# STATINS AND METFORMIN IN DIGESTIVE TRACT CANCERS

Pattern of use and its association with disease progression

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## SCIENTIFIC CURRICULUM VITAE

### Publications

- **Lacroix, O.**, Couttenier, A., Vaes, E., Cardwell, C. R., De Schutter, H., & Robert, A. (2018). Impact of metformin on gastric adenocarcinoma survival: A Belgian population-based study. *Cancer epidemiology*, 53, 149-155.

- **Lacroix, O.,** Couttenier, A., Vaes, E., Cardwell, C. R., De Schutter, H., & Robert, A. (2019). Statin use after diagnosis is associated with an increased survival in esophageal cancer patients: a Belgian population-based study. *Cancer Causes & Control*, 30(4), 385-393.

- Couttenier, A., **Lacroix, O.**, Silversmit, G., Vaes, E., De Schutter, H., & Robert, A. (2019). Beta-blocker use and mortality following ovarian cancer diagnosis: a population-based study. *Cancer epidemiology*, *62*, 101579.

- Couttenier, A., **Lacroix, O.**, Vaes, E., Cardwell, C. R., De Schutter, H., & Robert, A. (2017). Statin use is associated with improved survival in ovarian cancer: A retrospective population-based study. *PloS one*, *12*(12), e0189233.

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- Couttenier, A., **Lacroix, O.**, Vaes, E., De Schutter, H., & Robert, A. "Statin use is associated with improved survival in ovarian cancer: a retrospective population-based study" GRELL Ascension Reunion, 2018. Trento (Italy)

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- Lacroix, O., Couttenier, A., Vaes, E., De Schutter, H., & Robert, A. "Is statin use associated with an increased survival after esophageal cancer diagnosis? A Belgian population-based study" GRELL Ascension Reunion, 2018. Trento (Italy)

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*“What is success?*

*To laugh often and much; to win the respect of intelligent people and the affection of children; to earn the appreciation of honest critics and endure the betrayal of false friends; to appreciate the beauty; to find the best in others; to leave the world a bit better, whether by a healthy child, a garden patch or a redeemed social condition; to know even one life has breathed easier because you have lived. This is to have succeeded!”*

Ralph Waldo Emerson

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SUMMARY

With the epidemiological transition and the decreasing mortality from cardiovascular diseases, the incidence of cancers has increased worldwide. As a consequence, cancer is expected to become the leading cause of death and the most important barrier to life expectancy. In the future, it is estimated that the major burden of cancers will affect mainly middle and low-income countries. Accessible, affordable and high-quality treatments for all patients with cancer are therefore needed.

Digestive tract cancers are a heterogeneous group of cancers among the most commonly diagnosed ones. Upper gastrointestinal cancers are generally associated with poor survival, whereas lower digestive tract cancers showed better prognosis.

Metformin and statins are among the most commonly prescribed drugs for chronic condition management. Metformin and statins are the first-line treatments for type II diabetes and dyslipidaemia, respectively. These two drugs represent affordable treatments, widely available.

Preclinical evidence had suggested that cancer outcomes might be impacted by these two treatments. More specifically, experimental studies had shown that metformin could reduce gastric cancer cell proliferation and potentiate the action of some cancer treatments such as cisplatin. Other preclinical studies had shown that statins inhibit oesophageal cancer cell proliferation and increase their apoptosis.

Epidemiological studies evaluating the association between the use of these two drugs and digestive tract cancer progression have reported similar results but were however limited in their number or were subject to missing important prognostic factors such as cancer stage.

Aiming to investigate these two associations at the Belgian population level, the Belgian cancer registry database coupled with several other databases was used in the present research. The Belgian permanent sample was also used to access a Belgian sample of individuals without cancer, for comparison purposes.

**Sections 6 and 7** present the main findings of our research project, in assessing oesophageal cancer and gastric adenocarcinoma survival in association with statins and metformin, respectively.

In esophageal cancer patients (**Section 6**), statins use was associated with a significant 13% reduction in mortality from esophageal cancer (adjusted HR=0.87, 95%CI [0.78-0.97]). Considering all-cause mortality, statins use was associated with a significant 16% reduction in deaths (adjusted HR=0.87, 95%CI [0.78-0.97]). After

conducting several sensitivity analyses, no significant variation was observed in these findings, there was however no gradient in the association.

In diabetic patients diagnosed with gastric adenocarcinoma (**Section 7**), metformin use was associated with a bigger reduction in all-cause mortality (adjusted HR= 0.73, 95%CI [0.52-1.01]) compared to gastric cancer-specific mortality (adjusted HR=0.86, 95%CI [0.56-1.33]). The results of the analyses were however sensitive to changes in the sample size depending on the type of sensitivity analysis conducted. Also, the association between metformin use and gastric adenocarcinoma mortality didn't show any gradient.

The above-mentioned results need however to be discussed and interpreted with the results of Sections 4 and 5.

In **section 4**, the pattern of statins and metformin use have been presented in patients diagnosed with gastrointestinal tract cancer. Comparisons were made with individuals without cancer in the Belgian permanent sample.

Despite their cancer diagnosis, patients with digestive tract cancers were characterized by a higher volume of metformin and statin prescriptions, particularly at a younger age. Initiation of those two drugs increased before diagnosis and reached a peak in the first following month. This increase was mainly driven by contact with hospital health care providers.

In **section 5**, an estimation of digestive tract cancer patients' adherence to metformin and statins was calculated.

Non-adherence to both medications was common in patients with digestive tract cancer and adherence declined following the cancer diagnosis. Non-adherence was higher in patients using metformin than in patients using statins (74% versus 46%, respectively). Patients diagnosed with colorectal cancer were the biggest adherers compare to others. Non-adherence was associated with increasing age, female patients, initiation of the drug after diagnosis, and the occurrence of surgery.

Among statins users, those having comorbidities such as cardiovascular diseases or diabetes had a higher adherence. In diabetic patients using metformin, radiotherapy was associated with lower adherence.

Based on our results, none of the two studied medications were associated with poorer outcomes in the two studied digestive tract cancers. For the management of the chronic conditions they have been approved for, metformin and statins could continue to be administered to patients who can benefit from their administration. However, there is also a need to study the quality of life of patients with digestive tract cancer taking chronic medications such as statins and metformin.

Those encouraging results, cannot be used to establish a causal association between metformin and statins consumption and the observed cancer outcome. More studies are needed, specifically to assess the specificity of the association by considering important confounders such as BMI, socio-economic status and consumption of alcohol and tobacco. To further discuss the results from prescription volumes, drug adherence and dose-response analyses, studies evaluating the agreement between the measure of the daily defined dose (DDD) and the daily prescribed drug in Belgium are required.

LIST OF ABBREVIATIONS

| | |
|---|---|
| **A** | |
| **AMP** | Adenosine monophosphate |
| **AMPK** | AMP-activated protein kinase |
| **APC** | Adenomatous polyposis coli |
| **ATC** | Anatomical Therapeutic Chemical classification |
| **ATP** | Adenosine triphosphate |
| **B** | |
| **BC** | Before Christ |
| **Bcl-2** | B-cell lymphoma-2 |
| **BCR** | Belgian cancer registry |
| **BWK** | Belgian Work against Cancer |
| **C** | |
| **Cag-PAI** | Cytotoxin-associated gene A pathogenicity island |
| **CBSS** | Crossroad bank for social security |
| **CDH1** | Cadherin-1 |
| **CI** | Confidence interval |
| **CIN** | Chromosomally unstable |
| **CROSS** | Chemotherapy for oesophageal cancer followed by surgery |
| **CSC** | Cancer stem cell |

**D**

| | |
|---|---|
| **DDD** | Defined daily dose |
| **DNA** | Deoxyribonucleic acid |

**E**

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|---|---|
| **EBV** | Epstein-Barr Virus |
| **ECF** | Epirubicin, cisplatin and 5-Fluorouracil |
| **ECX** | Epirubicin, cisplatin and capecitabine |
| **EMT** | Epithelial-mesenchymal transition |
| **EPS** | Echantillon Permanent(e) Steekproef |

**F**

| | |
|---|---|
| **FLOT** | Fluorouracil, leucovorin, oxaliplatine, docetaxel |
| **FPP** | Farnesyl pyrophosphate |
| **FU** | Fluorouracil |

**G**

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|---|---|
| **GGPP** | Geranylgeranyl pyrophosphate |
| **GS** | Genomically stable |
| **GTPases** | Guanosine triphosphatases |

**H**

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|---|---|
| **HDL** | High-density lipoprotein |
| **HER** | Epidermal growth receptor |

| | |
|---|---|
| **HMG-COA** | 3-hydroxy-3-methylglutaryl coenzyme A |
| **HR** | Hazard rates ratio |

**I**

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|---|---|
| **IMA** | Intermutualistic agency |
| **IARC** | International agency for research on cancer |

**K**

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|---|---|
| **KM** | Kaplan-Meier |

**L**

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|---|---|
| **LDL** | low-density lipoprotein |

**M**

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|---|---|
| **MAGIC study** | Medical research council adjuvant gastric infusional chemotherapy study |
| **MAPK** | Ras/Raf/Mitogen activated protein kinase |
| **MPRm** | Medication possession ratio modified |
| **MSI** | Microsatellite unstable |
| **mTOR** | Mammalian target of rapamycin |

**N**

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|---|---|
| **NCR** | National Cancer Registry |

**O**

| | |
|---|---|
| **OR** | Odds ratio |

| | |
|---|---|
| **P** | |
| **p-EGFR** | Phosphorylated epidermal growth factor receptor |
| **PET** | Positron emission tomography |
| **p-IGF-1R** | Phosphorylated insulin-like growth factor-1 receptor |
| **S** | |
| **SSIN** | Social security identification number |
| **STK-11** | Serine threonine kinase 11 |
| **SAMS** | Statins-associated muscle symptoms |
| **SToP** | Stomach cancer pooling |
| **T** | |
| **TP53** | Tumour protein 53 |
| **V** | |
| **VLVD** | Very low-density lipoprotein |
| **W** | |
| **WHO** | World Health Organisation |
| **WSR** | World Standardised Rates |

## 1.1 CANCER

« *We tend to think of cancer as a "modern" illness because its metaphors are so modern. It is a disease of overproduction, of fulminant growth—growth unstoppable, growth tipped into the abyss of no control* »

Siddhartha Mukherjee,
**The emperor of all maladies: a biography of cancer**

Cancer is probably one of the oldest diseases in humans with paleo-pathologists indicating that tumours were already present in prehistoric animals, before the apparition of men on earth.[1]

The Edwin Smith papyrus written 3000 years BC contained the first written trace of cancer and more specifically a breast tumour.[1] More than 1500 years later, the Ebers papyrus referred to possible tumours of stomach and colon.[1]

The term cancer itself was derived from the Greek 'karkinos' (*Καρκινός*) that means "crab" and was first given by Hippocrates (460-375 BC) who compared cancer growth to a moving crab.[1] At the time of Hippocrates cancer was thought to occur from an imbalance in body secretions called humours, particularly at an advanced age.[1]

It is only after the spreading of microscope use and the establishment of the cell theory in 1838, that Rudolph Virchow (1821-1902) with its popular "*omnis cellula e cellul*a" (i.e. each cell derived from an existing cell ) put an end to the humoral theory in oncology.[2]

Nowadays, cancers are known to be a "heterogeneous group of diseases with common underlying pathology characterized by uncontrolled cellular growth and division".[3] It is also recognized that this disease is caused by the accumulation of alterations in signalling pathways that control cell metabolism and proliferation.[4] In humans, there are more than 200 different types of cancers and they can either be benign (*i.e.* circumscribed and confined to the primary site of origin) or malignant (*i.e.* able to invade and metastasize) .[3,5] Cancers can be inherited (approximately 10% of all cancers) or sporadically acquired through spontaneous mutation of the DNA in genes.

It is known that genes involved in inherited cancers can also be mutated in sporadic cancers; however, the opposite is not true. Inherited cancers tend to appear mostly at a younger age and are often bilateral while sporadic cancers affects older patients and are mostly unilateral. [3]

### 1.1.1 The eight Hallmarks of a cancer

A tumour of approximately 1 cm of diameter is resulting from the proliferation of one to $10^9$ aberrant cells.[4] In order to achieve and maintain this characteristic, cancer cells must have some capabilities enabling them to be more than insular masses. Thus, during the tumour development, succession of genetic alterations confers eight hallmarks that are essential to the tumour growth (**Figure 1 and Figure 2)**. [6]

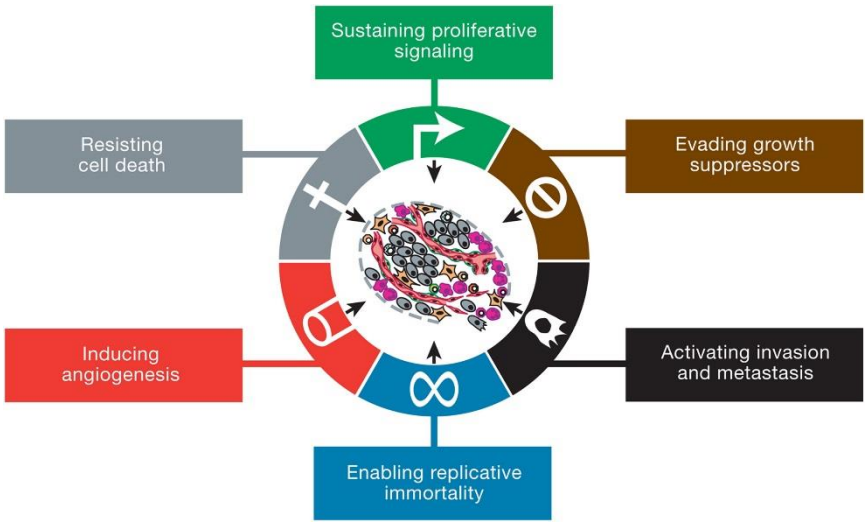



**Figure 1**. The Hallmarks of a cancer (Hanahan and Weinberg, 2011)[6]

**Sustaining proliferative signalling**

The capacity of cancer cells to deregulate growth-promoting signals is one of the fundamental traits of a cancer.

In normal tissues, proliferative signals, mainly transmitted by growth factors like insulin growth factor (IGF), are carefully controlled and homeostasis is maintained.

In cancer cells however, these signals are activated and/or sustained by different yet ingenious mechanisms. For example, some tumours produce growth factors by themselves, or may trigger normal cells to produce growth factors.[6]

Some cancer cells also have the capability to activate downstream components of signalling pathways of the growth factor receptors that will make them independent of growth factor stimulation. This downstream activation can occur after somatic mutation for example.[6] Once they have activated their proliferative signal, the inactivation of negative feedback loops is induced, as another way to sustain their uncontrolled growth.[6]

The mutation of some genes involved in cell growth and known as proto-oncogenes leads to the creation of oncogenes. Mutations in proto-oncogenes are dominant, which means that only one copy of the gene need to be mutated to activate oncogenes. Oncogenes have been associated with growth factors, with growth factor receptors and also with intracellular growth signalling coding.

**Evading growth suppressors**

To elude mechanisms that negatively regulate cells proliferation is another fundamental trait of a cancer.

In normal tissues, some genes like tumour protein 53 (*TP53)* are known to be involved in the recognition of DNA damages and/or the control of cell-division process. Those genes are called tumour suppressor genes and Alfred G. Knudson (1922-2016), an American paediatrician who was studying retinoblastoma, discovered their role in 1975. [3,6]

Knudson proposed his two-hit model where he mathematically demonstrated that in order to be inactivated, both alleles of tumour suppressor genes need to be mutated.[3,7] This inactivation can occur when an inherited defective copy of the gene is transmitted by a parent (the first hit) and a sporadic mutation occurs in the second copy of the gene (the second hit). Those inherited cancers occur in younger patients because only one sporadic mutation is necessary to inactivate the genes. He demonstrated that the same genes can also be mutated in sporadic cancer, however two mutations are necessary, and this explains why those cancers appeared at a later age.

Other mechanisms like contact inhibition that ensure the creation of confluent cell monolayers, can also be corrupted in cancer tissues through the liver kinase B1 (LKB1) inhibition for example.[6]

LKB1 also known as serine/threonine kinase 11 (STK-11) is a tumour suppressor gene involved in intracellular energy homeostasis and in cell polarity regulation.[8] Inactivation of LKB1, among other things, will be associated with cell polarity disorders which will facilitate invasion and metastasis.[8] Mutated LKB1 is associated with Peutz-Jeghers syndrome, a tumour susceptibility syndrome characterized by the presence of multiple polyps predominantly in the gastrointestinal (GI) tract.[3] This syndrome is characterized among others things by an increased risk of gastrointestinal tract cancers.[3]

**Resisting cell death**

Cell death can occur through several mechanisms and can prevent cancer development. Therefore, cell death can be used for cancer treatment, but it can also be used by cancer cells to proliferate.

The first cell death mechanism is called apoptosis or programmed cell death and it is induced when DNA damage or insufficient survival factors are detected in a cell. In normal tissues, apoptosis occurs through the action of tumour suppressor genes (like TP53) or pro-apoptotic proteins.[6] Apoptosis is one of the most important barriers to cancer progression.

In cancer tissues, as mentioned in the previous section, tumour suppressor genes can lose their function because of mutation. This is a way for tumours to evade apoptosis. A different way to evade this cell death process is by upregulating anti-apoptotic signals or by down-regulating pro-apoptotic signals.[6] For example, the anti-apoptotic proteins of the B-cell lymphoma-2 (Bcl-2) have been shown to be upregulated in over half of all cancer types.[9]

The second mechanism by which cell death can occur is autophagy. During this process, cells can break some small organelles into small catabolites that are then reused for example in case of nutrient deficiency.

In some cases, inhibition of autophagy is associated with the development of more cancers, suggesting that autophagy plays a role in the prevention of cancer.[6] However, in some cancers, autophagy is used by tumour cells to shrink into a state of reversible dormancy that could potentially explain cancer relapse.[6]

The third mechanism causing cell death is necrosis. During necrosis, cells explode and release their content into the extracellular environment. This release creates an inflammatory response that can lead to proliferation of neighbouring cells.[6]

**Enabling replicative immortality**

Normal cells can pass through a limited number of cell replications mainly because of the limited length of their telomeres. Telomeres are a repetition of a complex of hexanucleotide (TTAGGG) at the end of chromosomal DNA.[3] They have the ability to protect this region of DNA from fusions that could be dangerous for the cell.[3,6]

In normal cells, telomeres shorten progressively as the cell replicates, leading to a state of senescence or crisis in which the cell either is maintained into a non-proliferative state or dies.

Telomerase is an enzyme specialized in adding telomeres segment to the end of chromosomal DNA in some situations. High activity of telomerase is seen in embryonic stage, in pluripotent stem cells but also in human cancer cells. This phenomenon is associated with a resistance to both senescence and crisis or apoptosis. [6]

**Inducing angiogenesis**

Angiogenesis is the process in which new vessels are created, sprouting from an existing one. Normally in adults, this process occurs in specific situations, *e.g.* after an injury or in women during their menstrual cycle. At another time, angiogenesis is shut down.

In cancers, even if some tumours are hypovascularized, most tumours display a hypervascularisation. This hypervascularisation results from the activation of angiogenesis that allows the tumour to acquire nutrients and oxygen and to expand.[6]

The blood vessels resulting from this process are however, like their neighbouring cells aberrant.[6] They are characterised by "precocious capillary sprouting, convoluted and excessive vessel branching, distorted and enlarged vessels, erratic blood flow, microhaemorrhage, leakiness, and abnormal levels of endothelial cell proliferation and apoptosis". [6]

**Activating invasion and metastasis**

Metastasis is one of the specific traits of malignant tumour and approximately 90% of all cancers deaths are caused by metastasis.[3,5]

Metastasis can be defined as "the dissemination of cancer cells from primary tumours and their subsequent seeding […] in distant tissues" whereas invasion, refers to the "local invasion of primary tumour cells into surrounding tissues".[5]

The so-called invasion-metastasis cascade begins with invasion, followed by intravasation that is the passage of primary tumour cells into the circulatory system.[5,6] Once the cells break through the circulatory system and manage to survive, they can go through extravasation, or the passage through vascular wall into the parenchyma of distant tissue.[5,6] The cascade is then finalized by the proliferation of those cells in the final tissue (*a.k.a.* the colonization).[5,6]

During the carcinogenesis, tumour cells acquired some characteristics that allow them to circulate into the body. For example, some tumours exhibit a downregulation or an inactivation of E-cadherin. This protein is known to be a major actor of cellular adhesion and therefore it downregulation allows cancerous cells to acquire the mobility they need in the invasion-metastasis cascade .[6]

The main process that allows epithelial cells to acquire those characteristics is called the epithelial-mesenchymal transition (EMT). This process will transform epithelial cells through the activation of some transcription factors such as Snail, Slug, Twist and Zeb1 into mesenchymal cells able to disseminate and to resist apoptosis.[5,6]

Also, during the EMT step, some tumours will acquire the ability to become cancer stem cells (CSCs). This sub-population of cancerous cells is undifferentiated and such cells have the ability to initiate tumours in other parts of the body.[5]

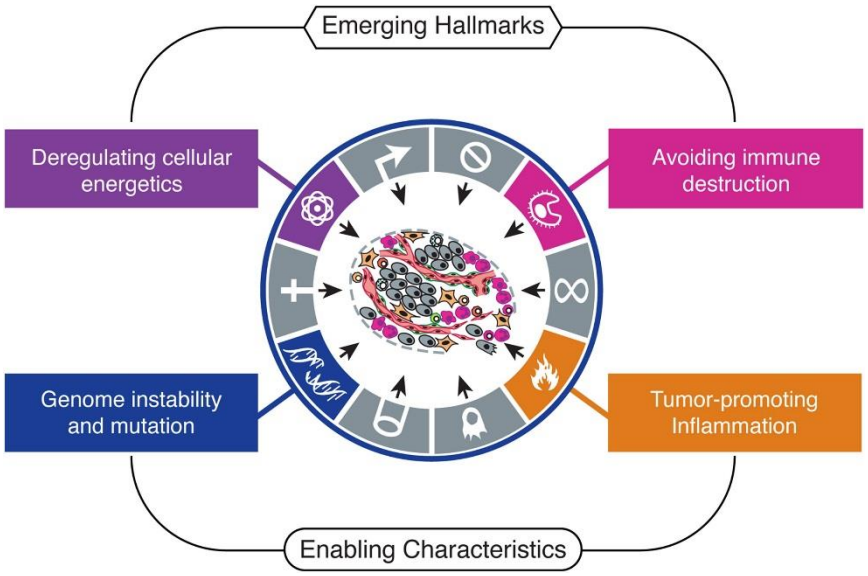



**Figure 2**. Emerging Hallmarks (Hanahan and Weinberg, 2011)[6]

**Emerging hallmarks: Deregulating cellular energetics**

In order to proliferate, cancer cells need to adapt their metabolism to fuel their proliferative state.

An interesting example of this adaptation is the modification of the glycolysis in cancer cells.

In normal cells and in aerobic conditions, glucose is first catabolised through glycolysis into pyruvate in the cytosol. Then, the pyruvate is used in the mitochondria through the Krebs cycle and the electron transport chain.[6] The result of this glucose catabolism is a high production of energy and a low rate of lactate production.[6]

In anaerobic conditions, the first part of the glucose catabolism is the same. However, instead of using pyruvate into the mitochondria, a high quantity of lactate will be produced and exported outside of the cell.[6]

In cancerous cells and even in presence of oxygen, the cell will proceed to an anaerobic glycolysis. This is called the Warburg effect.[6] The produced lactate can also be used by other tumour cells.

As the energy yielded in anaerobic glycolysis is low, cancerous cells will compensate by increasing their glucose import, for example by upregulating glucose transporters like GLUT1.[6] Thus in cancers, the tumour will be characterised by a high consumption of glucose. This is the theory behind positron emission tomography (PET) where F-fluorodeoxyglucose (FDG), a radiolabelled analogue of glucose, is administered to patients in order to visualize high metabolic activity of tumours.

**Emerging hallmarks: Avoid immune destruction**

An emerging tumour hallmark that needs to be further investigated is the ability of tumours to escape immune surveillance.[6] Indeed, it has been shown trough experimentation in mice and clinical epidemiology that immunity plays a non-negligible role in oncogenesis.[6]

First, immunocompromised mice have been shown to develop more frequently and more rapidly tumours.[6] In addition, tumours from immunocompromised mice transplanted to immunocompetent syngeneic hosts were unable to metastase.[6]

Second, the incidence of virus-induced cancers is increased in immunocompromised patients.[6] In immunocompromised patients who have been transplanted, cancers originated from apparently healthy donors have been able to develop in the transplanted organ. [6]

### 1.1.2 Epidemiology of cancer

#### *1.1.2.1 Incidence*

As pointed out in previous sections, cancer is a genetic disease and a disease of aging.

Between 1980 and 2015, worldwide life expectancy has increased from about 62 years to 72 years old.[10] This increase in life expectancy is associated with a greater number of older people in populations and it is one of the hallmarks of the epidemiological transition.[11] Through this transition, and also because of a decreasing mortality from cardiovascular diseases, incidence of cancers has increased worldwide.[12]

In 2018, it was estimated that there was 18 million new cases of cancers worldwide.[12] Lung cancer was the most commonly diagnosed cancer followed by breast cancer, prostate cancer, and colorectal cancer.[12]

In future, the number of diagnosed cancers will continue to increase; with a 50% increase between 2012 and 2030, its incidence is projected to reach 21.6 million.[13]

Regionally, depending on the socioeconomic development of countries, differences in cancer incidence still persist even if in the long run they are set to disappear (**Figure 3**).[12]

In developed countries, the incidence of most tumours is either stabilized or decreased, depending on the aetiology of cancer and the extent to which early detection and screening are used.[3] Associated with cancer screening, over-diagnosis tends to increase the burden of some cancers with longer latency periods (*e.g.* prostate cancer, thyroid cancer).

It has been projected that in future, a major burden of cancer will affect mainly middle and low-income countries, as their currently young population will experience an increase in life expectancy.[3] An aging of population combined with a greater exposure to certain lifestyle risk factors like cigarette smoking, physical inactivity or poor diet will result in an increase in cancer incidence for those countries.[3,12] A cancer transition will occur during which infection-associated cancers like cervix, stomach or liver cancers will decrease and will give way to cancers associated with the "westernization" of lifestyle.[12]

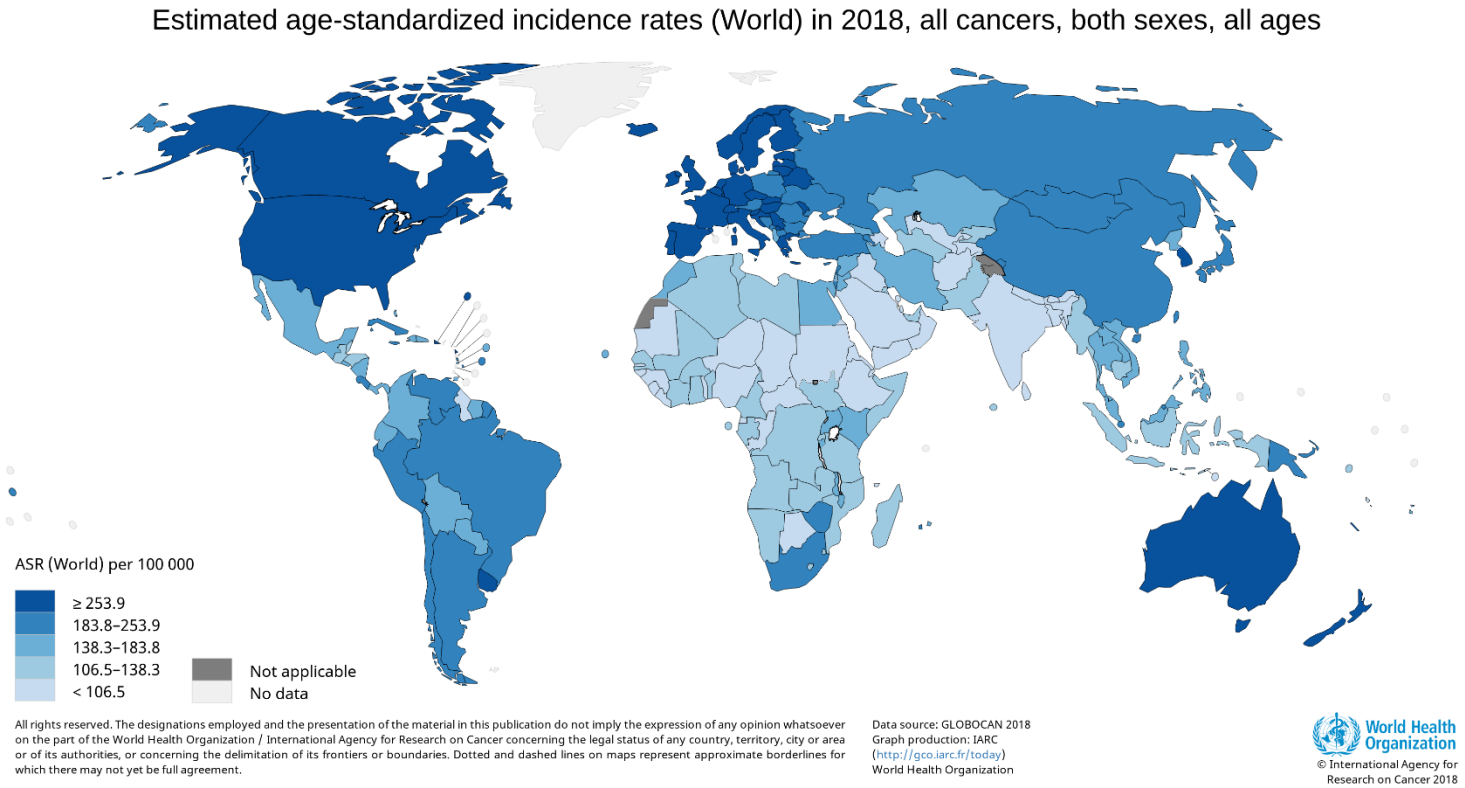



**Figure 3.** Worldwide estimated age-standardized incidence rates in 2018 for all cancers. (source: IARC)[14]

#### *1.1.2.2 Mortality*

Cancer is expected to become the leading cause of death worldwide and the most important barrier to life expectancy. [12]

In 2015, non-communicable diseases were the leading cause of death worldwide representing 71% of all deaths.[15] Cancers were, behind cardiovascular deaths, the second leading cause of death worldwide in that category.

In 2018 worldwide, it was estimated that there were approximately 9.6 million cancer deaths. Asia accounted for more than half of the cases whereas Europe, America and Africa represented 20%, 14% and 7% of cancer deaths, respectively (**Figure 4**).[12] The bigger percentage of cancer deaths in Asia was linked to the higher number of people living there.[12]

Lung cancer was the first cause of death by cancer in both sexes followed by colorectal, stomach and liver cancer.[12]

If over-diagnosis affects mainly developed countries and contributes to increase their cancer burden, under-diagnosis is a problem of low and middle income countries where limited medical resources and limited access to health care contribute to increase cancer mortality.[3]

There are differences in cancer incidence between countries. However, globally there is a low variation in cancer mortality (**Figure 4**).[3,12] In low-or middle income countries, cancers are generally associated with a high fatality rate even if there are less cancer diagnosed.[3,12]

Improvement in cancer mortality is associated with both a reduction in cancer incidence and an improvement in cancer survival.[12,13]

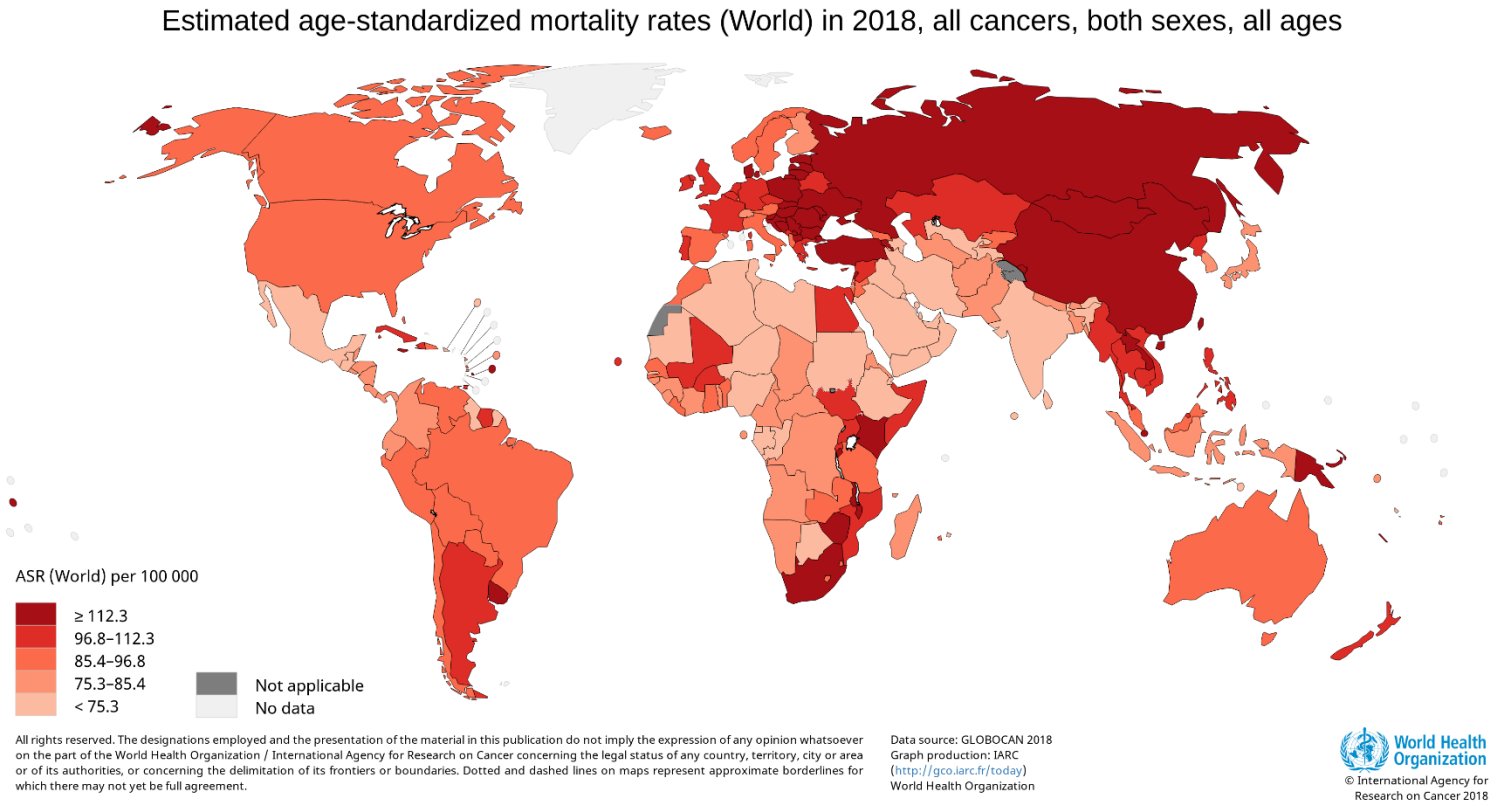



**Figure 4.** Worldwide estimated age-standardized mortality rates in 2018 for all cancers. (source: IARC)[16]

## 1.2 DIGESTIVE TRACT CANCERS

*"All Disease Begins in The Gut."*

Hippocrates

### 1.2.1 The digestive tract

The digestive tract is a single continuous tube divided in a series of hollow organs running from the mouth to the anus.[17] Each organ is delimited by a series of sphincters from the lower oesophageal sphincter to the anal sphincter.[17]

Associated with other organs like the liver and the pancreas, it constitutes the digestive system. It normally includes the mouth, the oesophagus, the stomach and the intestines.

The digestive tract represents the largest surface in the human body (ca. 400m²) far ahead of the skin and the lungs.[18] Considered as the external part of the body, it is in direct contact with all agents orally ingested.

The main functions of the digestive tract are absorption of nutrients and excretion.[19] In addition, the digestive tract also plays a role in blood glucose, water and electrolyte regulation and in immunological defense.[17]

### 1.2.2 Cancer of the digestive tract

In 2018, worldwide, digestive tract cancers such as oesophagus, stomach, colon and rectum accounted for more than 20% of all cancers and deaths by cancer.

Europe-wide, according to the latest estimates, the majority of those cancers were still associated with a poor prognosis, with a 5-year relative survival ranging from 57% in colon cancers, 25% in stomach cancers to 12% in oesophageal cancers. [20,21]

In Belgium in 2017, digestive tract cancers accounted for approximately 15% of all cancers diagnosed.[22] The majority of the cases were localised in the colon (n=5.795, 52%), followed by rectum (n=2.246, 20%), oesophagus (n=1.516, 14%) and stomach (n=932, 8%).[23] In the same year, the 5-years relative survival associated with those cancers ranged from 25% for oesophageal cancers, 40% for stomach cancers to more than 70% for colorectal cancers. [23] In Europe, Belgium has been consistently reported to be among the countries with the best survival for those cancers.[13,20,21] It seems that lifestyle and risk factors differences such as smoking behaviour, co-morbidities, cancer stage and/or subtype could partially explain these differences.[20]

In the present work, the focus has been laid on oesophageal and stomach cancers, two digestive tract cancers among the most commonly diagnosed that have a poor survival. On the opposite, colorectal cancer is characterised by a relatively good prognosis and have been extensively studied in numerous papers. Therefore, it has not been studied in the present work.

### *1.2.2.1 Oesophageal cancer*

Oesophageal cancer was first described by Avenzoar (1070-1162) , an Arab physician who practiced in Spain and who died himself from a cancer when he was 92 years old.[1] He described oesophageal symptoms as beginning with "mild pain and difficulty to swallow".[24] This is still, with involuntary weight loss, one of the few symptoms of this cancer. Unspecific symptoms are the reason why oesophageal tumours are often diagnosed at an advanced stage, contributing also to its poor relative survival.

Oesophageal cancer is characterized by two main histological subtypes -*i.e.* oesophageal adenocarcinoma and squamous cell carcinoma. Other subtypes account for less than 1-2%.[3] Oesophageal adenocarcinoma and squamous cell carcinoma differ by their geographical incidence, and primary risk factors.

Epidemiology of oesophageal cancer

In 2018 worldwide, oesophageal cancer was one of the most frequent cancer, representing 3.2% of all cancers and 5.3% of all cancer deaths.[12]

In both subtypes, men are over-represented with a male to female ratio of 3:1 for squamous cell carcinoma and 6:1 for oesophageal adenocarcinoma.[25] Those ratios however, vary by region.

Worldwide, oesophageal squamous cell carcinoma is the most prevalent subtype, especially in Eastern Asia and in Eastern and Southern Africa.[25] In the oesophageal cancer belt, the highest risk-region, 90% of patients diagnosed with oesophageal cancer have a squamous cell carcinoma.[25]

In Western countries, adenocarcinoma is the most common subtype.[25,26]

In Belgium in 2017, there were 1,516 new diagnosed cases (24 % women and 76% men) and 793 related deaths (26% women and 74% men).[23] Over time, oesophageal cancer incidence was stable in men but it was slightly increased in women (**Figure 5**). The increase in women incidence has been associated with an increase in incidence of squamous cell carcinoma related to smoking habits.[27]

In future, the yearly number of oesophageal cancer new cases is expected to increase up to 1,790 incident cases in 2025.[28]

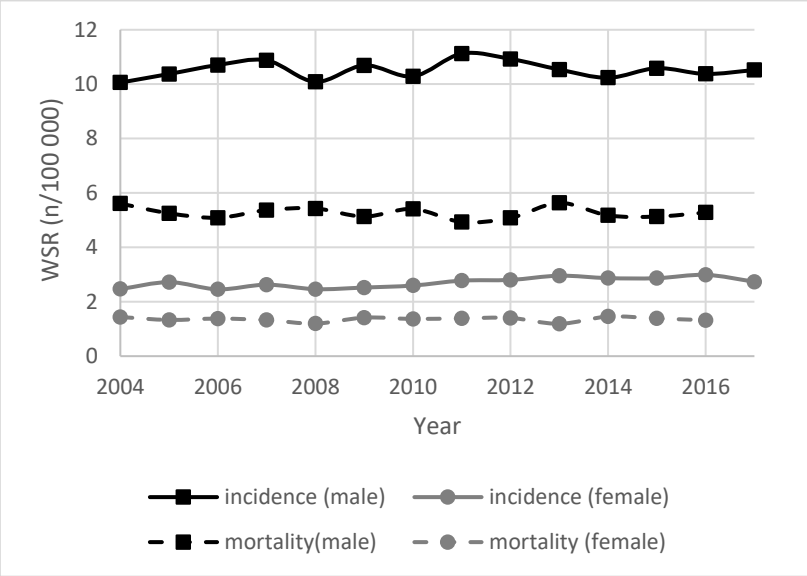



**Figure 5**. World Standardised incidence (2004-2017) and mortality (2004-2016) rate for oesophageal cancer (C15.0-C16.0) by sex in Belgium (Source: BCR)

## Main risk factors

*Oesophageal squamous cell carcinoma*

The main risk factors for oesophageal squamous cell carcinoma are related to lifestyle. Alcohol and tobacco consumption in whatever form are the major factors. The size of the association varies according to population.[25] For example, in developed countries cigarette smokers have a 3-to 9 fold risk of developing squamous cell carcinoma whereas in developing countries, the strength of the association decreases to an approximate relative risk of 1.5.[29]

Regarding alcohol consumption, differences still exist between regions of high prevalence where a 1.6-5.3 fold risk was estimated, and regions of lower prevalence where greater associations were observed (6-9 fold risk for Europe and North-America, respectively).[29]

*Oesophageal adenocarcinoma*

The principal risk factors for oesophageal adenocarcinoma are mainly reflux and obesity.[25]

Gastroesophageal reflux disease is the most important risk factor for oesophageal adenocarcinoma with population-based studies indicating a 6 fold risk of developing this type of cancer in patients with heartburn for at least 30 years.[26]The association was stronger in younger patients than in older.[26]

In patients with chronic gastroesophageal disease, Barrett's oesophagus, the pre-malignant stage of oesophageal adenocarcinoma, is susceptible to develop and to become invasive.

Obesity is a risk factor for oesophageal adenocarcinoma, but it could also be associated with gastroesophageal reflux as extensive intra-abdominal adipose tissues might cause gastric compression which in turn will cause the disruption of the lower oesophageal sphincter and will lead to reflux.[26] However, obesity itself with the associated inflammation and metabolic change, is susceptible to be a risk factor for many cancers.

## Treatment

A multidisciplinary board team is mandatory for oesophageal cancer treatment planning.[30]The choice of the treatment is determined by the stage of the disease, the location of the tumour, comorbidities, organ function and performance status (PS) of the patient.

For localised tumours (stage I tumours), surgery is the treatment of choice with possibility of endoscopic resection in very early stage.[30] When surgery is not possible (because of patient comorbidities for example), definitive combined chemo-radiotherapy has been shown to be superior to radiotherapy alone.[30,31]

In locally advanced tumour (stage II to III), surgery alone is not recommended and preoperative therapy is clearly associated with better survival rate.[30]

*Squamous cell oesophageal carcinoma*
In locally advanced squamous cell carcinoma, the standard therapy has been established after the CROSS Study (Chemo-radiotherapy for Oesophageal Cancer Followed by Surgery Study) that has shown a survival benefit from adding neoadjuvant chemo radiotherapy to surgery (HR 0.48, 95% CI 0.28–0.83).[25,32]The CROSS regimen is a combination of carboplatin, paclitaxel and radiotherapy.[25]

*Oesophageal adenocarcinoma*
Oesophageal adenocarcinomas are less sensitive to radiotherapy than squamous cell carcinomas.

When surgery is possible, doublet neoadjuvant chemotherapy with cisplatin and 5-fluorouracil (5-FU) has been associated with an increased survival.[25] Also, perioperative (pre- and postoperative) chemotherapy with cisplatin plus 5-FU or Epirubicin plus cisplatin plus 5-FU is an alternative treatment that has been shown to increase 5-years relative survival in two randomized controlled trials .[25]

In the CROSS study a survival benefit from adding neoadjuvant chemo radiotherapy to surgery was also observed in the subgroup of oesophageal adenocarcinoma even if the magnitude of the effect was not as important as it was in squamous cell carcinoma.[25]

Prognostic factors
Prognostic factors for oesophageal cancers include tumour stage, tumour subsite and histology, patients' performance status and comorbidities, and health-related quality of life (HRQoL).[20,25]

Other factors such as tobacco smoking and access to optimum care have also been reported to be associated with survival.[20]

Most of the sus-mentioned factors contribute to the concept of patient's frailty that is defined as "a complex, multidimensional, and cyclical state of diminished physiologic reserve that results in decreased resiliency and adaptive capacity and increased vulnerability to stressors."[33]Frailty is linked with advanced age but can also be a concern in younger patients, particularly in some cancers settings where frailty has been associated with an increased mortality after surgery.[33]

Even if associated with frailty, social vulnerability has also been pointed as an important prognostic factor.[33]

In patients undergoing surgery, a meta-analysis has shown better survival rate in high-volume hospitals compared to low-volume hospitals (HR 0.82, 95% CI 0.75–

0.90).[25,34] It seems that surgeon volume is more important than hospital volume.[25,34,35] After oesophagectomy, women have been reported to have consistently better survival than men, in squamous cell carcinoma.[36] This sex disparity is however not visible in individuals with adenocarcinoma. [36] Also, in individuals undergoing oesophagectomy, weight loss and low BMI are generally associated with a poorer prognosis.[37]

#### *1.2.2.2 Gastric cancer*

The first evidence of gastric cancer was found in the *Ebers papyrus*, dated around 1500 BC. [38] One of the most famous patients with gastric cancer was the French emperor Napoleon Bonaparte who died in exile in 1821 in St. Helena from a sporadic gastric tumour, after his defeat at Waterloo.[39]

Gastric cancer is characterized by two main anatomical subtypes, *i.e.* true gastric adenocarcinomas (non-cardia gastric cancers) and gastro-oesophageal adenocarcinomas (cardia gastric cancer).[31] This classification based on anatomical location is of clinical importance due to difference in aetiology, incidence, and treatment.[31]

Histologically, gastric cancers are mainly adenocarcinomas (i.e. neoplasia of epithelial tissue from glandular origin). However, they represent a heterogeneous group with differences in cell differentiation, histogenesis, and molecular pathogenesis.[31] The most common classifications used in this cancer are the Lauren and the WHO classification.

At the molecular level, four tumour subgroups were identified by the Cancer Genome Atlas: positive for Epstein-Barr virus tumours, microsatellite unstable (MSI) tumours, genomically stable tumours (GS), and chromosomally unstable tumours (CIN).[31] In future, molecular classification of gastric tumours might be of clinical relevance to personalize treatment.[31]

<u>Epidemiology of gastric cancer</u>

In 2018, gastric cancer was among the most frequently diagnosed cancers worldwide, with an estimated 1,000,000 new cases.[12] It was estimated that nearly one in twelve cancer deaths concerned this cancer, making it the third leading cause of cancer death.[12] The majority of the cases were diagnosed in Asia and the highest incidence rate was from the republic of Korea.[3] In Japan and South-Korea, two countries with high incidence of gastric cancers, screening for gastric cancer has been implemented.[40]

The number of cases of non-cardia gastric cancer has been decreasing whereas cardia gastric cancer with characteristics similar to oesophageal adenocarcinoma increased in high income countries.[12]

Similarly to oesophageal cancer, in both subtypes, men are over-represented with an incidence rate twice as high in men as in women.[12]

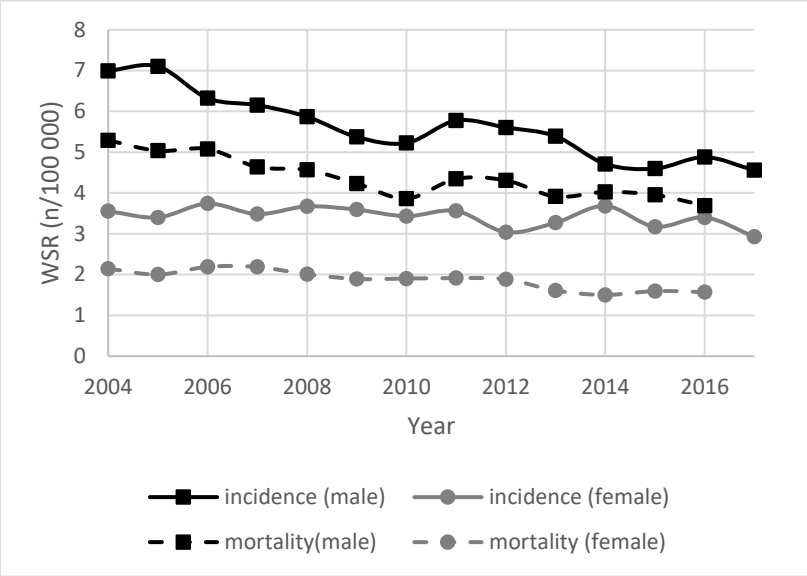



**Figure 6**. World Standardised incidence (2004-2017) and mortality (2004-2016) rate for gastric cancer (C16.1-C16.9) by sex in Belgium (source: BCR)

In Belgium, in 2017 there were 932 new cases (42 % women and 58% men) and 713 related deaths (38% women and 62% men). Over time, gastric cancer incidence and mortality decreased both in men and in women (**Figure 6**). In future, the number of new cases diagnosed each year is expected to decrease in Belgium [28].

## Risk factors of gastric cancer

*Helicobacter pylori*

The most important known risk factor for non-cardia gastric adenocarcinoma is *Helicobacter pylori*.[3,31] *Helicobacter pylori (H. pylori)* is a Gram-negative bacterium that has been associated with the development of gastritis and of peptic ulcer.[3,41] Patients with chronic *H. pylori* infection tend to develop gastric atrophy followed by metaplasia, which might evolve towards dysplasia and adenocarcinoma.[31]

The story of *H. pylori* begun in 1984 when Barry Marshall and Robin Warren identified spiral bacteria in the stomach of patients with gastritis and peptic ulcer.[42] It was only in 1991 that the first prospective study was conducted on 22 000 men and it concluded that individuals with *Helicobacter pylori* antibodies had a threefold-risk to develop gastric cancer. [42] In 1994, the WHO recognized *H. pylori* as a first class carcinogen for non-cardia gastric adenocarcinoma.[3,42]

Worldwide it is estimated that 50% of the population is infected by this bacterium but only 1-2% will develop gastric cancer.[43] The carcinogenicity of *H. pylori* has been associated with the presence of the cytotoxin-associated gene A pathogenicity island (cag-PAI) in the bacterium.[3,43] Compared to cag-PAI negative strains, positive strains are associated with a higher risk of developing gastric adenocarcinoma.[3]

*Epstein-Barr virus*

In 2014, the cancer Genome Atlas research network had included *Epstein-Barr* virus (EBV) positive as one of the four gastric adenocarcinoma molecular subtypes.[3,42] The mechanism behind this association remains unclear. It was estimated that approximately 9-10% of gastric adenocarcinomas were positive for EBV.[44]

*Smoking*

Similarly to the majority of the gastrointestinal tract cancers, smoking is associated with an increased risk of both cardia and non-cardia gastric cancers. [3] In the recent Stomach cancer Pooling (StoP) Project combining 23 epidemiological studies, the risk of gastric cancer was higher in smokers (OR=1.20; 95% CI 1.09-1.32) and it increased with the number of cigarettes per day or with the duration of smoking. The risk decreased with time after tobacco cessation and became similar to non-smokers 10 years after stopping.[45]

*Inherited susceptibility*

Familial aggregation of gastric cancer has been observed in approximately 10% of the cases but truly hereditary gastric cancers represented 1-3% of all gastric cancers. Hereditary gastric cancers are categorized into three syndromes: hereditary diffuse gastric cancers (mutation in E-cadherin/CDH1 gene) , gastric adenocarcinoma and proximal polyposis of the stomach, and familial intestinal gastric cancers.[31,43]

Other inherited predisposing syndromes included Li-Fraumeni syndrome (with mutations in TP53), familial adenomatous polyposis (mutation in APC), and Peutz-Jeghers syndrome (mutations in STK11).[3,31,43]

## Treatment

A multidisciplinary approach is also mandatory for gastric cancer treatment planning.[40] The choice of the treatment is determined by the stage of the disease, comorbidities, organ function, and performance status (PS).[46]

Surgical resection is the only curative therapeutic option for gastric cancer, specifically at early stages.[31,40] Resection by endoscopy is only recommended for non-ulcerated tumours confined to the mucosa, well differentiated, and with less than 2 cm of diameter.[40] For other tumours, surgery is required.

From stage IB to stage III tumours, radical gastrectomy with perioperative treatment is recommended.[40]

Since the MAGIC (Medical Research Council Adjuvant Gastric Infusional Chemotherapy ) phase III study, the use of perioperative chemotherapy with a combination of epirubicin, cisplatin, and 5-fluorouracil (5-FU), also known as ECF regimen, or epirubicin, cisplatin, and capecitabine (the ECX regimen) has been largely accepted as a standard in Europe.[40] Capecitabine has not been found to be inferior to 5-FU and could therefore be used instead.

Recently, a German study has shown however that the inclusion of taxanes to the combination of platinum and 5-FU was associated with a better survival than the classical ECF/X regimen (hazard ratio (HR) 0.77; 95% confidence interval (CI; 0.63 to 0·94)).[47] The so-called FLOT regimen (fluorouracil, leucovorin, oxaliplatin, docetaxel) wasn't associated with a higher toxicity.[47]

In advanced stage chemotherapy, a platinum (cisplatin or oxaliplatin) associated with a fluoropyrimidine doublet (5-FU, capecitabine, tegafur/gimeracil/oteracil (S-1)) has been associated with a better overall survival and quality of life.[46]

Targeted therapy also emerged from intrinsic gastric cancer heterogeneity. After the molecular classification of the cancer genome Atlas program, a particular chromosomally instable subtype of gastric cancer has gained some attention. HER-2 positive gastric cancer which, as its name suggests, is characterized by an overexpression of the human epidermal growth receptor-2 (HER-2), is an aggressive tumour accounting for 10-15% of gastric cancer cases. [3,40] This subtype of tumour responded favourably to the addition of trastuzumab to the cisplatin and fluoropyrimidine doublet and therefore the HER-2 status is of clinical importance and should be assessed in gastric cancer patients.[3,40,46]

Prognostic factors

Tumour staging is crucial for determining treatment interventions such as surgical resection in early stages cancers. But such surgery is the only curative option for gastric cancer; tumour stage is therefore the most important prognostic factor in gastric cancers.[31,40]

Increasing age and presence of comorbidities were also reported to be associated with higher mortality in gastric cancer patients.[48] Indeed, as with oesophageal cancers, patients' frailty has been associated with survival outcome in this cancer.[33,49] A recent study conducted in China, has shown that patients preoperative frailty was associated with increased postoperative complications and mortality.[33,49] Beside surgical outcome, frailty plays an important role in the decision-making process surrounding the management of oncological patients.[33]

Depending on cancer's histology, grade of differentiation and patients age, sex disparities in survival have been reported in gastric cancer cases.[50] In a recent South-Korean study, younger women with advanced gastric cancer and signet ring cell carcinoma were shown to have a poorer prognosis after gastrectomy.[50]

Molecular classification of gastric cancer subtypes might also be associated with survival. For example, chromosomally unstable tumours representing around 50% of all gastric cancers, are frequently associated with a poor survival.[31] HER-2 positive gastric cancers have also been associated with a poor prognosis.[31] Incidence of gastric cancer molecular subtypes has however been reported to be associated with sex disparities.[44]

## 1.3 COMORBIDITIES IN ONCOLOGY

*"Illness is the night-side of life, a more onerous citizenship. Everyone who is born holds dual citizenship, in the kingdom of the well and in the kingdom of the sick. Although we all prefer to use only the good passport, sooner or later each of us is obliged, at least for a spell, to identify ourselves as citizens of that other place*."

Susan Sontag,
**Illness as Metaphor**

Multimorbidity or comorbidity refers to "the simultaneous existence of more than one pathophysiologic condition or clinical entity in an individual".[51] There are however, some small differences between the two terms.

While comorbidity refers to several co-occurring conditions in reference to a primary chronic condition, the multimorbidity term doesn't give priority to a specific condition.[52] It is the complex wording of "index disease" that differentiates comorbidity from multimorbidity. Usually, index disease refers to the main condition under study and comorbidities refer to additional conditions existing at the time of the index disease diagnosis.[53,54] Comorbidities cannot be consequences of the index disease.[53,54]

In oncology, comorbidities refer to disorders existing alongside cancer.[55] As the incidence of cancer and comorbidities increases with age, it is common to find comorbidities in patients with cancer.[55] In oncology, half of the patients over 65 years old are expected to have at least one chronic condition that may affect their treatment regimen.[51]

The prevalence of comorbidities in oncology ranges from 0.4% to 90% and may vary by type of cancer.[55] Because of shared risk factors like smoking, comorbidities will be more frequent in some cancers like lung or colorectal cancer. However, some cancers like prostate cancer will not share this particularity.[55] The presence of comorbidities in patients with cancer is often associated with a poorer prognosis.[55] And the impact of comorbidities on mortality is even higher in cancer with a poor survival.[55] A recent study on gastric and colorectal patients, has shown that comorbidities have a negative prognostic impact on overall survival of patients and should be assessed as risk factors for mortality in survival analysis.[48]

The reasons for this adverse impact are various. First, the higher mortality in patients with cancer and comorbidities is thought to be directly linked to the comorbidities mortality. [55]

Second, it has been shown that patients with comorbidities and cancer are less likely to receive curative treatment for their cancer and are at higher risk of suffering from the toxicity of cancer treatment.[55]

Last but not least, some authors hypothesized that comorbidities are risk factors for cancer recurrence.[55]

Nowadays, cardiovascular diseases are recognised as the most common disorders worldwide and the leading causes of death.[15] Cardiovascular diseases are a heterogeneous group of diseases affecting the heart and/or the blood vessel. Atherosclerosis plays a major role in myocardial infarction and in circulating thrombus causing ischemic stroke, peripheral artery disease or renal disorders.[56] Risks factors for atherosclerosis included among others diabetes mellitus and dyslipidaemia, two more and more common chronic diseases.[57–59]

### 1.3.1 Diabetes mellitus

Diabetes mellitus are defined as "a group of metabolic diseases characterized by hyperglycaemia from defects in insulin secretion, insulin action or both".[60]

Insulin is a hormone produced by pancreatic islet β-cells that is responsible of the reduction of blood glucose. Under normal conditions, insulin is released when blood glucose rate increases, for example after a meal. It stimulates the transformation of glucose into fatty acids that will be stored in adipose tissues and decreases the liver's glycogenolysis. This induces a reduction of glycaemia to maintain normoglycemia.

Globally, 9.3% of adults aged 20-79 years in 2019 were living with diabetes and 50% of them were unaware of their condition.[61] In Belgium, in 2019 the prevalence of diabetes was estimated to be around 6.8% in adults aged 20-79 years.[61]

Broadly, there are two main types of diabetes. Type I diabetes represents approximately 10% of all diabetes and is characterized by a total deficiency in insulin.[60] It is a chronic autoimmune disease associated with a destruction of β-cells in pancreas by T-lymphocytes.[57] Type I diabetes results from complex interactions between environmental factors, metabolic factors, and genomic factors.[57]

Type II diabetes accounts for approximately 90% of all diabetes and is characterized by insulin resistance and a relative insulin deficiency.[60] It is a metabolic disease beginning with an insulin insensitivity in the liver, the muscles and the adipose tissues.[62] This insensitivity leads to an increased rate of circulating blood glucose caused by the uninterrupted production of glucose in the liver together with a decreased consumption of glucose by muscles.[62] In response to this hyperglycaemia, the pancreatic islet β-cells produce more insulin and eventually become dysfunctional.[62] In advanced stages of the disease, the produced insulin is no longer sufficient or efficient to allow normoglycemia. Type II diabetes results from interactions between genetic, epigenetic and lifestyle factors but can be prevented by lifestyle modifications in many cases.[62] For example, in the Nurses' Health Study, BMI was the most important risk factor for type II diabetes with 61% of the cases attributed to obesity.[62]

Increasing healthy lifestyle habits such as weight loss, increasing physical activity, healthy diet and smoking cessation are the first recommended measures for the management of diabetes mellitus.[63] Drugs used in the management of both diseases can be categorized into five groups: insulin sensitizers such as metformin and pioglitazone; insulin providers such as insulin, sulfonylureas, and meglitinides; incretin-based therapies such as GLP1-RAs and DPP4 inhibitors; gastrointestinal glucose absorption inhibitors such as acarbose; and renal glucose reuptake inhibitors such as SGLT2 inhibitors.[63]

In both types of diabetes, the main macrovascular complications are cardiovascular diseases that do not seem to be reduced by intensive blood glucose control. [57,58] Both types of diabetes mellitus are independent risk factors for vascular events such as coronary heart disease, ischaemic stroke, and vascular death.[63]

For more than a century, a link between diabetes and an increased risk of cancer has been pointed out.[64,65]In an article published in 1914, M. Greenwood (1880–1949) an epidemiologist, physiologist and medical statistician, discussed from a statistical point of view the correlation observed between death rate of cancer and diabetes in an American dataset.[66]

More recently, in June 2009 four papers published simultaneously in the journal of the European association for the study of diabetes, have started the controversy about insulin glargine use and increase risk of cancer.[65] This led to the writing of a consensus report from European and American cancer and diabetes associations in 2010.[64] They agreed that diabetes and cancers were diagnosed within the same individuals more frequently than would be expected by chance, and even after adjustment for age.[64] The lifetime risk of having both diseases has been estimated at 15%.[67]

The association between diabetes and an increase in cancer mortality continues to be debated nonetheless. Indeed, even though several studies have shown an increased cancer mortality in diabetics' patients with cancer, it is not clear if this association is simply explained by the excess age-adjusted mortality of diabetes, or if it is a reverse causation, or if it is a causal association.[64,68] Two cohorts of patients, one from Italy and the other one from the United States, gave similar results with 30% increased risk of dying from cancer in diabetic patients when compared to non-diabetics.[68,69]

In their consensus report of 2010, European and American cancer and diabetes Associations suggested to study the association between cancers and diabetes in specific sites (and not in all cancers combined) as some but not all cancers might be associated with diabetes.[64] In their recommendations, they also highlighted the necessity to study this association in less common cancers as data in those cancers are limited.[64]

### 1.3.2 Dyslipidaemia

In the blood, there are six different types of lipoproteins involved in the transport of lipids to tissues.[59]

Chylomicrons, very low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), low-density lipoproteins (LDL), lipoprotein (a) (Lp(a)) and high-density lipoproteins (HDL) differed by their density, their composition and their use. They are mainly constituted of cholesterol, triglycerides, phospholipids and apolipoproteins, the latter being different depending on the type of lipoproteins.[59]

Dyslipidaemia can be characterized by an imbalance of low-density cholesterol levels and high-density cholesterol (hypercholesterolemia).[70] It can also refer to an increase in triglycerides level (hypertriglyceridemia) or to an increase in both cholesterol and triglycerides (mixed hyperlipidaemia).[70]

An increase in any low-density lipoproteins with type B apolipoproteins on their surface is associated with the building of atherosclerotic plaque and acute atherosclerotic cardiovascular disease.[59]

Plasma LDL-Cholesterol represents an estimation of the circulating cholesterol carried by LDL lipoproteins.[59] LDL lipoproteins represent the majority of apolipoprotein B-containing particles. An increase in LDL-Cholesterol has been consistently associated with an increased risk of acute atherosclerotic cardiovascular disease and the relationships has been established as continuous.[59] Also, circulating level of triglycerides, mostly carried by VLDL another apolipoprotein B-containing lipoprotein, have been associated with the risk of acute atherosclerotic cardiovascular disease.[59]

Increased HDL-Cholesterol level have been associated with a decreasing risk of atherosclerotic cardiovascular disease in observational studies.[59] However, there is no evidence from randomised trial that increasing HDL-Cholesterol reduces the risk of atherosclerotic cardiovascular disease.[59]

In the United States, it is reported that 50% of adults have elevated LDL-C and the latest estimations in Europe showed similar prevalence.[70,71]

As for diabetes mellitus, lifestyle changes such as weight loss, increasing physical activity, healthy diet and smoking cessation are the first recommended measures for the management of dyslipidaemia.[59] Therapeutic management of this condition includes statins, fibrates, cholesterols absorption inhibitors such as Ezetimibe, bile acids sequestrants such as Colestipol, Kexin type 9inhibitors (also known as PCSK9 inhibitors), Lomitapide , Mipomersen, n-3 fatty acids, Nicotinic acid and Cholesteryl ester transfer protein inhibitors.[72]

Clinical studies have linked obesity to increased risk of several cancers, such as liver or colon cancers.[73] However, even if obesity and hyperlipidaemia are associated, increased circulating lipids level as a risk factor for cancer is controversial. Several epidemiological studies tend to suggest that cancers could be associated with both, an increased cholesterol level or a decreased cholesterol level.[74,75]

The temporality of this association is also discussed as cancers might alter lipids metabolism and might therefore precede dyslipidaemia .[73] Indeed, tumours by altering several oncogenic and tumour suppressor factors like TP53 or mTOR are able to impact the cholesterol biosynthesis.[73]

## 1.4 PHARMACOEPIDEMIOLOGY

"*The specificity of drugs decreases over time*"

Sir John Vane, 1993

As stated in previous sections, patients with cancer are generally older and have co-occurring medical conditions that need to be treated with additional drugs. Drugs are "substances (other than food) that are intended to affect the structure or any function of the body and are used in the diagnosis, cure mitigation, treatment, or prevention of disease".[3] They can be used for acute condition, sporadically over a lifetime or chronically for years.[3]

Polypharmacy or the use of several drugs is common among elderly patients with and without cancer.[76] This combination of treatments can be associated with several undesirable outcomes like adverse drug events, drug–drug interactions, and reduced adherence to drugs.[76] Moreover, special populations like elderly are often underrepresented in clinical studies and that is where pharmacoepidemiologic studies come into play.[77]

The term *pharmacoepidemiology* derived from the combination of two other terms: "*pharmaco"* and "*epidemiology"*. Pharmacology is defined as the "study of the effects of drugs" whereas epidemiology is defined as "the study of the distribution and determinants of diseases in human populations".[77] Pharmacoepidemiology is "the study of the use, and effect of drugs and other medical devices in a large number of people".[77]

Historically, pharmacoepidemiology was developed for the study of adverse drug effects.[77,78] In the 1960s, after several drug crises like the in utero exposition to thalidomide, it became evident that adverse effects and later adverse events should be monitored.[77] Thus, the first indication of pharmacoepidemiologic studies was post marketing studies. It was only between the 90s and 2000s that pharmacoepidemiology extended its field to beneficial drug effects. [77]

Comorbidities are chronic illnesses that need to be treated during a long time and so the long-term effects of drugs used in these diseases are important to address. Premarketing studies are generally conducted in relatively short periods and therefore long-term effects of drug exposition cannot be assessed.

Pharmacoepidemiologic studies allow cost-effective post marketing research in order to assess adverse and beneficial drug effects in large numbers of patients, including those who are often excluded from premarketing studies (elderly, children or pregnant women).[77] When data are available, this type of studies is also more time-efficient than *de novo* studies. [77]

The use of computerised databases as a source of data has been implemented in pharmacoepidemiologic studies since 1980 in North America.[77] Linking several health related databases, such as claims and registry databases, allow to overcome the needs for large sample and follow-up time in postmarketing studies. Depending on the objectives of the study, pharmacoepidemiologic studies may require different source of data.[77] Claims data represent one of the best alternative for studying drug exposure while population-based medical records -such as cancer registries- are among the best alternatives for disease data .[3,77]

The WHO has also developed several tools to improve pharmacoepidemiologic studies at an international level. For example, in 1981, the WHO recommended the use of the Anatomical Therapeutic Chemical (ATC) classification as a tool for drug utilization monitoring and research in drug utilisation studies.[79] This classification tool allows the identification of each drug with a code of seven elements representing five different levels of information. The different levels of information in the ATC classification are illustrated for metformin in **Table 1**.

**Table 1.** The Anatomical Therapeutic Chemical (ATC) classification system illustrated for metformin

| **The five ATC classification levels** |
|---|
| **1st level:** anatomical main group<br>Ex: **A:** Alimentary tract and metabolism |
| **2nd level:** therapeutic subgroup<br>Ex: **A10:** Drugs used in diabetes |
| **3rd level:** pharmacological subgroup<br>Ex: **A10B:** Blood glucose lowering drugs (excl. insulins) |
| **4th level:** chemical subgroup<br>Ex: **A10BA:** Biguanides |
| **5th level:** chemical substance<br>Ex: **A10BA02**: Metformin |

Beside the ATC classification system, the Defined Daily Dose (DDD) unit has also been defined by the WHO as "the assumed average maintenance dose per day for a drug used for its main indication in adults".[79] Each drug defined by an ATC code has a specific DDD representing an average prescribed dose per day, based on the assessment of available information in several countries.[79]

### 1.4.1 Drug repurposing

Drug repurposing (or drug repositioning) defined as the application of a drug for another indication than the one it was originally approved for, has received increasing interest in the past few years. [80] This term has been first described by Ashburn and Thor in 2004 and has been used since then by several other authors.[81]

Compared to the process of finding *de novo* drugs, drug repositioning could reduce the development risks associated with the standard way of drug discovery.[81] With the advances made in the medical and pharmaceutical fields, it happened that a drug previously approved for its specific activity on a certain pathway might also be active in another target. [82]

In the standard process, when a potential drug candidate has been identified, there is a long procedure of validation, where the pharmacokinetics, pharmacodynamics, the toxicity of the compound have to be assessed together with safety.[83]

Indeed, before commercialising a drug, this drug has to be tested in humans through the standard three phases of clinical trial i.e. phase I, phase II and phase III. In oncology, early access to the market due to unmet medical need or innovative clinical trial such as baskets trial are however possible but these case will not be discussed here.[84]

The typical Phase I study in oncology, involved a small number of cancer patients who do not benefit from standard therapy anymore. In other medical fields or when the drug toxicity is considered acceptable for healthy volunteers, the phase I study is conducted in healthy patients. In this step, the pharmacokinetic and pharmacodynamics together with the appropriate dose, the safety and the toxicity of the drug will be assessed. Drug efficacy is generally not an endpoint of phase I studies.

In standard phase II studies in oncology the safety and the efficacy of the drug are tested. The patients are generally cancer patients with a specific cancer, and they may or may not have previous anti-cancer therapy. In other medical fields, phase II studies will represent the first drug administration in sick individuals.

The last step of the standard clinical trial phase is the phase III clinical study. This phase also aims to test the safety and efficacy of the drug. It includes a large number of patients and is characterized by rigorous predefined inclusions criteria and statistical methods. The most common design used for oncological trial is double blind randomised controlled trial that can either test the superiority, the equivalence or the non-inferiority of the drug compared to standard care. Endpoint of phase III studies involves among others, overall survival (OS), progression free survival (PFS) and disease-free survival (DFS).

Approximately 90% of drug candidates will not be approved at the end of the classical process.[85] Also, the cost of a drug development starting from the traditional preclinical process has been estimated around 1-2 billion dollars. In comparison, drug repurposing is thought to reduce the time and the cost of the drug development process.[85] For example, drug repurposing is estimated to cost around 40-80 million dollars.[85]

### 1.4.2 Drug repurposing in oncology

In oncology, an increasing number of currently used drugs (200 in 2017) have shown evidence of an anticancer activity.[86] Those drugs are often well known and have been used for many years with information available regarding their posology, safety and pharmacodynamics. [86]

In this field, drug repurposing could be an alternative way to reduce difficulties arising from the cost and the length of new drug development. It is also evident that given the poor survival linked to the majority of digestive cancers and the limited efficacy of the available cancer treatment, a modest improvement in cancer outcomes could be beneficial to both the patient and the health economy.

One of the most popular cases of drug repurposing in oncology was illustrated by thalidomide. This drug first used in the 50s for its sedative activity in pregnant women was found to be associated with a previously rare birth defect called phocomelia. This "disaster" led to some regulatory actions regarding drug safety like the Kefauver Harris amendments in the US that require for each drug, proof of efficacy and preclinical pharmacologic and toxicological study .[77] Also, the US food and drug administration's (FDA) explicit approval has been specifically required before drug marketing.[77] Later, in 2008, the same drug acquired European marketing authorization for the treatment of multiple myeloma in oncology.[77]

An interesting fact about the regulatory changes made for example in the US market was that in order to market supposedly safer and more effective drugs, they prolonged the approval process of new drugs and increased the cost of drug research and developpement.[77]

As previously mentioned, the study of drug repurposing is facilitated by the growing trend of recording computerized health care data, for example through cancer registries.[87] Electronic pharmacy records in observational pharmacoepidemiologic studies are considered the gold standard.[3] This kind of data source allows conducting time- and cost-effective observational studies of cancer risks and outcomes that could complement randomized clinical trials' findings. Observational studies are interesting to generate or strengthen hypotheses about drug effects.[77] They can be conducted before the assessment of preclinical models to generate hypothesis or after to strengthen them. After the hypothesis generation, and the assessment of the drug effect in preclinical models, the drug has to pass through clinical testing if clinical safety and/or efficacy need to be evaluated. But when safety is well established, observational studies are useful for assessing a potential benefit of the drug in other diseases. When observational studies show a potential effect, the pharmaceutical industry has an open window to consider a new Phase III trial in order to prove the

drug benefit. Of course, the decision for conducting such trial for repurposing the drug strongly depends upon a cost benefit analysis.

## 1.5 METFORMIN AND STATINS

### 1.5.1 Metformin

Metformin (or 1,1-dimethylbiguanide hydrochloride) is the first-line oral treatment from the biguanides family used to treat type II diabetes.[58] This drug derives from *Galega officinalis* (also known as French lilac) that was first used in traditional medicine in Medieval Europe.[88] Actually, there is even a reference to metformin in the Eber Papyrus. In 1772, the botanist Sir John Hill recommended *Galega officinalis* for the relief of thirst and frequent urination, two common symptoms of diabetes.[88]

*Galega officinalis* was actually found in the mid-1800s to be rich in guanidine and other related compounds. Biguanides result from the fusion of two guanidines originally synthesized by Bernhard Rathke in 1879.[88]

Metformin, as a treatment for diabetes, was officially introduced in the UK and other European countries in 1958.[88] It's only in 1994 that the drug was approved and introduced in 1995 in the USA.[88] An overview of some metformin's characteristics is shown in **Table 2.**

**Table 2**. Metformin overview

| | **ATC code** | **DDD *(mg)*** | **Standard daily dose *(mg)**** | **Half-life *(h)*** ** | **Number of intakes** |
|---|---|---|---|---|---|
| **Metformin** | A10BA02 | 2000 | 1000-3000 | 6.5 | 2-3 |

DDD: defined daily doses; ATC: Anatomical Therapeutic Chemical classification
*in Belgium
** The half-life of elimination is the time it takes to clear half of the drug from plasma

#### *1.5.1.1 Mechanisms in diabetes*

Metformin was not designed to target a particular pathway or disease mechanism, and even after more than a half-century of use, its molecular mechanisms of action remain partially unknown.[89]

It is known that approximately 70% of the administered dose is absorbed from the small intestine and is excreted unchanged in the urine. Its plasmatic concentration ranges from 8 to 24 µmol/L.[89]

Metformin targets the liver where it improves blood glucose level by reducing hepatic gluconeogenesis.

Metformin is absorbed into the hepatocytes through the organic cation transporter-1 (OCT1). Then the drug is accumulated into the cell and more specifically into mitochondria to concentrations up to 1000-fold higher than in the extracellular

medium. [89] Metformin is known to inhibit the complex I of the respiratory chain and consequently prevents adenosine triphosphate (ATP) production.

ATP is the energy exchange unit of the cells and this molecule is highly needed in gluconeogenesis (six ATP is needed in order to product one molecule of glucose). By preventing ATP production, metformin increases the cytoplasmic ADP: ATP and AMP:ATP ratio, leading to the activation of AMP-activated protein kinase (AMPK). AMPK is the cellular energy censor that is activated when cellular energy balance is compromised. The activation of AMPK, is followed by the activation of catabolic pathway generating ATP and the inhibition of metabolic process consuming ATP.[89] This will cause the inhibition of protein, lipid and glucose synthesis. [90]

Metformin uptake is also associated with alteration of the appetite regulation through several mechanisms, including the gut-brain axis.[91] Its well-documented weight loss effect is indeed thought to be associated with a decrease in caloric intake rather than an increase in energy expenditure.[91]

#### *1.5.1.2 Mechanisms in cancers*

Metformin is thought to inhibit cancer through several mechanisms that can be direct (insulin independent) and indirect (insulin dependent).[90]

Metformin through the activation of AMPK appears to inhibit the mammalian target of rapamycin (mTOR) pathway. MTOR pathway is involved in many processes to regulate protein synthesis and is consequently implicated in cell growth and proliferation. In cancer, overexpression of mTOR is associated with tumour progression, resistance to chemotherapy, and poorer prognosis.[90]

The inhibition of mTOR (mediated by metformin) in cancer stem cells is one of the possible explanations of metformin effect in improving response to chemotherapy.[90] Indeed, cancer stem cells have been associated with resistance to both chemo and radiotherapy.

The activation of AMPK also leads to G1-phase cell cycle arrest by inhibiting cyclin-D1 protein and to the inhibition of lipogenesis.[90] G1-phase cell cycle arrest is associated with the inhibition of proliferation in cancerous cells and the inhibition of lipogenesis deprives the cells of essential fatty acids for their proliferation.

Other signalling pathways, like the Ras/Raf/Mitogen activated protein kinase (MAPK) seem to be involved in the Metformin indirect effect on cancer.[90]

In epithelial cancer, metformin also suppresses epithelial-mesenchymal transition.

Metformin also reduces circulating insulin and IGF-1 level, two growth factors that have been independently associated with cancer risk and progression.[90]

#### *1.5.1.3 Adverse side effects*

Metformin administration is mainly associated with gastrointestinal side effects occurring in 20 to 30% of patients with approximately 5% of them being unable to tolerate the treatment.[89,92] The most frequent gastrointestinal adverse events are diarrhoea followed by nausea.[93] Gastrointestinal side effects of metformin are not fully understood yet, but high concentration of metformin in intestinal enterocytes seems to be a possible explanation. Indeed, gastrointestinal side effects seem to be reduced in slow-release formulation of metformin.[89] Another explanation of metformin gastrointestinal side effects relies on the modification of the microbiome of metformin users. More specifically, an imbalance in the bacterial diversity has been shown in patients treated with metformin, with an increase in the bacterium *Akkermansia muciniphila*.[89]

The less frequent (<10 cases per 100 000 patient-years) but more dangerous adverse effect is lactic acidosis that is associated with 30 to 50% mortality rates.[94] The phenomenon is linked to an increased arterial lactate level (>5.0 mmol/L) and low blood pH (<7.35).[94] Lactate is an intermediary product of the glycolysis that can accumulate during hypoxic condition.[94] Generally, lactic acidosis is associated with high metformin plasma level (> 5µg/mL). A high plasmatic level of metformin can occur if there is an imbalance between an increased lactate production and an impaired metabolism or a reduced clearance.[94] This imbalance tends to occur in patients with impaired renal or hepatic function (decreased clearance) and in patients with an increased production of lactate, as in sepsis for example.[94]

### 1.5.2 Statins

Statin is a generic term including members of the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-COA) reductase inhibitors family. The first statin was mevastatin and was discovered by Endo et al. who were searching for a drug that could affect bacterial growth by inhibiting HMG-COA.[95] This statin, however, has never been commercially available.

The first generations used in clinical practice were lovastatin, fluvastatin and pravastatin , followed by the second generations including simvastatin, atorvastatin, cerivastatin, rosuvastatin, and pitavastatin.[95] Five statins are available in Belgium (**Table 3**).

**Table 3**. Statins available in Belgium

| | **ATC code** | **DDD *(mg)*** | **Standard daily dose *(mg)**** | **Half-life *(h)*** ** | **Number of intakes** |
|---|---|---|---|---|---|
| **Simvastatin** | C10AA01 | 30 | 10-40 | 2-5 | 1 |
| **Pravastatin** | C10AA03 | 30 | 10-40 | 1-3 | 1 |
| **Fluvastatin** | C10AA04 | 60 | 40-80 | 1-3 | 1-2 |
| **Atorvastatin** | C10AA05 | 20 | 40-80 | 7-20 | 1 |
| **Rosuvastatin** | C10AA07 | 10 | 5-40 | 20 | 1 |

**DDD**: defined daily doses; **ATC**: Anatomical Therapeutic Chemical classification
*in Belgium
****** The half-life of elimination is the time it takes to clear half of the drug from plasma

Statins are amphiphilic. They enter into the liver cells either directly by membrane interactions, through passive diffusion, for lipophilic agents like simvastatin, fluvastatin or atorvastatin or by intermediates for hydrophilic agents like pravastatin. Rosuvastatin is thought to be both lipophilic and hydrophilic.[96,97] Lipophilic statins are therefore less hepatoselective than hydrophilic ones and can diffuse in other cells. Statins are mainly metabolised by the cytochrome P450 family. Simvastatin, lovastatin, and atorvastatin are metabolised through the cytochrome P450 3A4 whereas fluvastatin is metabolised through cytochrome P450 2C9.[97]

#### *1.5.2.1 Mechanisms in cardiovascular prevention*

Statins are used in primary and secondary prevention of coronary heart disease in patients with dyslipidaemia.

Approximately 30% to 80% of the dose depending on the type of statin is absorbed in the intestines.[96] Except pravastatin and partially rosuvastatin, statins will undergo a first pass liver metabolism.[96]

Statins act on the mevalonate pathway by selectively inhibiting the HMG-COA reductase. The active component of statins is structurally similar to HMG-CoA. This active site binds to HMG-CoA reductase causing the inhibition of the cholesterol biosynthesis in the liver.[96,97] The decreasing intrahepatic cholesterol is associated with an increase in expression of LDL-receptors (LDL-R) in cell surface, enhancing the clearance of the circulating LDL-cholesterol in the blood.[59,96,97] The effect on circulating LDL-cholesterol varies according to the type of statins. In moderate intensity treatment, a reduction of 30 to 50% in the LDL-cholesterol is expected. The reduction can increase to more than 50% of the baseline value in high intensity treatment.[59]

Statins also reduce by 10-20% the triglycerides level by a mechanism that remains partially unknown. It seems that a decreasing synthesis of VLDL associated with an increase in VLDL uptake might partially explain the effect of statins on triglycerides.[59] Atorvastatin, rosuvastatin and pitavastatin are the most efficient statins for triglycerides reduction.[59]

Pleiotropic effects of statins on the cardiovascular system have been suggested but are debated. The most cited effects are improvements in endothelial function, stabilization of atherosclerotic plaques, anti-inflammatory and antioxidant effect of statins. [59,97]

#### *1.5.2.2 Mechanisms in cancers*

Statins effects on cancer cells have been observed in several cellular processes depending on the cancer type and the type of statins. It seems that the first evidence of statin in cancer prevention arises from unexpected results of randomized controlled trials comparing statins safety to other lipids-lowering agents in cardiovascular diseases.[98] In those studies, statins were consistently associated with an increase in non-cardiovascular mortality.[98] Thankfully, this association finally was thought to result mainly from accidents.[98] Intensive preclinical, clinical and observational studies arising from this concern even lead to the opposite conclusion that statins could have a beneficial effect on cancer.[98]

Interestingly, the first statin produced, mevastatin, produced by Sankyo and later the second one, lovastatin, produced by Merck were both discontinued due to rumours about treated dogs developing cancer.[99]

Statins, by inhibiting HMG-CoA reductase enzyme, decrease the level of mevalonic acid, and therefore the level of cholesterol and isoprenoid intermediates.

First, it is important to note that cholesterol is an essential component of cell membranes and is also the precursor of bile acids, lipoproteins, and steroid hormones.[75,100] For their membrane synthesis, tumours have been reported to be highly dependent on lipids produced through the mevalonate pathway.[100]

Isoprenoids intermediates in the mevalonate pathway include farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP).[100] They play an important role in protein prenylation. Protein prenylation is a process allowing the activation of proteins like Rho and Ras also known as small guanosine triphosphatases (GTPases).[100,101] Those proteins are involved among other things in cell proliferation, differentiation and survival and are often overexpressed in many cancers. For example, mutations in Ras gene leading to constant activation of the protein Ras are present in 20 to 30% of human cancers.[101]

By inhibiting isoprenoids production, statins are able to reduce the expression of GTPase.[95,101] Inhibiting GTPases activity is a treatment strategy that is now under study in cancers, with for example the development of Farnesyl-transferase Inhibitors.[100]

Statins -by decreasing the level of cholesterol- might also negatively affect the cell cycle in G1, G2 or S phase, and therefore inhibit cell proliferation.[95,101]

Additionally, several studies have shown that statins induce apoptosis in several cancer cell lines by activating all the related known mechanisms.[95,101] This included for example the decreasing expression of the anti-apoptotic proteins Bcl-2 or the increasing activity of caspase-3.[95,101]

#### *1.5.2.3 Adverse side effects*

The most common adverse effect induced by statins is statins-associated muscle symptoms (SAMSs). It is estimated that SAMSs represent 72% of all adverse side effects related to statins used. SAMSs included myalgia, myopathy, myositis and rhabdomyolysis in the most severe forms. In observational studies, 10 to 15% of patients have been affected.[59,96,97] Rhabdomyolysis, the most severe form of statin myopathy involving muscle necrosis and myoglobinuria is less frequent with approximately 1 to 3 cases per 100 000 patient-years. The prevalence of SAMSs is lower for hydrophilic statins because of their hepatoselectivity.

Statin use has been associated with a dose-dependent 20 to 30% increase in risk of diabetes. [96] The risk is higher with stronger statins, in elderly, in overweight patients or with patients showing an insulin resistance.[59,97] The risk of diabetes, however is considered acceptable given the risk reduction in cardiovascular diseases. [59]

An important modulator of adverse events in statins users is drug interactions. As statins are metabolised in the liver through the cytochrome P450, all drugs metabolised by the same enzymes will potentially interact with them. It involves anti-infective agents such as erythromycin or itraconazole, calcium antagonists such as amlodipine or cyclosporine, and grapefruit juice. The combination of statins with those drugs might enhance the risk of SAMSs because drug interactions can result in an increased blood concentration of statins.

In the light of the preceding theoretical background, the primary aim of this thesis was to evaluate the association between metformin and statins, two commonly prescribed non-oncological drugs, and digestive tract cancers survival at a population level in Belgium.

In order to achieve this objective, two population-based studies investigating metformin and statins use in gastric and oesophageal cancers were conducted, respectively. The results are presented in **sections 6** and **7**.

The secondary aim of this thesis was to assess the pattern of use and the adherence to these two medications around cancer diagnosis in patients with digestive tract cancer.

In order to achieve those objectives, two descriptive studies were conducted, using the Belgian cancer registry and the Belgian permanent sample (EPS) for comparison among a cancer free population. This work is presented in **sections 4** and **5**.

In research, the quality of the collected information and the way of managing this information are of crucial importance.

The quality of the collected data might depend on the source of the data but also on the way of these data have been collected. However, even with high quality data, it is possible to conduct inadequate studies and to draw inappropriate conclusions.

In the sections below, the different data sources of this thesis together with some basic epidemiological and statistical notions are described.

## 3.1 THE BELGIAN CANCER REGISTRY

In cancer surveillance and control, registries are central. They are the main providers of information allowing the assessment of cancer burden (e.g. incidence, survival, mortality and prevalence), together with treatments and preventives interventions outcomes (e.g. the impact of mass screening ).[102]

When they specifically refer to a geographically well-defined population, they are referred to as population-based registries. In Europe the first population-based cancer registries appeared in Nordic countries between 1940s and 1950s.[3] Since then it is estimated that cancer registries cover almost 50% of the European population but with great disparities in cancer coverage amongst regions.[103]

In the last 50 years, population-based registries have allowed for example to draw a representation of disparities in cancer burden among European countries with poor survival or unexpected higher mortality/incidence ratios in Eastern European regions.[103] They also contribute to identify cancer risk factors in big cohort studies like the EPIC (European Prospective Investigation into Cancer and Nutrition) Study for diet or ESCAPE (European Study of Cohorts for Air Pollution Effects) study for air pollution.[103]

The Belgian Cancer Registry (BCR) foundation, a national population-based cancer registry created in 2005 is meticulously collecting data on the Belgian national level from the incidence year 2004 onwards. Between 1983 and 2004, it was the Belgian National Cancer Registry (NCR), a department of the Belgian Work against Cancer (BWK), that was in charge of the registration of cancer cases in Belgium.[102] The cancer cases were obtained from the seven Belgian organisations for health insurance but their incidence was underestimated.[102]

In 2003, a royal decree allowing the refunding of the multidisciplinary oncological consultation and fixing the standards for recognition of the oncological care programs was published.[102] From then, and in order to benefit from financial incentives, hospitals were encouraged to register every cancer diagnosed.[102]

In 2006, shortly after the discontinuation of the NCR and the creation of the BCR, the Health Law of December 13th was voted, allowing the BCR to use the national social security number (INSZ/NISS) as the unique identifier of the patient.[102] This law also made compulsory the registration of cancers to the BCR and involved hospitals within the process, through the information from the oncological care programs, together with the pathological anatomy laboratories, and haematology departments.[102]

Beside standard tumours and some patients' characteristics, the BCR is also allowed to collect details on treatments and cancer related medical procedures from the seven official Belgian health insurance companies through the intermutualistic agency (IMA) (**Figure 7**). Since 2002, the IMA collected information regarding reimbursed

therapeutic and diagnostic procedures from hospitals and public pharmacies. As health insurance in Belgium is mandatory, the medical claims database can be considered as comprehensive for the Belgian population. The period for which the treatment information is available in the BCR database spans from one year before diagnosis to five years after. These treatment-related data are also used to obtain an estimation of three comorbidities in the cancer population: diabetes, cardiovascular diseases and respiratory diseases. [104]

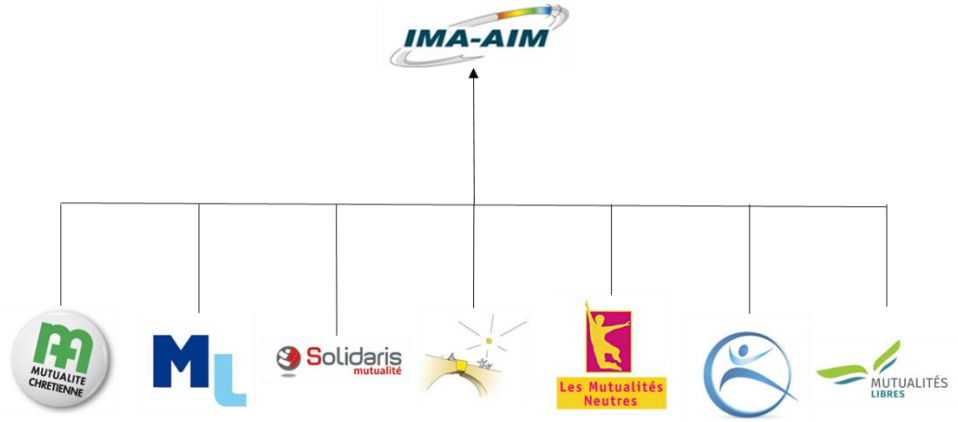



**Figure 7.** The seven health insurances in Belgium

In order to link the collected information to other national databases on vital status, date of death, and cause of death , the BCR uses the national social security identification number (SSIN) as a unique patient identifier.[105,106] Vital status are obtained by linking the Belgian cancer registry database to the data of the Crossroad Bank for social security (CBSS) that collects data on social security for the Belgian population. As death certificates do not contain the SSIN, the BCR deterministically couples its data to the regional table of deaths by using a combination of date of birth, date of death, sex and residence. The different national databases linked with the Belgian cancer registry data are illustrated in **Figure 8**.

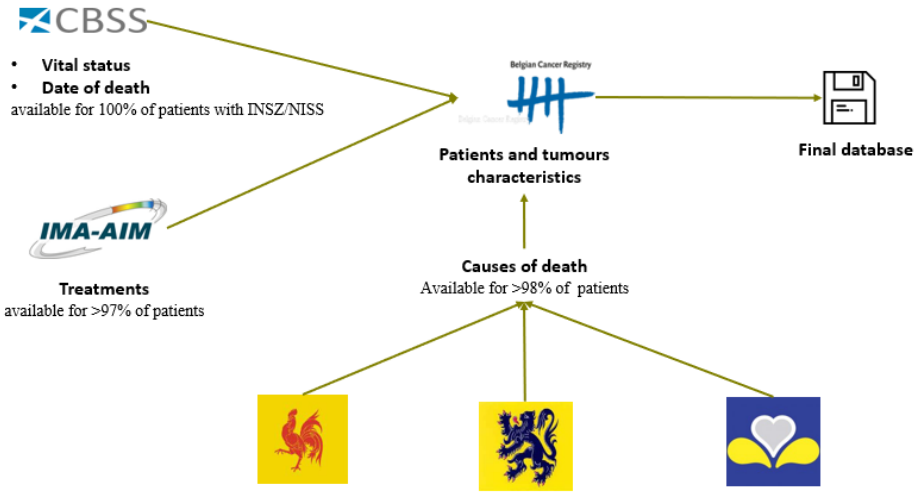



**Figure 8.** Belgian cancer registry database linkage with other national databases

The BCR database offers a unique opportunity to study pharmacoepidemiology in cancer at a population level in Belgium. The completeness of the database has been estimated to be higher than 95%, meaning that more than 95% of all incident cancers are thought to be included in the database of the BCR.[27] The remaining 5% are probably related to elderly patients with a very poor prognosis at diagnosis or outpatients with a clinical diagnosis only.[27]

**Did you know?**

The Belgium Health care system is characterized by mandatory health insurance, independent medical practice, free choice of physician by the patient, and in most of the cases, fee-for-service payment of providers with reimbursement.[107,108]

Health insurance companies, also called mutualities are in the number of seven and were originally created by merging small mutualities into national unions depending on their political and ideological background.[108] As a result, the National Alliance of Christian mutualities was founded in 1906, the National Union of Neutral mutualities in 1908, the National Union of Socialist mutualities in 1913, the National Union of Liberal mutualities in 1914 and the Union of the Free and Professional mutualities in 1920.[108]

The auxiliary sickness and invalidity insurance fund is a public social security institution created for people not wishing to be affiliated to the above mentioned mutualities. A specific mutuality was also created for the members of the statutory staff of the Belgian Railways and their families

## 3.2 THE BELGIAN PERMANENT SAMPLE

In order to compare cancer patients to the general population in Belgium, a representative sample is needed.

The Belgian permanent sample (EPS) was created in 2007 by the Intermutualistic agency (IMA) to monitor health care consumption and expenditure in Belgium. The EPS is a 2.5% random selection of the total population affiliated to health insurance in Belgium. This random selection is enriched by a 5% sample of individuals older than 65 years in order to better study the consequences of the population's ageing. **Table 4** shows a comparison of the distribution of some common characteristics between EPS and the general population for the year 2014.

The data inside the EPS are updated every 31st December, meaning that if a patient dies or is lost to follow-up during a specific year, he/she is deleted from the database the next year. The EPS does not contain information regarding patient diagnosis. However, similarly to the BCR database, the EPS database contains information regarding participants characteristics (age, place of residence, estimated comorbidities, year of death, etc.), healthcare consumption (by procedure and delivery date) and drug consumption (by ATC, CNK code and delivery date).

**Table 4.** Comparison between the total Belgian population and the permanent sample for the year 2014

| **Variables** | **Population (1st January 2014)** | **EPS (year 2014)** | | |
|---|---|---|---|---|
| | | **Total** | **Sample** | **Oversampling** |
| **N** | 11150516 (100) | 333120 (100) | 280496 (84) | 52624 (16) |
| **Men** | 5474309 (49) | 160883 (48) | 137896(49) | 22987 (43) |
| **Age category** | | | | |
| **<18** | 2268745 (20) | 55944 (17) | 55944 (20) | NA |
| **[18-64]** | 6887933 (62) | 171067 (51) | 171067 (61) | NA |
| **≥65** | 1993838 (18) | 106109 (32) | 53485 (19) | 52624 (100) |
| **Region** | | | | |
| **Flanders** | 6410705 (58) | 192233 (58) | 160690 (57) | 31543 (60) |
| **Brussels** | 1163486 (10) | 31284 (9) | 27337 (10) | 3947 (8) |
| **Wallonia** | 3576325 (32) | 104094 (31) | 87794 (31) | 16300 (31) |
| **Strangers** | - | 5064 (2) | 4267 (2) | 797 (2) |
| **missing** | - | 445 (0) | 408 (0) | 37 (0) |

**Data are n (%)**

## 3.3 SURVIVAL ANALYSIS

« *Death is certain, the time is not* »
Jaqen H'ghar

Survival analysis represents a set of statistical methods designed to study the time to the occurrence of a specific event when the event does not occur in all participants. These methods were initially designed to study the time to death occurrence, which explains the name.

To conduct a survival analysis, the best observation plan is prospective. Starting from a group of individuals at a given starting point, who will be followed for a suitable period of time, any event of interest that may occur and its time of occurrence will be noted. There are several methods to analyze survival data and some are complementary.

In order to better understand what follows, some definitions are needed.

Let us suppose that we follow **n** people:

- Let **T be** the random variable representing the lifetime of a person taken at random

Then S (t), the **survival function at time t** will be defined as the probability that the person's lifetime is greater than t:

$S(t) = Pr(T > t)$

The **cumulative distribution function or mortality** F (t), is the probability to die before time t:

$F(t) = Pr(T \leq t) = 1- S(t)$

The **density function of lifetime** is the derivative of F (t).

$$f(t) = \frac{dF(t)}{dt} = \lim_{\Delta t \to 0} \frac{\Pr\{t < T \leq t+\Delta t\}}{\Delta t}$$

The **hazard rate** $\lambda$ (t) is the probability to die a few moments after t **for someone** who lived at least until t, over the period, when the period tends to 0:

$$\lambda(t) = \lim_{\Delta t \to 0} \frac{\Pr\{t < T \leq t+\Delta t \mid T > t\}}{\Delta t}$$

This function aims to quantify the instantaneous risk that an event occurs at a time t. Paul D. Allison explains that as the time is continuous, the probability of occurrence of an event exactly at time t is 0.[109] However, we can estimate the probability of an event occurring between a time t and $t + \Delta t$.[109] This probability is conditional to the fact of having survived beyond time t mainly because if an individual is already dead, he is no longer at risk of knowing the event.[109] Thus focus will be laid on individuals who are still alive at the beginning of the period $[t, t + \Delta t]$.[109] Finally, as we try to

have the probability of occurrence of the event at time t, we will try to reduce at most $\Delta t$ until it tends to 0 hence the limit.[109] The unit of $\lambda(t)$ is person-year.

Hazard and survival are linked:

$$\rightarrow \lambda(t) = \lim_{\Delta t \to 0} \frac{\Pr\{t < T \leq t+\Delta t \mid T > t\}}{\Delta t}$$

$$\rightarrow \lambda(t)\, dt = \Pr(t < T \leq t+\Delta t \mid T > t)$$

$$= \frac{\Pr(t < T \leq t+\Delta t)}{\Pr\{T > t\}}$$

$$= \frac{f(t)dt}{S(t)}$$

$$= \frac{dF(t)}{S(t)}$$

$$= \frac{d(1-S(t))}{S(t)}$$

$$= -d(\ln(S(t))$$

$$\rightarrow -\ln(S(t)) = \int_0^t \lambda(u)\, du$$

$\int_0^t \lambda(u)\, du$ is called the **cumulative hazard function**, usually noted H(t).

$$\rightarrow S(t) = e^{-\int_0^t \lambda(u)\, du}$$

In time-to-event analysis, all individuals will not necessarily experience the event of interest because the study will stop before the occurrence of the event or because they will be lost to follow-up. These individuals will be referred to as right censored observations. It means that their time to event is incomplete and that the only information we have about the real time to event is that it exceeds the time of censoring.

An important condition for the validity of all survival analyses is that the censoring should be independent of lifetime. If, for example, all the censorings are lost to follow up and are advanced cases of the disease with a small survival, the analyses will result into a biased estimation of the survival. This is the independent censoring assumption.

### 3.3.1 The Kaplan Meier estimate of a survival curve

The Kaplan Meier method was develop by Edward L. Kaplan and Paul Meier and published in June 1958 in one of the most cited scientific papers.[110]

The history behind the Kaplan-Meier method is compelling and deserves to be known. Edward L. Kaplan and Paul Meier were both mathematicians graduated from Princeton University. John W. Tukey was their PhD supervisor.

In 1952, Paul Meier had only just obtained his doctorate in statistics. He was teaching at Johns Hopkins University and at the same time working on the variance of failures dispersed in time. A year later, in 1953, Edward L. Kaplan was working for Bell Telephone Laboratories and was interested in studying the lifetime of vacuum tubes in the repeaters in telephone cables buried in the ocean.

Both, Edward L. Kaplan and Paul Meier worked independently and submitted their work to the Journal of American Statistical Association (JASA). JASA's editor, suggested to merge the two separate manuscripts. It took them four years of correspondence to finally publish the merged paper because of differences in their approaches.

Their method is now a standard way to report patient survival in research and particularly in oncology.[110]

In the Kaplan-Meier method, the survival time is divided into time intervals delimited by each time-point corresponding to the occurrence of the event of interest (typically death). Within each interval, the conditional survival -*i.e.* The probability to survive during this interval knowing that you survive the previous interval- is estimated. The conditional survival is calculated by dividing the number of people alive at the end of the interval by the number of people at risk at the beginning of the interval. Then the Kaplan-Meier estimator is the "product limit estimator" of these conditional survivals. The idea behind the product limit estimator is that in order to survive at time $t_j$ you need first to survive to the time point before $t_j$ (that is $t_{j-1}$):

$$\hat{S}(t_j) = Pr(T > t_j) = Pr(T > t_j \cap T > t_{j-1})$$

$$= Pr(T > t_j \mid T > t_{j-1}) * \underbrace{\mathbf{Pr(T > t_{j-1})}}$$

$$\mathbf{= Pr(T > t_{j-1} \cap T > t_{j-2})}$$

$$\mathbf{= Pr(T > t_{j-1} \mid T > t_{j-2}) * Pr(T > t_{j-2})}$$

$$\mathbf{= \ldots}$$

Censored patients are considered alive up to the point of censoring but are not considered at risk in the next time interval.

The result is often represented as a steps curve with the time to event on the abscissa and the survival in ordinate (**Figure 9**). Each drop of the curve represents the time at which at least one event occurs, and the height of the drop is proportional to the number of events and to the number of people remaining at risk.

The Kaplan-Meier method is a semi parametric method because the times of death are not considered in the estimate, but only their rank.

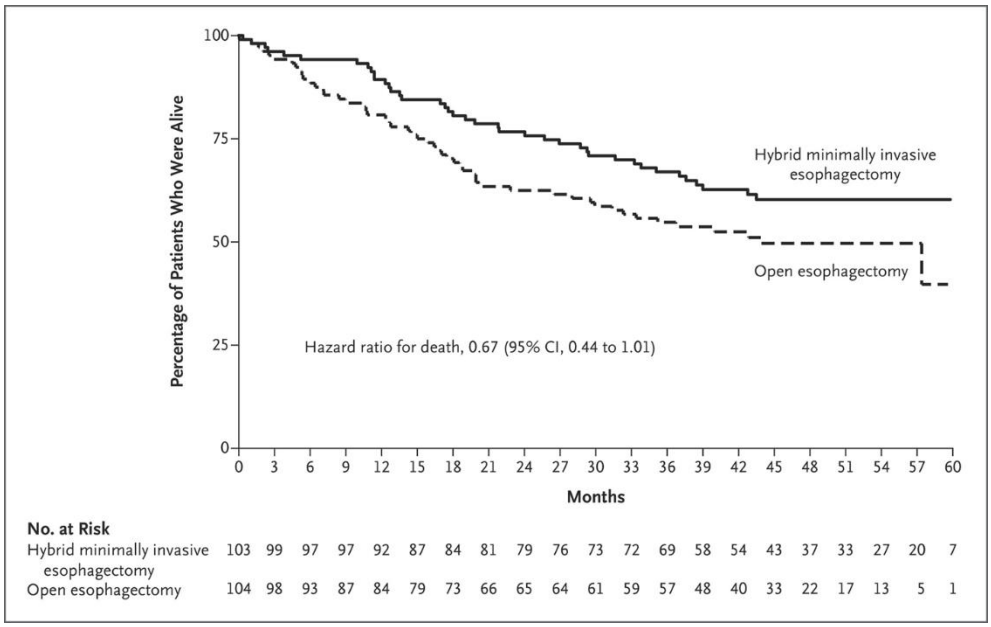



**Figure 9**. Example of Kaplan-Meier curve in an oncological study. (source: Mariette, 2019)[111]

### 3.3.2 Overall survival and specific survival

As seen above, the Kaplan-Meier (KM) method is an easy and commonly used tool for estimating survival in the medical field. Survival is a key measure for cancer patients who want to know their prognosis but also for clinicians, researchers, and policy makers. Depending on the question asked, the measure of survival may however vary.

One of the most used and understood survival measures in oncology might be overall survival, also called observed survival or crude survival.[112] In oncology, overall survival is defined as the probability of remaining alive after a specific period beginning at cancer diagnosis.[112] The endpoint event of overall survival is death from any cause.[112] It is a measure known to be reliable, easily available and determined unequivocally. For these reasons, it remains the gold standard for the assessment of clinical benefit in patients with cancer.[113]

As pointed in the previous sections, cancer patients are usually old with multiple comorbidities. It is therefore obvious that they might also die from other causes like cardiovascular diseases before having time to die from their cancer. Those other causes of death are called competing causes of death. In the presence of competing risks, the standard product limit estimator is biased because the idea behind its construction -i.e. that each censored individual would have experienced the event of interest with sufficient follow-up time- becomes false in cancer specific survival analysis. Modelling competing risks can be handled by using specific methods such as proportional subdistribution hazards model that will not be developed here.

In cancer-specific survival, the endpoints are cancer specific deaths and people dying from competing causes of death are censored.[112] Cancer specific survival relies however, on the quality of the cause of death registration.[112,113] In cancer registries, the causes of death are derived from death certificates for economic reasons and for convenience. The physician who has to report the direct cause of death as well as the underlying causes of death completes the death certificate. His/her report might be complicated in presence of several comorbidities, especially when he/she cannot access the full medical file and when no autopsy report is available.[113] Comparative studies using death certificates and autopsy reports have shown 15% to 35% of disagreement.[113] Therefore, cancer-specific survival is thought to have a certain level of subjectivity while this is not the case with overall survival.[113]

For this reason the recommendations for every study using death certification for cause specific death registration are to report both overall and cause-specific survival.[113]

### 3.3.3 Modeling factors associated with survival: The Cox model

The Cox regression is a generalized linear model. Beginning with the word regression and from a frequentist point of view, a linear regression is a function that describes how on average a dependent variable (usually named Y) will be affected by the levels of one (in simple regression) or a set of external or independent variables (in multiple regression) usually named X.[114] In survival analysis, Y will typically be the selected measure of survival and X will be a predictor or a set of predictors known to potentially affect survival.

A model is a mathematical representation of the variability of a dataset that allows among other things to make prediction on, or to describe the data, taking into account the joint effect of several risk factors.[115] The use of a model may however be conditional upon the respect of a set of assumptions necessary to ensure the validity of the outcome.

One of the motivations behind the use of a regression model is the existence of potential confounders that could influence the value of the outcome.[116] A confounder is a variable related to the risk factor and the outcome that can distort the association between both variables. In randomized trial, the randomization allows to get rid of the issue of confounders, however in observational studies, confounding is an important issue. A way to manage confounding is to adjust the model for (or control) confounders. Adjustments are made by including the potential confounders as dependent variables in the regression model.

We have previously defined the hazard rate $\lambda(t)$. In the Cox regression model, the regression is about hazard, i.e. the instantaneous risk that an event occurs.

The model for an individual ***i*** at time ***t*** is usually described as:

$$\lambda_i(t) = \lambda_0(t)\, e^{(\beta_1 x_{i1} + \cdots + \beta_k x_{ik})}$$

This means that for a specific individual ***i*** at time ***t***, the probability to die a few moments after ***t*** if he lived at least until ***t*** depends on a baseline hazard function $\lambda_0(t)$ that is the same for all individuals and on a linear function of a set of ***k*** predictors $(x_{i1}, \ldots, x_{ik})$.

$\lambda_0(t)$ represents the value of the instantaneous risk of death when all the predictors are equal to zero, *i.e.* when no predictor influences the hazard function. No assumption is made on the mathematical form of $\lambda_0(t)$ and the only part of the model that will be estimated is the coefficients $\beta$ associated with each predictor. This is the reason why Cox model is often referred to as a semi-parametric model.

It is interesting to note that the only part of the model depending on time t is $\lambda_0(t)$. All coefficients $(\beta_{1\ldots}\ \beta_k)$ are independent of time, which implies that the association between predictors X and the occurrence of death doesn't change over time. For two

patients with $X_1$ and $X_2$ covariates, the ratio of their hazards does only depend on the regression coefficients and the values of the predictors $X_1$ and $X_2$. This assumption, called the proportional hazards assumption, is fundamental in Cox modelling.

To verify if this assumption holds, several methods can be used, including the visual examination of the "log minus log" curves, the evaluation of Schoenfeld residuals or the identification of possible interactions between covariates and time in the model. In the present work, Schoenfeld residuals were used to verify proportional hazards assumptions.

In order to estimate the unknown β regression parameter, Cox proposed the use of the partial likelihood function which accounts only for non-censored survival times and eliminates the baseline hazard $\lambda_0(t)$ function.

Let's considered a sample with ***n*** independent individuals (***i*** =1, …, n) in which we have ***r*** uncensored observation and ***n-r*** censored observations. $\vec{\beta}$ is the vector of the parameters in the model and $\vec{x}$ the vector of the ***k*** predictors associated.

For each event, the partial likelihood is calculated by estimating the probability that a specific patient ***j*** dies at time $t_i$ given the fact that a death occurred at time $t_i$ among the survivors at $t_{i-1}$. This probability is calculated by dividing the hazard of the patient ***j*** by the sum of the hazards of all patients at risk of death at time $t_i$.

Then the partial likelihood for the proportional hazard model can be expressed as the product of likelihoods for all the events that are observed:

$$PL = \prod_{j=1}^{r} \frac{e^{\vec{\beta}.\vec{x}_j}}{\sum_{k=1}^{n_i} e^{\vec{\beta}.\vec{x}_k}}$$

In Cox's proportional hazard model, β regression parameters are estimated by maximizing this partial likelihood generally using Newton-Raphson algorithms or other maximizing algorithms.

When two or more observations have the same time of event -i.e. tied observations, the aforementioned expression of the partial likelihood is no more valid. Ties are generally handled by using the Breslow's approximation if there are few ties. If the data are heavily tied, other approximations such as the Efron's one, are preferred.

In SAS software, PROC PHREG has been implemented to perform survival analysis based on the Cox proportional hazards model. In PROC PHREG, as in most statistical software, the maximization of the partial likelihood is based on a Newton-Raphson algorithm. Ties are handled by using the Breslow's approximation by default. Other types of approximations can be specified using the TIES=EFRON/EXACT/DISCRETE option in the MODEL statement.

The interpretation of a Cox model relies on the β coefficients that are indicators of the association between death occurrence and the predictor of interest.

Let's consider a cox model with only one predictor. Then the general model will be:

$\lambda_i(t) = \lambda_0(t)e^{\beta x_i}$

$$\begin{cases} \lambda_i(t) = \lambda_0(t) & \text{when } x_i=0, \\ \lambda_i(t) = \lambda_0(t)e^{\beta} & \text{when } x_i=1, \end{cases}$$

The instantaneous relative risk (if the proportional hazard assumption is hold), also called hazards ratio (HR) for the predictor x at time t will be:

$HR= \frac{\lambda_1(t)}{\lambda_0(t)} = e^{\beta} \rightarrow \ln(HR) = \beta$

This is also why this model is called log-linear model for relative hazards. And as seen above, the HR for an increase of one unit of the predictor is given by the exponential of the coefficient in the Cox model. When multiple predictors are included in the regression model, the interpretation is the same except that the value of other predictors are kept constant.

When β is negative, then the HR will be lower than 1, meaning a reduction in the hazards rate when the predictor increases by one unit. In other words, increasing values of the predictor is associated with longer survival times.[116] The association between the predictor and the outcome is said protective.

When the value of β is positive, the HR will be greater than 1, meaning an increase in the hazards rate when the predictor increases by one unit. Increasing values of the predictor are associated with shorter survival times.[116] The association between the predictor and the outcome is then said deleterious.

If β is equal to zero, then the HR will be equal to 1, meaning that the hazards rates are the same when the predictor increases by one unit.

### 3.3.4 Cox regression with time dependent variables

We have seen in the previous section the general case of predictors that are invariant and fixed over time. In some cases, however, the predictor x could be time-dependent. A time-dependent variable is a predictor whose values vary with time.[116] Such variables can be included in a Cox regression model for example to control for immortal time bias (**section 3.3.1**).

There are different ways of integrating time dependent variables in a Cox regression model depending on the type of predictor.

If the predictor is binary and cannot change back from 1 to 0, an indicator of the predictor $x_i$(t) which takes on value 0 before exposure and 1 subsequently can be implemented in the regression.[116] For unexposed individuals, $x_i$(t) retains its original value of 0. Thus, in an unadjusted model, the hazard at time t can be written as

$$\lambda_i(t) = \lambda_0(t)\, e^{\beta x_i(t)}$$

With

$$\begin{cases} \lambda_i(t) = \lambda_0(t) & \text{when the individual is not exposed (i.e. } x_i(t)=0) \\ \lambda_i(t) = \lambda_0(t)\, e^{\beta} & \text{when the individual is exposed (i.e. } x_i(t)=1) \end{cases}$$

Modelling an interaction term with time is another way to implement time dependent variables in Cox proportional hazards regression.[116] It is a way to check for non-proportional hazards when conducting Cox regression.[116] In such case, the predictor itself is modelled as a linear function of time.

When the variable is measured occasionally, the last observation carried forward approach or the two stages approach can be used, keeping in mind that both have their limitations.[116] Two stages approach consists first in modelling the time dependant variable for each individual and in then using its expected value at time t in the final model.[116] The last observation carried forward approach uses the most recent measurement before time t, but it is more likely to produce biased results.[116]

In SAS PHREG procedure, there are two equivalent ways to handle time-dependant covariates, the programming statements method and the counting process method.[109] In the programming statements method, there is one line per patient and the time dependant covariate is define using multiple variables. [109] In the counting process, multiple line per patients are created , each line representing an interval of time during which the time-dependant covariate remains constant.[109]

In the counting process method, all the programming is made during the construction of the data set and required more coding than the programming statements methods.[109] On the other hand, in the programming statements method, the programming needs to be done in every PROC PHREG statement and relies on the

creation of inaccessible temporary data sets that make it difficult to check for programming mistakes.[109]

In the present work, the counting process method was used and for example in the main analysis, two line per patients were created in the exposed group. The first line representing the immortal time where the patient was considered unexposed and the second line representing the exposed time.

## 3.4 POTENTIAL BIAS IN PHARMACOEPIDEMIOLOGICAL STUDIES

When conducting a study in general, several kinds of situations could arise from the results of the analysis. Beside no association or true causal association, one could be confronted to spurious associations. Spurious associations can arise either by chance or by bias. Bias are systematic errors due to an inadequate experimental design. Contrary to random error, bias tends to drive the results in a similar direction.

Observational studies of post diagnosis use of drug and mortality are prone to analytic challenges like reverse causation, confounding, selection and information bias.[117] These errors can affect the outcome of studies and can lead to erroneous associations. Below we will discuss frequent bias occurring in observational studies and the way to reduce their amplitude.

### 3.4.1 Immortal time bias

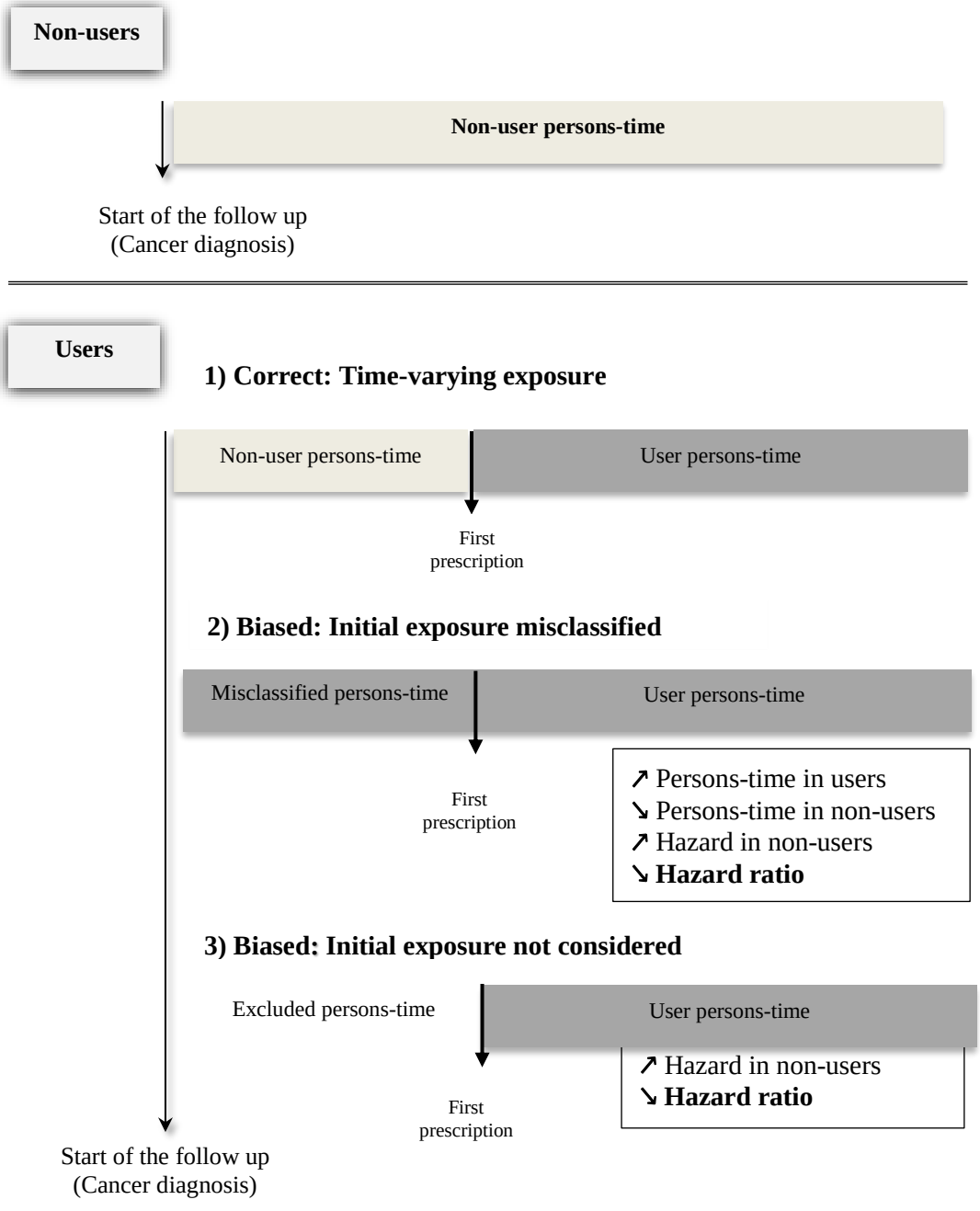



**Figure 10**. Immortal time bias

In an observational study aiming at determining if a specific drug is associated with the occurrence of a specific event (death, cancer...), immortal time is the period between the beginning of the follow-up and the first drug prescription in the users' group (**Figure 10**). During this period, the user is by definition immortal because, in order to receive its first prescription, the user could not experience the event of interest (otherwise he would have been classified as non-user).

Immortal time bias is a frequent bias occurring when unexposed time is either misclassified as exposed time or suppressed from the study.[3,118] In this bias, unexposed person-time between the beginning of the follow-up and initial exposure is not taken into account or is instead misclassified as exposed person-time. This bias will cause the incidence rate of the exposed group to be low while it will increase the incidence rate of the unexposed group leading to an underestimation of the rate ratio.[118] Immortal time bias can be avoided by using time-varying exposure.[3]

### 3.4.2 Prevalent users bias

Prevalent users bias occurs when most of individuals in the users group have been using the drug of interest prior to their entry into the study.[3] Two types of problems could arise from this type of bias. First, prevalent users are not representative of random users of the drug of interest. They represent a subset of the user population having survived the introduction period of the therapy and who tend to be more adherent.[119]

Second, baseline characteristics that are covariate at the study entry could be affected by the treatment.[119]

One way to avoid prevalent users bias is to restrict the analysis to new users.[3] However, when evaluating the association between drug use and cancer outcome, restricting the analysis to new users could lead to small sample size and healthy user's bias.

### 3.4.3 Confounding by indication bias

Confounding by indication (or contraindication) is an important issue in studies examining the association between drug use and cancer outcome. It can occur if the initial indication of the medication is also a risk factor for the outcome of interest. [3]

Typically, this bias is encountered in studies investigating diabetes medication use in a context of cancer risk or death. As diabetes is associated with both cancer risk and deaths, the results observed from the analyses could be biased by differences in cancer risk or survival between patients with and without diabetes.[55,64]

Confounding by indication can be avoided by restricting the analysis to individuals with the drug's indication.[3]

# 4 PATTERN OF STATIN AND METFORMIN USE IN GASTROINTESTINAL CANCER PATIENTS IN BELGIUM

Cancer risk increases with age as for each 10-years increase, the overall incidence of cancer is more than doubled.[3] As chronic conditions are also more common among older people, those two conditions are therefore frequently associated.[55] Cancers are mainly associated with long induction periods, therefore contact with healthcare providers due to cancer symptoms might lead to concomitant disease discovery and chronic treatment initiation before or after cancer diagnosis.[120] Also, cancer symptoms could be misinterpreted as other benign illness symptoms.[120]

These two phenomena lead to an increase in prescriptions around cancer diagnosis and this may have implications for pharmacoepidemiologic studies. For example, the increase of prescriptions prior cancer diagnosis could lead to reverse causation bias in risk studies assessing the association between drug exposure and cancer risk.[120] Also, in pharmacoepidemiologic studies on drugs' beneficial and adverse effects, new users of medication may be less likely to benefit from or to be harmed by the studied drug.

While the impact of comorbidities on cancer have been extensively studied and discussed, literature about the impact of cancer on comorbidities management and treatment is scarce. More specifically, studies on chronic medication utilisation pattern around cancer diagnosis are scarce.

Several studies have investigated patterns of chronic medication use and their appropriateness toward end-of-life in patients with cancer[121,122] but to our knowledge few studies have investigated patterns of chronic conditions medications initiation near cancer diagnosis.[120,123] Two studies were conducted on the Danish population.

In the first published study, the authors have shown that incident use of drug increased in the month prior to diagnosis.[120] However, the observed increase was specific to the type of cancer and drug. For example, prescriptions of drugs used to treat cancer symptoms like pain or constipation were more likely to increase before cancer diagnosis.[120]

In the second study focusing on patients with ovarian cancer, the authors have shown an increase in new drug use before diagnosis that peaked in the first month after diagnosis.[123]When they specifically focused on preventive drugs such as statins, an increase in new prescriptions, although limited, was seen before diagnosis. Moreover, only very limited changes in the post diagnosis use of these drugs were observed.

In the present study, we aimed to investigate the pattern of initiation and use of metformin and statins around cancer diagnosis at the Belgian population level. We use the Belgian nationwide cancer registry and the health insurance companies' database to investigate in a quantitative way the onset of new prescriptions and the

crude estimates of exposure to statins and metformin treatments in the period around digestive tract cancer diagnosis.

## 4.1 METHODS

### 4.1.1 Study population

The patients with gastrointestinal cancers were extracted from the Belgian cancer registry (BCR) database. Gastrointestinal tract cancers were defined as first primary oesophageal cancers (ICD10 C15.0-16.0), gastric cancers (ICD10 C16.1-C16.9) and colorectal cancers (ICD10 C18.0-C20.9) diagnosed between January 2004 and December 2014.

Diagnostic and therapeutic procedures obtained from the Intermutualistic agency (IMA) were linked to the BCR database using the patient's specific national social security identification number (SSIN).

Individuals with previous cancer diagnosis, not residing in Belgium at the time of diagnosis, with an uncertain date of diagnosis, with no national social security identification number (SSIN), lost to follow-up at the date of cancer incidence, or missing from the medical claims (IMA) database, without a multidisciplinary oncological consultation and/or chemotherapy and/or radiotherapy were excluded from the analysis.

For comparison purposes, the Belgian permanent sample (EPS) was used to estimate metformin and statins use in a cancer-free population. The Belgian permanent sample is a 2.5% random selection of the total population affiliated to health insurance in Belgium. This random selection is enriched by a 5% sample of individuals older than 65 years in order to better study the consequence of the population ageing. As health insurance is mandatory in Belgium, the Belgian permanent sample is considered to be representative of an ageing Belgian population. In the EPS, the cancer-free population was defined by excluding individuals with a multidisciplinary oncological consultation and/or chemotherapy and/or radiotherapy.

### 4.1.2 Analysis

Drug consumption was referred to as the number of defined daily Doses by 1000 inhabitants per day (DDD/THID). A DDD is defined by the World Health Organisation as "the assumed average maintenance dose per day for a drug used for its main indication in adults".[79] DDD per 1000 inhabitants per day is the WHO recommended measure when reporting chronic drug exposure.[79] This measure is defined by the number of the drug DDDs' that are used on average in a representative group of 1000 inhabitants, on any given day of the analysed years.[79]

New drug users were identified as those with a first ever filling prescription of metformin or statin in the Belgian cancer registry database. Incidence rates (IR) of metformin and statins were calculated from 6 months before cancer diagnosis to two years after. To do this, within each month we divided the number of new users by

the population at risk (*i.e.* the number of person-months who haven't had a prescription of that drug at the beginning of this month and who were still alive). Data were analysed using SAS enterprise guide released 9.3 software.

## 4.2 RESULTS

### 4.2.1 Drug consumption

#### *4.2.1.1 Statins*

From the 82,336 patients identified with digestive tract cancer, 32,604 (40 %) were statin users. The characteristics of individuals included in the analyses are presented in **Table S1** in supplementary material.

The volume of statins used in patients with digestive tract cancer in the five years following diagnosis was 236.81 DDDs per 1000 patients per day (DDD/TID). The highest consumption was in patients with colorectal cancer (**Figure 1.**) In the EPS sample, the prescribed volume of statins was 123.75 DDDs per 1000 inhabitants per day. Patients with cancer had a higher consumption of statins compared to the general and non-cancerous patients.

Globally, men have higher volume of statins prescriptions than women (**Figure S1**). The volume of statins prescriptions increased with age until 80 years before decreasing (**Figure S1**). Comparing patients with cancer to patients without, the higher volume of prescriptions observed in patients with digestive tract cancers was driven by the youngest patients. In patients younger than 70 years old, the difference in volume of statins prescriptions between cancer cases and non-cancer cases was the highest (**Figure S1**).

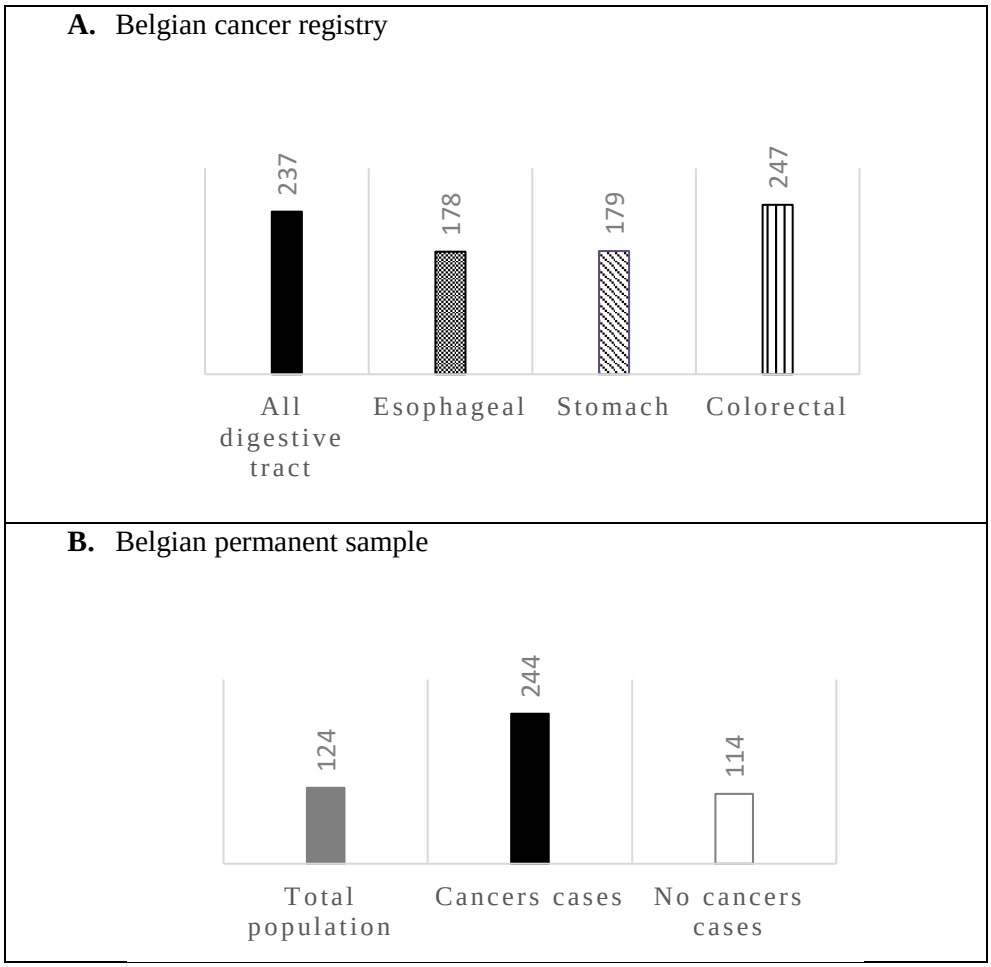



**Figure 1.** Statins consumption in DDD per thousand persons-day (DDD/TID) in individuals with digestive tract cancer in Belgium between 2004 and 2014 (**Panel A**) and in the Belgian permanent sample (**Panel B**)

#### *4.2.1.2 Metformin*

From the 82,336 patients identified with digestive tract cancer, 11,628 (14.1 %) were considered diabetics in the year before diagnosis of whom 8,923 (76.7%) were metformin users. The characteristics of individuals included in the analyses are presented in **Table S2** in supplementary material.

The volume of metformin used in patients with digestive tract cancer was 49.96 DDD/TID. Similarly to statins use, the highest volume of prescribed metformin was seen in patients with colorectal cancer **(Figure 3.)**. In the EPS sample, the volume of prescribed metformin was 24.96 DDD/TID. Patients with cancer had higher volume of prescribed metformin (45.1 DDD/TID) than patients without cancer (23.3 DDD/TID) **(Figure 4)**.

Similarly to statins, men have higher volume of metformin prescriptions than women (**Figure S2**). The volume of metformin prescriptions increased with age until 80 years before decreasing (**Figure S2**). Again, younger patients were at origin of the higher volume of prescriptions observed in patients with digestive tract cancers (**Figure S2**).

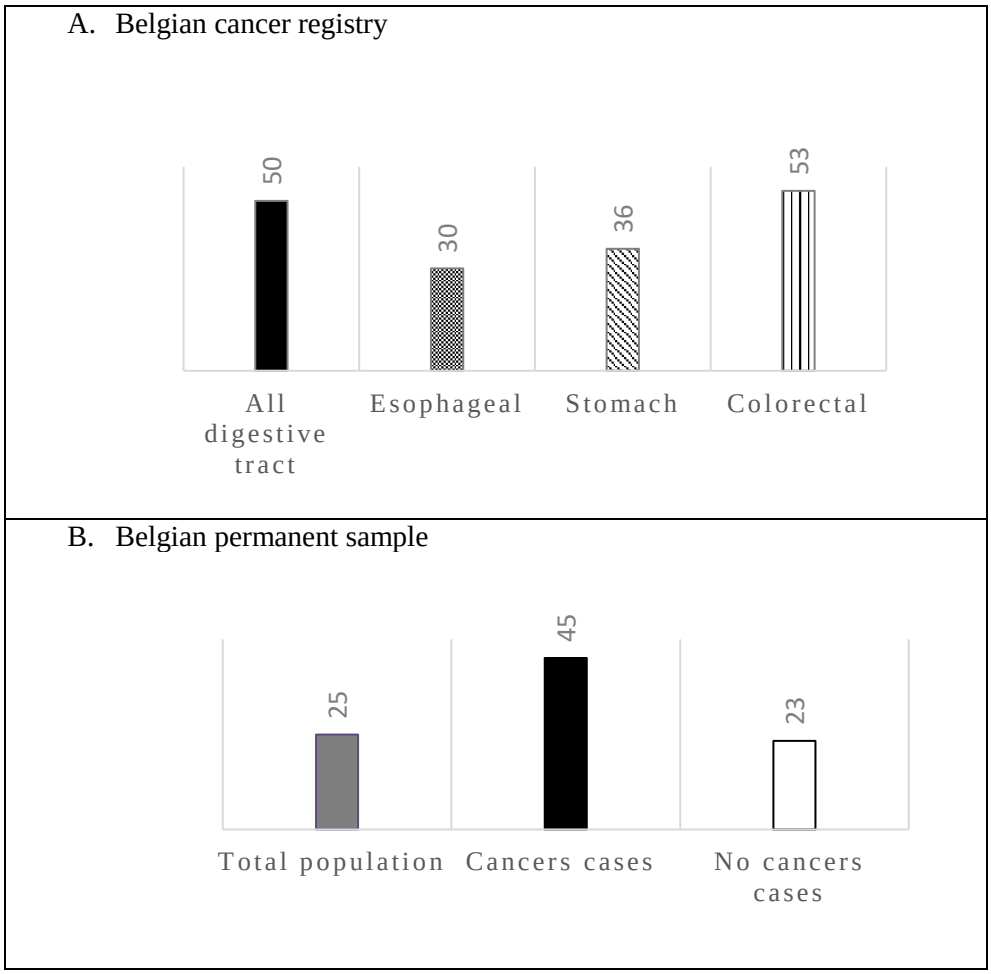



**Figure 2.** Metformin consumption in DDD per thousand persons-day (DDD/TID) in individuals with digestive tract cancer in Belgium between 2004 and 2014 (**Panel A**) and in the Belgian permanent sample (**Panel B**)

### 4.2.2 Drug initiation

#### *4.2.2.1 Statins*

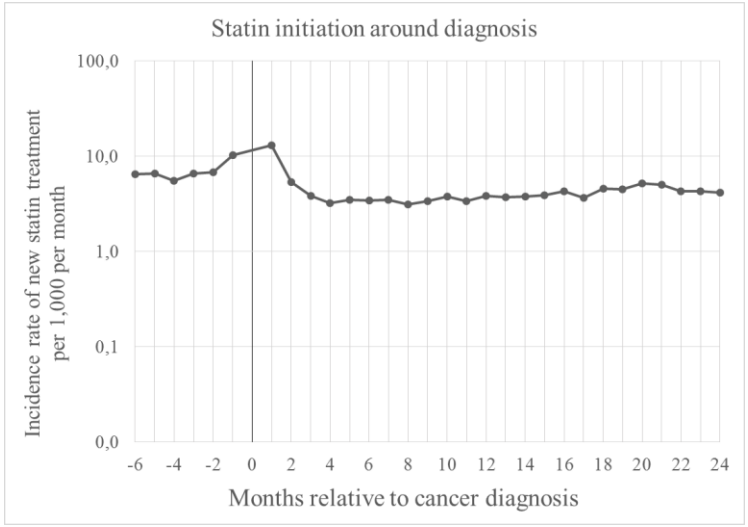
From the 32,604 statins users, 25.648 (79%) initiated their treatment before their cancer diagnosis. From those patients, 17% (n=4,442) stopped their treatment before diagnosis.

A slightly higher number of statins users initiated their treatment after diagnosis (n=6,884 (21%)) (**Table 1**). Their initiation occurred later after diagnosis with half of the patients initiating statins therapy between 9 months and one year and a half following cancer diagnosis depending on the type of cancer **(Table 1)**.

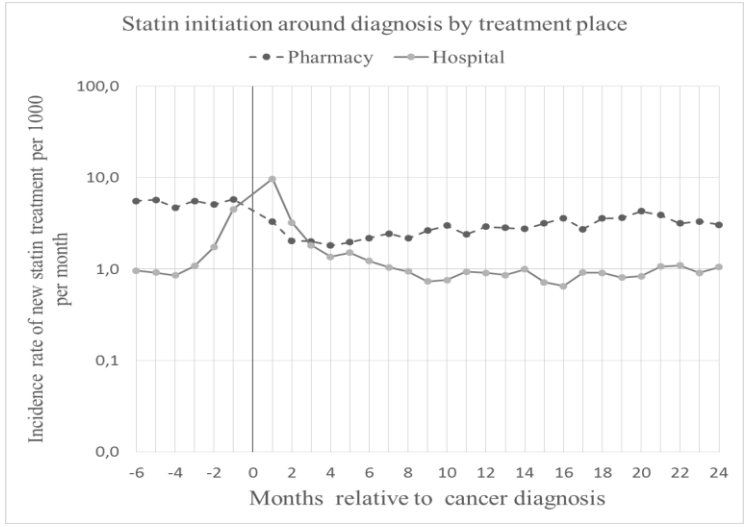
**Table 1.** Statins initiation around diagnosis in patients with digestive tract cancers diagnosed between 2004 and 2014 in Belgium

| | Oesophageal | Stomach | Colorectal | Total |
|---|---|---|---|---|
| | N=10,280 | N=7,444 | N=64,612 | N=82,336 |
| **Statin ever use** | **3,547** | **2,627** | **26,430** | **32,604** |
| **Initiation before diagnosis** | **3,048** | **2,226** | **20,374** | **25,648** |
| **N prescriptions** | 773 | 615 | 3,054 | 4,442 |
| Mean ± SD | 5±3 | 5±3 | 5±3 | 5±3 |
| Median (IQR) | 5 (3-6) | 5 (3-7) | 5 (3-6) | 5 (3-6) |
| Range | 22(1-23) | 21(1-22) | 29(1-30) | 29(1-30) |
| Stop before or at diagnosis | 773 | 615 | 3,054 | 4,442 |
| **Initiation at diagnosis** | **7** | **9** | **56** | **72** |
| **N prescriptions** | | | | |
| Mean ± SD | 7±7 | 8±7 | 10±8 | 10±8 |
| Median (IQR) | 5 (2-10) | 5 (2-12) | 9 (4-15) | 8 (3-15) |
| Range | 63 (1-64) | 100 (1-101) | 201 (1-202) | 201 (1-202) |
| Only prescription at diagnosis | 2 | 1 | 16 | 19 |
| **Initiation after diagnosis** | **492** | **392** | **6,000** | **6,884** |
| **Days to treatment initiation** | | | | |
| median | **282** | **411** | **605** | **578** |
| IQR | 66-724 | 65-890 | 232-1,083 | 198-1,055 |
| **Post-diagnosis users** | **2,772** | **2,011** | **23,360** | **28,143** |
| 1 prescription after diagnosis | 580 | 408 | 2,788 | 3,776 |
| Prescriptions at the same date | 9 | 5 | 25 | 39 |

From 6 months before to 2 years after diagnosis, there was an increase in statin initiation around diagnosis, peaking in the first month after diagnosis and driven by hospital prescriptions **(Figure 3A** and **Figure 3B)**.




**A**




**B**

**Figure 3**. Incidence rate of statins around cancer diagnosis in individuals with digestive tract cancer in Belgium, diagnosed between 2004 and 2014 (**Panel A**) and with source of dispensation (**Panel B**)

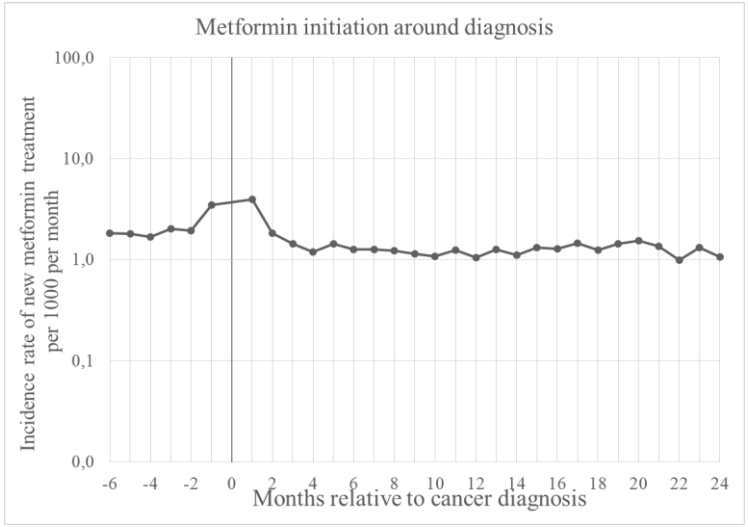
#### *4.2.2.2 Metformin*

From the 11,628 diabetics patients with a diagnosed digestive tract cancer, 8,923 (77%) used metformin at least once during the follow up. The majority of them initiated their treatment before their cancer diagnosis (n=8,294 (93%)). Metformin initiation after diagnosis was less common (n=623 (7%)) and was made between 8 months and more than one year after diagnosis for half of the patients, depending on the type of cancer (**Table 2**). The majority of metformin users still use their treatment after the cancer diagnosis (n=7,441 (83%)) even if 18% stop their treatment before the diagnosis of cancer (**Table 2**).

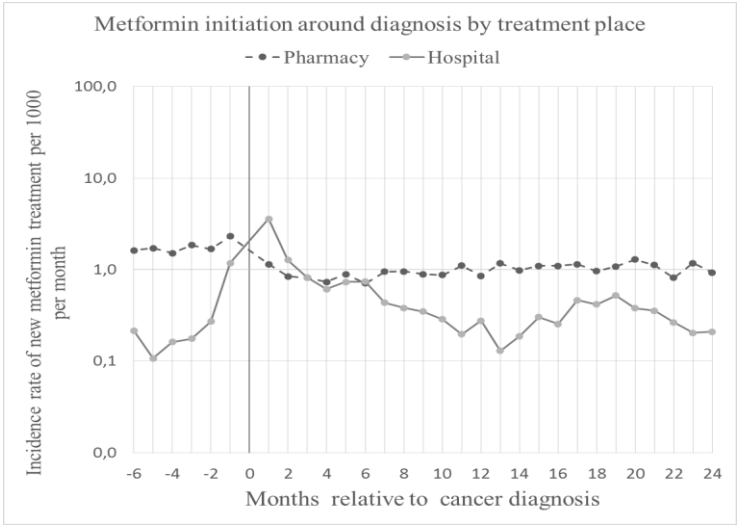
**Table 2.** Metformin initiation around diagnosis in patients with digestive tract cancers and diabetes diagnosed between 2004 and 2014 in Belgium

| | Oesophageal | Stomach | Colorectal | Total |
|---|---|---|---|---|
| | N=10,280 | N=7,444 | N=64,612 | N=82,336 |
| **Diabetics** | 1,370 | 1,106 | 9,152 | 11,628 |
| **Metformin ever use** | **1,029** | **814** | **7,080** | **8,923** |
| **Initiation before diagnosis** | **976** | **772** | **6,549** | **8,294** |
| **N prescriptions** | | | | |
| Mean ± SD | 8±5 | 8±5 | 8±5 | 8±5 |
| Median (IQR) | 7 (4-11) | 8 (5-12) | 7 (4-11) | 7 (4-11) |
| Range | 33 (1-34) | 29 (1-30) | 38 (1-39) | 38 (1-39) |
| Stop before or at diagnosis | 227 | 205 | 1,050 | 1,482 |
| **Initiation at diagnosis** | **-** | **1** | **5** | **6** |
| **N prescriptions** | | | | |
| Mean ± SD | 9±10 | 10±10 | 17±14 | 15±14 |
| Median (IQR) | **5 (2-12)** | **5(2-14)** | **14 (6-25)** | **12 (4-23)** |
| Range | **72 (1-73)** | **61 (1-62)** | **145 (1-146)** | **145 (1-146)** |
| Only prescription at diagnosis | **-** | **-** | **-** | **-** |
| **Initiation after diagnosis** | **53** | **41** | **529** | **623** |
| Days to treatment initiation | | | | |
| median | **275** | **227** | **476** | **449** |
| IQR | 68-781 | 79-777 | 151-930 | 125-910 |
| **Post-diagnosis users** | **802** | **609** | **6,030** | **7,441** |
| 1 prescription after diagnosis | 122 | 105 | 450 | 677 |
| Prescriptions at the same date | 4 | 3 | 4 | 11 |

Focusing on metformin initiated between 6 months before and 2 years after diagnosis, an increase in metformin initiation was also observed around cancer diagnosis peaking in the first month after diagnosis (**Figure 4A**). As in statins users, the increase in metformin initiation was mainly driven by hospital prescriptions (**Figure 4B**).




A




B

**Figure 4**. Incidence rate of metformin around cancer diagnosis in individuals with digestive tract cancer in Belgium, diagnosed between 2004 and 2014 (Panel A) and with source of dispensation (Panel B)

## 4.3 DISCUSSION

In this nationwide study, metformin and statins were both largely prescribed in patients with cancer and more specifically in digestive tract cancers. The initiation rate of both treatments increased near diagnosis and peaked in the month after. The observed increase and the resulting spike in the month following diagnosis were probably driven by hospital prescriptions. Outside hospitals, the initiation rate of statins and metformin dropped after cancer diagnosis.

The estimates of this study have shown a statins consumption of 123.75 Defined Daily Dose per one thousand inhabitants per day (DDD/TID) in the Belgian population. A previous study has shown an increasing consumption of statins from 50.9 DDDs in 2000 to 112.7 DDD in Belgium in 2012.[124] Considering that our estimates were made using a random sample enriched by individuals older than 65 years, the consumption of statins in the general population could have been slightly overestimated. Moreover, with the ageing of the population, the increase in chronic condition prevalence and the modification in dyslipidaemia management guidelines, statins consumption has been expected to increase between 2012 and 2014.[59,124]

Regarding metformin, the present study estimated a consumption of 24.96 DDD/TID. No estimate has been previously published in the Belgian population. However, estimates from other European regions gave similar estimates with a consumption of 24.32 DDD/TID in 2015 in Poland and 23.68 DDD/TID in 2014 in Granada (Spain).[125,126] Outside the European region, another study has reported a consumption of 20 DDD/TID in 2012 in Australia.[127]

In patients with digestive tract cancer and more generally in patients diagnosed with cancer, higher volumes of metformin and statins have been documented in the present study. The estimates indicated an approximate two-fold higher volume of prescribed metformin and statins in cancer patients that was further confirmed in patients with digestive tract cancer. To our knowledge, it is the first time that the volume of chronic conditions drug consumption is reported in patients with cancer. Higher volumes of DDD/TID could be the reflection of higher doses prescribed in cancer population, due for example to an advanced stage of the chronic condition. It could also reflect the greater proportion of individuals treated for diabetes or dyslipidaemia in patients with digestives tract cancers.

Despite cancer diagnosis, an increase in initiation of preventive treatments such as statins or metformin has been shown in this study. This finding is consistent with two others Danish studies.

The first published study had shown that incident use of drugs increased in the month prior to diagnosis depending on the type of cancer and drug.[120] For example and as

expected, prescriptions of drugs used to treat cancer symptoms such as pain were more likely to increase before cancer diagnosis.[120] The second study on ovarian cancer patients, had shown an increase in new drug use before diagnosis that peaked in the first month after diagnosis.[123] Focusing on preventive drugs such as statins, an increase in new prescriptions, although limited, was seen before diagnosis. Moreover, only very limited changes in the post diagnosis use of these drugs was observed.

The pattern of metformin and statins initiation in the present study was similar to both previously published studies. However, they did not have the opportunity to study drug initiation depending on the source of prescription. In the present study, the increase was only seen in hospitalisation prescriptions.

Due to the methodology and the source of data used in this study, our results should be interpreted with caution for several reasons.

First, the results on the consumed volume of statins and metformin in this study are only valid if there is good agreement between the actually prescribed dose and the DDD. The DDD for metformin is 2000mg per day. For statins, the DDDs vary according to the type of statins ranging from 10 to 60 mg per day. To our knowledge, no study has investigated the concordance between DDDs and the dose prescribed by physicians in Belgium for metformin and statins. However, a study conducted in the US has reported an underestimation of the exposure using the DDD method for metformin and statins.[128] This could mean that compared to the DDD defined by the World Health Organisation, lower doses of both medications are actually prescribed by physicians so people are exposed longer with the same amount of drug. In their study, the authors found that in the case of metformin, the average daily prescribed dose for the first prescription was 1000mg in their cohort contrasting with a DDD of 2000mg.[128] In Belgium the prescription recommendations for metformin are ranging from 1000mg to 3000mg per day.[129]

Second, in the Belgian cancer registry, only data on prescriptions occurring in the year before diagnosis are available for pre-diagnosis exposure. Some prevalent users might therefore have been, misclassified as initiating therapy in the year before diagnosis whereas they had initiated their therapy long before. This phenomenon could also partially explain the spike observed around diagnosis in hospitals. Chronic medication drugs used in hospital do not belong to patients, they come from the hospital pharmacy and, are charged daily and directly to patients' health insurance. Therefore, patients with a "stock" of pills will be charged again by hospital and may appear as initiating their treatment whereas they are prevalent users.

Moreover, it would be interesting to conduct a nested matched case-control study with BCR (cancer patients) and EPS (population persons) databases to compare

incidences rates and DDD/TID after removing bias due to age, sex or regional differences.

Our study has several strengths arising from the use of a nationwide registry database for the identification of the digestive tract cancer cases. The Belgian cancer registry also has a legal basis to use the national social security number (INSZ/NISS) as a unique patient's identifier and can therefore use this number to link its database to diagnostic and therapeutic procedures stemming from the IMA. As health insurance in Belgium is mandatory, the IMA database containing all the treatments and medical procedures claims from the seven Belgian health insurance companies, can be considered as comprehensive. Also, statins and metformin are only available with a medical prescription at pharmacy. Therefore, it is quite unlikely that over-the-counter use could have happened.

From a methodological point of view, drug consumption was assessed using the Anatomical Therapeutic Chemical (ATC) for international comparison and standard methods for estimates drug use across population such as the Defined Daily Dose per one thousand inhabitants per day (DDD/TID) were used.

## 4.4 CONCLUSIONS

In conclusion, in this Belgian population-based study, an increase in metformin and statin initiation peaking one month after diagnosis was observed around digestive tract cancer diagnosis. Also, despite a diagnosis of digestive tract cancer, the prescribed volume of the two drugs was higher than in the general population, reaching more than twice the latter. These findings might contribute to the elaboration of pharmacoepidemiologic studies design, by enhancing the general knowledge on chronic conditions medication usage in a cancer setting.

## 4.5 SUPPLEMENTARY MATERIAL

**Table S1.** Characteristics of statins users in patients with digestive tract cancer diagnosed between 2004 and 2014 in Belgium

| | **Total** | Statins users | | |
|---|---|---|---|---|
| | | Ever | Pre-diagnostic | Post diagnostic |
| | **82,336** | **32,604** | 25,648 | 28,143 |
| **Age (**Mean ±SD) | **69±12** | **71±10** | 71±9 | 71±10 |
| **Men —n (%)** | 47,365 (58) | 19,348 (59) | 15,166 (59) | 16,925 (60) |
| **Grade of differentiation—n (%)** | | | | |
| Poorly | **16,581 (20)** | **6,005 (18)** | 4,992 (20) | 4,910 (18) |
| Moderately | **39,878 (48)** | **16,457 (50)** | 12,750 (50) | 14,549 (52) |
| Well | **12,511 (15)** | **5,320 (16)** | 4,131 (16) | 4,712 (17) |
| Unknown/missing | **13,368 (16)** | **4,822 (15)** | 3,775 (15) | 3,972 (14) |
| **Localisation—n (%)** | | | | |
| Oesophagus | **10,280 (13)** | **3,547 (11)** | 3,048 (12) | 2,772 (10) |
| Stomach | **7,444 (9)** | **2,627 (8)** | 2,226 (9) | 2,011 (7) |
| Colorectum | **64,612 (79)** | **26,430 (81)** | 20,374 (79) | 23,360 (83) |
| **Combined stage—n (%)** | | | | |
| I | **14,925 (20)** | **6,980 (21)** | 5,324 (21) | 6,479 (23) |
| II | **20,645 (27)** | **8,717 (27)** | 6,502 (25) | 7,878 (28) |
| III | **21,394 (28)** | **8,478 (26)** | 6,602 (26) | 7,430 (26) |
| IV | **17,331 (23)** | **5,635 (17)** | 4,998 (20) | 4,118 (15) |
| NA | **1,120 (2)** | **494 (2)** | 378 (2) | 421 (2) |
| **missing** | **6,921 (8)** | **2,300 (7)** | 1,844 (7) | 1,817 (7) |
| **Treatment—n (%)** | | | | |
| Surgery | **59,963 (73)** | **24,944 (77)** | 19,187 (75) | 22,300 (79) |
| Chemotherapy | **49,657 (60)** | **18,897 (58)** | 14,786 (58) | 16,336 (58) |
| Radiotherapy | **21,567 (26)** | **8,120 (25)** | 6,226 (24) | 7,123 (25) |
| **Comorbidities —n (%)** | | | | |
| Cardiovascular diseases | **41,816 (51)** | **22,283 (68)** | 19,264 (75) | 19,285 (69) |
| Respiratory diseases | **4,495 (6)** | **1,966 (6)** | 1,617 (6) | 1,641 (6) |
| Diabetes | **11,628 (14)** | **7200 (22)** | 6,248 (24) | 6,296 (22) |

**Table S2.** Characteristics of metformin users in patients with digestive tract cancer and diabetes diagnosed between 2004 and 2014 in Belgium

| | **Total** | **Diabetics** | **Metformin users** | | |
|---|---|---|---|---|---|
| | | | **Ever** | **Pre-diagnostic** | **Post diagnostic** |
| | **82,336** | **11,628** | **8,923** | 8,294 | 7,441 |
| **Age (**Mean ±SD) | **69±12** | **72±10** | **72±9** | 71±9 | 71±9 |
| **Men —n (%)** | 47,365 (58) | **6,987 (60)** | **5,442 (61)** | 5,056 (61) | 4,573 (62) |
| **Grade of differentiation—n (%)** | | | | | |
| Poorly | **16,581 (20)** | **2,259 (25)** | **1,714 (19)** | 1,613 (20) | 1,386 (19) |
| Moderately | **39,878 (48)** | **5,676 (49)** | **4,416 (49)** | 4,065 (49) | 3,771 (51) |
| Well | **12,511 (15)** | **1,827 (16)** | **1,405 (16)** | 1,311 (16) | 1,202 (16) |
| Unknown/missing | **13,368 (16)** | **1,866 (16)** | **1,388 (16)** | 1,305 (16) | 1,082 (15) |
| **Localisation—n (%)** | | | | | |
| Oesophagus | **10,280 (13)** | **1,370 (12)** | **1,029 (12)** | 976 (12) | 802 (11) |
| Stomach | **7,444 (9)** | **1,106 (10)** | **814 (9)** | 772 (9) | 609 (8) |
| Colorectum | **64,612 (79)** | **9,152 (79)** | **7,080 (79)** | 6,546 (79) | 6,030 (81) |
| **Combined stage—n (%)** | | | | | |
| I | **14,925 (20)** | **2,111 (18)** | **1,694 (19)** | 1,567 (19) | 1,501 (20) |
| II | **20,645 (27)** | **3,034 (26)** | **2,334 (26)** | 2,144 (26) | 2,038 (27) |
| III | **21,394 (28)** | **3,028 (26)** | **2,354 (26)** | 2,165 (26) | 2,009 (27) |
| IV | **17,331 (23)** | **2,329 (20)** | **1,737 (19)** | 1,659 (20) | 1,298 (17) |
| NA | **1,120 (2)** | **163 (1)** | **134 (2)** | 129 (2) | 111 (2) |
| **missing** | **6,921 (8)** | **963 (8)** | **670 (8)** | 630 (8) | 484 (7) |
| **Treatment—n (%)** | | | | | |
| Surgery | **59,963 (73)** | **8,429 (72)** | **6,655 (75)** | 6,147 (74) | 5,756 (77) |
| Chemotherapy | **49,657 (60)** | **6,448 (55)** | **5,116 (57)** | 4,760 (57) | 4,341 (58) |
| Radiotherapy | **21,567 (26)** | **2,704 (23)** | **2,094 (23)** | 1,929 (23) | 1,791 (24) |
| **Comorbidities disease—n (%)** | | | | | |
| Cardiovascular diseases | **41,816 (51)** | **9,324 (80)** | **7,176 (80)** | 6,707 (81) | 5,985 (80) |
| Respiratory diseases | **4,495 (6)** | **835 (7)** | **607 (7)** | 565 (7) | 487 (7) |

**Figure S1.** Statins consumption in DDD per thousand persons-day (DDD/TID) in individuals with digestive tract cancer in Belgium between 2004 and 2014 by sex and age categories.

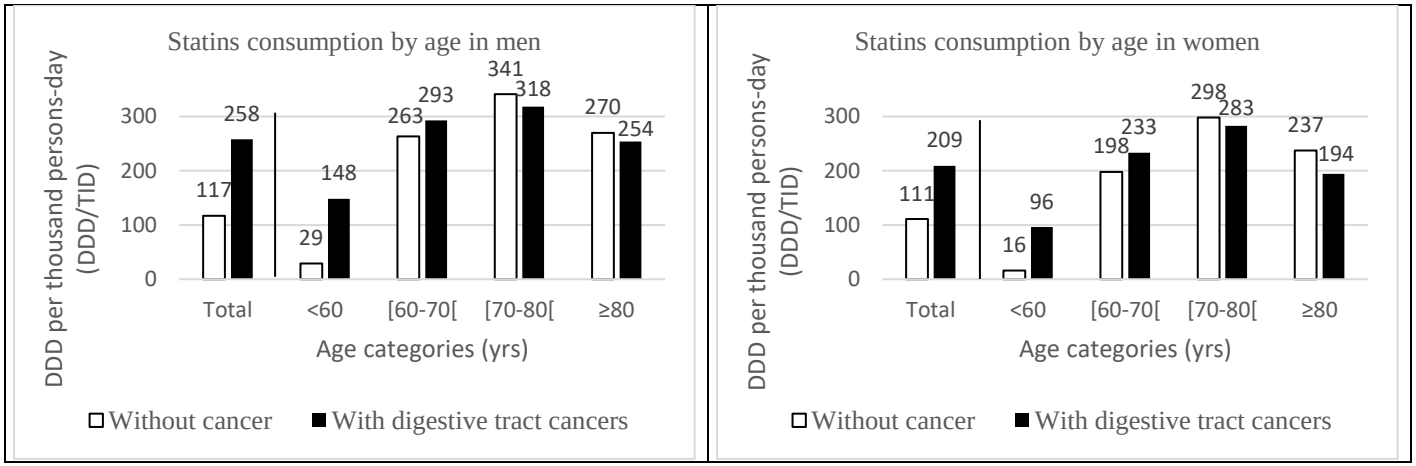
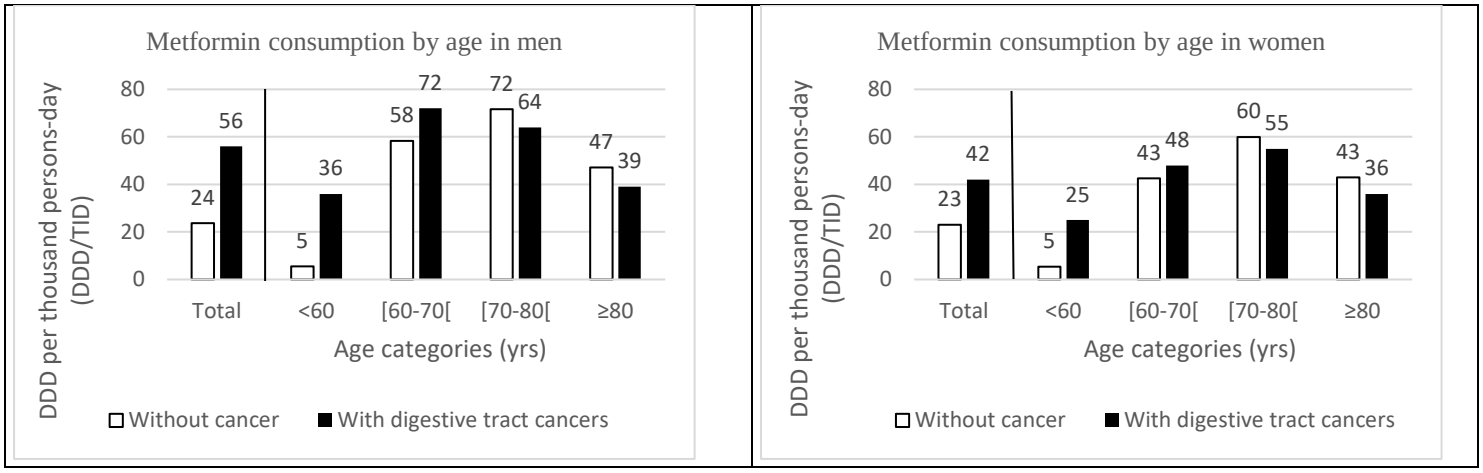


**Figure S2.** Metformin consumption in DDD per thousand persons-day (DDD/TID) in individuals with digestive tract cancer in Belgium between 2004 and 2014 by sex and age categories.



# 5 ADHERENCE TO STATINS AND METFORMIN IN GASTROINTESTINAL CANCER PATIENTS IN BELGIUM

With advances in cancer detection and treatment, cancer survival has increased, and cancer is now considered as a chronic disease. The majority of cancer survivors are expected to be in their 60s or more.[130]

Comorbidities are more frequent in older patients and more particularly in older patients with cancer.[131] The presence of comorbidities in those patients is often associated with poorer prognosis explained in part by comorbidities' mortality.[55]

Adherence is defined as "the extent to which a person's behaviour -taking medication, following a diet, and/or executing lifestyle changes- corresponds with the agreed recommendations from a healthcare provider".[132] Successful adherence to chronic condition treatments is crucial in managing these conditions.[133]

In clinical research in general, poor adherence can mask a difference between treatments if not considered. Adherence disparities can lead to compliance bias that will distort the results. In clinical trials, this issue is less relevant because volunteers are expected to be motivated to take the medication.[77] In observational studies, however, patients are representative of those treated in real clinical practice and it is estimated that only 50% of them are adherent to their chronic therapy.[77,134] In pharmacoepidemiologic studies on drugs' beneficial and adverse effects particularly, adherence is essential in order to properly interpret the findings of those studies.[77] Adherence might also be useful to determine how much medication must be taken to observe the desired outcome.[77]

With the growing trend of using administrative databases in pharmacoepidemiologic studies, indirect methods to assess adherence have emerged.[132] However, studies on adherence to chronic medications in cancer patients are still scarce. They generally focus on patients with good prognosis' cancer such as breast or prostate cancers and little is known about cancers with a poorer prognosis like oesophageal or gastric cancer.[133,135,136]

In the present study, we aimed to evaluate adherence to statins and to metformin, two chronic-conditions medications around digestive cancer diagnosis and to identify factors associated with patients' adherence after their diagnosis.

## 5.1 METHODS

### 5.1.1 Study population

Patients with gastrointestinal tract cancers were extracted from the Belgian cancer registry (BCR) database. Gastrointestinal tract cancers were defined as first primary oesophageal cancers (ICD10 C15.0-16.0), gastric cancers (ICD10 C16.1-C16.9) and colorectal cancers (ICD10 C18.0-C20.9) diagnosed between January 2004 and December 2014.

Diagnostic and therapeutic procedures stemming from the Intermutualistic agency (IMA) were linked to the BCR database using the patient's specific national social security identification number (SSIN).

Individuals with a previous cancer diagnosis, not residing in Belgium at the time of diagnosis, with an uncertain date of diagnosis, with no national social security identification number (SSIN), lost to follow-up at the date of cancer incidence, or missing from the medical claims (IMA) database were excluded from the analysis.

For comparison purposes, the Belgian permanent sample was used to estimate adherence in a cancer-free population. The cancer-free population was defined by excluding individuals with a multidisciplinary oncological consultation and/or chemotherapy and/or radiotherapy. In the BCR database, the same characteristics were added to the inclusion criteria.

### 5.1.2 Adherence outcome

The primary outcome was adherence to metformin and statins defined as a modified medication possession ratio (MPRm) of 80% or more. The modified MPR was calculated by dividing the sum of all defined daily doses (DDD) received between the first and the last prescription by the number of days between those two dates.[137,138]

$$MPR_m = \frac{\sum received\ Defined\ Daily\ Doses}{\sum days\ between\ first\ and\ last\ prescription}$$

The DDD is defined by the World Health Organisation as the assumed average maintenance dose per day for a drug used in its main indication in adults.[79] The DDDs in the last prescription were not taken into account in the calculation.

### 5.1.3 Analysis

Descriptive statistics were used to assess patients and tumour characteristics. Factors associated with metformin and statins adherence were assessed through univariate and multivariable logistic regression. A stepwise backward elimination procedure was used for finding factors independently associated with adherence. All variables were included in the initial model; then, using the likelihood ratio test, the contribution of each variable in the model was tested and the less contributing variable was eliminated if not significant. The results are reported using odds ratios (ORs) and their 95% confidence intervals (CI). Data were analysed using SAS enterprise guide release 9.3 software. The statistical significance level was set to 0.05.

## 5.2 RESULTS

### 5.2.1 Study population

From the 82,336 patients identified with digestive tract cancers, 32,604 (40 %) were statin users, 11,628 (14%) were diabetics patients of whom 8,923 (77%) were metformin users.

Statins users before and after diagnosis were 25,648 and 28,143 representing 79% and 86% of the total users, respectively. MPR was calculable for 22,872 (89%) pre-diagnostic and 24,328 (86%) post-diagnostic users due to unique prescription or prescriptions occurring at the same date.

In diabetics' patients, metformin users were 8,294 before diagnosis and 7,441 after diagnosis representing 92% and 83% of the total users, respectively. MPR was calculable for 7,701 (93%) pre-diagnostic and 6,753 (91%) post-diagnostic users for the same reasons as statins users.

The Belgian permanent sample represented 394,252 individuals, of whom 7.8 % (n=30,899) were considered to have a cancer. From the remaining 363,353 individuals without cancer, statins users represented 22% (n=81,435). Diabetics patients represented 10% (n=35,153) of individuals without cancer, of whom 79% (n=27,870) where metformin users.

The modified MPR was calculable in 71,987 (88%) statins users and in 24,749 (88%) diabetics' metformin users.

The characteristics of individuals included in the analyses are presented in **Table S1** and **S2** in supplementary material.

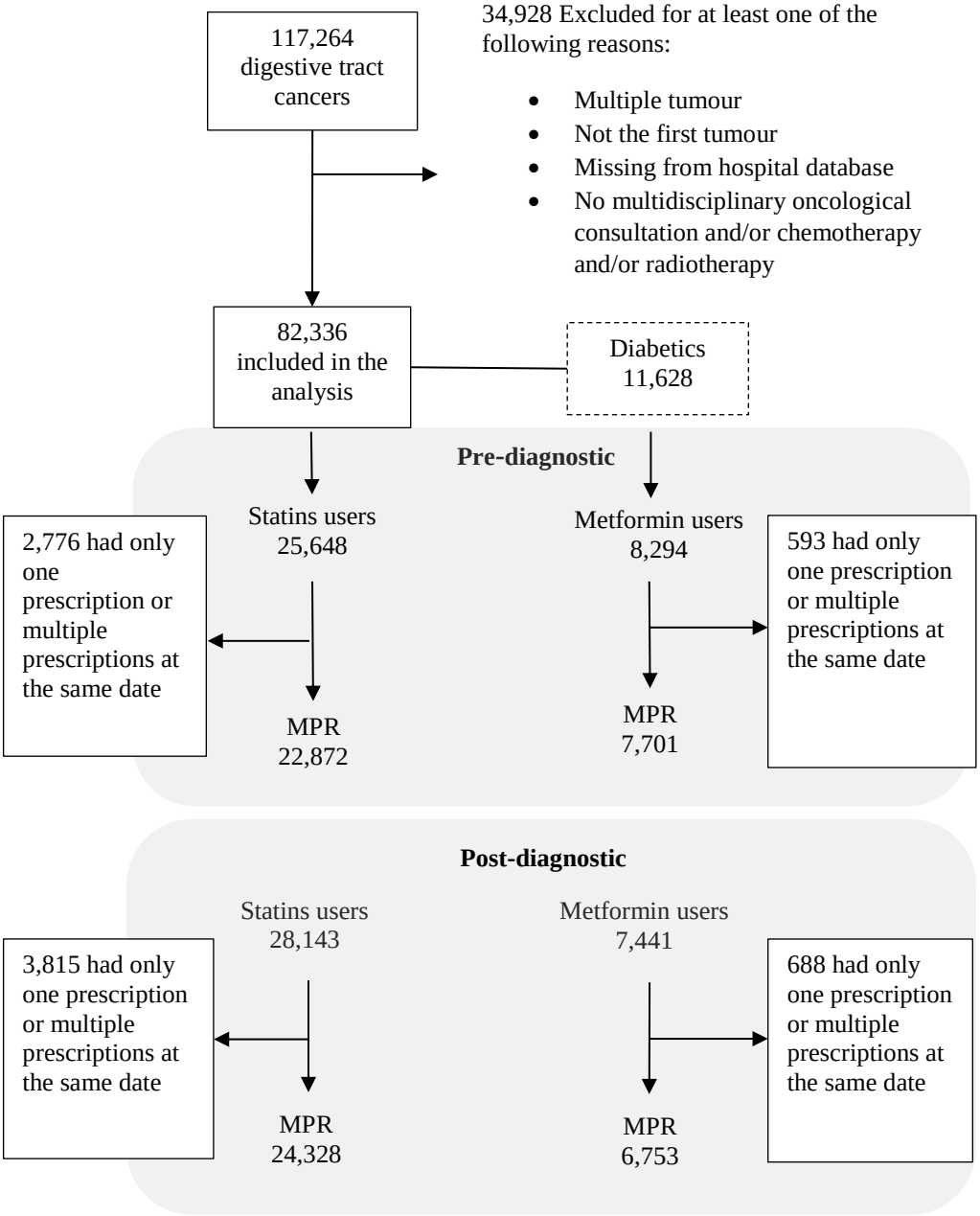



**Figure 1.** Flow of patients diagnosed between 2004 and 2014 in Belgium with gastrointestinal tract cancers and involved in adherence assessment

### 5.2.2 Statins and metformin adherence around diagnosis

The patients' adherence to statins and metformin drops after cancer diagnosis. Pre-diagnosis adherence was higher than the adherence in a non-cancerous population (**Figure 2, Figure 3**). The median adherence of patients without cancer was closer to the post-diagnosis one in cancer patients (**Figure 2, Figure 3**).

In statins users, the MPR drop from a median of 0.90 (IQR, 0.63-1.28) before diagnosis to a median of 0.74 (IQR, 0.49-1.12) after diagnosis (**Figure 2**). As expected, the proportion of adherent to statins also falls below 50 % after cancer diagnosis (57% before to 46% after diagnosis) (**Figure 2**).

In diabetic metformin users, the MPR drop from a median of 0.69 (IQR, 0.44-0.92) before diagnosis to a median of 0.55 (IQR, 0.34-0.81) after diagnosis (**Figure 3**). Similarly to statins users, the proportion of adherent to metformin also falls from 39% before to 26% after diagnosis (**Figure 3**).

This drop in adherence for both medications was also seen in prevalent users (i.e. patients who were already statins users before diagnosis (**Figure S1, Figure S2)**.

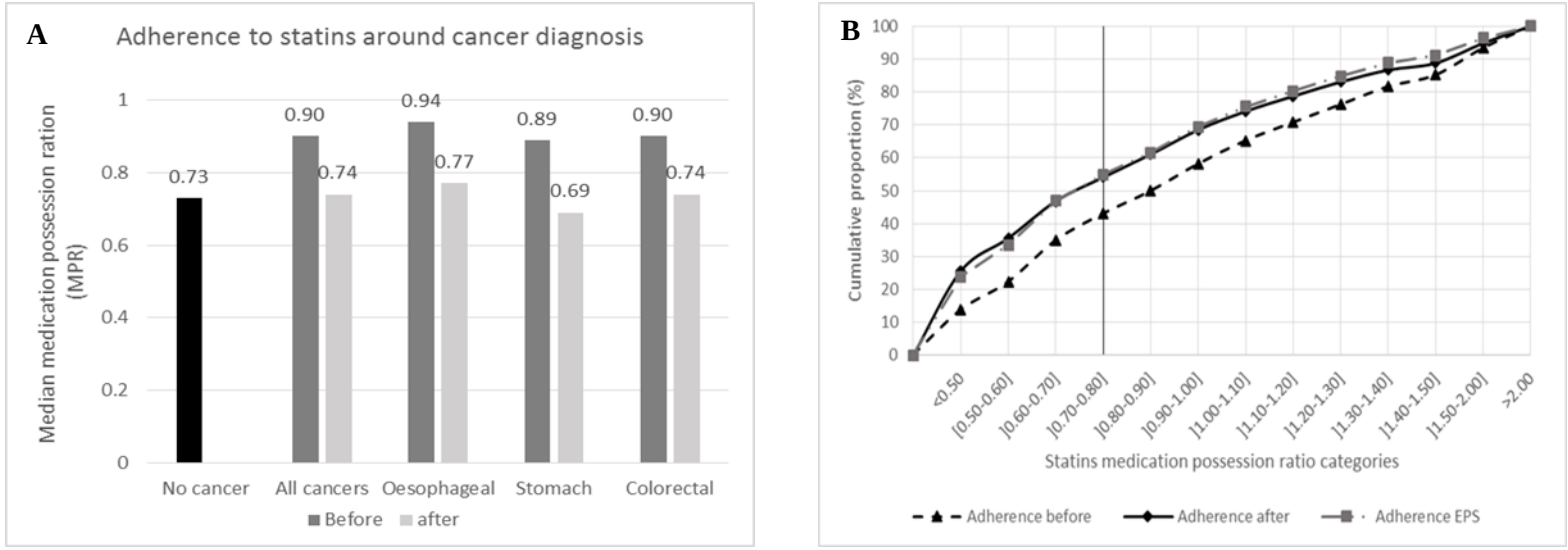



**Figure 2:** Adherence to statins before and after diagnosis in patients diagnosed with digestive tract cancer between 2004 and 2014 in Belgium.

Panel **A** shows statins median medication possession ratio in the cancer free population from the permanent sample and in digestive tract cancer patients. For digestive cancers, median adherence before and after diagnosis are represented for each cancer.

Panel **B** shows the cumulative proportions of individuals in each statins medication possession ratio category. The black lines represent adherence before (dashed line) and after (plain line) diagnosis in patients with digestive tract cancer. The lighter line represents the cumulative proportion in the cancer free population.

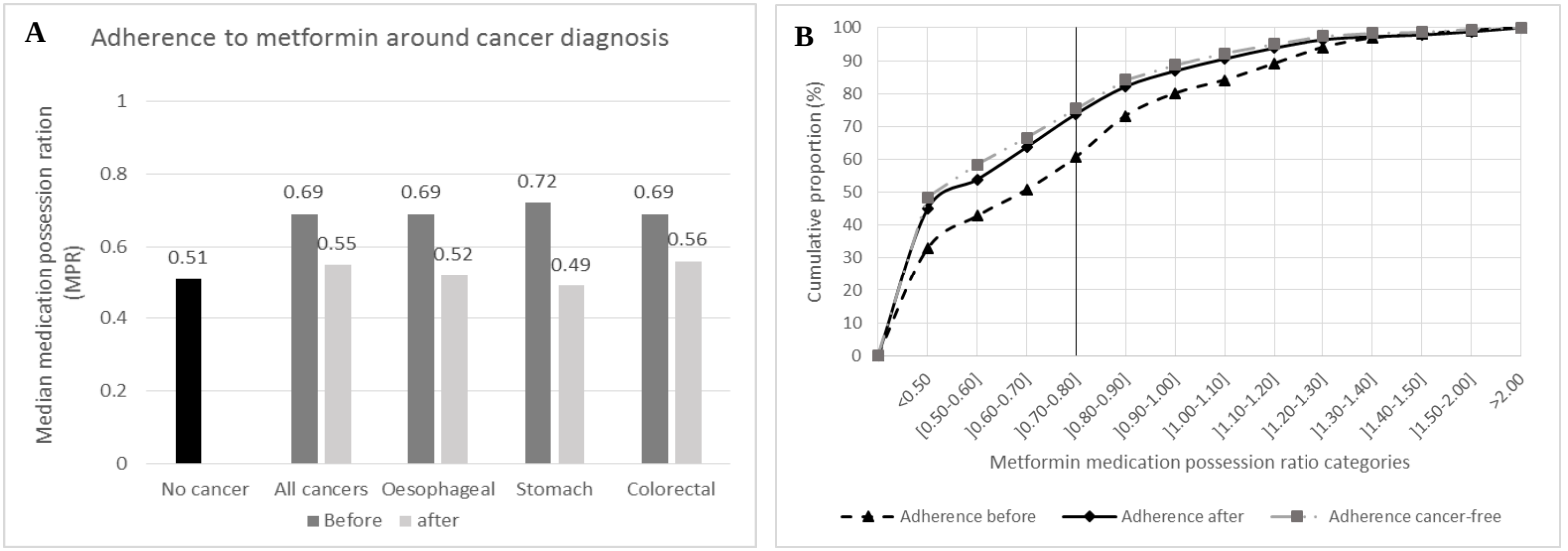



**Figure 3:** Adherence to metformin before and after diagnosis in patients diagnosed with digestive tract cancer between 2004 and 2014 in Belgium.

Panel **A** shows metformin median medication possession ratio in the cancer free population and in digestive tract cancer patients. For digestive cancers, median adherence before and after diagnosis are represented for each cancer.

Panel **B** shows the cumulative proportion of individuals in each metformin medication possession ratio category. The black lines represent the cumulative proportion before (dashed line) and after (plain line) diagnosis in patients with digestive tract cancer. The lighter line represents the cumulative proportion in the cancer free population.

### 5.2.3 Factors associated with adherence after cancer diagnosis

#### *5.2.3.1 Statins*

In statins users, patient related factors independently associated with adherence were age at diagnosis, sex, cardiovascular comorbidities and diabetes. The proportion of statins adherent decreased with increasing age at diagnosis (adjusted OR=0.88, 95%CI, 0.86-0.91) and in women when compared to men (adjusted OR =1.22, 95%CI, 1.15-1.28 for men) (**Table3**).

In multivariable model, treatment related factors associated with adherence were statins prevalent user's status and surgery. Prevalent statin users were more likely to be adherents after cancer diagnosis when compared to new users (adjusted OR=1.19, 95%CI, 1.12-1.28) (**Table3**). Patients who underwent cancer surgery, however, were less likely to be adherent to statins (adjusted OR=0.91, 95%CI, 0.85-0.98) (**Table3**).

Regarding tumour related factors, only tumour localisation was associated with adherence to statins with lower adherence in oesophageal cancer and gastric cancer when compared to colorectal cancer (adjusted OR =0.96, 95%CI 0.87-1.05, for oesophageal cancer, and adjusted OR =0.84, 95%CI, 0.76-0.93 for gastric cancer) (**Table3**). No significant interaction with cancer localisation was observed.

**Table 1**. Unadjusted and adjusted odds ratio of adherence to statins after a gastrointestinal tract cancer diagnosis.

| | | | OR (95% CI) | | | |
|---|---|---|---|---|---|---|
| | Total | Adherents n (%) | Unadjusted | *p* [a] | Adjusted [b] | *p* [a] |
| **Age (10years)** | 24,328 | 11,188 (46.0) | 0.92 (0.90-0.95) | <0.0001 | 0.88 (0.86-0.91) | <0.0001 |
| **Prior statin use** | | | | <0.0001 | | <0.0001 |
| No | 5,011 | 2,043 (40.8) | reference | | reference | |
| Yes | 19,317 | 9,145 (47.3) | 1.31 (1.23-1.39) | | 1.19 (1.12-1.28) | |
| **Sex** | | | | <0.001 | | <0.0001 |
| Women | 9,654 | 4,093 (42.4) | reference | | reference | |
| Men | 14,671 | 7,095 (48.3) | 1.27 (1.21-1.34) | | 1.22 (1.15-1.28) | |
| **Grade of differentiation** | | | | 0.56 | | |
| Poorly | 4,097 | 1,844 (45.0) | reference | | | |
| Moderately | 12,775 | 5,905 (46.2) | 1.05 (0.98-1.13) | | | |
| Well | 4,164 | 1,913 (45.9) | 1.04 (0.95-1.13) | | | |
| Unknown/missing | 3,292 | 1,526 (46.4) | 1.06 (0.96-1.16) | | | |
| **Localisation** | | | | 0.003 | | 0.004 |
| Oesophagus | 2,183 | 1,059 (48.5) | 1.10 (1.01-1.21) | | 0.96 (0.87-1.05) | |
| Stomach | 1,598 | 684 (42.8) | 0.88 (0.79-0.97) | | 0.84 (0.76-0.93) | |
| Colorectal | 20,547 | 9,445 (46.0) | reference | | reference | |
| **Combined stage** | | | | 0.43 | | |
| I | 5,853 | 2,747 (46.9) | 1.06 (0.97-1.15) | | | |
| II | 6,939 | 3,128 (45.1) | 0.98 (0.90-1.07) | | | |
| III | 6,529 | 3,012 (46.1) | 1.02 (0.94-1.11) | | | |
| IV | 3,173 | 1,447 (45.6) | reference | | | |
| NA | 365 | 167 (45.8) | 1.01 (0.81-1.25) | | | |
| missing | 1,469 | 687 (46.8) | 1.05 (0.92-1.18) | | | |
| **Surgery** | | | | <0.001 | | 0.007 |
| Yes | 19,649 | 8,927(45.4) | 0.90 (0.84-0.95) | | 0.91 (0.85-0.98) | |
| **Chemotherapy** | | | | 0.02 | | |
| Yes | 14,032 | 6,545 (46.7) | 1.07 (1.01-1.12) | | | |
| **Radiotherapy** | | | | 0.55 | | |
| Yes | 6,125 | 2,837 (46.4) | 1.02 (0.96-1.08) | | | |
| **Cardiovascular diseases** | | | | <0.001 | | <0.0001 |
| Yes | 17,120 | 8,340 (48.7) | 1.45 (1.37-1.54) | | 1.43 (1.35-1.52) | |
| **Respiratory diseases** | | | | 0.004 | | |
| Yes | 1,391 | 693 (49.8) | 1.17 (1.05-1.31) | | | |
| **Diabetes** | | | | <0.001 | | <0.0001 |
| Yes | 5,624 | 2,883 (51.3) | 1.32 (1.24-1.40) | | 1.22 (1.14 -1.29) | |

[a] Likelihood ratio test

[b] Adjusted for age, Sex, Prior statin use, localisation, surgery, cardiovascular diseases and diabetes.

Adherent is defined as a medication possession ratio >0.80

#### *5.2.3.2 Metformin*

In diabetic patients using metformin, patient related factors independently associated with adherence were age at diagnosis and sex. Similarly to statins, the proportion of adherents to metformin decreased with increasing age at diagnosis (adjusted OR=0.79, 95%CI, 0.74-0.84) and in women when compared to men (adjusted OR =1.21, 95%CI, 1.07-1.35 for men) (**Table 4**).

In multivariable model, treatment related factors associated with adherence were metformin prevalent user's status, surgery and radiotherapy. Prevalent metformin users were more likely to be adherent to metformin after cancer diagnosis when compared to new users (adjusted OR=1.40, 95%CI, 1.11-1.76) (**Table 4**). However, patients who underwent cancer surgery or radiotherapy were less likely to be adherent to statins (adjusted OR=0.81, 95%CI, 0.71-0.92 for surgery and adjusted OR=0.77, 95%CI, 0.67-0.88 for radiotherapy) (**Table 4**).

None of the tumour related factors included in the multivariable model was associated with metformin adherence (**Table 4**). No significant interaction with cancer localisation was observed.

**Table 2**. Unadjusted and adjusted odds ratio of adherence to metformin after a gastrointestinal tract cancer diagnosis

| | Total | Adherents n (%) | OR (95% CI) Unadjusted | *p* [a] | Adjusted [b] | *p* [a] |
|---|---|---|---|---|---|---|
| **Age (10years)** | 6,753 | 1,766 (26.2) | 0.79 (0.74-0.84) | <0.0001 | 0.79 (0.74-0.84) | <0.0001 |
| **Prior statin use** | | | | <0.0001 | | <0.01 |
| No | 492 | 98 (19.9) | reference | | reference | |
| Yes | 6,261 | 1,668 (26.6) | 1.46 (1.16-1.83) | | 1.40 (1.11-1.76) | |
| **Sex** | | | | <0.001 | | <0.01 |
| Women | 2,592 | 603 (23.3) | reference | | reference | |
| Men | 4,161 | 1,163 (28.0) | 1.28 (1.14-1.43) | | 1.21 (1.07-1.35) | |
| **Grade of differentiation** | | | | 0.99 | | |
| Poorly | 1,225 | 322 (26.3) | reference | | | |
| Moderately | 3,480 | 909 (26.1) | 0.99 (0.86-1.15) | | | |
| Well | 1,105 | 289 (26.2) | 0.99(0.83-1.20) | | | |
| Unknown/missing | 943 | 246 (26.1) | 0.99 (0.82-1.20) | | | |
| **Localisation** | | | | 0.61 | | |
| Oesophagus | 676 | 172 (25.4) | 0.95 (0.79-1.14) | | | |
| Stomach | 501 | 123 (24.6) | 0.91 (0.74-1.12) | | | |
| Colorectal | 5,576 | 1471 (26.4) | reference | | | |
| **Combined stage** | | | | 0.05 | | |
| I | 1,396 | 358 (25.6) | 0.84 (0.70-1.00) | | | |
| II | 1,887 | 455 (24.1) | 0.77 (0.65-0.91) | | | |
| III | 1,843 | 500 (27.1) | 0.90 (0.77-1.06) | | | |
| IV | 1,109 | 324 (29.2) | reference | | | |
| NA | 102 | 24 (23.5) | 0.75 (0.46-1.20) | | | |
| missing | 416 | 105 (25.2) | 0.82 (0.63-1.06) | | | |
| **Surgery** | | | | 0.02 | | <0.01 |
| Yes | 5,326 | 1,358 (25.5) | 0.86 (0.75-0.97) | | 0.81 (0.71-0.92) | |
| **Chemotherapy** | | | | <0.01 | | |
| Yes | 3,997 | 1,096 (27.4) | 1.18 (1.05-1.32) | | | |
| **Radiotherapy** | | | | 0.04 | | <0.001 |
| Yes | 1,655 | 401 (24.2) | 0.88 (0.77-0.99) | | 0.77 (0.67-0.88) | |
| **Cardiovascular diseases** | | | | 0.96 | | |
| Yes | 5,431 | 1,421 (26.2) | 1.45 (1.37-1.54) | | | |
| **Respiratory diseases** | | | | 0.05 | | |
| Yes | 436 | 97 (22.3) | 0.80 (0.63-1.01) | | | |

[a] Likelihood ratio test
[b] Adjusted for age, Sex, Prior statin use, surgery and radiotherapy.
Adherent defined as a medication possession ratio >0.80

## 5.3 DISCUSSION

In this large study on patients diagnosed with gastrointestinal tract cancers, the adherence to metformin and statins was low and declined following the diagnosis of cancer. In both metformin and statins, factors associated with adherence after cancer diagnosis included age at diagnosis, sex, the prevalent users' status and the occurrence of surgery. More specific to statins users, comorbidities such as cardiovascular disease and diabetes were positively associated with adherence. In diabetics' patients using metformin, radiotherapy was negatively associated with adherence.

Studies on adherence to chronic medication in a cancer setting are scarce. The few ones published tend to show a negative association between adherence to both types of medication and cancer diagnosis.

A recent study on breast cancer patients has reported rate of non-adherence to glucose lowering therapy as high as 75% in the second year after cancer diagnosis.[139]Another study showed similar results with an adherence rate to diabetes medications of 24.6% , 27%, 24%, 32% during breast cancer treatment, one year after, two years after and three years after cancer treatment, respectively.[140] Those results strengthened our present findings of 26% of adherence (or 74% of non-adherence) in the five years following digestive tract cancer diagnosis.

Regarding statins use, a previously cited study reported a 39% rate of non-adherence in breast cancer survivors.[139] Another one, focusing on stage I to III breast cancers found a non-adherence rate of 29%.[141] In the present study a higher rate (54%) of non-adherence to statins has been observed. This difference could be attributed to more aggressive cancer and the inclusion of advanced stage disease.

Concerning the impact of cancer on medication adherence, a recent study has shown a decrease in adherence to statins following breast, colorectal or prostate cancer diagnosis.[135] In those three cancers having a relatively good prognosis, the impact of cancer diagnosis was already visible. In another study investigating adherence to glucose lowering therapy in diabetics' patients, results were similar with a higher decrease in individuals with advanced cancer stage, gastrointestinal or lung cancer.[142]

Only few studies have investigated chronic medications' adherence in a cancer setting and so, even fewer studies have attempted to identify factors associated with this behaviour.

In two studies, age has been negatively associated with adherence.[133,139] Younger age together with advanced age have been pointed out to be at higher risk of non-adherence. In older patients, reasons for non-adherence are multiple, like frailty, less family and social support, or transportation difficulties to refill drugs.[133]

In individuals without cancer, adherence to statins is reported to be associated with gender; women being less adherent than their male counterparts.[143,144] Reasons for medication non-adherence to statins include the misconception of women being less at risk of cardiovascular disease, or the poor adherence observed in families' caregivers who are often women.[144]

In cancer patients, most studies that have assessed factors associated with treatment adherence focused on women with breast cancer, making the impact of gender difficult to establish.[133,139–141] However, in a recent study evaluating statins use in cancer survivors, women had a higher rate of statins discontinuation compared to men.[145] In diabetic patients, to our knowledge, only two published studies have investigated adherence to oral antidiabetics in a cancer setting.[140,142] One of these focused only on women survivors of breast cancer and the other one reported a decreased adherence following cancer diagnosis but did not investigate the factors associated with this decrease.

Similarly to statins users in the present study, greater adherence in individuals with cancer and higher comorbidities index score have been reported in breast cancer patients.[139] This association could be explained by the fact that patients with multiple chronic conditions might be more prompted to accept targeted interventions complementing their primary care services.[139] Those supportive resources could be a reason behind a better adherence in those patients.

As expected, cancer treatments such as surgery in both types of medication or radiotherapy in diabetic metformin users were associated with lower adherence rate. This is not surprising given the fact that drug discontinuation is sometimes needed before and after surgery. For example, metformin is discontinued before surgery and until the patient restarts eating. Also considering that both drugs are orally administered, it seems logical that they will not be given following a digestive tract surgery until the patient restarts eating. Moreover, radiotherapy toxicity in digestive tract cancer might potentially reduce the maximum tolerated dose of metformin and therefore may explain the lower adherence in this subcategory of patients.

Adherence is a difficult concept to study and therefore our study may suffer some limitations.

Regarding the choice of methods, there are several ways of assessing patients' adherence with direct or indirect methods. Direct methods are conducted by dosing pharmacological and biochemical markers in patients.[134] These methods are the most accurate ones but they are not commonly used in general practice because of their invasiveness and their cost.[132] Moreover, patients knowing that they are observed might change their habits, increasing their adherence. This phenomenon is known as an information bias called the Hawthorne effect.

Regarding indirect measurement, there is no gold standard to define adherence. The numerous indirect methods to assess adherence in observational studies have all their limitations and are often discordant. Self-report, electronic measures, pharmacy refill

and claims data are among the most popular indirect measures of adherence. These methods are quite simple to implement and inexpensive.[132] However, like the MPR in our study, they are only proxies of drug administration that do not reflect drug ingestion but drug dispensation.

Second, like all indirect adherence measures, MPR is usually calculated with the sum of drug days' supply.[137] The days' supply represents the "determined number of days that each prescription will last if used according to the prescribing instructions".[128] This information is included in most US pharmacy databases but is generally missing elsewhere like in Belgium.[128] Instead of using the days' supply, the use of the DDD has been demonstrated to be superior to other alternatives. [128,146] The use of the DDD is, however, associated with an underestimation of the exposure for both metformin and statins, so the real adherence rate might actually be higher than the one in the present study.[128]

Third, for legal reasons, it was not possible to link the database of patients with cancer to the one without cancer. Therefore, conducting any analysis comparing the adherence of patients with and without cancer was practically impossible to conduct.

Despite the above-mentioned limitations, our study has major strengths such as the use of a national population-based registry for the identification of the digestive tract cancers cases. Moreover, the Belgian cancer registry has the legal authorisation to use the national social security number (INSZ/NISS) as a unique identifier of the patient and can use this number to link its database to the one of the Intermutualistic Agency (IMA). The IMA is responsible, among other thing, for the centralisation of the treatments and medical procedures claims from the seven Belgian health insurance companies. As health insurance in Belgium is mandatory, the IMA database can be considered to extend to the whole Belgian population.

## 5.4 CONCLUSIONS

In this large population-based study, digestive tract cancer diagnosis was associated with a low rate of adherence to metformin and statins therapy. Cancer diagnosis was moreover associated with a decrease in adherence in both medications. The factors associated with adherence following and digestive tract cancer diagnosis may vary by type of treatment but not by type of cancer. These findings might be of interest for physicians to identify individuals at risk of non-adherence to chronic-conditions medications in cancer patients but also in interpreting pharmacoepidemiologic studies on chronic medications in a cancer setting.

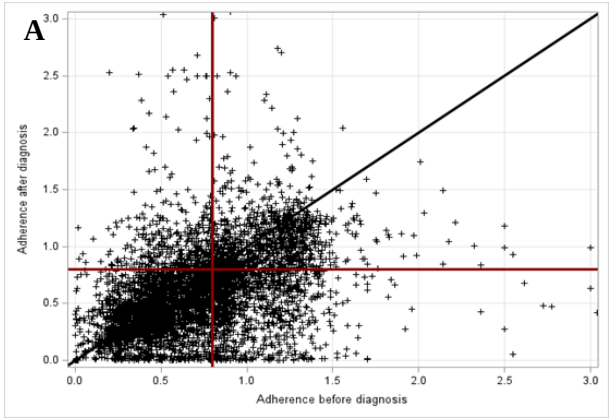
## 5.5 SUPPLEMENTARY MATERIAL

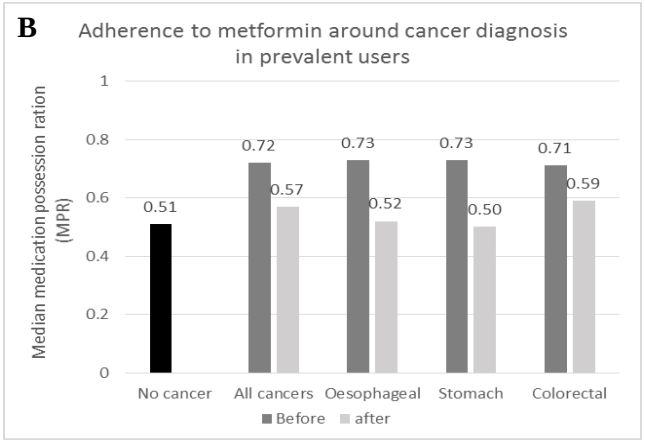
**Table S1.** Characteristics of statins users by availability of modified medication possession ratio (MPRm)

| | Total | Ever | Pre-diagnostic | | | Post diagnostic | | |
|---|---|---|---|---|---|---|---|---|
| | | | Total | MPR missing | MPR not missing | Total | MPR missing | MPR not missing |
| | **82,336** | **32,604** | 25,648 | 2,776 | 22,872 | 28,143 | 3,815 | 24,328 |
| **Age (**Mean ±SD) | **69±12** | **71±10** | 71±9 | 70±11 | 72±9 | 71±10 | 70 ± 11 | 71±9 |
| **Prior statin use — n (%)** | **25,648 (31)** | **25,648 (79)** | 25,648 (100) | 2,776 (100) | 22,872 (100) | 21,206 (75) | 1,889 (50) | 19,317 (79) |
| **Men —n (%)** | 47,365 (58) | 19,348 (59) | 15,166 (59) | 1.635 (59) | 13,531 (59) | 16,925 (60) | 2,251 (59) | 14,674 (60) |
| **Grade of differentiation—n (%)** | | | | | | | | |
| Poorly | **16,581 (20)** | **6,005 (18)** | 4,992 (20) | 537 (19) | 4,455 (20) | 4,910 (18) | 813 (21) | 4097 (17) |
| Moderately | **39,878 (48)** | **16,457 (50)** | 12,750 (50) | 1,370 (49) | 11,380 (50) | 14,549 (52) | 1,774 (47) | 12,775 (53) |
| Well | **12,511 (15)** | **5,320 (16)** | 4,131 (16) | 432 (16) | 3,699 (16) | 4,712 (17) | 548 (14) | 4,164 (17) |
| Unknown/missing | **13,368 (16)** | **4,822 (15)** | 3,775 (15) | 437 (16) | 3,338 (15) | 3,972 (14) | 680 (18) | 3,292 (14) |
| **Localisation—n (%)** | | | | | | | | |
| Oesophagus | **10,280 (13)** | **3,547 (11)** | 3,048 (12) | 342 (12) | 2,706 (12) | 2,772 (10) | 589 (15) | 2,183 (9) |
| Stomach | **7,444 (9)** | **2,627 (8)** | 2,226 (9) | 249 (9) | 1,977 (9) | 2,011 (7) | 413 (11) | 1,598 (7) |
| Colorectum | **64,612 (79)** | **26,430 (81)** | 20,374 (79) | 2,185 (79) | 18,189 (80) | 23,360 (83) | 2,813 (74) | 20,547 (85) |
| **Combined stage—n (%)** | | | | | | | | |
| I | **14,925 (20)** | **6,980 (21)** | 5,324 (21) | 496 (18) | 4,828 (21) | 6,479 (23) | 626 (16) | 5,853 (24) |
| II | **20,645 (27)** | **8,717 (27)** | 6,502 (25) | 738 (27) | 5,764 (25) | 7,878 (28) | 939 (25) | 6,939 (29) |
| III | **21,394 (28)** | **8,478 (26)** | 6,602 (26) | 696 (25) | 5,906 (26) | 7,430 (26) | 901 (24) | 6,529 (27) |
| IV | **17,331 (23)** | **5,635 (17)** | 4,998 (20) | 588 (22) | 4,410 (19) | 4,118 (15) | 945 (25) | 3,173 (13) |
| NA | **1,120 (2)** | **494 (2)** | 378 (2) | 42 (2) | 336 (2) | 421 (2) | 56 (2) | 365 (2) |
| **missing** | **6,921 (8)** | **2,300 (7)** | 1,844 (7) | 216 (8) | 1,628 (7) | 1,817 (7) | 348 (9) | 1,469 (6) |
| **Treatment—n (%)** | | | | | | | | |
| Surgery | **59,963 (73)** | **24,944 (77)** | 19,187 (75) | 2,065 (74) | 17,122 (75) | 22,300 (79) | 2,651 (70) | 19,649 (81) |
| Chemotherapy | **49,657 (60)** | **18,897 (58)** | 14,786 (58) | 1,671 (60) | 13,115 (57) | 16,336 (58) | 2,304 (60) | 14,032 (58) |
| Radiotherapy | **21,567 (26)** | **8,120 (25)** | 6,226 (24) | 744 (27) | 5,482 (24) | 7,123 (25) | 998 (26) | 6,125 (25) |
| **Comorbidities —n (%)** | | | | | | | | |
| Cardiovascular diseases | **41,816 (51)** | **22,283 (68)** | 19,264 (75) | 1,533 (55) | 17,731 (78) | 19,285 (69) | 2,165 (57) | 17,120 (70) |
| Respiratory diseases | **4,495 (6)** | **1,966 (6)** | 1,617 (6) | 155 (6) | 1,462 (6) | 1,641 (6) | 250 (7) | 1,391 (6) |
| Diabetes | **11,628 (14)** | **7200 (22)** | 6,248 (24) | 492 (18) | 5,756 (25) | 6,296 (22) | 672 (18) | 5,624 (23) |

**Table S2.** Characteristics of metformin users by availability of medication possession ratio (MPRm)

| | **Diabetics** | **Ever** | **Pre-diagnostic** | | | **Post diagnostic** | | |
|---|---|---|---|---|---|---|---|---|
| | | | **Total** | **MPR missing** | **MPR not missing** | **Total** | **MPR missing** | **MPR not missing** |
| | **11,628** | **8,923** | 8,294 | 593 | 7,701 | 7,441 | 688 | 6,753 |
| **Age (**Mean ±SD) | **72±10** | **72±9** | 71±9 | 71±10 | 71±9 | 71±9 | 73 ± 9 | 71±9 |
| **Prior metformin use—n (%)** | **8,294** | **8,294** | 8,294 (100) | 593 (100) | 7,701 (100) | 6,812 (92) | 551 (80) | 6,261 (93) |
| **Men —n (%)** | **6,987 (60)** | **5,442 (61)** | 5,056 (61) | 342 (58) | 4,714 (61) | 4,573 (62) | 412 (60) | 4,161 (62) |
| **Grade of differentiation—n (%)** | | | | | | | | |
| Poorly | **2,259 (25)** | **1,714 (19)** | 1,613 (20) | 93 (16) | 1,520 (20) | 1,386 (19) | 161 (23) | 1,225 (18) |
| Moderately | **5,676 (49)** | **4,416 (49)** | 4,065 (49) | 296 (50) | 3,769 (49) | 3,771 (51) | 291 (42) | 3,480 (52) |
| Well | **1,827 (16)** | **1,405 (16)** | 1,311 (16) | 101 (17) | 1,210 (16) | 1,202 (16) | 97 (14) | 1,105 (16) |
| Unknown/missing | **1,866 (16)** | **1,388 (16)** | 1,305 (16) | 103 (17) | 1,202 (16) | 1,082 (15) | 139 (20) | 943 (14) |
| **Localisation—n (%)** | | | | | | | | |
| Oesophagus | **1,370 (12)** | **1,029 (12)** | 976 (12) | 57 (10) | 919 (12) | 802 (11) | 126 (18) | 676 (10) |
| Stomach | **1,106 (10)** | **814 (9)** | 772 (9) | 48 (8) | 724 (9) | 609 (8) | 108 (16) | 501 (7) |
| Colorectum | **9,152 (79)** | **7,080 (79)** | 6,546 (79) | 488 (82) | 6,058 (79) | 6,030 (81) | 454 (66) | 5,576 (83) |
| **Combined stage—n (%)** | | | | | | | | |
| I | **2,111 (18)** | **1,694 (19)** | 1,567 (19) | 105 (18) | 1,462 (19) | 1,501 (20) | 105 (15) | 1,396 (21) |
| II | **3,034 (26)** | **2,334 (26)** | 2,144 (26) | 155 (26) | 1,989 (26) | 2,038 (27) | 151 (22) | 1,887 (28) |
| III | **3,028 (26)** | **2,354 (26)** | 2,165 (26) | 155 (26) | 2,010 (26) | 2,009 (27) | 166 (24) | 1,843 (27) |
| IV | **2,329 (20)** | **1,737 (19)** | 1,659 (20) | 123 (21) | 1,536 (20) | 1,298 (17) | 189 (28) | 1,109 (16) |
| NA | **163 (1)** | **134 (2)** | 129 (2) | 9 (2) | 120 (2) | 111 (2) | 9 (1) | 102 (2) |
| **missing** | **963 (8)** | **670 (8)** | 630 (8) | 46 (8) | 584 (8) | 484 (7) | 68 (10) | 416 (6) |
| **Treatment—n (%)** | | | | | | | | |
| Surgery | **8,429 (72)** | **6,655 (75)** | 6,147 (74) | 430 (73) | 5,717 (74) | 5,756 (77) | 430 (63) | 5,326 (80) |
| Chemotherapy | **6,448 (55)** | **5,116 (57)** | 4,760 (57) | 355 (60) | 4,405 (57) | 4,341 (58) | 344 (50) | 3,997 (59) |
| Radiotherapy | **2,704 (23)** | **2,094 (23)** | 1,929 (23) | 142 (24) | 1,787 (23) | 1,791 (24) | 136 (20) | 1,655 (25) |
| **Comorbidities disease—n (%)** | | | | | | | | |
| Cardiovascular diseases | **9,324 (80)** | **7,176 (80)** | 6,707 (81) | 417 (70) | 6,290 (82) | 5,985 (80) | 554 (81) | 5,431 (80) |
| Respiratory diseases | **835 (7)** | **607 (7)** | 565 (7) | 45 (8) | 520 (7) | 487 (7) | 51 (7) | 436 (7) |



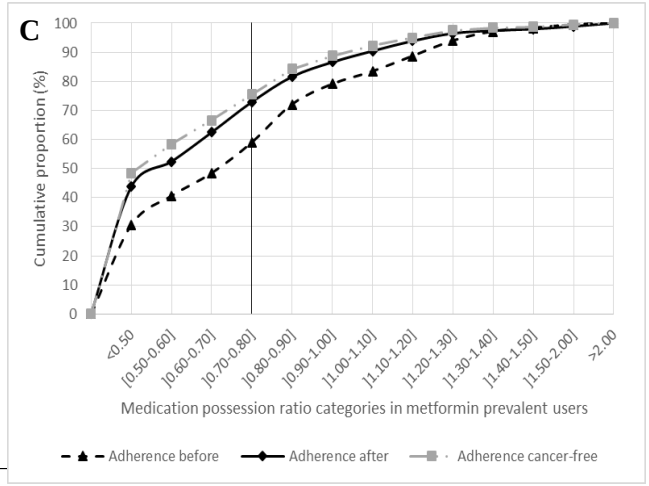
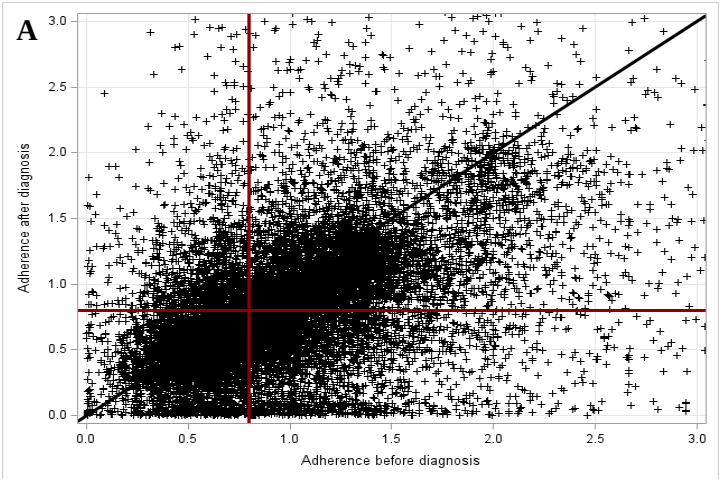
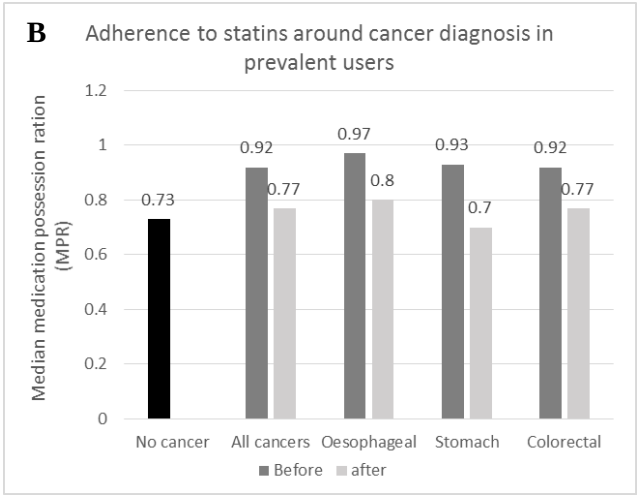




**Figure S1.** Adherence before and after diagnosis in metformin prevalent users diagnosed with digestive tract cancers between 2004 and 2014 in Belgium.

Panel **A** shows values of the medication possession ratio before and after cancer diagnosis among prevalent metformin users.
Panel **B** shows statins median medication possession ratio in the cancer free population from the permanent sample and in digestive tract cancer patients. For digestive cancers, median adherence before and after diagnosis are represented for each cancer.
Panel **C** shows the cumulative proportion of individuals in each statins medication possession ratio category. The black lines represent adherence before (dashed line) and after (plain line) diagnosis in patients with digestive tract cancer. The lighter line represents the cumulative proportion in the cancer free population.



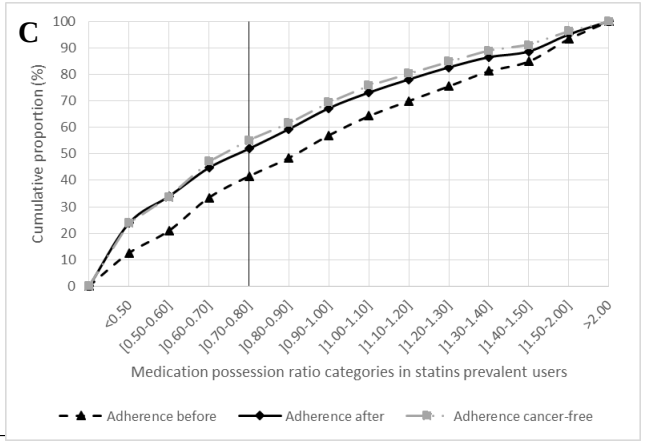




**Figure S2:** Adherence before and after diagnosis in statins prevalent users diagnosed with digestive tract cancers between 2004 and 2014 in Belgium

Panel **A** shows values of the medication possession ratio before and after cancer diagnosis among prevalent metformin users.
Panel **B** shows statins median medication possession ratio in the cancer free population from the permanent sample and in digestive tract cancer patients. For digestive cancers, median adherence before and after diagnosis are represented for each cancer.
Panel **C** shows the cumulative proportion of individuals in each statins medication possession ratio category. The black lines represent adherence before (dashed line) and after (plain line) diagnosis in patients with digestive tract cancer. The lighter line represents the cumulative proportion in the cancer free population.

## *Statin use after diagnosis is associated with an increased survival in oesophageal cancer patients: A Belgian population-based study*

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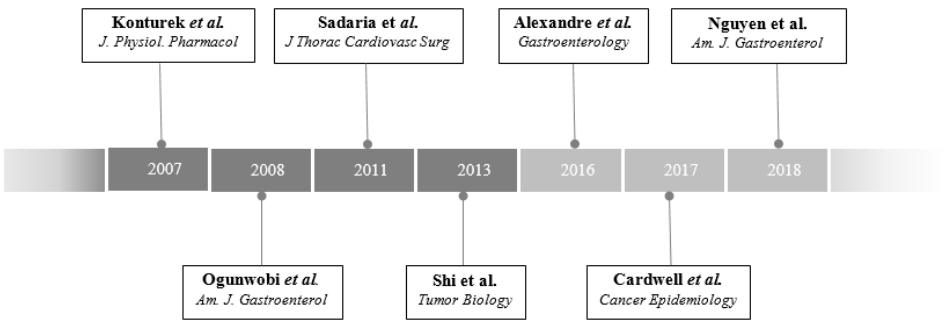


**Figure 11.** Previous publications timeline on statins and oesophageal cancer.

**Table 5**. Characteristics of the preclinical studies on statins an oesophageal cancer

| *Year* | *Authors (journal)* | *Cancer type (cancer cells)* | *Type of statins* | *Type of study* | *Dosage* | *Results* |
|---|---|---|---|---|---|---|
| 2007 | **Konturek *et al* (***J. Physiol. Pharmacol*****)** | Oesophageal adenocarcinoma (EO-19 cells) | Simvastatin | In vitro | 1μM-30μM | Incubation with simvastatin was associated with a decrease in COX-2 expression and an induction of apoptosis (through an increase in Bax expression and a decrease in Bcl-2 expression) |
| 2008 | **Ogunwobi *et al.*** *(Am. J. Gastroenterol)* | Oesophageal adenocarcinoma (OE33, BIC-1 cells) | Simvastatin Lovastatin Pravastatin | In vitro | 1μM–100μM | Statins inhibit proliferation and induce apoptosis in oesophageal adenocarcinoma cells by inhibiting Ras farnesylation and the ERK and Akt signalling pathways. |
| 2011 | **Sadaria *et al.*** *(J Thorac Cardiovasc Surg)* | Oesophageal adenocarcinoma (FLO-1 cells) | Simvastatin Atorvastatin Pravastatin | In vitro | 10, 30, or 50μM | Simvastatin treatment was associated with a significant decrease in oesophageal adenocarcinoma cells viability and proliferation while increasing apoptosis.<br>Atorvastatin therapy had smaller association than simvastatin, whereas pravastatin had no association with these cell growth and metastatic potential. |
| 2013 | **Shi *et al.*** *(Tumor Biology)* | Oesophageal squamous cell carcinoma (KYSE180, Caes17)<br>An immortalized non-cancerous cell line (SHEE) | Lovastatin | In vitro<br>In vivo | <u>In vitro:</u><br>0, 5, and 10μM<br><br><u>In vivo:</u><br>20 mg/kg (5 days/week for 4 weeks) | Lovastatin selectively inhibited the growth of the two cells line by inhibiting the mevalonate pathway, while it exerted less potent effects on non-tumorigenic SHEE cells.<br>Lovastatin treatment was associated with cell cycle arrest and apoptosis in KYSE180 cells.<br>In mouse xenograft tumour model, lovastatin therapy after 18 days was associated with a 60% tumour reduction. |

**Table 6.** Characteristics of the previous population-based cohort studies on statins and oesophageal cancer.

| *Year* | *Authors (journal)* | *Cancer type* | *Type of statins* | *Size of the study* | *Exposure* | *Year* |
|---|---|---|---|---|---|---|
| 2016 | **Alexandre *et al.*** *(Gastroenterology)* | Oesophageal or Oesophago-gastric junction cancers, diagnosed between January 1, 2000, and November 30, 2009 in the UK | Simvastatin Atorvastatin Pravastatin Rosuvastatin Fluvastatin | 4,445 patients | time-dependent | −Oesophageal cancer–specific mortality (HR=0.62; 95%(CI), 0.44–0.86)<br>- All-cause mortality (HR=0.67; 95% CI, 0.58–0.77)<br>No effect in patients with oesophageal squamous cell carcinoma. |
| 2017 | **Cardwell *et al.*** *(Cancer Epidemiology)* | Oesophageal cancer (ICD code C15) diagnosed between January 2009 and December 2012 in Scotland | Simvastatin Atorvastatin Pravastatin Rosuvastatin Fluvastatin | 1,921 patients | time-dependent | − Oesophageal cancer-specific mortality<br>(HR = 0.93, 95% CI, 0.81-1.07)<br>- All-cause mortality<br>(HR=0.94, 95% CI, 0.82-1.07)<br>No differences in the association between statin use and oesophageal cancer-specific mortality by histologic subtypes |
| 2018 | **Nguyen *et al.*** *(Am. J. Gastroenterol.)* | First primary oesophageal cancer diagnosed between 2002 and 2016 in the US | Simvastatin Lovastatin Atorvastatin Fluvastatin Pravastatin Rosuvastatin | 11,750 patients | time-dependent | - Adenocarcinoma:<br>Cancer specific mortality (HR=0.79; 95% CI 0.70–0.88) All-cause mortality<br>(HR=0.80; 95% CI 0.74–0.86).<br>-Squamous cell carcinoma:<br>cancer specific<br>(HR=0.77; 95% CI 0.63–0.92) All-cause mortality<br>(HR=0.83; 95% CI 0.74–0.95) |

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ORIGINAL PAPER

# Statin use after diagnosis is associated with an increased survival in esophageal cancer patients: a Belgian population-based study

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## Abstract

**Purpose** Preclinical studies have shown that statins reduce proliferation in esophageal cancer. Three recent observational studies have shown encouraging results but suffered from limitations. This work aimed to assess at the Belgian population level whether statin usage was associated with a decreased mortality in esophageal cancer patients.

**Methods** We conducted an observational, population-based study by linking data of the Belgian Cancer Registry (BCR) with medical claims data coming from health insurance companies and mortality records collected by regional governments for patients diagnosed with esophageal cancer between 2004 and 2014. Using time-dependent Cox regression models, hazard ratios (HRs) and 95% confidence intervals (CI) for overall and cancer-specific mortality were calculated.

**Results** Of 6,238 patients with stage I-III esophageal cancer, post-diagnostic use of statins was found in 1,628 (26%) patients. Statins use after diagnosis was associated with a reduction in overall mortality (adjusted HR = 0.84, 95% CI [0.77; 0.92]) and cancer-specific mortality (adjusted HR = 0.87, 95% CI [0.78; 0.97]). Similar association were also seen for pre-diagnostic statin use in overall (adjusted HR = 0.83, 95% CI [0.76–0.91]) and cancer-specific analysis (adjusted HR = 0.86, 95% CI [0.77–0.96]).

**Conclusions** In this large cohort of Belgian patients with esophageal cancer, statins use after diagnosis was associated with a decreased mortality.

**Keywords** Esophageal cancer · Statin · Survival · Epidemiology · Pharmacoepidemiology

## Introduction

In 2015, esophageal carcinoma was the 11th most common cancer and the 6th most common cause of cancer death worldwide [1]. This cancer is characterized by two main histological subtypes—i.e. esophageal adenocarcinoma and esophageal squamous cell carcinoma—with different epidemiologic profiles. In Western countries, the predominant subtype is adenocarcinoma: mostly associated with reflux, obesity and male sex [2, 3]. In the Asian highest risk region, called the esophageal cancer belt, the squamous cell subtype is more prevalent, which has mainly been associated with tobacco and alcohol consumption [2, 4, 5].

Despite an improvement in the prognosis of this cancer, the 5-year relative survival remains poor (around 20% in Belgium) [2, 6]. Therefore, any new treatment modalities that would improve survival would be of significant importance.

Statins are lipid-lowering agents used for primary and secondary prevention of atherosclerotic cardiovascular

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diseases [7]. As cardiovascular diseases remain a leading cause of morbidity and mortality, statins are amongst the most widely used medications with more than 200 million people taking these drugs [7–9].

Statins reduce low-density lipoprotein (LDL) by selectively inhibiting the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the initial enzyme involved in the cholesterol biosynthesis. This inhibition also affects the production of isoprenoid intermediates, which play an important role in many physiological processes [10]. As carcinogenesis is included in those processes, it has been assumed that the pleiotropic effects of statins could be extended to the field of oncology [10].

In a more specific way, several preclinical studies have shown that statins reduce esophageal cancer cells growth and proliferation, and increased apoptosis [11–14]. More recently, three epidemiological studies have shown some interesting results. The first one demonstrated a large significant reduction in esophageal cancer-specific and overall mortality, limited to the adenocarcinoma subtype [15]. The second study found little evidence of a reduction in mortality with statins use after diagnosis and showed no differences between histologic subtypes [16]. However, important prognostic factors, such as cancer stage were not considered in these two studies. The third study showed that statin use was associated with an increase in survival but possible reverse causation was addressed by Authors [17].

In the present study, we aimed to investigate if statin use after diagnosis was associated with cancer-specific and overall mortality of esophageal cancer at the Belgian population level. Based on previous studies, we hypothesized that statin use after diagnosis might be associated with a decreased mortality for this cancer type.

## Materials and methods

### Study design

As described in another report, our data set resulted from the linkage of several databases [18]. First, the cancer cases were derived from the Belgian cancer registry's (BCR) database. The BCR is a population-based cancer registry covering more than 95% of the Belgian population since 2004. A complete description of the BCR role, objective and data flow is available elsewhere [19]. Vital status was provided by the crossroads bank for social security (CBSS). Diagnostic and therapeutic procedures stemmed from the Intermutualistic agency (IMA). Both vital status and therapeutic procedures were linked to the BCR database using the patient's specific national social security identification number (SSIN). Regional authorities provided causes of death data that were probabilistically coupled to the BCR database.

Patients with stage I to III esophageal adenocarcinoma or squamous cell carcinoma diagnosed between 1 January 2004 and 31th December 2014 were identified from the BCR database. Patients with prior history of cancer (except for non-melanoma skin cancer) were excluded. Further exclusion criteria referred to individuals not residing in Belgium at the time of diagnosis, with an uncertain date of diagnosis, with no national social security identification number (SSIN), lost to follow-up at the date of cancer incidence, or missing from the medical claims (IMA) database. In the main analysis, patients who died in the first 6 months after their diagnosis were also excluded as drug use during this time is unlikely to exert an effect on cancer death.

### Exposure data

In Belgium, five different types of statins are available for clinical use: simvastatin, fluvastatin, pravastatin, rosuvastatin and atorvastatin. Therefore, statin use was defined as a prescription of any of those subtypes. Post-diagnostic use of statins was defined as a time-varying covariate to avoid immortal-time bias. Patients were considered non-users before their first post-diagnostic statins prescription. Then, they were considered exposed after a 6-months lag period until the end of follow-up. The 6-months lag period was used to remove prescriptions occurring prior to death as they may reflect end of life treatment, thus avoiding reverse causation bias.

In sensitivity analyses, pre-diagnostic use of statins was defined as a dispense of any of the statins mentioned above recorded from 1 month before diagnosis.

### Covariates

Patient- and tumor-related covariates included gender, age at diagnosis, comorbidities, stage, morphology (adenocarcinoma and squamous cells carcinoma) and cancer treatments at 6 months (surgery/radiotherapy/chemotherapy).

Comorbidities in the year prior to diagnosis were derived from claims data including in and outpatient-dispensed medication, according to a previously described methodology [18, 20].

Concomitant medications were defined as the presence of at least one prescription given in the same time-period than statins for the following drugs: beta-blocker, angiotensin-converting inhibitor (ACEi) or angiotensin receptor blocker (ARB), metformin and insulin.

### Outcome measures

The primary outcome was overall mortality with a follow-up until 1st July 2016. In the cancer-specific analysis, patients were followed until 1st January 2014. Patients who died after this date were censored. Cancer-specific deaths were defined as those with an underlying cause of death coded with ICD-10 C15.0-C16.9 for esophageal and gastric cancer or C26.9-C26.8 for malignant neoplasm of overlapping lesion of the digestive system.

### Statistical analysis

The baseline characteristics of statins users and non-users were compared using Chi square test. In post-diagnosis analyses, we investigated overall and cancer-specific mortality using Cox proportional hazard regression with time-dependent exposure. Patients were followed from 6 months after cancer diagnosis until death, end of follow-up or lost to follow-up.

Dose response analyses were carried out using cumulative post-diagnosis number of prescriptions or defined daily doses (DDDs) using time-varying co-variables. In these analyses, statin users were first classified as non-users before the first post-diagnosis prescription, they became light users after their first prescription and heavy users at the date when they tread over the 12th prescription or the 365th DDD.

The main analysis was also performed to compare each type of statins separately to statins non-users.

Subgroup analyses were conducted by histological subtype, sex, and cancer treatment within 6 months after diagnosis. In secondary analyses, we investigated the association between pre-diagnostic statin use in the year prior to diagnosis without excluding those patients with < 6 months of follow-up after diagnosis. A simplified analysis using statin prescriptions in the first 6 months after esophageal cancer diagnosis in patients with more than 6 months of follow-up was also conducted. This kind of analysis allows controlling for immortal time bias without using time-varying covariates [21]. Indeed, statin use is only assessed within the 6 months after diagnosis in 6 months' survivors, therefore, there is no survival bias.

All analyses were adjusted for sex, age, year of diagnosis, comorbidities, cancer treatment within 6 months after diagnosis, and cancer histology. An adjusted analysis was also conducted with concomitant medication used (angiotensin-converting enzyme inhibitors, angiotensin 2-receptor blockers, beta-blockers, insulin and metformin) as time-varying covariate.

Sensitivity analyses regarding the length of the lag were also conducted. First, the main analysis was reproduced without lag (and without excluding deaths after cancer diagnosis) then, with a 3-months lag (excluding deaths in the 3 months after diagnosis) and finally with a 12-months lag (excluding deaths in the first year after diagnosis).

In all analyses, censoring was conducted at 5 years after diagnosis. All analyses were carried out with SAS Enterprise Guide statistical release 9.3 software.

## Results

### Patient cohort

A total of 6,238 patients who met the inclusion criteria were identified from the BCR database with a diagnosis of esophageal cancer between 2004 and 2014. In cancer-specific analyses 71 patients were excluded because they had no cause of deaths.

A flowchart showing the number of patients included in each analysis is provided in Fig. 1.

From the 5,234 patients included in the overall analysis, 3,015 (57.6%) died from any cause within 5 years after diagnosis. In cancer-specific analysis, 2,003 (78.2%) deaths were considered due to esophageal cancer. The median follow-up time was 2.39 years (IQR 1.22–4.85). The median time to the first post diagnosis prescription was 43 days (Interquartile range (IQR) 17–121.5).

From the 6,238 patients who met the inclusion criteria, 1,939 (31.1%) were statins users in the year prior to their diagnosis. After diagnosis, 391 (20.1%) patients who were previous users stopped using statins.

Patients' characteristics by post-diagnostic statin use are shown in Table 1. Post-diagnosis statin users were older, diagnosed more recently, with mainly adenocarcinoma subtype and had more associated comorbidities. They also tended to use more concomitant medications and underwent less surgery/chemotherapy/RT treatments for their cancer. Similar observations were made when comparing pre-diagnosis statin users to non-users (not shown).

### Post-diagnosis statin use

In post-diagnosis analyses, statin use was independently associated with a significant 16% decrease in overall mortality (adjusted HR = 0.84; 95% CI 0.77–0.92) and a 13% decrease in cancer-specific mortality (adjusted HR = 0.87; 95% CI 0.78–0.97) (Table 2).

No significant dose–response associations were observed with either the number of prescriptions or the number of DDDs for overall mortality. In cancer-specific analysis, despite the apparent decrease in mortality from light to heavy users when considering DDDs, the mortality difference between those two groups did not reach a significant level (> 365 vs. ≤ 365DDDs, HR = 0.93; 95% CI 0.76–1.15).

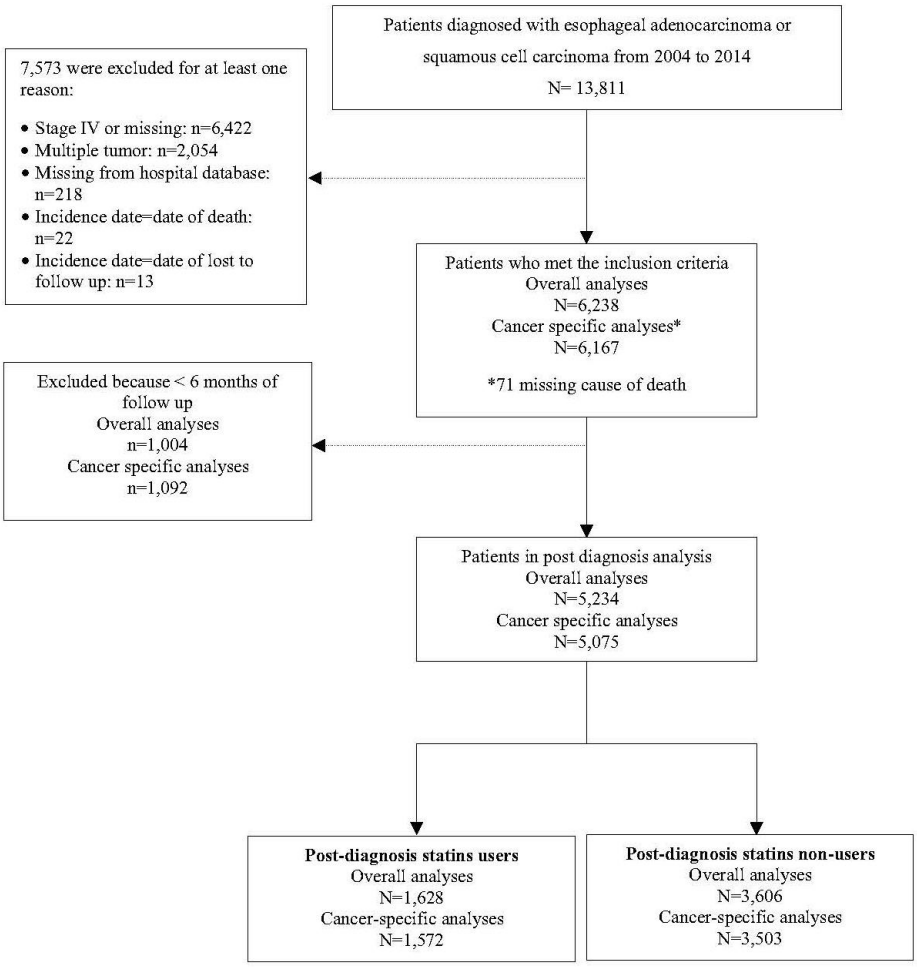


**Fig. 1** Flow of study participants

Post-diagnosis use of each type of statin except rosuvastatin and fluvastatin was associated with a decreased overall and cancer-specific mortality compared to statin non-users (Supplementary Table I).

## Sensitivity and subgroup analysis

Analysis of post-diagnosis statin use gave similar results after additional adjustment for concomitant medications (adjusted HR for overall mortality = 0.83; 95% CI 0.76–0.91 and adjusted HR for cancer-specific mortality = 0.86; 95% CI 0.77–0.96).

In addition, reassuringly, the simplified analysis of statin use in the 6 months following diagnosis gave similar results as the time-varying analysis for overall mortality (adjusted HR = 0.86; 95% CI 0.79–0.93) and cancer-specific mortality (adjusted HR = 0.86; 95% CI 0.78–0.96).

Statins use before diagnosis was associated with a 16% decrease in overall mortality (adjusted HR = 0.84; 95% CI 0.78–0.90) and 18% decrease in cancer-specific mortality (adjusted HR = 0.82; 95% CI 0.75–0.90).

Sensitivity analyses regarding the length of the lag period are shown in Table 3. The association between post-diagnosis statin use and mortality, was still observed when no lag was used (adjusted HR for overall mortality = 0.81; 95%

**Table 1** Statin use before and after esophageal cancer diagnosis, Belgium, 2004–2014

| Characteristics | Statin after diagnosis ($n=5,234$)[a] | | |
|---|---|---|---|
| | Users ($n=1,628$) | Non-users ($n=3,606$) | $p$ value |
| Age (years) | | | <0.001 |
| Mean ± SD | 68 ± 9 | 64 ± 12 | |
| Age category $n$ (%) | | | <0.001 |
| <60 | 327 (20) | 1,362 (38) | |
| 60–69 | 601 (37) | 1,083 (30) | |
| 70–79 | 521 (32) | 782 (22) | |
| 80–89 | 174 (11) | 362 (10) | |
| ≥ 90 | 5 (0) | 17 (0) | |
| Sex $n$ (%) | | | 0.19 |
| Men | 1,262 (78) | 2.735 (76) | |
| Year of diagnosis $n$ (%) | | | <0.001 |
| <2009 | 522 (32) | 1,540 (43) | |
| ≥2009 | 1,106 (68) | 2,066 (57) | |
| Grade of differentiation $n$ (%) | | | 0.10 |
| Poorly | 528 (32) | 1,200 (33) | |
| Moderately | 581 (36) | 1,352 (38) | |
| Well | 251 (15) | 467 (13) | |
| Unknown/missing | 268 (17) | 587 (16) | |
| Morphology $n$ (%) | | | <0.001 |
| Squamous cell | 513 (32) | 1,385 (38) | |
| Adenocarcinoma | 1,115 (68) | 2,221 (62) | |
| Combined stage $n$ (%) | | | <0.001 |
| I | 565 (35) | 987 (27) | |
| II | 476 (29) | 1,061 (29) | |
| III | 587 (36) | 1.558 (43) | |
| Cardiovascular disease $n$ (%) | | | <0.001 |
| Yes | 1,158 (71) | 1,429 (40) | |
| Respiratory diseases $n$ (%) | | | 0.002 |
| Yes | 134 (8) | 215 (6) | |
| Diabetes $n$ (%) | | | <0.001 |
| Yes | 372 (23) | 346 (10) | |
| Concomitant medication use $n$ (%) | | | |
| ACEi or ARB | 863 (53) | 884 (25) | <0.001 |
| Beta-blocker | 1,165 (72) | 1,720 (48) | <0.001 |
| Metformin | 303 (19) | 270 (7) | <0.001 |
| Insulin | 669 (41) | 1,100 (30) | <0.001 |
| Cancer therapy at 6 months $n$ (%) | | | <0.001 |
| Surgery with CT or RT | 467 (29) | 1,161 (32) | |
| Surgery alone | 463 (28) | 898 (25) | |
| CT or RT without surgery | 477 (29) | 1,182 (33) | |
| No treatment | 221 (14) | 365 (10) | |

*ACEi* angiotensin conversing inhibitor, *ARB* angiotensin receptor blocker, *CT* chemotherapy, *RT* radiotherapy

[a]Statins use after diagnosis in individuals with more than 6 months of follow-up

CI 0.75–0.87 and adjusted HR for cancer-specific mortality = 0.81; 95% CI 0.74–0.88) and with a 3 months' lag in individuals living more than 3 months (adjusted HR for overall mortality = 0.83; 95% CI 0.76–0.90 and adjusted HR for cancer-specific mortality = 0.83; 95% CI 0.75–0.91). When a 12 months' lag in individuals living more than 1 year was applied, the association remained similar for overall mortality (adjusted HR = 0.87; 95% CI 0.79–0.97) but did not reach significance for cancer-specific mortality (adjusted HR = 0.94; 95% CI 0.83–1.08).

In the subgroup analysis, interaction tests did not show a difference between statin use and mortality by histologic subtypes (overall mortality: $p_{\text{forinteraction}}=0.60$ and cancer-specific mortality: $p_{\text{forinteraction}}=0.89$), sex (overall mortality: $p_{\text{forinteraction}}=0.54$ and cancer-specific mortality: $p_{\text{forinteraction}}=0.60$) or cancer treatment in the 6 months after diagnosis (Supplementary Figure I).

## Discussion

In this large, population-based cohort of 5,234 patients with incident esophageal cancer, post-diagnosis statins use was associated with a 16% decrease in overall mortality and a 13% decrease in cancer-specific mortality. Similar associations were seen when investigating pre-diagnosis statin use. However, there were no dose–response relationships when considering the cumulative number of prescriptions or the cumulative number of DDDs and there was no apparent difference between the two main histological subtypes.

Our findings match with previous results regarding statins use and cancer outcome. Indeed, statins have been consistently associated with a decreasing mortality in cancer [22, 23]. In gastrointestinal cancer particularly, meta-analyses of observational studies have associated statins use with a decreased mortality in colorectal cancer [22, 24]. Statins have also been tested in phase II and III randomized trials for advanced gastric cancer with no improvement seen in survival outcome [25, 26]. Those studies, however, focused on metastatic patients not amenable for curative resection.

The anti-tumor effect of statins is thought to occur through the inhibition of the mevalonate pathway as cancer cells are known to be highly dependent on the mevalonate pathway metabolites. More specifically cholesterol, an important component of cell membranes, is mainly obtained by cancer cells through this pathway [27]. In addition, small guanosines triphosphatases (GTPases) involved in carcinogenesis such as Ras, Rho, Rab, Arf and Ran are dependent on prenylation, one of the steps in the mevalonate pathway [27].

In esophageal adenocarcinoma cells specifically, simvastatin, lovastatin, atorvastatin and pravastatin, which are subtypes of the statin family, have been associated with

**Table 2** Post-diagnosis statin use and overall or cancer-specific mortality in patients with esophageal cancer between 2004 and 2014 in Belgium

| Post-diagnosis statins use as time varying covariate[a] | Event rate (%) | Person Years | Unadjusted | | | Adjusted[b] | | |
|---|---|---|---|---|---|---|---|---|
| | | | HR | 95% CI | *p* value | HR | 95% CI | *p* value |
| Overall mortality ($n=5,234$) | | | | | | | | |
| Non-users | 2,235/3,606 (62.0) | 10,915 | 1 | | | 1 | | |
| Users | 780/1,628 (47.9) | 3,498 | 0.92 | 0.85–1.00 | 0.04 | 0.84 | 0.77–0.92 | <0.001 |
| Prescriptions | | | | | 0.006 | | | <0.001 |
| 1–12 | 710/1,286 (55.2) | 3,091 | 0.90 | 0.82–0.98 | | 0.83 | 0.76–0.91 | |
| >12 | 70/342 (20.5) | 408 | 1.25 | 0.98–1.60 | | 1.07 | 0.84–1.37 | |
| Defined daily doses | | | | | 0.12 | | | <0.001 |
| 1–365 | 544/866 (62.7) | 2,097 | 0.92 | 0.84–1.01 | | 0.84 | 0.76–0.93 | |
| >365 | 236/762 (31.0) | 1,402 | 0.91 | 0.79–1.05 | | 0.86 | 0.74–0.99 | |
| Cancer-specific mortality ($n=5,075$) | | | | | | | | |
| Non-users | 1,508/3,503 (57.2) | 10,551 | 1 | | | 1 | | |
| Users | 495/1,572 (32.7) | 3,383 | 0.88 | 0.80–0.98 | 0.02 | 0.87 | 0.78–0.97 | 0.01 |
| Prescriptions | | | | | 0.05 | | | 0.04 |
| 1–12 | 468/1,244 (39.8) | 2,989 | 0.88 | 0.79–0.98 | | 0.87 | 0.78–0.97 | |
| >12 | 27/328 (8.2) | 395 | 0.93 | 0.63–1.38 | | 0.88 | 0.59–1.31 | |
| Defined daily doses | | | | | 0.02 | | | 0.03 |
| 1–365 | 371/832 (44.6) | 2,032 | 0.91 | 0.81–1.02 | | 0.88 | 0.78–1.00 | |
| > 365 | 124/740 (16.8) | 1,352 | 0.80 | 0.66–0.96 | | 0.83 | 0.68–1.00 | |

*p* value are for likelihood ratio test comparing model with and without the specific variable

[a]Analyses included a lag of 6 months in individuals living more than 6 months

[b]Adjusted for age (continuous), sex, year of diagnosis (<2009 and ≥2009), stage, cancer treatment, morphology (adenocarcinoma or squamous cell carcinoma) and comorbidities (cardiovascular, diabetes and respiratory)

inhibition of cancer cell proliferation [11, 12]. Simvastatin and pravastatin have also been associated with an increased apoptosis through the inhibition of prenylation step [11]. In esophageal adenocarcinoma a decrease in the oncoprotein Ras activity was observed after treatment with simvastatin [11]. This oncoprotein is known to be highly correlated to the mevalonate pathway [27]. Simvastatin and Atorvastatin were also involved in the inhibition of metastasis [12].

In esophageal squamous cell carcinoma, only lovastatin was studied. In those cancerous cells, lovastatin decreased cancer cells growth by inhibiting the upregulated mevalonate pathway, thus leading to a decreasing activity of the oncoprotein Ras [13].

Consistently, simvastatin, pravastatin and atorvastatin were the three statins subtypes available in Belgium that were associated with lower overall and esophageal cancer specific mortality in the present study, thereby aligning with the pre-clinical studies.

At the population level, three studies have previously investigated statin use after esophageal cancer diagnosis.

The first used the United Kingdom General Practice Research Database (GPRD), the world's largest electronic database of prospective demographic, lifestyle and medical data in primary care [15]. This study found a 38% decreased risk of esophageal cancer-specific mortality and a 37% decrease risk of overall mortality in post-diagnosis statin users in a population of 4,445 patients [15]. However, their conclusion relied on unlagged analysis, without adjustment on cancer stage, and on a substantial amount of missing data for treatment. In comparison, and even with unlagged prescription, the magnitude of the effect was lower in our analysis when considering esophageal cancer-specific mortality. In overall mortality however, our findings were similar: they showed a 15% reduction of mortality, and our results suggest a 16% reduction.

The differences in cancer-specific analysis could be explained by differences in cancer-specific deaths definition. They considered as cancer specific deaths, those in which esophageal cancer was listed in part one of death certificate while we also include gastric cancer deaths (C15) and malignant neoplasm of other and ill-defined digestive organs (C26).

Pre-diagnosis statin use was also investigated as a prescription for a minimum of 2 months between 6 and 18 months. The authors show a reduction of 14% in overall mortality and a non-significant 9% reduction for cancer-specific mortality. In comparison, we found a 16% reduction of overall mortality and an 18% reduction for cancer-specific mortality. Again, our definition of cancer-specific deaths allowed us to have more events. In addition, in our study

**Table 3** Sensitivity analysis regarding the length of the lag period in either overall or cancer-specific mortality in patients with esophageal cancer between 2004 and 2014 in Belgium

| Medication usage | Event rate (%) | Person Years | Unadjusted | | | Adjusted[d] | | |
|---|---|---|---|---|---|---|---|---|
| | | | HR | 95% CI | *p* | HR | 95% CI | *p* |
| Post diagnosis statins use as time varying covariate | | | | | | | | |
| Overall mortality | | | | | | | | |
| without lag ($n=6,238$)[a] | | | | | | | | |
| Non-users | 2,951/4,303 (68.6) | 10,298 | 1 | | | 1 | | |
| Users | 1,066/1,935 (55.1) | 4,389 | 0.89 | 0.83–0.95 | <0.001 | 0.81 | 0.75–0.87 | <0.001 |
| 3 months lag ($n=5,794$)[b] | | | | | | | | |
| Non-users | 2,644/4,006 (66.0) | 10,699 | 1 | | | 1 | | |
| Users | 930/1,788 (52.0) | 3,923 | 0.91 | 0.84–0.98 | <0.01 | 0.83 | 0.76–0.90 | <0.001 |
| 1 year lag ($n=4,242$)[c] | | | | | | | | |
| Non-users | 1,489/2,881 (51.7) | 10,933 | 1 | | | | | |
| Users | 535/1,361 (39.3) | 2,756 | 0.95 | 0.86–1.05 | 0.30 | 0.87 | 0.79–0.97 | 0.01 |
| Cancer-specific mortality | | | | | | | | |
| without lag ($n=6,167$)[a] | | | | | | | | |
| Non-users | 2,033/4,266 (47.7) | 9,979 | 1 | | | 1 | | |
| Users | 682/1,901 (35.8) | 4,249 | 0.83 | 0.77–0.91 | <0.001 | 0.81 | 0.74–0.88 | <0.001 |
| 3 months lag ($n=5,669$)[b] | | | | | | | | |
| Non-users | 1,814/3,935 (46.1) | 10,361 | 1 | | | 1 | | |
| Users | 590/1,734 (34.0) | 3,795 | 0.85 | 0.78–0.94 | <0.001 | 0.83 | 0.75–0.91 | <0.001 |
| 1 year lag ($n=4,081$)[c] | | | | | | | | |
| Non-users | 962/2,763 (34.8) | 10,544 | 1 | | | 1 | | |
| Users | 328/1,318 (24.9) | 2,668 | 0.92 | 0.81–1.05 | 0.21 | 0.94 | 0.83–1.08 | 0.39 |

[a]Analyses include all individuals

[b]Analyses include all individuals with more than 3 months of follow-up

[c]Analyses include all individuals with more than 1 year of follow-up

[d]Adjusted for age (continuous), sex, year of diagnosis (<2009 and ≥2009), stage, cancer treatment, morphology (adenocarcinoma or squamous cell carcinoma) and comorbidities (cardiovascular, diabetes and respiratory)

pre-diagnosis users were defined as those with a prescription of any kind of statin between 1 and 12 months before the diagnosis.

The second study used the Scottish Cancer Registry database and was based upon 1,921 newly diagnosed esophageal cancer patients [16]. The authors concluded on little evidence of a reduction in esophageal cancer-specific mortality while their main analyses showed a non-significant 7% reduction of mortality. Statins use before diagnosis was, however, associated with a 12% reduction in overall mortality and an 11% reduction in cancer specific mortality. In comparison, our effect size was bigger but we also have included more patients. Moreover, the authors were able to adjust their analyses for deprivation, but were unable to adjust for cancer stage.

In a large cohort study including 11,750 patients diagnosed with esophageal cancer during 15 consecutive years in the United States, statin use after esophageal cancer diagnosis was associated with a 10% decrease in mortality [17]. As the two-previous study, they included all stage patients, and so metastatic patients with a poor survival were also included. That can explain the smaller effect size and differences in analysis with different lag. Indeed, for each lagged analysis they suppressed dead patients according to the lag duration. For example, in the 6-months lag analysis, suppressed patients with less than 6 months of follow-up, should be mainly stage IV patients.

It is interesting to note that despite little methodological differences with the three previous studies, our results are headed in the same direction.

Our result may have some limitations. First, some potentially important confounders e.g. body mass index (BMI), smoking, alcohol and socioeconomic statuses were missing from our database.

BMI is a recognized risk factor for esophageal adenocarcinoma. Moreover, in some studies, BMI was also associated with survival outcome in esophageal cancers patients and this association might be modified by smoking status [28]. However, distinction should be made considering the time of BMI assessment. If a high BMI is

considered as a risk factor for adenocarcinoma, weight loss before surgery in esophageal cancers and low BMI in the first 6 months after surgery in adenocarcinoma were associated with poor prognosis [29, 30]. This phenomenon, well known in the cardiovascular literature, is called the obesity paradox [31]. Therefore, healthier patients (that is patients with a higher BMI in the present context) may have been prescribed selectively statin, leading to a better survival: this type of bias is a selection bias also called confounding by indication. This could also be one of the reasons why no dose–response was seen.

Socioeconomic status is a consistent risk factor for esophageal squamous cell [5]. Patients with low income or lower educational level might be less health-conscious and more likely to adopt bad lifestyle habits, being therefore, more at risk for squamous cell carcinoma, a tumor highly associated with tobacco and alcohol consumption [32]. Moreover, patients with lower socioeconomic status might present with advanced stage of cancer and therefore suffer from a worse prognosis. Thus, and as in the current study statin users were more likely to be diagnosed with adenocarcinoma than squamous cell carcinoma, they might represent a healthier population with higher socioeconomic status. This could contribute to spurious association between statin use and an improved survival.

Prevalent user bias is frequently cited in pharmacoepidemiological studies and restricting analysis to the subset of new users is a way to avoid it [33]. In our study, only 23% of post-diagnosis users were new users. We did not analyze this subgroup of patients to avoid healthy users' bias as they are healthy enough to initiate a preventive cardiovascular treatment after their cancer diagnosis, and therefore, are not representative of the general population of esophageal cancer patients.

One of the main strength of this study relies on the fact that we used the Belgian national cancer registry containing all cases of cancer diagnosed by hospital or laboratories. Patients were selected over 11 consecutive incidence years resulting in a large population-based set of more than 6,000 esophageal cancer cases.

To avoid immortal time bias, statin use after diagnosis was used as a time-varying variable, allowing participants to be considered as non-users until they receive their first post-diagnostic statins prescription. Immortal time bias is a frequent issue in observational study leading to an overestimation of the studied effect [34].

As concomitant medications could possibly confound association with survival, we also conducted an additional analysis taking into account the post-diagnosis use of metformin, insulin, beta-blockers, angiotensin conversing inhibitor and angiotensin receptor blocker as time varying covariates. In a reassuring way, this analysis showed results similar to the main one.

We also adjusted for cancer stage that is an important prognostic factor in esophageal cancer but was missing in two of the three previous studies [15, 16]. We choose to exclude patients with stage IV cancer because they have a poor 5-years relative survival (< 10%) and they are less likely to use or continue statins after the cancer diagnosis.

Health insurance data allowed to avoid recall bias and provided precise information regarding statin exposure. As in Belgium, health insurance is compulsory and covers all reimbursed medication, it seems unlikely to miss statin prescriptions as they are reimbursed medication only available with medical prescription. We also benefit from excellent data related to cancer incidence and characteristics thanks to the Belgian population-based cancer registry.

In summary, we used specific methods in this study to control for frequent important biases in observational studies. Robust verification of cancer case and deaths was allowed with the use of a nationwide cancer registry database. Moreover, it is also the first study conducted in Belgium investigating the association between statin use and esophageal cancer mortality.

According to our results, statin use in esophageal cancer patients with good prognosis could be maintained as it was not associated with poorer prognosis. However, further studies are needed to explore a potential antineoplastic effect of statins before initiating such medication. We believe this study could add information to the general topic of statin use and esophageal cancer progression.

## Conclusion

In this large, population-based esophageal cancer cohort, post diagnosis statin use was associated with significant decrease in overall and cancer-specific mortality. Other large observational studies are, however, needed to confirm these findings before conducting randomized controlled trials.

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## 6.1 SUPLEMENTARY MATERIAL

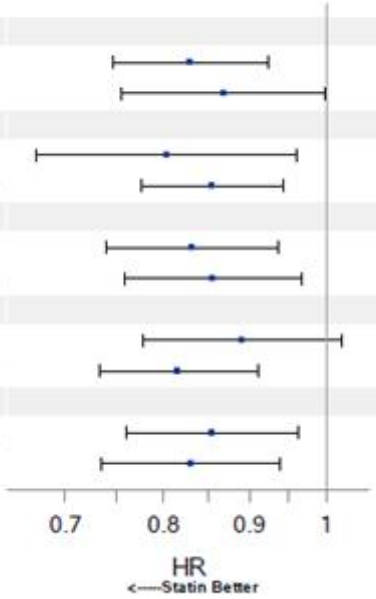
**Supplementary Table 1.** Post diagnosis statin use by statin subtype and overall mortality or cancer-specific mortality in patients with oesophageal cancer between 2004-2014 in Belgium

| | | | | Unadjusted | | | Adjusted | |
|---|---|---|---|---|---|---|---|---|
| **Medication usage** | **Event rate (%)** | | **Person Years** | **HR** | **(95%CI)** | **p** | **HR** | **(95%CI)** | **p** |
| **Overall mortality** | | | | | | | | | |
| **Post diagnosis statins use as time varying covariate[1]** | | | | | | | | | |
| **statin non-users** | 2,235 /3,606 | (62.0) | 10,318 | 1 | | | 1 | | |
| Simvastatin | 418 / 860 | (48.6) | 1,812 | 0.94 | (0.85-1.04) | 0.24 | 0.86 | (0.77-0.96) | 0.007 |
| **statin non-users** | 2,235 /3,606 | (62.0) | 9,623 | 1 | | | 1 | | |
| Pravastatin | 80 /176 | (45.5) | 401 | 0.78 | (0.63-0.98) | 0.03 | 0.71 | (0.56-0.89) | 0.003 |
| **statin non-users** | 2,235 /3,606 | (62.0) | 9,914 | 1 | | | 1 | | |
| Atorvastatin | 182 /451 | (40.4) | 987 | 0.74 | (0.63-0.86) | <0.001 | 0.66 | (0.57-0.77) | <0.001 |
| **statin non-users** | 2,235 /3,606 | (62.0) | 9,756 | 1 | | | 1 | | |
| Rosuvastatin | 141 /297 | (47.5) | 590 | 0.92 | (0.78-1.09) | 0.33 | 0.87 | (0.73-1.04) | 0.13 |
| **statin non-users** | 2,235 /3,606 | (62.0) | 9,473 | 1 | | | 1 | | |
| Fluvastatin | 11/16 | (68.8) | 29 | 1.37 | (0.76-2.47) | 0.30 | 1.20 | (0.66-2.17) | 0.55 |

| **Supplementary Table 1 (suite).** Post diagnosis statin use by statin subtype and overall mortality or cancer-specific mortality in patients with oesophageal cancer between 2004-2014 in Belgium | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | **Unadjusted** | | | **Adjusted** | | |
| **Medication usage** | **Event rate (%)** | | **Person Years** | **HR** | **(95%CI)** | **p** | **HR** | **(95%CI)** | **p** |
| **Cancer-specific mortality** | | | | | | | | | |
| **Post diagnosis statins use as time varying covariate[1]** | | | | | | | | | |
| **statin non-users** | 1,508 /3,503 | (43.0) | 9,975 | 1 | | | 1 | | |
| Simvastatin | 266 / 828 | (32.1) | 1,761 | 0.90 | (0.79-1.03) | 0.13 | 0.88 | (0.77-1.01) | 0.06 |
| **statin non-users** | 1,508 /3,503 | (43.0) | 9,309 | 1 | | | 1 | | |
| Pravastatin | 49 /170 | (28.8) | 390 | 0.72 | (0.54-0.96) | 0.02 | 0.69 | (0.52-0.92) | 0.01 |
| **statin non-users** | 1,508 /3,503 | (43.0) | 9,592 | 1 | | | 1 | | |
| Atorvastatin | 112 / 438 | (25.6) | 950 | 0.69 | (0.57-0.84) | <0.001 | 0.67 | (0.55-0.81) | <0.001 |
| **statin non-users** | 1,508 /3,503 | (43.0) | 9,439 | 1 | | | 1 | | |
| Rosuvastatin | 91 /286 | (31.8) | 564 | 0.86 | (0.69-1.06) | 0.16 | 0.88 | (0.70-1.09) | 0.24 |
| **statin non-users** | 1,508 /3,503 | (43.0) | 9,167 | 1 | | | 1 | | |
| Fluvastatin | 8/15 | (53.3) | 28 | 1.50 | (0.75-3.01) | 0.25 | 1.58 | (0.79-3.17) | 0.20 |

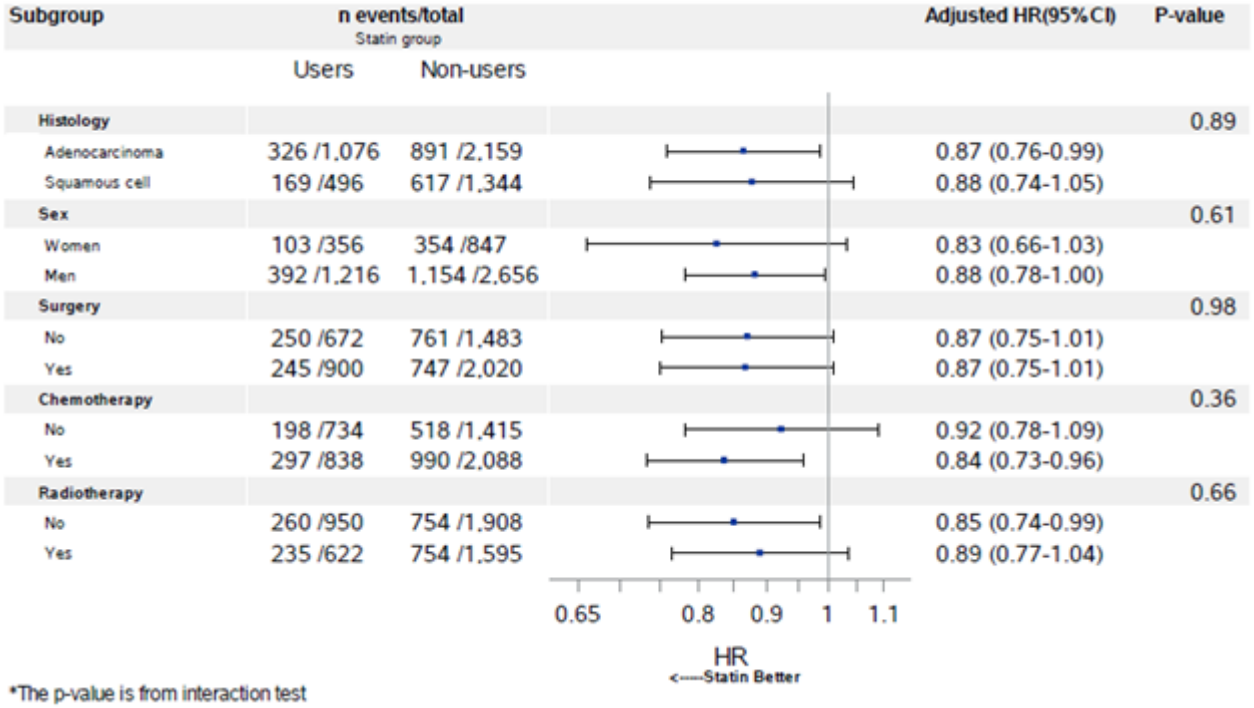
A. Overall mortality

| Subgroup | n events/total (Statin group) Users | Non-users | Adjusted HR(95%CI) | P-value |
|---|---|---|---|---|
| **Histology** | | | | 0.61 |
| Adenocarcinoma | 510 /1,115 | 1,324 /2,221 | 0.83 (0.75-0.92) | |
| Squamous cell | 270 /513 | 911 /1,385 | 0.87 (0.76-1.00) | |
| **Sex** | | | | 0.54 |
| Women | 162 /366 | 524 /871 | 0.80 (0.67-0.96) | |
| Men | 618 /1,262 | 1,711 /2,735 | 0.86 (0.78-0.94) | |
| **Surgery** | | | | 0.73 |
| No | 403 /698 | 1,159 /1,547 | 0.83 (0.74-0.94) | |
| Yes | 377 /930 | 1,076 /2,059 | 0.86 (0.76-0.97) | |
| **Chemotherapy** | | | | 0.31 |
| No | 304 /751 | 750 /1,449 | 0.89 (0.78-1.02) | |
| Yes | 476 /877 | 1,485 /2,157 | 0.82 (0.73-0.91) | |
| **Radiotherapy** | | | | 0.73 |
| No | 414 /978 | 1,082 /1,955 | 0.86 (0.76-0.96) | |
| Yes | 366 /650 | 1,153 /1,651 | 0.83 (0.74-0.94) | |




*The p-value is from interaction test

B. Cancer-specific mortality




**Supplementary figure 1.** Subgroup analysis

***Impact of metformin on gastric adenocarcinoma survival: a Belgian population-based study***

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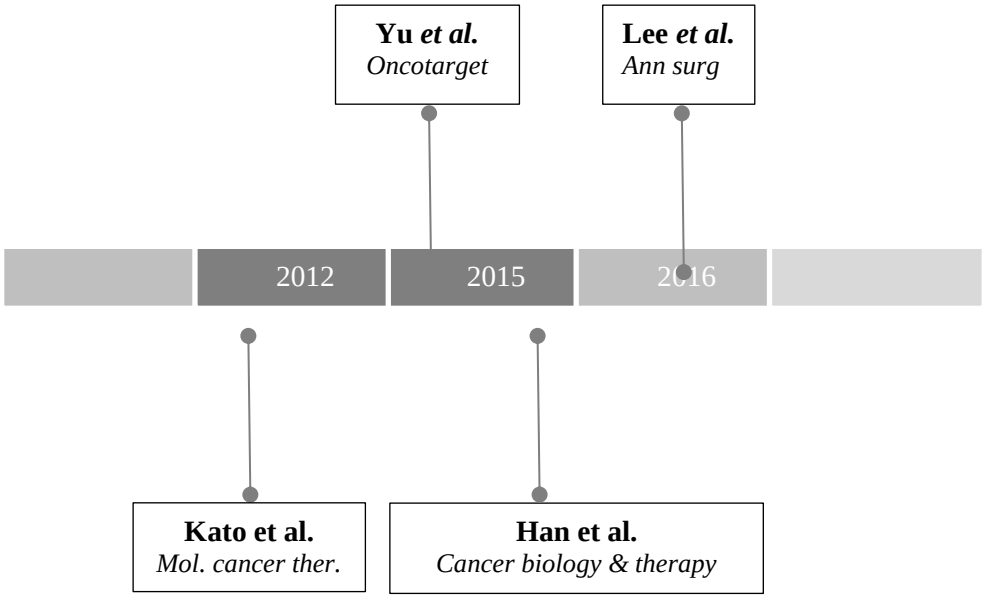


**Figure 12.** Previous publications timeline on metformin and gastric cancer.

**Table 7**.Characteristics of the preclinical studies on metformin and gastric cancer.

| *Year* | *Authors* | *Cancer type (cancer cells)* | *Size of the study* | *Type of study* | *Treatment* | *Results* |
|---|---|---|---|---|---|---|
| 2012 | **Kato *et al.*** (Mol. cancer ther.) | Gastric adenocarcinoma (MKN1, MKN45, MKN74) | In vitro: NA<br><br>In vivo: 30 mice | *In vitro*<br>*In vivo* | In vitro: 0, 1, 5, and 10mmol/L<br><br>In vivo: intraperitoneal injection at 1 mg/body or 2 mg/body per day 5 times a week, for 4 weeks | The three cancerous cells line shown a decreased proliferation when treated with metformin *in vitro* and in a dose-dependent manner<br><br>In MKN74 cells, metformin inhibits gastric cancer cells proliferation and tumour growth by decreasing specific cyclin/Cdk complexes like cyclin D1/Cdk6 both *in vitro* and *in vivo*. Those complexes are involved into the cell cycle and their inhibition lead to G1 cell-cycle stop. In the same cell line, metformin decreased the expression of phosphorylated epidermal growth factor receptor (p-EGFR) and phosphorylated insulin-like growth factor-1 receptor (p-IGF-1R). |

| | | | | | | |
|---|---|---|---|---|---|---|
| 2015 | **Yu *et al.*** *(Oncotarget)* | Gastric adenocarcinoma (AGS, N87, MKN28, MGC803, BGC823, HGC27, and MKN45) | <u>In vitro:</u> NA<br><br><u>In vivo:</u> 5 mice | In vitro<br>In vivo | <u>In vitro:</u> 0, 10, 20, or 50mM<br><br><u>In vivo:</u> intraperitoneal injection of metformin 250 mg/kg<br><br>Metformin (250 mg/kg, intraperitoneal, *q.d.*) with or without rapamycin (2.5 mg/kg, intraperitoneal, *q.d.)*<br><br>Cisplatin treatment (4 mg/kg, intraperitoneal) with metformin (250 mg/kg) | Metformin inhibited the proliferation of the seven gastric cancers cells lines dose-manner dependently.<br>N87 and MKN45 metformin treated cells exhibits an activation of AMPK in a dose depend manner associated with a decreased phosphorylated mTOR and its downstream targets S6, 4EBP1, and P70S6K.<br>In addition, BGC823 metformin treated cells also shown an up regulation of PTEN and CDKN1. Genes associated with tumour invasion like DCN, CLDN1, FN1 MMP7 WFDC1 and UDB were downregulated.<br>Mice treated with metformin exhibited a 47% and 52% reduction in the weight and volume of tumour, respectively compared to non-treated mice. Additional effect was seen in combination with cisplatin and rapamycin. |

| | | | | | | |
|---|---|---|---|---|---|---|
| 2015 | **Han *et al.*** *(Cancer biology & therapy)* | Gastric adenocarcinoma (MKN28, SGC7901, BGC823)<br><br>An immortalized non-cancerous cell line (GES-1) | <u>In vitro:</u><br>NA<br><br><u>In vivo:</u><br>30 mice | In vitro<br>In vivo | <u>In vitro:</u><br>5mM metformin<br><br><u>In vivo:</u><br>0mg/kg<br>20 mg/kg,<br>40 mg/kg,<br>60 mg/kg<br>metformin<br>intraperitoneal, *q.d.*,<br>for 4 weeks | Metformin selectively induces apoptosis in human gastric<br>cancer cell lines, but not the noncancerous one<br>Metformin might induce apoptosis in human gastric cancer cell lines by an AMPK/mTOR-mediated inhibition of survivin |

**Table 8**. Characteristics of the previous observational studies on metformin and gastric cancer.

| *Year* | *Authors* | *Cancer type* | *Size of the study* | *Type of study* | *Exposure* | *Results* |
|---|---|---|---|---|---|---|
| 2016 | **Lee *et al.*** ***(****Ann surg)* | Stages I to III gastric cancer with confirmed pathology and who underwent curative gastrectomy | 1,974 patients | A single-institution retrospective cohort | NO Time-dependent variable<br><br>and<br><br>Time-dependent variable for cumulative metformin use | - All-cause mortality: HR=0.584, 95% (CI), 0.369–0.926<br><br>- Gastric cancer–specific mortality: HR=0.57, 95%CI, 0.334–0.975<br><br>- Recurrence-free survival: HR=0.633, 95% CI, 0.410–0.977<br><br>- By cumulative 6 months of metformin<br>All-cause mortality: HR=0.870, 95% CI, 0.801–0.945)<br><br>Gastric cancer–specific mortality: HR=0.865, 95% CI, 0.782–0.958<br><br>Recurrence-free survival: HR=0.864, 95% CI, 0.797–0.937 |

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# Impact of metformin on gastric adenocarcinoma survival: A Belgian population based study

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ABSTRACT

*Background:* Preclinical studies have shown anticancer activities of metformin in gastric cancer and a recent epidemiological study showed a decrease in recurrence and mortality of gastric cancer in metformin users. This study aimed to assess the impact of metformin on gastric cancer survival in diabetic patients at a Belgian population level.

*Methods:* We conducted an observational, population-based study by linking data of the Belgian Cancer Registry with medical claims data coming from the health insurance companies for patients diagnosed with stage I to III gastric adenocarcinoma between 2006 and 2012. Information on gastric cancer-specific deaths was retrieved from mortality records collected by regional governments. Time-dependent Cox regression models were used to calculate hazard ratios (HRs) and 95% confidence intervals (CI) for overall survival (OS) and cancer-specific mortality (CSS).

*Results:* In our population of 371 patients, a reduction in all-cause mortality was observed in metformin users (adjusted HR = 0.73, 95% CI: [0.52; 1.01], p = 0.06) but not for cancer specific mortality (adjusted HR = 0.86, 95% CI: [0.56; 1.33], p = 0.50). Pre-diagnosis exposure to metformin was associated with a significant improvement in OS (adjusted HR = 0.75, 95% CI: [0.57; 0.98], p = 0.04) that was not significant for CSS (adjusted HR = 0.89, 95% CI: [0.62; 1.28], p = 0.52). Moreover, no dose-response relationship between metformin use and either all-cause or cancer-specific mortality was observed.

*Conclusion:* In the first population based study of metformin use in gastric cancer adenocarcinoma patients with previous diabetes, our findings suggest that metformin use might improve overall mortality. However, no such association was found for cancer-specific survival. Additional studies in other populations are required.

## 1. Introduction

Gastric cancer is one of the most common cancers worldwide with more than 950 000 new cases diagnosed in 2012 [1]. Despite a recent decline in incidence in some European countries, gastric cancer remains one of the leading causes of cancer death globally, with approximatively 720 000 stomach cancer deaths in 2012 alone [1,2]. Similar to most European countries, in Belgium gastric cancer incidence is approximatively 5.4 per 100 000 men and 3.3 per 100 000 women and the five-year overall survival is approximatively 35% and 44% for men and women, respectively [3].

Diabetes and cancer have been studied intensely during the last decade and researchers focused on investigating the existence of a link between these two chronic conditions [4–6]. It has been shown that diabetes increases the risk of stomach, liver, pancreatic, colon, and rectum cancer [7,8]. Also, diabetes has been reported to be associated with premature death from several cancers [9].

Metformin is the most widely prescribed first-line treatment for type II diabetes and has a favourable safety profile, even in those without type II diabetes [10–13]. Experimental studies have shown that metformin can exert an anti-cancer effect on human cancer cells [14]. Evidence from observational and clinical studies, have shown

*Abbreviations:* BCR, Belgian cancer registry; IMA, intermutualistic agency; NNSS, national number for social security; ICD, international classification of disease; DDD, defined daily dose; OS, overall survival; CSS, cancer specific survival; HR, hazard ratio; CI, confidence interval; BMI, body mass index

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metformin to be inversely associated with cancer risk and progression however results from individual studies have not been consistent [12,14–20].

For gastric cancer in particular, experimental studies demonstrate that metformin can inhibit human gastric cancer cell proliferation and metastasis and can inhibit tumour growth and enhance the effect of rapamycin and cisplatin in mouse models [21–24].

Randomized clinical trials are the gold standard for assessing treatment efficacy and safety. However, in the field of pharmacoepidemiology, such studies suffer from limitations compared to observational studies such as selection bias and short follow up durations [25]. Therefore, well conducted observational studies are a good alternative to confirm preclinical hypotheses.

To date, only one single-institution study in South Korea has investigated metformin use and mortality from gastric cancer and results were encouraging: a 14% decrease in recurrence and a 13% decrease in mortality risk for diabetic gastric cancer patients for each 6 month of cumulative use of metformin after gastrectomy [26].

Despite promising preclinical evidence, data on metformin use and gastric cancer mortality remain scarce and such pharmacoepidemiological studies haven't been conducted at a population level yet. Therefore, the current study aimed to examine the impact of metformin use on overall and cancer specific survival in 371 diabetic patients with gastric adenocarcinoma, at the Belgian population level. Based on previous evidence, we hypothesized that metformin use might increase survival in diabetic patients with gastric cancer.

## 2. Material and methods

### 2.1. Data sources

The Belgian Cancer Registry (BCR) is a population-based registry covering more than 95% of the Belgian population from 2004 onwards [3]. In addition to detailed patient and tumour characteristics collected through standard cancer registrations, the BCR has authorization to collect medical claims data from the health insurance companies. These data are gathered on a national level by the Intermutualistic Agency (IMA) and can be linked to the BCR data using the national number for social security (NNSS). Available IMA information covers all reimbursed diagnostic and therapeutic procedures and pharmaceuticals for in- as well as out-patient dispenses for a period ranging from one year before until five years after diagnosis for each cancer patient.

Vital status is also retrieved based on NNSS from the Belgian Crossroads bank for Social Security (BCSS). Causes of death are extracted from death certificate data collected by the regional governments and probabilistically linked to the BCR data (> 98% successfully linked).

Informed written consent was not needed for this study, because the use of BCR data for scientific purposes is regulated by Belgian law since 2006 [27].

### 2.2. Study subjects

All patients diagnosed between the 1st January 2006 and the 31st December 2012 with stage I–III gastric adenocarcinoma and previous diabetes were selected from the BCR database (International Classification of Diseases (ICD), 10th revision: C16.1–C16.9). Cancers of the gastro-oesophageal junction were excluded as they were considered to be oesophageal cancers.

In addition, patients who died in the first 6 months after diagnosis were excluded as drug use during this time is unlikely to exert an effect on cancer death.

A patient was defined to be diabetic if he had a record of anti-diabetic medications (ATC code "A10") dispensed with a total sum of > 30 daily defined doses (DDD) in the year prior to diagnosis.

Additional exclusion criteria were: presence of a previous tumour (apart from non-melanoma skin cancer), not residing in Belgium at the time of diagnosis, an uncertain date of diagnosis, no national number for social security (NNSS), lost to follow up at the date of cancer incidence, or missing from the medical claims (IMA) database.

Cardiovascular and respiratory comorbidities in the year prior to diagnosis were also derived from claims data including in-and outpatient dispensed medication, according to a previously described methodology [28]. A patient was defined to have cardiovascular or respiratory disease if cardiovascular medications (ATC code "C01–C04", "C07–C09" and "B01" with exclusion of heparin) or respiratory medications (ATC code "R03") with a total sum of > 180 DDDs and > 80 DDDs respectively were dispensed in the year prior to diagnosis.

### 2.3. Outcome

The primary outcome was overall survival and follow-up was until July 1st, 2015. In the cancer-specific survival analysis, patients were followed until January 1st, 2014. Patients who died after this date were censored. Cancer specific deaths were defined as those with an underlying cause of death coded with ICD-10 C16.1–C16.9 for gastric cancer or C26 for malignant neoplasm of other and ill-defined digestive organs.

### 2.4. Covariates

Information available from the BCR included data on cancer diagnosis and other demographic and clinical information: age in categories (< 70years, ≥70years), sex, year of diagnosis (from 2006 to 2012 recoded into a binary variable: 2006–2008 and 2009–2012), and combined stage (stage I–III).

Metformin use, as well as cancer treatments in the 6 months after diagnosis were derived from prescription records provided by the IMA. Cancer treatment categories included primary surgery, peri-operative treatment (chemotherapy, radiotherapy or both), primary chemotherapy and/or radiotherapy, and no treatment.

### 2.5. Statistical analysis

Users and non-users of metformin were compared using Pearson chi square test or Fisher exact test where the former was invalid.

For post-diagnosis metformin use, time-dependent cox regression models were used to calculate adjusted and unadjusted hazards ratios (HR) with 95% confidence intervals (CI). Patients became metformin users only after they were dispensed a metformin prescription, therefore avoiding immortal-time bias [29]. Before this prescription, patients were considered as non-users.

Drug use was lagged by 6 months after diagnosis to remove prescriptions occurring immediately prior to death as they may reflect palliative care. Sensitivity analyses were performed to study the effect of varying the length of this lag.

Dose response effects were explored in two types of time-varying analyses. Firstly, we investigated increasing number of prescriptions. A patient was classified a non-user if prior to the first metformin prescription. Light use was classified as use from the first prescription until the 6th prescription after diagnosis. A patient was considered a heavy user if he had the 6 or more metformin prescriptions after diagnosis.

Secondly, we investigated increasing number of defined daily doses (DDDs). In DDD analyses, patients were classified as non-users if they had less than 1 DDD after the diagnosis. Then, assuming that one DDD corresponds to one day of metformin use, we classified light users as 1–182.5 DDDs, and heavy users as more than 182.5 DDDs.

In secondary analysis, we investigated the association between pre-diagnosis metformin use in the year prior to diagnosis without excluding those with less than 6 months of follow-up after diagnosis.

In a simplified post-diagnosis use, we compared metformin users to non-users in the first six months after the cancer diagnosis in

individuals living more than six months. This method controls for immortal time bias without the use of time varying covariates [30].

As metformin usage might be associated with possible benefits on cardiovascular outcomes [31,32], an additional analysis was conducted only for cardiovascular deaths including diabetes related deaths (ICD10 codes I00-I99, E14) and censoring other causes of deaths. Note that, in the cardiovascular specific analyses no adjustments for comorbidities were conducted as all cardiovascular deaths occurred in patients with cardiovascular but no respiratory disease.

In all analyses, censoring was conducted at 5 years after diagnosis. All models were adjusted for age at diagnosis, sex, incidence year, cancer stage, cancer treatments and cardiovascular and respiratory comorbidities.

All analyses were performed using the SAS Enterprise Guide statistical release 9.3 software. The statistical significance level was set at 0.05.

## 3. Results

### 3.1. Baseline characteristics

A total of 2552 participants with gastric cancer met the initial inclusion criteria (Fig. 1). Within these patients, 371 (15%) were identified as diabetics. The main analysis included 298 patients who had more than 6 months of follow-up. The median follow-up time was 48.6 months (95% CI, 36.1–58.3). During the follow up, 180 (60.4%) patients died. According to the death certificates, 114 (63.3%) deaths were due to cancer and 92 (51.1%) deaths were due to gastric cancer. Patient and tumour characteristics according to metformin use are summarized in Table 1.

The median age of patients was 75.0 years (Interquartile range (IQR): 66.0–80.0). Patients were more often diagnosed at stage I cancer (43.3%, n = 129), with poorly differentiated disease (46.3%, n = 138) and when specified, tumours localised in the pyloric antrum (29.9%, n = 89). Within diabetic patients with more than 6 months of follow up, 228 (76.5%) had a history of metformin use. The latter group was younger than the non-metformin users (median age (IQR): 74.0 (65.0–80.0) years for users versus 78.0 (69.0–82.0) years for non-users, p = 0.05).

There was no difference between metformin users and non-users in terms of sex, year of incidence, grade of differentiation, stage or comorbidities including cardiovascular and respiratory disease.

### 3.2. Post diagnosis use of metformin and survival

In unadjusted time dependent analysis, metformin use was associated with a significant increase in overall survival with a HR of 0.72 (95% CI: [0.52; 0.98], p = 0.04). After adjustment for age, sex, stage, year of diagnosis, cancer treatment and comorbidities, the reduction in hazards all-cause mortality remained (HR = 0.73, 95% CI: [0.52; 1.01], p = 0.06) (Table 2).

For metformin use and cancer specific survival, the association was not significant (HR = 0.80, 95% CI: [0.53; 1.21], p = 0.29), even after adjustment for potential confounders (adjusted HR = 0.86, 95% CI: [0.56; 1.33], p = 0.50) (Table 2).

There was no dose-response association observed between metformin use and mortality from all causes or from gastric cancer in analyses of increasing considering number of prescriptions or DDDs (for instance, adjusted HR for 1–6 prescriptions = 0.64, 95% CI: [0.44; 0.93], p = 0.02 and adjusted HR for more than 6 prescriptions = 0.92, 95% CI: [0.59; 1.43], p = 0.71) (Table 2).

### 3.3. Secondary and subgroup analysis

Tables 3 and 4 show results from sensitivity analyses for overall and cancer specific analysis, respectively. Using a time-varying definition for metformin use and no lag time, overall survival was associated with a significant increase in survival for metformin users (adjusted

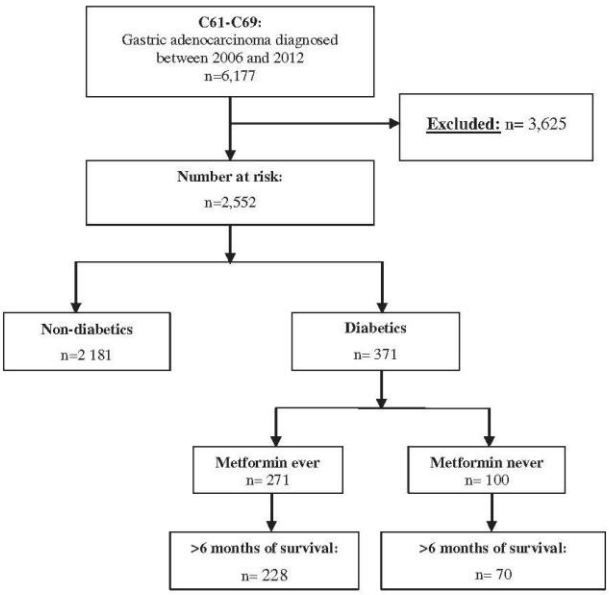


Fig. 1. Flowchart of Belgian patients with stage I–III gastric adenocarcinoma and diabetes.

**Table 1**
Baseline characteristics of patients with gastric adenocarcinoma and diabetes by metformin use in patients with more than 6 months' follow-up after diagnosis.

| Characteristic | Total (N = 298) | Metformin Use | | p-value |
|---|---|---|---|---|
| | | Ever (n = 228) | Never (n = 70) | |
| **Sex—n (%)** | | | | 0.20 |
| Male | 169 (56.7) | 134 (58.8) | 35 (50.0) | |
| **Age category—n (%)** | | | | 0.14 |
| ≤69 years | 98 (32.9) | 80 (35.1) | 18 (25.7) | |
| ≥70 years | 200 (67.1) | 148 (64.9) | 52 (74.3) | |
| **Year of incidence—n (%)** | | | | 0.14 |
| < 2009 | 118 (39.6) | 85 (37.3) | 33 (47.1) | |
| ≥2009 | 180 (60.4) | 143 (62.7) | 37 (52.9) | |
| **Grade of differentiation—n (%)** | | | | 0.73 |
| Poorly | 138 (46.3) | 106 (46.5) | 32 (45.7) | |
| Moderately | 84 (28.2) | 62 (27.2) | 22 (31.4) | |
| Well | 22 (7.4) | 16 (7.0) | 6 (8.6) | |
| Unknown/missing | 54 (18.1) | 44 (19.3) | 10 (14.3) | |
| **Combined stage—n (%)** | | | | 0.07 |
| I | 129 (43.3) | 99 (43.4) | 30 (42.9) | |
| II | 87 (29.2) | 60 (26.3) | 27 (38.6) | |
| III | 82 (27.5) | 69 (30.3) | 13 (18.6) | |
| **Vital Status—n (%)** | | | | 0.11 |
| Alive | 140 (47.0) | 113 (49.6) | 27 (38.6) | |
| Dead | 158 (53.0) | 115 (50.4) | 43 (61.4) | |
| **Cardiovascular disease—n (%)** | | | | 0.96 |
| Yes | 222 (74.5) | 170 (74.6) | 52 (74.3) | |
| **Respiratory diseases—n (%)** | | | | > 0.99 |
| Yes | 16 (5.4) | 12 (5.3) | 4 (5.7) | |
| **Cancer therapy within 6mths—n (%)** | | | | 0.46 |
| None | 37 (12.4) | 25 (11.0) | 12 (17.1) | |
| Surgery alone | 154 (51.7) | 117 (50.9) | 37 (52.9) | |
| Primary CT and/or RT | 19 (6.4) | 15 (6.6) | 4 (5.7) | |
| Surgery and others treatment | 88 (29.5) | 71 (31.6) | 17 (24.3) | |

HR = 0.69, 95% CI: [0.53; 0.91], p = 0.01). Metformin use was also associated with an increase in overall survival when the lag time was set to 3 months (adjusted HR = 0.75, 95% CI: [0.55; 1.01], p = 0.06) or 1 year (adjusted HR = 0.67, 95% CI: [0.46; 1.00], p = 0.05). With a lag time of 2 years however, the estimate was similar but was no longer significant (adjusted HR = 0.70, 95% CI: [0.41; 1.18], p = 0.18) (Table 3). In cancer specific analysis, these associations were no longer significant (Table 4). Cardiovascular mortality risk appeared to be reduced in metformin users but results were non-significant (adjusted HR = 0.39, 95% CI: [0.14–1.10], p = 0.08).

In analysis of metformin use in the year before diagnosis, a significant protective association was observed for all-cause mortality (adjusted HR = 0.75, 95% CI: [0.57; 0.98]; p = 0.04) (Table 3). This significant survival advantage was not found for cancer specific mortality (adjusted HR = 0.89, 95% CI: [0.62; 1.28], p = 0.52) (Table 4).

In the simple analysis of post-diagnosis metformin use in the 6 months after diagnosis, no significant associations were found for all-cause mortality (adjusted HR = 0.76, 95% CI: [0.55; 1.06], p = 0.10) or cancer specific mortality (adjusted HR = 0.84, 95% CI: [0.55; 1.29]; p = 0.43) (Tables 3 and 4).

## 4. Discussion

In this population-based study, we sought to assess whether metformin use is associated with an improvement in survival in patients with diabetes and stage I–III gastric adenocarcinoma.

We observed a borderline 27% reduction in the overall mortality risk. However, no significant association was observed for cancer specific mortality. Caution is required in the interpretation of the findings for all-cause mortality as results were borderline significant and no dose-response association was observed for increasing metformin DDDs.

We aimed to avoid the potential biases which pharmacoepidemiological studies are prone to by using robust methods to investigate the association between metformin use and mortality. For example, metformin use was modelled as a time varying covariate in order to avoid immortal time bias in the definition of 'users', time lags were introduced for medication prescriptions to avoid bias due to end-of-life treatment, sensitivity analyses were carried out to investigate the impact of the choice of these time lags and all-cause mortality, cancer specific mortality and cardiovascular mortality were studied.

Reassuringly, the sensitivity analyses for different lag sizes on all-

**Table 2**
Association between metformin use and either all-cause mortality or cancer-specific mortality at 5 years in patients with diabetes and gastric adenocarcinoma diagnosed between 2006 and 2012 in Belgium.

| Medication usage | 5-yr event rate (%) | | Person Years | Unadjusted | | | Adjusted[a] | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | HR | (95% CI) | p | HR | (95% CI) | p |
| **All-cause mortality** | | | | | | | | | |
| **Post diagnosis metformin use as time varying covariate[b]** *(n = 298)* | | | | | | | | | |
| Non-users | 78/114 | (68.4) | 449 | 1 | | | 1 | | |
| Users | 80/184 | (43.5) | 471 | 0.72 | (0.52–0.98) | 0.04 | 0.73 | (0.52–1.01) | 0.06 |
| 1 to 6 prescriptions | 45/94 | (47.9) | 269 | 0.62 | (0.43–0.90) | 0.01 | 0.64 | (0.44–0.93) | 0.02 |
| More than 6 prescriptions | 35/90 | (38.9) | 202 | 0.92 | (0.60–1.40) | 0.68 | 0.92 | (0.59–1.43) | 0.71 |
| 1 to 182.5 DDDs | 46/87 | (52.9) | 256 | 0.65 | (0.45–0.94) | 0.02 | 0.65 | (0.47–0.92) | 0.02 |
| More than 182.5 DDDs | 34/97 | (35.1) | 215 | 0.84 | (0.55–1.30) | 0.43 | 0.89 | (0.57–1.40) | 0.61 |
| **Cancer-specific mortality** | | | | | | | | | |
| **Post diagnosis metformin use as time varying covariate[b]** *(n = 297)* | | | | | | | | | |
| Non-users | 45/113 | (39.8) | 415 | 1 | | | 1 | | |
| Users | 49/184 | (26.6) | 378 | 0.80 | (0.53–1.21) | 0.29 | 0.86 | (0.56–1.33) | 0.50 |
| 1 to 6 prescriptions | 34/99 | (34.3) | 227 | 0.82 | (0.52–1.28) | 0.39 | 0.86 | (0.54–1.36) | 0.52 |
| More than 6 prescriptions | 15/85 | (17.6) | 151 | 0.76 | (0.41–1.41) | 0.38 | 0.87 | (0.46–1.68) | 0.69 |
| 1 to 182.5 DDDs | 34/103 | (33.0) | 219 | 0.83 | (0.53–1.30) | 0.41 | 0.84 | (0.53–1.34) | 0.47 |
| More than 182.5 DDDs | 15/81 | (18.5) | 160 | 0.74 | (0.40–1.37) | 0.34 | 0.92 | (0.48–1.78) | 0.81 |

[a] Adjusted for age, sex, year of diagnosis, stage, cancer treatment, and comorbidities.
[b] Analyses include a lag of 6 months in individuals living more than 6 months.

**Table 3**
Sensitivity analysis on metformin use and all-cause mortality at 5 years in patients with diabetes and gastric adenocarcinoma diagnosed between 2006 and 2012 in Belgium.

| All-cause mortality | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Medication usage | 5-yr event rate (%) | | Person Years | Unadjusted HR | (95% CI) | p | Adjusted[a] HR | (95% CI) | p |
| **No lag**[b] *(n = 371)* | | | | | | | | | |
| Non-users | 118/154 | (76.6) | 365 | 1 | | | 1 | | |
| Users | 112/217 | (51.6) | 570 | 0.69 | (0.53–0.90) | 0.006 | 0.69 | (0.53–0.91) | 0.01 |
| **3 months lag**[c] *(n = 328)* | | | | | | | | | |
| Non-users | 93/129 | (72.1) | 412 | 1 | | | 1 | | |
| Users | 95/199 | (47.7) | 518 | 0.74 | (0.55–0.99) | 0.04 | 0.75 | (0.55–1.01) | 0.06 |
| **1 year lag**[d] *(n = 250)* | | | | | | | | | |
| Non-users | 56/92 | (64.1) | 498 | 1 | | | 1 | | |
| Users | 54/158 | (34.2) | 386 | 0.66 | (0.45–0.96) | 0.03 | 0.67 | (0.46–0.99) | 0.05 |
| **2 years lag**[e] *(n = 201)* | | | | | | | | | |
| Non-users | 32/70 | (45.7) | 574 | 1 | | | 1 | | |
| Users | 29/131 | (22.1) | 244 | 0.68 | (0.41–1.13) | 0.14 | 0.70 | (0.41–1.18) | 0.18 |
| **Metformin use before diagnosis**[f] *(n = 371)* | | | | | | | | | |
| Non-users | 87/122 | (71.3) | 282 | 1 | | | 1 | | |
| Users | 143/249 | (57.4) | 653 | 0.72 | (0.55–0.94) | 0.01 | 0.75 | (0.57–0.98) | 0.04 |
| **Metformin use after diagnosis**[g] *(n = 298)* | | | | | | | | | |
| Non-users | 68/112 | (60.7) | 344 | 1 | | | Ref | | |
| Users | 90/186 | (48.4) | 575 | 0.79 | (0.58–1.08) | 0.14 | 0.76 | (0.55–1.06) | 0.10 |

[a] Adjusted for age, sex, year of diagnosis, stage, cancer treatments, and comorbidities.
[b] No lag with no exclusion of deaths after diagnosis.
[c] Decreasing the lag to 3 months in time varying co-variate analysis in individuals with more than 3 months' follow-up.
[d] Increasing the lag to 1 year in time varying co-variate analysis in individuals with more than 1 years' follow-up.
[e] Increasing the lag to 2 year in time varying co-variate analysis in individuals with more than 2 years' follow-up.
[f] Previous users were individuals with at least one prescription in the year before diagnosis.
[g] Post diagnosis in the six months after diagnosis in individuals with more than 6 months' follow-up.

cause mortality as well as cancer-specific mortality showed similar protective effects (HR < 1) that once adjusted, did not reach a statistically significant level.

One might postulate that, at least in this diabetic population, metformin use was not found to significantly reduce cancer-specific mortality whilst suggesting a possible protective association for all-cause mortality could be explained by the fact that metformin is the first-line treatment for type II diabetes. Thus, metformin users could

**Table 4**
Sensitivity analysis on metformin use and gastric cancer specific mortality at 5 years in patients with diabetes and gastric adenocarcinoma diagnosed between 2006 and 2012 in Belgium.

| Cancer-specific mortality | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Medication usage | 5-yr event rate (%) | | Person Years | Unadjusted HR | (95% CI) | p | Adjusted[a] HR | (95% CI) | p |
| **No lag**[b] *(n = 367)* | | | | | | | | | |
| Non-users | 70/152 | (46.1) | 332 | 1 | | | 1 | | |
| Users | 67/215 | (31.2) | 477 | 0.72 | (0.51–1.01) | 0.06 | 0.75 | (0.53–1.07) | 0.11 |
| **3 months lag**[c] *(n = 326)* | | | | | | | | | |
| Non-users | 56/128 | (43.8) | 378 | 1 | | | 1 | | |
| Users | 58/198 | (29.3) | 426 | 0.80 | (0.55–1.17) | 0.25 | 0.86 | (0.58–1.28) | 0.46 |
| **1 year lag**[d] *(n = 249)* | | | | | | | | | |
| Non-users | 33/95 | (34.7) | 463 | 1 | | | 1 | | |
| Users | 31/154 | (20.1) | 295 | 0.73 | (0.44–1.20) | 0.21 | 0.84 | (0.50–1.42) | 0.52 |
| **2 years lag**[e] *(n = 169)* | | | | | | | | | |
| Non-users | 15/69 | (21.7) | 474 | 1 | | | 1 | | |
| Users | 15/100 | (15.0) | 173 | 0.83 | (0.40–1.74) | 0.63 | 0.99 | (0.46–2.16) | 0.99 |
| **Metformin use before diagnosis**[f] *(n = 367)* | | | | | | | | | |
| Non-users | 48/119 | (40.3) | 253 | 1 | | | 1 | | |
| Users | 89/248 | (35.9) | 556 | 0.83 | (0.58–1.17) | 0.29 | 0.89 | (0.62–1.28) | 0.52 |
| **Metformin use after diagnosis**[g] *(n = 297)* | | | | | | | | | |
| Non-users | 41/111 | (36.9) | 308 | 1 | | | 1 | | |
| Users | 53/186 | (28.5) | 485 | 0.81 | (0.54–1.21) | 0.30 | 0.84 | (0.55–1.29) | 0.43 |

[a] Adjusted for age, sex, year of diagnosis, stage, cancer treatments, and comorbidities.
[b] No lag with no exclusion of deaths after diagnosis.
[c] Decreasing the lag to 3 months in time varying co-variate analysis in individuals with more than 3 months' follow-up.
[d] Increasing the lag to 1 year in time varying co-variate analysis in individuals with more than 1 years' follow-up.
[e] Increasing the lag to 2 year in time varying co-variate analysis in individuals with more than 2 years' follow-up.
[f] Previous users were individuals with at least one prescription in the year before diagnosis.
[g] Post diagnosis in the six months after diagnosis in individuals with more than 6 months' follow-up.

have a better prognosis due to a lower morbidity from diabetes (confounding by indication) or possible benefits on cardiovascular outcomes [31,32]. This argument, while only a hypothesis, is strengthened by the results from the cardiovascular mortality analysis where a 60% reduction in cardiovascular mortality was observed for metformin users, albeit non-significant. However, this analysis could suffer from a lack of power due to low number of cardiovascular events. Similarly, the loss of statistical significance in the cause-specific analysis could also be explained by a lower number of events.

In our analysis, a lag of 6 month for metformin use was used and consistent results were found when varying this lag. The use of a lag for medication use is recommended in order to avoid reverse causation bias [33], however, as the lag time was increased, the number of events decreased, thus potentially leading to an underestimation of the hazard ratio and wider confidence intervals.

It is plausible that a number of limitations may have influenced our findings. We had no information on potential lifestyle confounders such as alcohol or tobacco use. Moreover, data about body mass index (BMI) or socioeconomic factors were unavailable. Therefore, residual confounding cannot be ruled out by unrecorded confounders. Also, our analyses were not adjusted for type of diabetes, diabetes duration, severity or glycemic control. Despite the use of a nation-wide gastric cancer cohort, the study was limited by a relatively small sample size. Therefore, subgroup analyses leading to further subdivision of the sample (e.g. by type of treatment) have not been conducted.

Lastly, patients included in the analysis were not all naïve to medication exposure before diagnosis and so differed from patients usually included in clinical trials. However, in the case of type II diabetes, it could be difficult to exclude patient with a history of metformin use as it is the first line treatment for this condition. Some clinical trials of metformin in cancer patients, have excluded diabetic patients in order to avoid previous exposure [34,35] however, in our study, the choice of focusing on a diabetic population allowed for a more homogenous cohort.

In addition, focusing on diabetic patients, also allowed to control partly for confounding by indication, as diabetes is associated with both metformin use and cancer outcome [36–38]. Confounding by indication is a frequent bias which occurs when the treatment is preferentially prescribed to a group of patient for a clinical reason and restriction is a method to reduce this bias [39].

Among the strengths of the study, we count the fact that this is the first study of metformin use and survival in gastric adenocarcinoma patients to be conducted at a population level. Only one clinical series study conducted in South Korea has assessed survival in relation to metformin use in gastric cancer patients after gastrectomy [26]. The authors showed that the cumulative use of metformin after gastrectomy in patients with type II diabetes was associated with an improved survival in stage I–III gastric cancer. They showed that for each cumulative 6 month of metformin use, there was a 13% reduction of cancer specific death and in all-cause deaths [26]. However, our study showed contradictory results, despite the similarity in the effect size in the cancer specific analysis. Also, there was no evidence of a dose-response relationship between metformin use and overall or cancer-specific mortality. The reason for these contradictory results may be due to population or tumours profiles differences and the fact that our analysis focused on post-diagnosis drug use and not on post-surgery exposure.

As describe, we used robust methods in this study in order to control for certain biases. We also used data from a nationwide cancer registry allowing for robust verification of cancer cases and deaths. Moreover, in Belgium, health insurance is mandatory; therefore, the information on dispensed drug use is comprehensive and avoids recall bias (without affecting the precision of our study).

So, in conclusion, despite of the fact that many biases were overcome by applying specific methods recommended in pharmacoepidemiological studies, it remains challenging to draw a conclusion about the effect of metformin use on survival of gastric cancer patients with diabetes.

Despite encouraging preclinical studies suggesting an anti-cancer effect for metformin in gastric cancer [21–24], only one previous study has been conducted. Therefore, our findings are important to contribute to the overall evidence base for metformin and gastric cancer progression, especially considering it is the only population-based study conducted. Larger (inter)nation-wide studies however are required to further elucidate the association between metformin use and mortality in gastric cancer patients.

## 5. Conclusion

In a nationwide study of gastric cancer patients with diabetes, our findings suggest that metformin use might reduce overall mortality, but not gastric cancer mortality. These findings, however need to be confirmed in other populations.

## Authorship contributions

- **Conception and design of the study**: Chris R. Cardwell, Olivia Lacroix.
- **Acquisition of data**: Evelien Vaes, Harlinde De Schutter.
- **Analysis and/or interpretation of data**: Olivia Lacroix, Alexandra Couttenier, Evelien Vaes, Harlinde De Schutter, Annie Robert, Chris R. Cardwell.
- **Manuscript preparation**: Olivia Lacroix.
- **Revision of the manuscript**: Olivia Lacroix, Evelien Vaes, Harlinde De Schutter, Chris R. Cardwell, Annie Robert.
- **Final approval of the version to be published**: Olivia Lacroix, Evelien Vaes, Harlinde De Schutter, Chris R. Cardwell, Annie Robert, Alexandra Couttenier.

## Conflict of interest

None.

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This thesis set out to analyze the use of metformin and statins, two commonly prescribed non-cancer drugs, in a digestive tract cancer setting.

The primary aim of this thesis was to investigate digestive tract cancer survival in relation with statins and metformin use. In order to achieve this part of the thesis, the Belgian cancer registry database was used, and two population-based studies were performed.

Secondary to this main objective, a descriptive part, assessing the pattern of use and the adherence to these two drugs using the Belgian cancer registry and the Belgian permanent sample databases, was carried out.

The key findings of the present work are summarized and discussed in the sections below.

## 8.1 Statins and metformin use and oesophageal and gastric cancer survival

As seen in **Section 6** and **Section 7**, the use of both metformin in gastric cancer and statins in oesophageal cancer, was associated with encouraging results in cancer outcome in pre-clinical studies. In both cases, only few observational studies have investigated cancer outcome in association with the two drugs.[147–150] In metformin and gastric cancer for example, only one population-based study conducted in South Korea was published at the time of the publication of our results.[147]

In both studies, the Belgian cancer registry database was used and linked to social security, health insurance and mortality databases. Cox regression models with time dependent variables were used and several sensitivity analyses were carried out.

### 8.1.1 Statins and oesophageal cancer

In oesophageal cancer patients who use statins (**Section 6**), a significant 13% and 16% reduction in mortality from oesophageal cancer and all causes of deaths have been measured, respectively. After conducting several sensitivity analyses no significant variation was observed in these findings. Moreover, the results from this article were concordant with other similar studies indicating a reduction in mortality ranging from 6% to 20% for all causes of death and from 7% to 23% for oesophageal cancer specific deaths.[148–150] The biggest effect was observed in the largest studies.[150,151]

The main limitation of this study was the lack of information regarding several confounders, such as BMI, smoking, alcohol and socioeconomic status.

Since the publication of the study on statins use in oesophageal cancer, a meta-analysis pooling the results of the four existing studies has been published.[152] On a total of 20,435 patients, statins used after a diagnosis of oesophageal cancer was significantly associated with a 19% decrease in all-cause mortality (random effects: HR=0.81, 95%CI: 0.75–0.89, P<.001; $I^2$=68.1%) and a 16% decrease in cancer specific mortality (fixed effects: HR=0.84, 95%CI: 0.78–0.89, P<.001; $I^2$=46.6%).[152]

At the time of the redaction of this thesis 79 interventional studies were registered on the clinicaltrials.gov website using statins in cancers patients and only one Spanish clinical study registered since 2009 with an incomplete status focused on oesophageal cancer.[153]

### 8.1.2 Metformin and gastric cancer

In diabetic patients with gastric adenocarcinoma (**Section 7**), a 14% reduction in mortality from gastric cancer was measured, albeit non-significant. Regarding overall survival, a 27% reduction in mortality associated with metformin use in diabetic patients was observed.

The results of the analyses were easily affected by variations in the sample size depending on the type of conducted sensitivity analyses. Indeed, these studies have been complicated by a small sample size due to a relatively rare cancer in Belgium and an additional restriction to diabetic patients (to avoid confounding by indication bias). Moreover, information regarding type of diabetes, diabetes duration, severity, or glycaemic control were missing.

The findings of this study were however, consistent with the single published study at this time, that have found a 13% decrease in mortality for each cumulative 6 months use of metformin after gastric cancer surgery.[147]

Since our publication, another study evaluating diabetes medications use in Chinese patients with breast, colorectal, lung, or gastric cancer has been published.[154] Within the 543 patients with gastric cancer, the use of metformin was associated neither with a better overall (HR= 0.95, 95%CI: 0.52-1.74) nor with a better cancer-specific (HR=0.90, 95%CI: 0.48-1.70) survival outcome.[154] Even after restricting the analysis to the 130 diabetic patients, the use of metformin was not associated with better overall (HR= 1.01, 95%CI: 0.48-2.12) or cancer-specific (HR=1.01, 95%CI: 0.46-2.22) survival. This study also suffered from reduction in sample size when restricting the analyses to diabetics' patients, with only 130 patients remaining in the analyses. However, authors, were able to adjust their analysis for diabetes medications mutually, (metformin, sulfonylureas and insulin), for BMI, and smoking status. This could explain the reduction in the effect size.

At the time of writing, 146 interventional studies evaluating metformin in several cancers were registered on the clinicaltrials.gov website. Only one study was recruiting for investigating metformin in association with vitamin C in gastric cancers.[155]

### 8.1.3 What's next?

For both studies, larger observational studies with comprehensive data on important confounders such as BMI, tobacco and alcohol, information on comorbidity severity and control (glycated hemoglobin, cholesterol level...) are required. Additionally, comorbidities should ideally be derived from medical diagnosis. It could also be

interesting to study the impact of a combination of treatments as they are common among patients with advanced disease.

## 8.2 Pattern of statins and metformin use in digestive tract cancer patients

In the previous part of the discussion, the main findings of this thesis, i.e. the better outcome in mortality associated with metformin and statins use, has been summarised. In order to further discuss these results, studies on drugs utilization were needed to provide an overview of how metformin and statins are used in the real world setting in cancer patients. This kind of information allowed to quantify the exposure to the two drugs and is of extreme importance.

Aiming to describe metformin and statins use in digestive tract cancers and in a representative sample of cancer-free individuals, the Belgian cancer registry database and the Belgian permanent sample were used. Volume of metformin and statins prescriptions were estimated by using the Anatomical Therapeutic Chemical (ATC) classification for international comparisons and standard methods for estimate drug use across population such as the Defined Daily Dose per thousand inhabitants per day (DDD/TID) were used.

In patients with digestive tract cancer, and despite their cancer diagnosis, elevated volumes of metformin and statins have been prescribed (**Section 4**). Compared to a representative sample of the Belgian general population, metformin and statins volume prescriptions were twofold higher in individuals with digestive tract cancer. Indeed, the volume of prescribed statins was estimated to be 114 and 237 DDD/TID in the cancer-free sample and in the digestive tract cancer population, respectively.

Considering the volume of prescribed metformin, the estimates in the present thesis were about 23 and 50 DDD/TID in the cancer-free sample and in the digestive tract cancer population, respectively.

The results found for the general population were in accordance with previous Belgian estimates for statins and to regional estimates for metformin.[124–126] In the cancer population, however, and to our knowledge, it's the first time that the volume of prescribed statins and metformin were reported. Yet, the results were expected, as the prevalence of comorbidities such as diabetes and dyslipidaemia are higher in cancer patients compared to the general population.[51,55,64] Another explanation could be that, as patients with cancer are older and prevalent users, they might be at higher doses of treatment for their comorbidities.

Interestingly, initiation of those two drugs increased before diagnosis and reached a peak in the first following month (**Section 4**). This increase in drugs' initiation preceding cancer diagnosis has already been documented in general cancers and in ovarian cancers.[120,123] It has been suggested that frequent contact with health care providers before cancer diagnosis might be associated with the discovery of undetected chronic conditions such as diabetes or in the present work

dyslipidaemia.[120] This explanation was further reinforced by the fact that the increase of drug initiation around diagnosis was mainly driven by hospital prescriptions while general practitioner prescriptions decreased.

These findings highlight a significant exposure of digestive tract cancer patients to metformin and statins around their cancer diagnosis but have to be interpreted with caution. The results on the prescribed volume of statins and metformin in this study are only valid if there is good agreement between the actually prescribed dose and the DDD. Moreover, in the Belgian cancer registry, only data on prescriptions occurring in the year before diagnosis are available for pre-diagnosis exposure.

### 8.2.1 What's next?

Studies investigating agreement between DDD and daily prescribed drug in cancer patients are needed to interpret the findings of this study. Also, an extended pre-diagnosis follow-up period would allow to better understand the pattern of drug initiation. Additionally, it could be interesting to conduct a nested matched case-control study with cancer patients and cancer-free individuals to compare incidences rates and DDD/TID after removing bias due to age, sex or regional differences.

## 8.3 Adherence to statins and metformin in patients with digestive tract cancer

In the previous sections, two findings were summarised and discussed. First, patients with digestive tract cancer were highly exposed to metformin and statins. And this exposition was further associated with better survival in patients with gastric or oesophageal cancer. To go deeper into the subject, adherence that is another type of measure to quantify exposure, is essential.[77] Adherence gave an indication on how patient behaviour corresponds to the medical advice. In some cases, adherence might also be useful to determine how much medication must be taken to observe the desired outcome.[77]

As described in **Section 5**, non-adherence to both medications was high in patients with digestive tract cancer and adherence declined after cancer diagnosis. Adherence was higher in patients using statins than in patients using metformin (54% versus 26%, respectively). Moreover, compared to patients with oesophageal and gastric cancers, patients with colorectal cancer were biggest adherers.

Factors associated with adherence were age at diagnosis, sex, prevalent users' status and the occurrence of surgery for both drugs. In statins users, comorbidities such as cardiovascular diseases and diabetes were positively associated with adherence. In diabetic patients using metformin, radiotherapy was negatively associated with adherence.

Low adherence could mean different things considering the fact that the consumption of the two drugs was estimated using the WHO's DDD methodology. As a reminder, a DDD is defined by the World Health Organisation as "the assumed average maintenance dose per day for a drug used for its main indication in adults".[79]

From a logical point of view low adherence means that the period during which the volume of drug has been prescribed is greater than the one normally expected. The expectations in the present thesis are based on the DDD's methodology established by the WHO. In the WHO methodology, one DDD is given in one day.

A possible explanation of low adherence could be that there is a discordance between DDD and real prescribed daily doses with DDD overestimating the prescribed doses in cancer patients. This is in agreement with a recent study conducted in the US that has reported an underestimation of the exposure using the DDD method for metformin and statins in the general population.[128] In this last study, patients using metformin, had an average daily prescribed dose for the first prescription equal to 1000mg contrasting with the DDD of 2000mg.[128]

If there is good agreement between the prescribed dose and the DDD, then it can be supposed that the adherence of patients with digestive tract cancer is effectively low.

And this might imply possible interventions to increase adherence in patients at risk of being non-adherers.

The most likely explanation would probably be a mix of the two situations with a discrepancy between DDDs and real prescribed dose and a high rate of non-adherence to treatment.

It might be interesting to discuss this finding together with the results of the **Section 4**. Even though the volumes of prescription of both drugs were higher in the cancer population, adherence which is in our case a derived measure of the volume of prescriptions, was low. Regarding the results of **Section 5**, it is most likely that the observed higher volume of metformin and statins prescriptions were the reflection of a higher number of people taking these drugs in cancer population rather than higher prescribed doses.

### 8.3.1 What's next?

Studies investigating agreement between DDD and daily prescribed drug in cancer patients are also needed to interpret the present findings. Alternatively, and for comparison purposes, further studies in countries with available data on the doses prescribed by physicians in cancer patients are needed. Linkage of the Belgian cancer registry to the Belgian permanent sample could also be of interest to identify cancer cases among the latter and to conduct matched studies.

## 8.4 Clinical implications of this thesis

Our results may have some implications for the clinical practice. In a reassuring way, none of the two studied medications were associated with poorer outcome in localised gastric or oesophageal cancer. Therefore, those medications could continue to be administered to patients who can benefit from it if there is no impact on their quality of life. This implies the necessity to also study the quality of life of patients with digestive tract cancer taking chronic medications such as statins and metformin.

The incidence of the main adverse effect of statins and metformin -gastrointestinal symptoms and myopathy- increases with age and in individuals with metabolic dysfunction. Therefore, it might be relevant to evaluate the clinical impact of those drugs in patients with cancer and more specifically with digestive tract cancer. A recent randomized study conducted in the US has shown that stopping statin medication therapy was associated with better quality of life in patients with advanced, life-limiting illness.[122] However, in the present thesis, the patients included in the survival analyses were diagnosed with localised disease with a better prognosis than the ones included in the American study. Moreover, it has been shown that the presence of chronic conditions in cancer patients is associated with poorer prognosis, partially explained by comorbidities' mortality.[55] Therefore managing those chronic conditions might be of clinical importance in patients with a relatively good prognosis, who are expected to live a sufficient time length to beneficiate from their treatment.

Quality of life studies could also be interesting to perform given the important exposure to both drugs that has been pointed out in the present thesis despite cancer diagnosis. Moreover, given that adherence to both drugs turned out to be low in patients with digestive tract cancers, these kinds of studies could be interesting to understand the mechanism behind low adherence.

Additionally, managing chronic conditions in cancer patients is an interesting topic that has been evaluated among the unmet needs of patients living with cancer and beyond. Indeed, the question "How do I manage my cancer alongside my other health conditions?" is one of many important questions raised by patients themselves in what has been called priority setting partnership research.[156] At the same time, multimorbidity and polypharmacy in cancer patients have been identified as one of the concerns of health professionals.[156] Priority setting partnership research is a process enabling patients and health professionals groups "to work together to identify and prioritize evidence uncertainties in particular areas of health care that could be answered by research".[157] Further research on this topic is therefore warranted given the common interest of all concerned parts.

## 8.5 Causal association and drug repurposing

To go further in this discussion, one might question the nature of the relationship between the use of both drugs and survival outcome in a digestive tract cancer. It could also be tempting to extrapolate the above-mentioned results, as proof of the possibility of drug repositioning. However, before considering drug repositioning, it would be interesting to discuss the causality of the observed association.

### 8.5.1 Causality of the observed associations between statins and metformin use and cancer outcome

One thing is sure, the present thesis lacks arguments for establishing the causal association between metformin and statins consumption and the observed cancer outcome. The nine criteria of Bradford Hill although criticized in the field of exposure-wide epidemiology have been widely used in epidemiology to differentiate association from causation.[158–160]

Regarding the criteria of experiment, consistency, coherence and plausibility of the association, amount of pre-clinical studies on statins and metformin use in digestives tract cancers are available to answer these points. Also, temporality of the association is generally not a problem when the studied outcome is death.

However, for the remaining criteria, it is more difficult to conclude.

Beginning with the biological gradient, the present thesis fails to show a dose-response association between the two drugs and cancer outcomes despite encouraging results in pre-clinical studies. The poor adherence to both drugs could partially explain this problem together with possible discrepancies between the real exposure and the measure used in the present thesis, *i.e.* DDDs. However, a major criticism arising from drug repurposing is the one regarding the dosage necessary to induce an effect in real patients with cancers. In preclinical studies investigating metformin or statins effects in cancerous cells, the concentrations used in cancers cells or injected to mice were generally higher than the ones used in clinical practice. For example, it is known that the plasmatic concentration of individuals taking metformin ranges between 8 and 24µmol/l.[89] In gastric cancer, metformin concentration used in pre-clinical studies ranges from 1mmol/L in vitro to 1mg/body in vivo.[161–163]

Beside biological gradient, the strength of the association needs also to be assessed through additional studies mainly for metformin in gastric cancers where the observational studies show inconsistent results. However, it is likely that the effect size in play here will be relatively small and that large effect size would reflect bias such as immortal-time bias.

The specificity of the association is also hard to establish as unmeasured confounders may explain the observed association in the present thesis. Specificity, means that one cause leads to a single effect, and Bradford himself recognized that one-to-one relationships are not frequent.[160] It is even more difficult to establish in oncology where one cause could impact several types of cancers.

To study the analogy of the association, it would be necessary to identify the specific mechanism of action by which metformin and statins could impact cancer outcome in humans. Part of this mechanism has been identified through pre-clinical studies, but more evidence is needed. Moreover, the specific mechanism of action of these drugs in their main indication could help to generate hypotheses. However, even in diabetic patients, the exact mechanism of action of metformin is still partially unknown.

### 8.5.2 Additional challenge for drug repositioning

In the previous part, the causality of the association between the use of the two studied drugs and the survival outcome of the two digestive tract cancers has been discussed. The Bradford-Hill criteria are hard to fill, even for well documented causal effects like smoking and lung cancer. However, even if efficacy of a commonly used drug was established in oncology, drug repositioning will require additional effort, especially if the generic form of the drug of interest exists.

Indeed, when the drug is under patent, companies can acquire patent for secondary clinical use. In Europe for example, patent extension can be possible only if "the new indication is registered within 8 years after the initial marketing authorisation and if there is a significant benefit compared with existing therapy".[86] In Europe, only the marketing authorisation holder can ask for a patent extension.[86] It means that even if well conducted academic research finds some interesting results, only the pharmaceutical companies have the power to make it accessible to the patients for another indication than the one originally set.[86]

When drugs are under patent protection, pharmaceutical companies have incentives to conduct additional studies in order to enlarge the field of the drug use. They can therefore recover the cost of research and development by selling the drug at a monopoly price, this is called the return on investment.[164]

However, many pharmaceutical companies avoid additional trials knowing that they could carry risk of discovering new adverse events and that drug could anyway be prescribed off-label by practitioners. This situation is illustrated by the case of Merck who was willing to repurpose rofecoxib, a cox2 nonsteroidal anti-inflammatory, in the prevention of colon polyp. During the required additional studies, they found a

new adverse event that was an increasing risk of cardiovascular event and the drug was withdrawn from the market.[164]

When the patent expires, what has been named “the problem of new use” happens.[164] Companies are no more willing to invest in the study of new indications knowing that in the case of generic treatment, other manufacturer might be able to benefit from any successful results.[86]

Several propositions like government funding of research, prize funds and the use of existing databases have been proposed in order to encourage studies of new use for old drugs. Other than financial incentives, some changes in the general implementation of repurposed drugs are also necessary.

## 8.6 Limitations and strengths of this thesis

The limitations of this thesis derive directly from the used data sources.

The first and maybe foremost one, was the lack of information regarding important known confounders in oncology such as alcohol or tobacco use, body mass index or clinically diagnosed comorbidities. For the latter, the use of an algorithm using drugs for the treatment of comorbidities as a proxy allowed to divert the issue.

Another limitation of the study is the period for which the treatments were available to study. For each patient in the Belgian cancer registry database, the period span from 1 year before the entry into the database to 5 years after. This period doesn't allow to fully study drug utilization in cancer survivors or even to make pharmacoepidemiologic study on cancer risk.

Despite these above-mentioned limitations, a non-negligible strength of the used database can be pointed out.

The Belgian administrative framework offers a unique opportunity to study cancers at a population-based level, through the foundation of the Belgian cancer registry. Moreover, by allowing the use of the national social security identification number (NSSIN) as a linking key to other databases, the Belgian legislator has thus simplified the data collection process.

As explained in previous parts of this thesis, each individual living in Belgium is obliged to subscribe to a health insurance (also called mutuality). Thanks to this obligation, the Intermutualistic agency is able to centralize from the seven mutualities all the details on reimbursed health care such as drugs delivered to the patient and health care procedures. By linking this information to the one present in the Belgian cancer registry database, through the NSSIN, comprehensive data on drug consumption in Belgian patients with cancer is available.

Moreover, this thesis generated several results of interest which led to the identification of further scope for pharmacoepidemiologic research in Belgium.

# 9 OVERALL CONCLUSION

At the beginning of the present thesis, the main objective was to study digestive tract cancers outcome in association with the use of common chronic medications, using data from the Belgian cancer registry. The studied drugs were metformin and statins, two of the most commonly used drugs worldwide that have been shown to interact with digestive tract cancer progression in pre-clinical studies. In order to achieve the main purpose of this thesis, two population-based studies were conducted in patients with gastric and oesophageal cancer. However, even though the use of these two drugs was associated with better survival outcome, the present thesis does not provide enough evidence for concluding on causality of the observed associations. This assertion is reinforced by two studies evaluating the pattern of adherence to these two drugs amongst digestive tract cancer patients. Those two studies have suggested poor adherence to the two selected drugs despite the high volume of prescriptions in this population.

The present thesis, however, illustrates how record linkage from existing databases could easily be used to conduct high quality epidemiological studies. More specifically, it shows the utility of cancer registry databases in conducting hypotheses generating studies that could improve health outcome of cancer patients.

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