)
Ever	151 (63.2)	117 (66.1)	169 (66.3)	437 (65.1)
Missing	13 (5.2)	6 (3.3)	20 (7.3)	39 (5.5)
Aspirin/NSAID use, n (%)				
Never	56 (42.4)	38 (44.7)	46 (43.8)	140 (43.5)
Ever	76 (57.6)	47 (55.3)	59 (56.2)	182 (56.5)
Missing	120 (47.6)	98 (53.6)	170 (61.8)	388 (54.6)
Heartburn symptoms status, n (%)				
Absent	35 (17.2)	34 (24.1)	47 (22.8)	116 (21.1)
Present	169 (82.8)	107 (75.9)	159 (77.2)	435 (78.9)
Missing	48 (19.0)	42 (23.0)	69 (25.1)	159 (22.4)
Total alcohol intake/week; *units*				
Mean [SD]	22.8 [32.6]	22.1 [17.1]	29.2 [51.4]	24.7 [36.7]
Median [Q1, Q3]	15.0 [4.75, 22.5]	20.0 [9.00, 28.0]	18.0 [6.00, 35.0]	18.0 [7.00, 30.0]
Missing	200 (79.4)	138 (75.4)	226 (82.2)	564 (79.4)
TNM, n (%)				
I	62 (28.4)	21 (13.2)	12 (11.8)	95 (19.8)
II	54 (24.8)	31 (19.5)	23 (22.5)	108 (22.5)
III	98 (45.0)	97 (61.0)	66 (64.7)	261 (54.5)
IV	4 (1.83)	10 (6.29)	1 (0.980)	15 (3.13)
Missing	34 (13.5)	24 (13.1)	173 (62.9)	231 (32.5)

Appendix B

List of publications

Publications related to my PhD:

Zamani, S. A., Killcoyne, S., Abbas, S., Secrier, M., Ng, A. W. T., Devonshire, G., Cheah, C., Grehan, N, Nutzinger, B., Redmond, A. M., Freeman, A., Coleman, H. G., the Oesophageal Cancer Clinical and Molecular Stratification (OCCAMS) Consortium, and Fitzgerald, R. C. (2023). Epidemiological and molecular similarity between prognostic phenotypes of oesophageal adenocarcinoma. Manuscript in preparation.

Abbas, S., Pich, O., Devonshire, G., **Zamani, S. A.**, Katz-Summercorn, A., Killcoyne, S., Cheah, C., Nutzinger, B., Lopez-Bigas, N., Fitzgerald, R. C, and Secrier, M. (2023). Mutational processes unveil bottlenecks that shape the evolution of oesophageal adenocarcinoma. Manuscript in revision.

Ng, A. W. T., McClurg, Wesley, B., **Zamani, S. A.**, Miremadi, A., Giger, O., ten Hoopen, R., Devonshire, G., Redmond, A. M., Grehan, N., Blasko, A. G., Li, X., Tavaré, S., the Oesophageal Cancer Clinical and Molecular Stratification (OCCAMS) Consortium, and Fitzgerald, R. C. (2023). Disentangling oncogenic amplicons in oesophageal adenocarcinoma. Manuscript in preparation.

Tan, W. K., Maroni, R., Offman, J., di Pietro, M., **Zamani, S. A.**, Sasieni, P. D., and

Fitzgerald, R. C. (2023). Modelling from a multicentre, pragmatic, randomised controlled trial in a reflux population identifies the target population for non-endoscopic screening for Barrett's oesophagus and oesophageal cancer. Manuscript in preparation.

Sawas, T.,* **Zamani, S. A.,*** Killcoyne, S., Dullea, A., Wang, K. K., Iyer, P. G. , Fitzgerald, R. C., and Katzka, D. A. (2022). Limitations of heartburn and other societies' criteria in Barrett's screening for detecting de novo esophageal adenocarcinoma. *Clinical Gastroenterology and Hepatology*, 20(8), 1709-1718. **Denotes equal contribution.*

Publications related to external work:

Zamani, S. A., Jones, G. S., McClain, K. M., Graubard, B. I., Liao, L. M., Zhang, X., McGlynn, K. A., and Petrick, J. L. (2023). Dietary polyunsaturated fat intake in relation to liver cancer incidence in the NIH-AARP Diet and Health Study. Manuscript in preparation.

Zamani, S. A., Ramírez, Y., Hyer, M., Graubard, B. I., Petrick, J. L., McGlynn, K. A. (2023). Use of non-statin cholesterol-lowering medications in relation to risk of primary liver cancer in the Clinical Practice Research Datalink. Manuscript in preparation.

Lacson J. C. A.,* **Zamani S. A.,*** Froes L. A. R., Mitra N., Qian L., Doyle S.H., Azizi E., Balestrini C., Bishop D. T., Bruno W., Carlos-Ortega B., Cuellar F., Cust A. E., Elder D. E., Gerdes A. M., Ghiorzo P., GrazziotinT. C., Gruis N. A., Hansson J., Hočevar M., Höiom V., Holland E. A., Ingvar C., Landman G., Larre-Borges A., Mann G. J., Molgo M., Moredo L. F., Olsson H., Out-Luiting J. J., Perić B., Pjanova D., Puig S., Salas-Alanis J., Schmid H., Wadt K. A. W., Newton-Bishop J. A., and Kanetsky P. A. (2021). Birth cohort-specific trends of sun-related behaviors among individuals from an international consortium of melanoma-prone families. *BMC Public Health*, 21(1): 692-707. **Denotes equal contribution*.