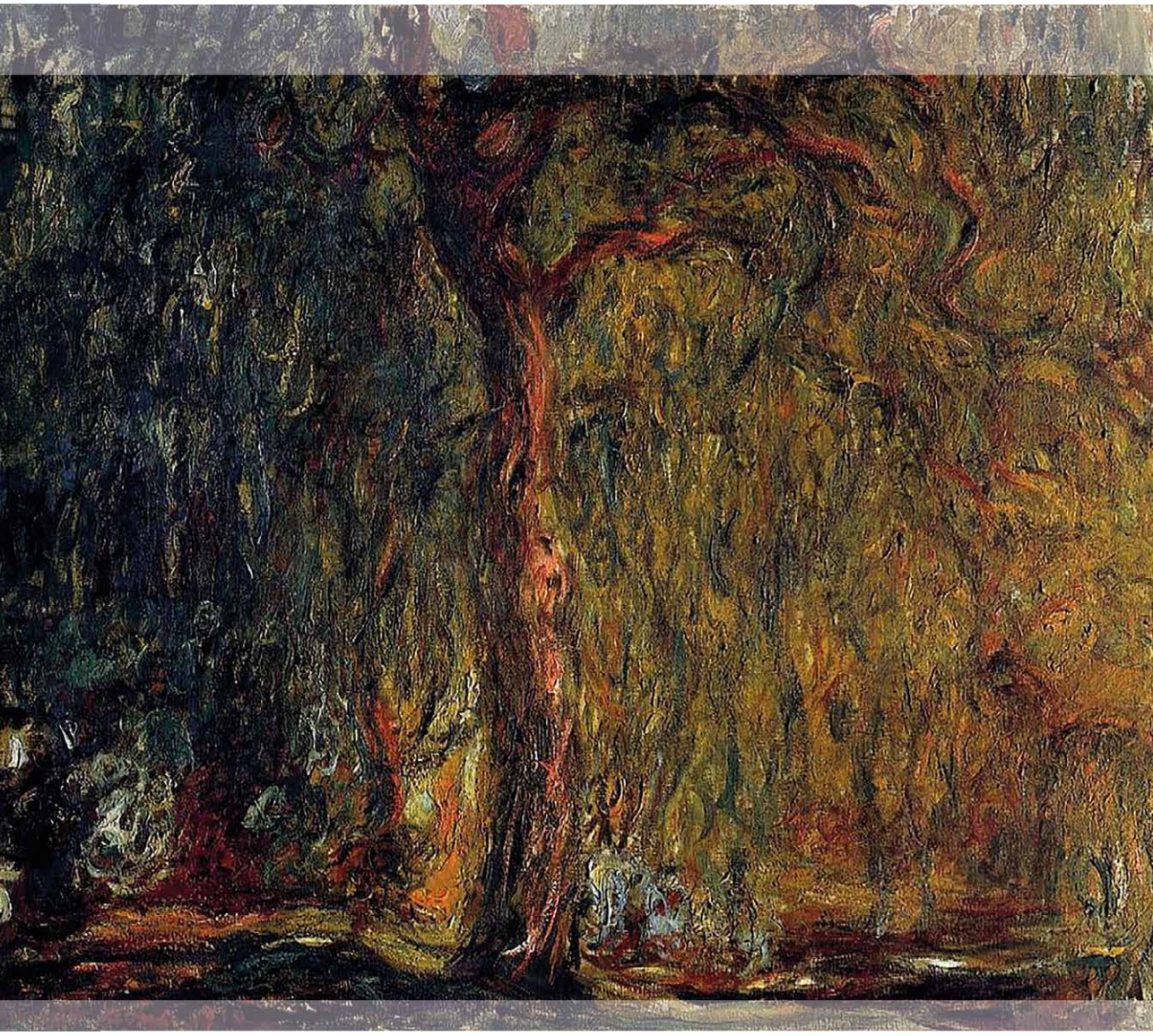
Renewing clinical applications for commonly used medications in gastrointestinal cancer

Frouws, M.A.; Frouws M.A.

RENEWING CLINICAL APPLICATIONS FOR COMMONLY USED MEDICATIONS IN GASTROINTESTINAL CANCER



MARTINE A. FROUWS

RENEWING CLINICAL APPLICATIONS FOR COMMONLY USED MEDICATIONS IN GASTROINTESTINAL CANCER

MARTINE A. FROUWS

Lay-out: Helga Daniels-Brokke
Cover design: Helga Daniels-Brokke
Schilderij 'De huilende wilg' van Claude Monet, geschilderd in 1918-1919
Printed by: VG Print BV

Renewing clinical applications for commonly used medications in gastrointestinal cancer
Thesis, Leiden University Medical Center, the Netherlands, 2017

ISBN: 978-90-9030423-6
This thesis is also available as an e-pub.
The research described in this thesis was written with a grant from NutsOhra.
Printing of the thesis was supported by Bayer B.V., Chipsoft, Pfizer.

RENEWING CLINICAL APPLICATIONS FOR COMMONLY USED MEDICATIONS IN GASTROINTESTINAL CANCER

Proefschrift
ter verkrijging van
de graad van Doctor aan de Universiteit Leiden,
op gezag van Rector Magnificus prof. mr. C.J.J.M. Stolker,
volgens besluit van het College voor Promoties
te verdedigen op donderdag 21 september 2017 klokke 16.15 uur.

door
Martine Aletta Frouws
geboren te Tilburg
in 1988

Promotor: Prof. dr. C.J.H. van de Velde

Copromotores: Dr. G.J. Liefers
Prof. dr. J.E.A. Portielje

Promotiecommissie: Prof. dr. H. Putter
Prof. Dr. R. Fodde (Erasmus Universiteit)
Prof. Dr. H. Morreau
Dr. M.P.P. van Herk-Sukel (PHARMO Institute for Drug Outcomes)

CONTENTS

RENEWING CLINICAL APPLICATIONS FOR COMMONLY USED MEDICATIONS IN GASTROINTESTINAL CANCER

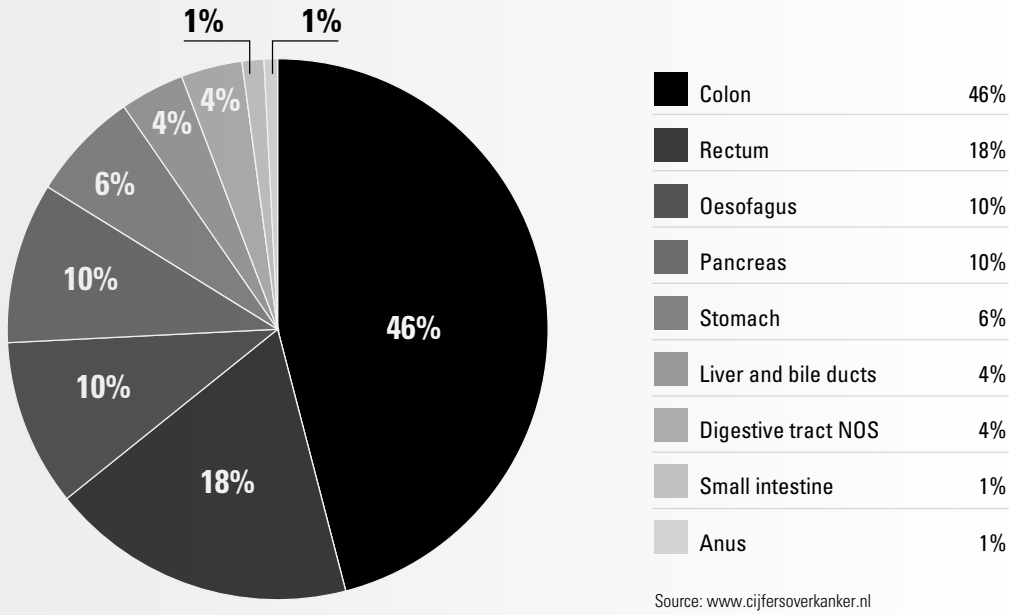
CHAPTER 1
GENERAL INTRODUCTION

Gastrointestinal cancer

Epidemiology

Globally, 20 million new cancer cases will be diagnosed in 2025.[1] Cancer of the gastrointestinal tract is the site of the most incident cancers and the source of more cancer mortality than any other organ system in the body.[2] In 2014, 17.4% of all diagnosed cancers and 25% of all cancer mortality in the United States was due to cancer of the digestive tract.[3]

Figure 1: Distribution of the incidence of gastrointestinal tract cancers in 2015 in the Netherlands



Source: www.cijfersoverkanker.nl

The common factor of gastrointestinal tumours is their origin; the gastrointestinal tract. Apart from their origin, gastrointestinal tumours differ in aetiology, morphology, pattern of symptoms, and location. Figure 1 shows the distribution of digestive tract tumours in 2015 in the Netherlands. The incidence of the various tumours of the gastrointestinal tract differs throughout the world. People in Asian countries suffer more from oesophageal and stomach cancer, whereas colorectal cancer is more common in North-America and the Western world.[2] This difference is not based on genetic or racial differences, but a result of environmental factors (e.g. diet and micro-organisms), and illustrates the importance of environment in gastrointestinal carcinogenesis. When people migrate to regions with a different incidence pattern, the organ-specific rates of cancer adjusts to this region within two generations.[2] Mortality of gastrointestinal malignancies in the Netherlands has improved over the past decade as is shown by figure 2.

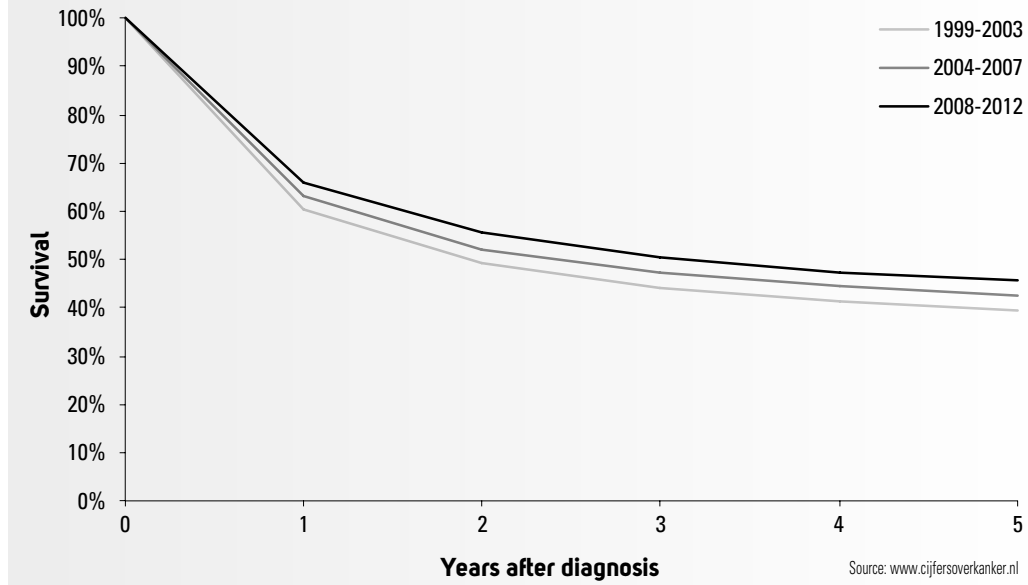
Current chemotherapeutic regimes in gastrointestinal cancer

Drugs that are currently used for (neo-)adjuvant therapy in gastrointestinal cancer in the Netherlands are shown in table 1:

Table 1

Current chemotherapy in the Netherlands[4]	Tumour type
5-Fluorouracil (5-FU) combined with leucovorin (Capecitabine when used oral)	Colorectal cancer Gastric cancer
Oxaliplatin	Colorectal cancer, gastric cancer
Epirubicin	Gastric cancer
Cisplatin	Gastric cancer
Carboplatin	Oesophageal cancer
Paclitaxel	Oesophageal cancer
Gemcitabine	Pancreatic cancer

Figure 2: Mortality in gastrointestinal cancer



Source: www.cijfersoverkanker.nl

Colorectal cancer

Epidemiology

As is shown by figure 1, colorectal cancer is the most common gastrointestinal malignancy in the Netherlands, and also in the Western world. Nine percent of all cancer deaths worldwide are considered due to colorectal cancer, with an absolute number of 694,000 deaths in 2012. Globally, 55% of colorectal cancers occur in more developed regions, however mortality is considerably lower in developed regions compared to undeveloped regions.[1] In 2015, 15.549 patients were diagnosed with colorectal cancer.[5] In the Netherlands, colorectal cancer is more common in male patients. As can be found in figure 3, survival is foremost determined by stage, with 90% of patients with stage I still alive after five years, as opposed to 10% of patients with stage IV disease.

Figure 3a: Survival of colon cancer patients in the Netherlands (2003-2009)

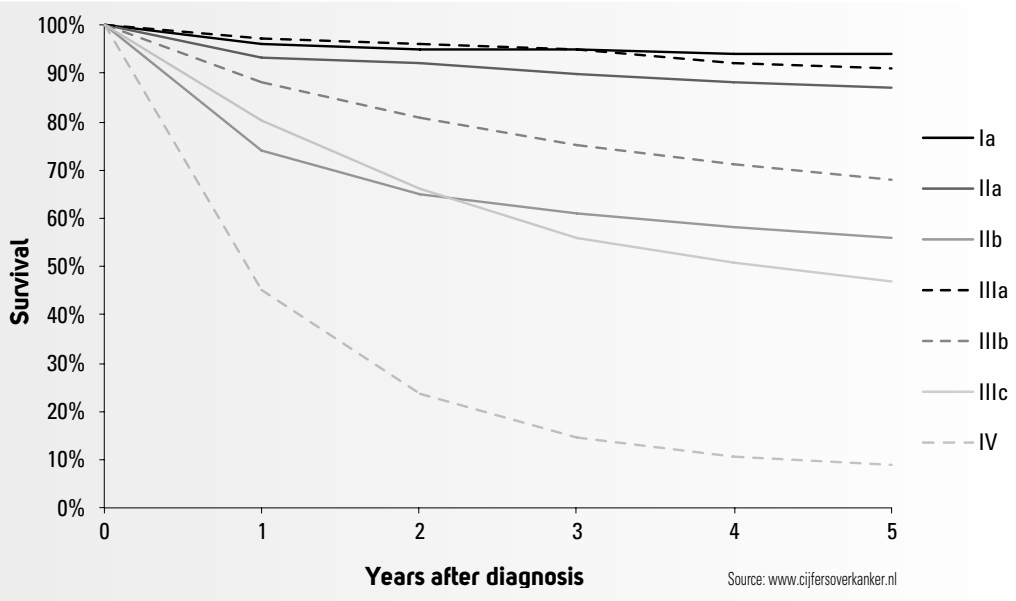
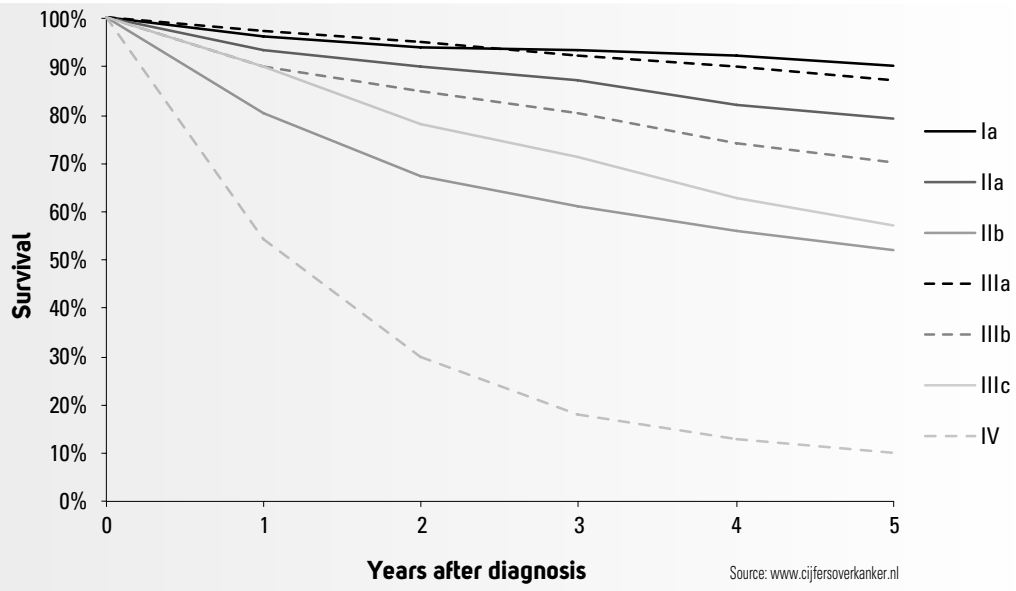


Figure 3b: Survival of rectal cancer patients in the Netherlands (2003-2009)



Treatment

Colon and rectum cancer are considered separate entities when considering curative treatment. The cornerstone of treatment in both types of cancer is surgery. Pre-operative, clinical staging in colorectal cancer requires a combination of CT (Computerized Tomography), MRI (Magnetic Resonance Imaging, only for rectal cancer), and endoscopy.

Based on the clinical stage, there are options for radiotherapy or chemoradiotherapy in the pre-operative phase in patients with stage II and III rectal cancer. After neo-adjuvant therapy, re-staging is performed and regular treatment is surgical resection according to TME (Total Mesorectal Excision) principles.[6] A new, upcoming, treatment option is so called 'watchful waiting' with close surveillance.[7] This treatment option is for patients who achieve complete clinical response after neoadjuvant therapy, and are monitored closely instead of undergoing a resection. In the Netherlands, adjuvant therapy is not part of standard treatment for patients with rectal cancer[4,8]

For colon cancer, treatment starts with surgical resection of the tumour, with the type of surgery being dependent on the location of the tumour in the colon. For patients with stage III or high risk stage II tumours, adjuvant therapy is indicated after surgery. Patients with stage II colon cancer are considered high risk in the case of perforation, T4 tumour, extramural vascular invasion, poorly differentiated tumours or when there are less than ten harvested lymph nodes. The absolute risk reduction is debated for the high risk stage II colon cancer.[4]

Health care costs and cancer medication

Due to higher incidence of cancer and increased survival of cancer patients, the volume of anticancer treatment is expanding.[9] Furthermore, the therapeutic options are growing. Where the only previous treatment option for colon cancer was 5-FU, nowadays capecitabine and oxaliplatin are additional treatment options and this has increased the costs of adjuvant colon cancer treatment with a 300-fold.[9]

The mean monthly costs of new anti-cancer medication is €40.000 in the Netherlands.[10] Expenditure on cancer medication in the Netherlands in 2013 was €733

Figure 4: Percentage of expensive cytostatic drugs of total cytostatic drugs in the Netherlands
(cancer medication is considered expensive as medication that exceeds a yearly national revenue of €2.5 million)

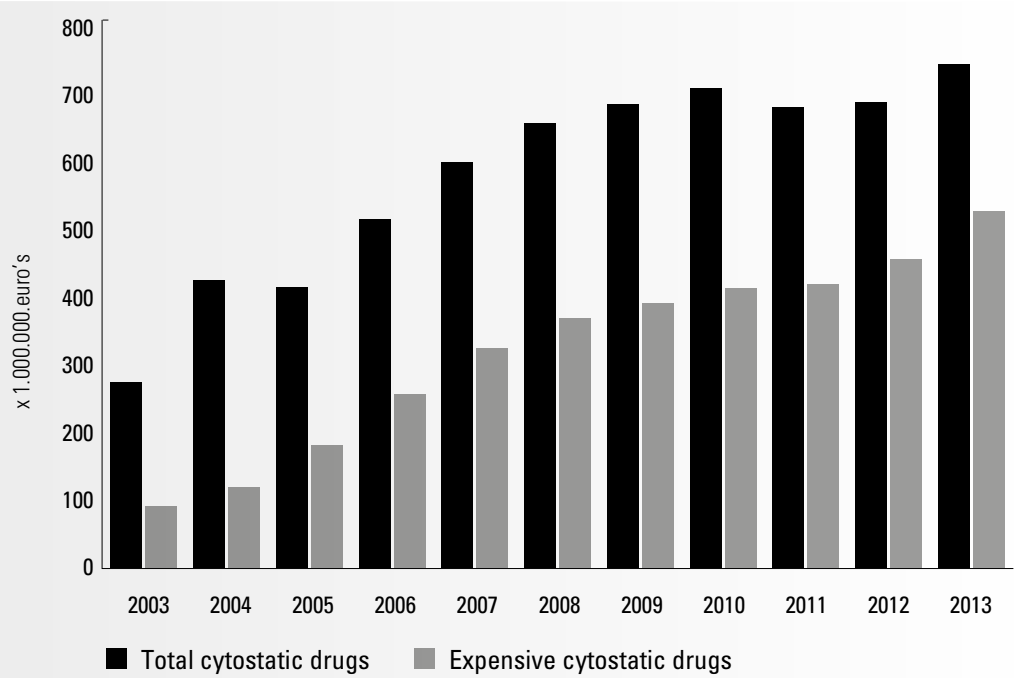


Figure adapted from the SCK Report 'Toegankelijkheid van dure kankergeneesmiddelen, Nu en in de toekomst'

million. Seventy-one percent (€519 million) of this amount was spent on expensive cancer medication. This amount has grown rapidly and is expected to grow exponentially in the coming years, as shown in figure 4.[10] This trend was also observed in the United States, were targeted therapies accounted for 63% of the total chemotherapy expenditures in 2011.[11] In the Netherlands, cancer medication is defined as expensive as medication with a yearly national revenue of €2.5 million. A national report addressing this issue entitled 'Accessibility of expensive cancer medication, now and in the future' was presented in the Netherlands in 2014. In this report covered the magnitude and effects of the current pattern of expenditure. This report concluded that the accessibility of expensive cancer medication can only be maintained when European countries collaborate and communicate about these issues. Due to the recent global financial crisis, almost all healthcare systems had to deal with a sobering budget, a factor that complicates the possibilities for the increasing costs.

Despite improvements over the last decades, global access to essential medication remains poor.[12] According to recent data, the availability was only 56-76% in low and middle-income countries. In the case medication was available, the affordability remains poor and anti-cancer drugs are becoming progressively more expensive.[13]

Many new drug therapies (mostly targeted therapies) were approved by the US Food and Drug Administration (FDA) the past decade. The median costs of approved cancer drugs has grown from less than $100 per month to about $10.000 per month in 2011.[14] These approvals were mostly based on surrogate endpoints, such as Progression-Free Survival. However, a study revealed that many of these drugs (57%) have an unknown effect on overall survival and that most cancer drugs have not been shown or do not improve clinically relevant endpoints. These results suggests that the FDA might be approving many costly, toxic drugs that do not improve overall survival.[15] Furthermore, molecularly targeted therapy is limited by a high failure rate and the small fraction of patients that can benefit.[16]

Drug repurposing

The urgency for new, effective, affordable anti-cancer medication that can be used on a global scale is high. Reusing the vast arsenal of already-approved drugs with a non-oncology primary purpose seems attractive. In theory, the repurposing of drugs should allow faster development at lower costs and, due to the wide experience, lower safety concerns. In addition, long-term data are mostly available. The barrier to introducing this as regular therapy seems relatively easy and low-effort. Considering that only 10% of developed candidate drugs will be used clinically, this must also seem attractive to pharmaceutical companies.[17]

Drugs that are considered for repurposing have several common characteristics:[17]

- Well-known drugs with many years of wide-spread clinical use, mostly available as off-patent
- Low toxicity profile
- Plausible mechanism of action
- Strong scientific evidence, both in vivo as in vitro
- Evidence at physiological dosing

One medicine that fulfils these criteria is aspirin (generic name: acetylsalicylic acid), reported in 1950 as world's most popular pain killing drug in the Guiness Book of Records.[18]

Aspirin

History of aspirin

Originating from the bark of a willow tree, aspirin was already used by Hippocrates 400 years B.C..[18] The Babylonians used both the bark and leaf of the willow tree to treat fever, pain and inflammation. It was not only reported to be used by the Babylonians, but the ancient Greeks, Aristotle and Roman physicians were also aware of the versatile effects of this drug.[18]

The commercial use of aspirin was initiated by Bayer. After the founding of Bayer in Germany in 1863 as a dye manufacturing company, the focus of the company advanced rapidly towards research on acetylsalicylic acid. A young chemist, Felix Hoffmann was hired and synthesized the first example of acetylsalicylic acid in 1897. In 1899 the drug was patented under the name of ASPIRIN (*A*-acetylation, *SPIR*- from the plant *Spiraea ulmaria* (meadowsweet), from which the salicylic acid was first isolated, *IN*- a common ending for medicines in that time).[18]

When aspirin was introduced on the market in 1900, the first (ironic) slogan of Aspirin was 'does not affect the heart'. The registered trademark of aspirin was lost by Bayer as soon as World War I ended, but interesting enough, only in the countries that emerged as winners of the war.

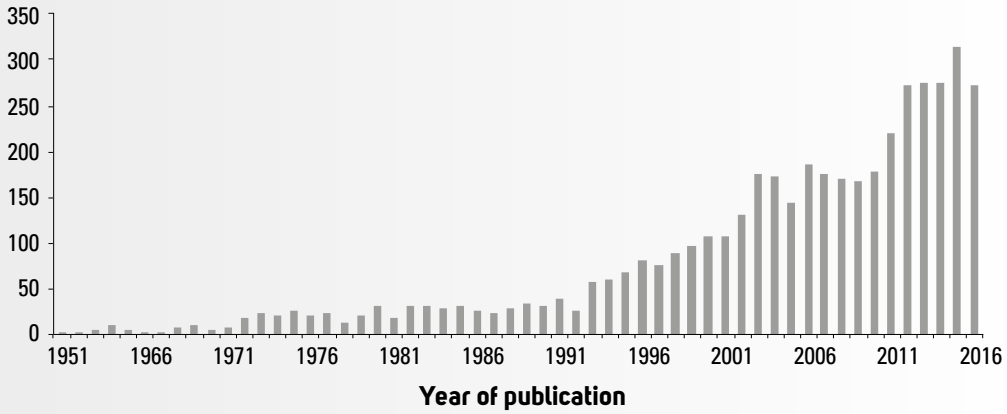
In 1948, a doctor from California, Lawrence Craven observed that none of his 400 patients, who were treated with aspirin, suffered from a heart attack. The involvement of aspirin in platelet aggregation was not discovered until 1967, a clinical experiment observed that patients taking aspirin have a longer coagulation time.[18]

Aspirin for cardiovascular prevention

Despite the registration of aspirin for cardiovascular disease by the FDA already in 1988, many unanswered questions remain regarding its protective effect in cardiovascular disease. Aspirin is already used globally as secondary prevention for cardiovascular disease.[19] The secondary prevention of atherosclerotic cardiovascular disease or myocardial infarction with aspirin has resulted in a 20% relative reduction in stroke and coronary events.[19] The absolute reduction of aspirin as secondary prevention for atherosclerotic cardiovascular disease is shown to be about 1-2% per year, with a greater reduction for non-fatal than fatal events.[19]

On the other hand, the net value of aspirin treatment in primary prevention of cardiovascular disease has not been proven.[19-22] Studies in that field continue to be published, and no decisive answer has been formulated yet. A recent review aimed to select certain subgroups that could benefit from primary prevention and the authors developed a decision making tool; the Aspirin-Guide. This tool has internal risk calculators to help clinicians with this difficult decision. The tool incorporated age- and sex-specific risk and additionally secondary considerations for colorectal cancer prevention.[19]

Figure 5: Number of publications on pubmed with search "Aspirin AND Cancer" (until november 2016)



Aspirin as cancer therapy

The repurposing of aspirin as anti-cancer medication was mentioned for the first time in 1972 when . aspirin was shown to reduce the number of metastases in a mouse model.[23] After the first publication of this effect, publications exponentially appeared on PubMed (figure 5).

The effect of aspirin on cancer is thought to be twofold. Both a reduced incidence of cancer as well as a mortality reducing effect of aspirin have been described.
The reduced incidence of cancer has been studied in many trials assessing the effect of aspirin in cardiovascular prevention. This evidence was summarized in a large meta-analysis by the United States Preventative Task Force (USTPF).[24] This meta analysis combined the existing randomised trials that have analysed the cancer outcomes of the patients in cardiovascular prevention trials that studied both primary and secondary prevention. The meta analysis demonstrated a significantly reduced incidence of gastrointestinal malignancies with the most pronounced effect in colorectal cancer (pooled rate ratio 0.60 (0.47-0.76).[24]

The only randomised clinical trial of aspirin in colorectal cancer so far, the CAPP-2 trial, studied the effect of aspirin in patients with hereditary colorectal cancer (Lynch syndrome). The study randomised between 600 mg aspirin and placebo and found a reduction in cancer incidence in a per-protocol analysis of Hazard Ratio (HR) 0.41 (95% C.I. 0.19-0.86). Currently, the CAPP-3 trial is recruiting to assess the optimal dose of aspirin in patients with Lynch syndrome (ISRCTN16261285).

Currently, the reduced incidence and improved cancer survival are considered separate issues in publications.

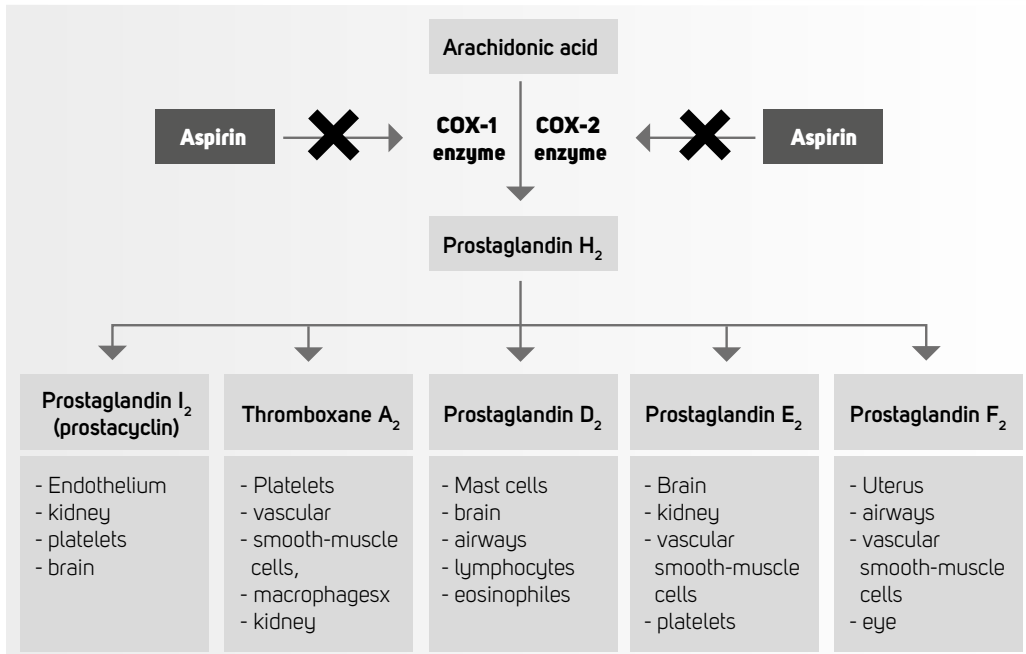
The evidence that aspirin could play a role to prevent cancer recurrence and improve survival after colorectal cancer comes from different study designs. Several meta-analysis of individual patient data studying the effect of aspirin in both primary as secondary cardiovascular disease prevention as numerous observational studies showing a beneficial survival in patients taking aspirin have been published.

Meta-analysis of large randomised trials have been completed and demonstrated that not all studies uniformly point towards a beneficial effect of aspirin on cancer mortality.[24-27] This may be due to heterogeneity of the studies with regard to study design, statistics, dose of aspirin, and selection of patients. This is discussed extensively in chapter seven of this thesis.
The most recent meta-analysis of Elwood et al pooled all current observational studies and showed a HR of 0.71 (95% CI 0.58-0.87) for both colorectal cancer specific survival and HR 0.80 (0.70-0.92) for overall survival.[26] An overview of current observational and randomised evidence can be found in chapter seven of this thesis.
One meta-analysis of individual patient data from several randomised controlled trials studying the effects of aspirin on cancer mortality showed that the mortality in patients with gastrointestinal cancer was significantly reduced in the group that used aspirin: HR 0.46 (95% CI 0.27-0.77).[28]
The meta-analysis of USTPF with individual patient data only from primary prevention trials found a reduced cancer mortality only in patients with colorectal cancer, but this effect was not found in patients with other types of cancer.[24]

Mechanism of action

The exact mechanism by which aspirin exerts its activity is not completely understood. As stated above, the mechanism of aspirin as platelet aggregation inhibitor was not discovered until the 1970's.[18] This illustrates the difficulties elucidating the working mechanisms of medication.

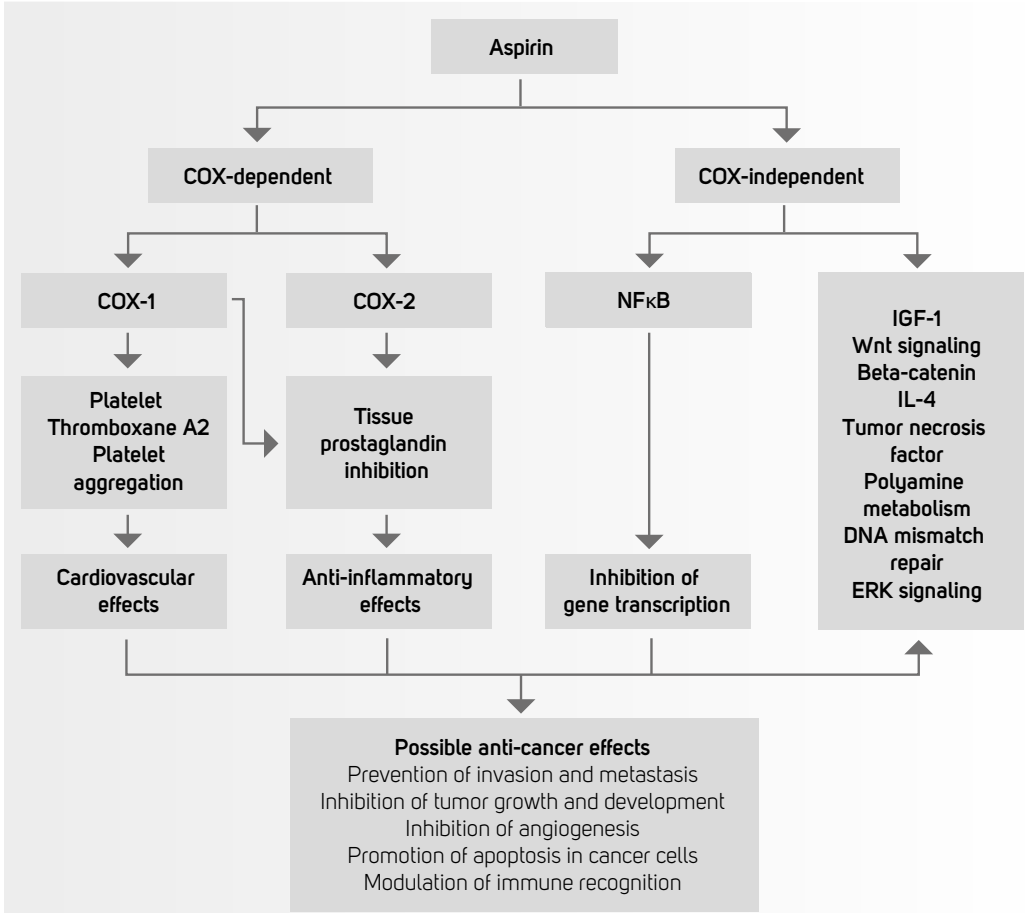
Figure 6: Mechanism of aspirin on COX-pathway and actions of prostanoids



Partly based on Fitzgerald, Nat Rev Drug Discov. 2003 Nov;2(11):879-90.

Aspirin is both a permanent non-steroidal anti-inflammatory drug (NSAID)[29] and a non-specific COX-inhibitor, of both COX-1 and COX-2.[30]

Figure 7: Overview of pathways responsible for the anti-cancer effects of aspirin.



Partly based on Langley et al, BJC 2011; 105; 1107-1113, and Alfonso et al, BJC 2014; 111, 61-67

Aspirin, an analgesic and anti-inflammatory agent, acts by indirectly inhibiting the production of prostanoids. Prostanoids are a group of active lipid mediators and include: prostaglandin E_2 (PGE_2), prostaglandin D_2 (PGD_2), prostaglandin I_2 (PGI_2, also called prostacyclin), prostaglandin F_2 (PGF_2)and thromboxane A_2 (TXA_2.).[31,32] The prostanoids have an important role in cellular responses and pathophysiologic processes, such as modulation of the inflammation response and its resolution gastrointestinal cytoprotection and ulceration, angiogenesis and cancer, haemostasis and thrombosis, atheroprotection and progression of atherosclerosis.[31] The tissues that are effected by the different prostanoids can be found in figure 6.

Multiple properties have been suggested to be responsible for the effect of aspirin on the prevention of cancer metastasis. TXA_2 and PGE_2, downstream mediators of the COX-pathway, are thought to be involved in tumourigenesis and metastasis, but also COX-independent mechanisms have been suggested (figure 7).[30,33]

The effect of aspirin is unique in the group of NSAID's because it acts by delivering its acetyl group mainly to platelet COX-1 and thereby completely inhibits TXA2 production.[34] Cells that have low levels of peroxides, such as platelets and epithelial cells of the lung and colon are particularly sensitive to aspirin and its COX-1 inhibition.[33]

Unfortunately, literature is sparse about the hypothesis that tumours that have developed while on aspirin therapy are less responsive to the effects of aspirin as anti-cancer therapy. The meta-analysis of Elwood et al. also refers to this phenomenon, and hints towards less sensitivity of tumours that are treated prediagnosis with aspirin.[26] This statement is however not supported with a reference. In a subgroup analysis in the meta-analysis of Elwood the group of patients that take aspirin both before and after diagnosis were analysed separately. No difference in effect was observed in the group of patients that already use aspirin at diagnosis vs. patients only starting aspirin after diagnosis. To our knowledge, there is one manuscript that assessed the effect of aspirin on tumour growth in mice.[35] This study showed that mice, that both did and did not receive aspirin before diagnosis, had a reduction of tumour growth when treated with aspirin after diagnosis.

Side effects

The risk of side effects when treating patients with low dose aspirin must be acknowledged. Common side effects of low-dose aspirin use are gastrointestinal symptoms (abdominal pain, dyspepsia, or nausea and vomiting) and increased bleeding tendency which can cause epistaxis, gastrointestinal bleeding or purpura.[36,37] Serious bleeding events have also been described, however the risk of serious haemorrhagic stroke with the use of aspirin is very rare (<0. 01%).[38] The risk of major gastrointestinal bleeding is as high as one or two in every 1,000 patients taking aspirin, and this increases with age.[39] Fatal bleeds however are not increased in groups of patients taking aspirin.

There are a few considerations that should be taken into account when studying the beneficial effect of aspirin in a cancer population. As stated above, aspirin as primary cardiovascular prevention is a controversial subject. When studying the risk:benefit ratio in a primary cardiovascular prevention setting, the acceptance of the risk of side effects is low, especially when the absolute benefit may be low. For secondary prevention in a cancer population, however, with patients at a relatively high risk of experiencing a recurrence, acceptance of side effects will generally be higher.

Outline of this thesis

Currently, there are multiple ongoing randomised clinical trials that assess the impact of aspirin on survival in colon cancer patients and it will take several years before these results will be published and implemented in daily practice (table 2).

The aim of this thesis was to provide epidemiological evidence that could lead to insights in the aetiology of commonly observed survival benefit in patients using non-anticancer drugs, focussing on aspirin.

As mentioned previously, the results from current studies vary largely, creating several knowledge gaps. The effect of aspirin on cancer has been investigated mostly in colorectal cancer. **Chapter two** studies the association between aspirin and non-aspirin NSAID's and survival in patients with oesophageal cancer, in a cohort with combined data of the Comprehensive Cancer Organisation (IKNL) and PHARMO cohort. In **chapter three** this analysis was extended to patients with other gastrointestinal cancers, to verify if the association was similar in other parts of the gastrointestinal tract. **Chapter four** focuses on *BRAF* and *KRAS* mutations in patients with colon cancer. Would it be possible to identify certain patient groups that benefit most from aspirin treatment? In **chapter five** we performed a study to find out if the effect of aspirin is an effect that may be found in many non-anticancer drugs, or that this effect is drug-specific. Many previous publications have suggested that metformin use is associated with increased overall survival, however with several methodological limitations. We aimed to study this association with proper statistical techniques .

Chapter six was performed to see whether we could provide more epidemiological evidence for the proposed anti-cancer effects of aspirin. The hypothesized mechanism behind the survival benefit of aspirin is platelet-mediated. Circulating Tumour Cells (CTC's) are physiologically surrounded by a cloak of thrombocytes, thereby guarding the CTC's from clearance by the immune system.[27] Aspirin, a thrombocyte aggregation inhibitor, maybe able to make this cloak disappear and then the CTC's become detectable to the immune system. Natural killer cells will clear the CTC's from the circulation and in that manner metastasis could be prevented.[27] However, there are more drugs that prevent thrombocyte aggregation, such as dipyridamole or clopidogrel. In this study we analysed if these drugs are also associated with an improved survival.

Chapter 7 is a critical appraisal of possible bias in the current retrospective studies of aspirin use for secondary cancer prevention. Could it be possible that the survival benefit associated with the use of aspirin is just a healthy user effect?

Finally an overall summary and discussion is provided in **chapter eight**.

Table 2: Overview of current ongoing trials for the effect of aspirin use after diagnosis in patients with colorectal cancer

Trial name and registration number	Type of cancer	Patients (n)	Inclusion criteria	Randomised treatment (incl treatment duration and dose)	Primary end-point	Participating countries	Start recruitment	Expected finished recruitment
Add-aspirin trial* (ISRCTN74358648)	Colorectal, stomach, oesophagus, prostate and breast cancer	2600 colorectal (total: 9920)	Stage II and III adenocarcinoma	100 mg, 300 mg of daily aspirin or placebo, during 5 years	5 yr DFS	UK	2016	2026
ALASCCA trial (NCT02647099)	Colorectal cancer	3900	Stage II and III PIK3CA mutated patients	160 mg of daily aspirin or placebo during 3 years	3 year time to recurrence	Sweden, Norway	2016	2021
ASCOLT trial (NCT00565708)	Colorectal cancer	1200	Dukes B or C	300 mg of daily aspirin or placebo during 3 years	3 yr DFS	Singapore, Australia, India, China, Hong-Kong, South-Korea, Malaysia, Taiwan, Saudi-Arabia, Indonesia and the Philippines	2012	2022
ASPIRIN trial (NCT02301286)	Colon cancer	1588	Stage II or III adenocarcinoma	100 mg of daily aspirin or placebo during 5 years	5 yr OS	Netherlands, Belgium, Portugal	2015	2022
SAKK 41/13 (NCT02467582)	Colorectal cancer	896	Stage II and III adenocarcinoma, PIK3CA mutated patients	300 mg of daily aspirin or placebo during 3 years	3 yr DFS	Switzerland, Hungary	2015	2018

** Only the specifications of the colorectal arm of the Add-aspirin trial are provided in this overview*

Abbreviations:
DFS: Disease-Free Survival
OS: Overall Survival
PIK3CA: phosphatidylinositol 3-kinase

Reference list

1. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. International journal of cancer 2015; 136(5): E359-86.
2. Alpers DH, Yamada T. Textbook of Gastroenterology. Chichester, West Sussex: Wiley-Blackwell; 2009.
3. American Cancer Society. Cancer Facts & Figures 2014. Atlanta: American Cancer Society, 2014.
4. www.oncoline.nl accessed on November 18 2016.
5. www.cijfersoverkanker.nl accessed November 17 2016.
6. Kapiteijn E, Marijnen CA, Nagtegaal ID, et al. Preoperative radiotherapy combined with total mesorectal excision for resectable rectal cancer. N Engl J Med 2001; 345(9): 638-46.
7. Beets GL, Figueiredo NF, Beets-Tan RG. Management of Rectal Cancer Without Radical Resection. Annu Rev Med 2016.
8. Breugom AJ, Swets M, Bosset JF, et al. Adjuvant chemotherapy after preoperative (chemo)radiotherapy and surgery for patients with rectal cancer: a systematic review and meta-analysis of individual patient data. Lancet Oncol 2015; 16(2): 200-7.
9. Chabner BA, Roberts TG, Jr. Timeline: Chemotherapy and the war on cancer. Nat Rev Cancer 2005; 5(1): 65-72.
10. Signaleringscommissie Kanker van KWF Kankerbestrijding. Toegankelijkheid van dure kankergeneesmiddelen Nu en in de toekomst, 2014.
11. Shih YC, Smieliauskas F, Geynisman DM, Kelly RJ, Smith TJ. Trends in the Cost and Use of Targeted Cancer Therapies for the Privately Insured Nonelderly: 2001 to 2011. J Clin Oncol 2015; 33(19): 2190-6.
12. Grover A, Citro B, Mankad M, Lander F. Pharmaceutical companies and global lack of access to medicines: strengthening accountability under the right to health. J Law Med Ethics 2012; 40(2): 234-50.
13. Organization. WH. World health statistics 2016: Monitoring health for the SDGs, sustainable development goals. 2016.
14. Bertolini F, Sukhatme VP, Bouche G. Drug repurposing in oncology--patient and health systems opportunities. Nat Rev Clin Oncol 2015; 12(12): 732-42.
15. Kim C, Prasad V. Cancer Drugs Approved on the Basis of a Surrogate End Point and Subsequent Overall Survival: An Analysis of 5 Years of US Food and Drug Administration Approvals. JAMA Intern Med 2015: 1-2.
16. Huang M, Shen A, Ding J, Geng M. Molecularly targeted cancer therapy: some lessons from the past decade. Trends Pharmacol Sci 2014; 35(1): 41-50.
17. Pantziarka P, Bouche G, Meheus L, Sukhatme V, Sukhatme VP, Vikas P. The Repurposing Drugs in Oncology (ReDO) Project. Ecancermedicalscience 2014; 8: 442.
18. Tsoucalas G, Karamanou M, Androutsos G. Travelling through Time with Aspirin, a Healing Companion. European Journal of Inflammation 2011; 9(1): 13-6.
19. Mora S, Manson JE. Aspirin for Primary Prevention of Atherosclerotic Cardiovascular Disease: Advances in Diagnosis and Treatment. JAMA Intern Med 2016; 176(8): 1195-204.
20. Puhan MA, Yu T, Stegeman I, Varadhan R, Singh S, Boyd CM. Benefit-harm analysis and charts for individualized and preference-sensitive prevention: example of low dose aspirin for primary prevention of cardiovascular disease and cancer. BMC Med 2015; 13: 250.
21. Baigent C, Blackwell L, Collins R, et al. Aspirin in the primary and secondary prevention of vascular disease: collaborative meta-analysis of individual participant data from randomised trials. Lancet 2009; 373(9678): 1849-60.
22. Antithrombotic Trialists C, Baigent C, Blackwell L, et al. Aspirin in the primary and secondary prevention of vascular disease: collaborative meta-analysis of individual participant data from randomised trials. Lancet 2009; 373(9678): 1849-60.
23. Gasic GJ, Gasic TB, Murphy S. Anti-metastatic effect of aspirin. Lancet 1972; 2(7783): 932-3.
24. Chubak J, Whitlock EP, Williams SB, et al. Aspirin for the Prevention of Cancer Incidence and Mortality: Systematic Evidence Reviews for the U.S. Preventive Services Task Force. Ann Intern Med 2016; 164(12): 814-25.
25. Ye XF, Wang J, Shi WT, He J. Relationship between aspirin use after diagnosis of colorectal cancer and patient survival: a meta-analysis of observational studies. Br J Cancer 2014; 111(11): 2172-9.
26. Elwood PC, Morgan G, Pickering JE, et al. Aspirin in the Treatment of Cancer: Reductions in Metastatic Spread and in Mortality: A Systematic Review and Meta-Analyses of Published Studies. PLoS One 2016; 11(4): e0152402.
27. Li N. Platelets in cancer metastasis: To help the "villain" to do evil. International journal of cancer 2016; 138(9): 2078-87.
28. Rothwell PM, Fowkes FG, Belch JF, Ogawa H, Warlow CP, Meade TW. Effect of daily aspirin on long-term risk of death due to cancer: analysis of individual patient data from randomised trials. Lancet 2011; 377(9759): 31-41.
29. Chia WK, Ali R, Toh HC. Aspirin as adjuvant therapy for colorectal cancer--reinterpreting paradigms. Nat Rev Clin Oncol 2012; 9(10): 561-70.
30. Langley RE. Clinical evidence for the use of aspirin in the treatment of cancer. Ecancermedicalscience 2013; 7: 297.
31. Bruno A, Dovizio M, Tacconelli S, Patrignani P. Mechanisms of the antitumoural effects of aspirin in the gastrointestinal tract. Best Pract Res Clin Gastroenterol 2012; 26(4): e1-e13.
32. FitzGerald GA. COX-2 and beyond: Approaches to prostaglandin inhibition in human disease. Nat Rev Drug Discov 2003; 2(11): 879-90.
33. Boutaud O, Sosa IR, Amin T, et al. Inhibition of the Biosynthesis of Prostaglandin E2 By Low-Dose Aspirin: Implications for Adenocarcinoma Metastasis. Cancer Prev Res (Phila) 2016; 9(11): 855-65.
34. Guillem-Llobat P, Dovizio M, Alberti S, Bruno A, Patrignani P. Platelets, cyclooxygenases, and colon cancer. Semin Oncol 2014; 41(3): 385-96.
35. Maity G, De A, Das A, Banerjee S, Sarkar S, Banerjee SK. Aspirin blocks growth of breast tumor cells and tumor-initiating cells and induces reprogramming factors of mesenchymal to epithelial transition. Lab Invest 2015; 95(7): 702-17.
36. Coyle C, Cafferty FH, Langley RE. Aspirin and Colorectal Cancer Prevention and Treatment: Is It for Everyone? Current Colorectal Cancer Reports 2016.
37. Whitlock EP, Williams SB, Burda BU, Feightner A, Beil T. Aspirin Use in Adults: Cancer, All-Cause Mortality, and Harms: A Systematic Evidence Review for the U.S. Preventive Services Task Force. Rockville (MD); 2015.
38. www.farmacotherapeutischkompas.nl. https://www.farmacotherapeutischkompas.nl/bladeren-volgens-boek/preparaatteksten/a/acetylsalicylzuur--als-trombocytenaggregatieremmer (accessed 23-11-2016.
39. Elwood PC, Almonte M, Mustafa M. Is There Enough Evidence for Aspirin in High-Risk Groups? Current Colorectal Cancer Reports 2012; 9(1): 9-16.

CHAPTER 2

THE EFFECT OF ASPIRIN AND NON-STEROIDAL ANTI-INFLAMMATORY DRUG USE AFTER DIAGNOSIS ON SURVIVAL OF ESOPHAGEAL CANCER PATIENTS

Jente van Staalduinen, Martine Frouws, Marlies Reimers, Esther Bastiaannet, Myrthe P.P. van Herk-Sukel, Valery Lemmens, Wobbe O. de Steur, Henk H. Hartgrink, Cornelis J.H. van de Velde, Gerrit-Jan Liefers

British Journal of Cancer 2016 Apr 26;114(9):1053-9

ABSTRACT

Background

Aspirin use has been shown to lower incidence and mortality in cancer patients. The aim of this population-based study was to determine the effect of postdiagnosis low dose aspirin use on survival of patients with esophageal cancer.

Methods

Patients with esophageal cancer (1998-2010) were selected from the Eindhoven Cancer Registry and linked with out-patient pharmacy data regarding aspirin and nonsteroidal anti-inflammatory drugs (NSAIDs). Users were subdivided in both prediagnosis and postdiagnosis or only postdiagnosis users. Parametric survival models with an exponential (Poisson) distribution were used with non-specific death as endpoint.

Results

In this study 560 patients were included. Overall, 157 patients (28.0%) were non-users, 293 patients (52.3%) pre- and postdiagnosis (89 aspirin and 204 NSAID users) and 110 patients (19.6%) only postdiagnosis users (16 aspirin and 94 NSAID users). Postdiagnosis aspirin use was associated with overall survival (RR 0.45 (95% CI 0.34-0.60; p<0.001); adjusted rate ratio was 0.42 (95% CI: 0.30-0.57; p<0.001). Postdiagnosis use of NSAIDs was associated with overall survival (RR 0.61 (95% CI 0.49-0.76; <0.001), however adjusted analyses did not show a significant association with a rate ratio of 0.84 (95%CI 0.66-1.07; p=0.2).

Conclusion

Our study shows that postdiagnosis aspirin use might be associated with a higher survival rate in esophageal cancer patients. A randomized clinical trial is needed to verify our observations of possible postdiagnosis aspirin use benefit.

INTRODUCTION

In 2008 an estimated 482,300 new esophageal cancer cases and 406,800 esophageal cancer deaths occurred worldwide.[1] Prognosis of patients with esophageal cancer is poor; the ten-year survival rate in all patients is approximately 14%.[2] Regular use of aspirin and other NSAIDs has been shown to have a chemo-preventive effect on the incidence of multiple cancers[3-6], including esophageal cancer.[7-9] In a meta-analysis by Corley D.A. et al. regular aspirin and NSAID use showed a protective association with esophageal cancer, with summary odds ratios (95% CI) of 0.50 (0.38-0.66) and 0.75 (0.54-1.00), respectively.[10] Also, regular use of aspirin has been shown to have therapeutic effects on the overall and cancer-specific survival of several types of cancers.[4, 5, 11]

Aspirin and NSAIDs are inhibitors of prostaglandin endoperoxide synthase 1 and 2 (PTGS1,2 also known as COX1,2); enzymes involved in the formation of prostaglandins. However, the exact biological mechanisms involved in the anti-cancer effects of aspirin are still unknown. Low-dose use of aspirin irreversibly inhibits the constitutive COX1 expression of circulating platelets. Only high-dose and frequent aspirin use is believed to be capable of inhibiting the induced COX2 expression in systemic tissues.[12]
The expression of COX2 has been shown to be upregulated in most esophageal tumors and has prognostic significance.[13, 14] Experimental data also showed that inhibition of COX2 expression inhibit cell proliferation and induces apoptosis in human esophageal squamous cell carcinoma in vitro.[15] Furthermore, aspirin has been demonstrated to have COX-independent effects on tumor cells.[16]

Consequently, aspirin and other non-steroid anti-inflammatory drugs could have an effect on cancer specific survival and overall survival. Cancer specific survival could be affected by taking aspirin after diagnosis due to the mimicking of adjuvant therapy in order to prevent metastases, but also on overall survival as the cancer related mortality of esophageal cancer is high. A preliminary experimental study showed a beneficial effect of postoperative use of aspirin on the survival of patients with esophageal cancer; the 5-year survival for the aspirin users was 51.2%, for the placebo group 41.0% and for the patients who used no tablets it was 42.3% (p=0.04 or p=0.029 when the last two groups were combined)[17], however, the results are still indecisive as no significant survival gain was observed in the any of the TNM staging groups. The effect of postdiagnosis use of aspirin and NSAIDs on overall survival of esophageal cancer has not been studied in a population-based study yet. Therefore, the aim of this observational study is to determine the effect of postdiagnosis use of aspirin and NSAIDs on the overall survival of patients with esophageal cancer, using a combined database of registered drug use and data from a regional cancer registry.[18]

METHODS

The Eindhoven Cancer Registry (ECR) is maintained by the Comprehensive Cancer Centre Netherlands and comprises information on newly diagnosed cancer patients in the southeastern part of the Netherlands. The ECR is served by 10 hospitals in an area of approximately 2,4 million inhabitants. Patients diagnosed with esophageal cancer between 1998 and 2010 were selected from the ECR with no exclusion criteria. Patients are informed about the registration and registered unless the patient has objected to be

registered. The Netherlands Cancer Registry is obliged to work according to the law about protection of privacy data; consent of the patients for this specific study was not applicable. The data from the ECR were linked to the central patient database of the PHARMO Database Network as described elsewhere.18 Data regarding the dispensing of aspirin and NSAIDs (a single dispensing for aspirin was usually for 90 days, for NSAIDs 30 days) were extracted from the Out-patient Pharmacy Database of the PHARMO Database Network. Linkage of cancer registry data with the municipal population registries, which document the vital status of their inhabitants, resulted in a reliable vital status of every patient.

Definition of user

Dispensings of aspirin and NSAIDs were extracted from the PHARMO Out-patient Pharmacy Database (see Supplementary Table 1). The majority of dispensings of low dose aspirin was 80 mg (98.1%); 30 mg aspirin was only dispensed 92 times from a total of 4835 dispensings (1.9%). Users were defined as patients who had at least one dispensing for aspirin or NSAIDs for at least 14 days. Patients were classified as non-users if they used any dispensed aspirin or NSAIDs for less than 14 days. Frequent users were defined as users that had ≥30 or ≥45 dispensings. Besides, we defined a subgroup of frequent

Table 1: Characteristics of the esophageal cancer patients included in the cohort

Variable	N	%
Sex		
Male	423	75.5
Female	137	24.5
Age		
<60	164	29.3
60-70	173	30.9
70-80	153	27.3
80+	70	12.5
Histological type		
SCC	190	33.9
AC	330	58.9
Squamo-adenocarcinoma	5	0.9
Other	35	6.3
Localization		
Upper third	23	4.1
Middle third	83	14.8
Lower third	420	75.0
GE-junction	16	2.9
Cervical	10	1.8
Unknown	8	1.4

Variable	N	%
Stage		
I	45	8.0
II	89	15.9
III	104	18.6
IV	186	33.2
Unknown	136	24.3
Grade		
I	28	5.0
II	150	26.8
III	215	38.4
Unknown	167	29.8
Surgery		
Yes	178	31.8
No	382	68.2
Chemotherapy		
Yes	177	31.6
No	383	68.4
Radiotherapy		
Yes	328	58.6
No	232	41.4

Abbreviations: SCC = squamous cell carcinoma, AC = adenocarcinoma, GE-junction = gastroesophageal junction

users who had more than 20 dispensings solely postdiagnosis, irrespective of the number of dispensings prediagnosis.

In order to divide the users into subgroups, the date of dispensing was compared with the date of diagnosis. Subsequently, users were subdivided in both prediagnosis (use of aspirin or NSAIDs at any time or duration before cancer diagnosis) and postdiagnosis users, only postdiagnosis users, and a group containing both groups (postdiagnosis users). Patients were defined as 'only postdiagnosis' if they started using medication for at least 14 days after diagnosis. Patients were classified as 'prediagnosis and postdiagnosis' users if they used a medication before diagnosis and still used that medication for at least 14 days after diagnosis. 'Postdiagnosis users' were defined as pre- and postdiagnosis users and only postdiagnosis users combined; thus it includes all postdiagnosis users, irrespective of when they started the aspirin or NSAIDs. Some patients (n=113) had both dispensings for aspirin and (other) NSAIDs; they were included in the aspirin group when the number of dispensings for aspirin exceeded the number of dispensings for NSAIDs and vice versa if the number of dispensings for NSAIDs exceeded the number of dispensings for aspirin.

Definition of follow-up time

Because the PHARMO Out-patient Pharmacy database comprises GP or specialist prescribed healthcare products dispensed by the out-patient pharmacy, drugs use of discharged patients, follow-up time started from 14 days after diagnosis (T0) of the esophageal cancer and ended at the last contact date or time of death. Consequently, all patients who died within 14 days before T0 were excluded. Time to first dispensing was defined as the time from T0 to the date of the first dispensing.

Survival analysis

In the overall survival analysis with time-dependent exposure of aspirin or NSAIDs, patients were defined as non-users from T0 to the date of first use of aspirin or NSAIDs and user from date of first use to the last contact or time of death. To analyze the association of aspirin or NSAID use on overall survival, parametric survival models with exponential (Poisson) distribution were used. Non-specific death was coded as event in the survival analyses. Univariable analyses were performed to assess the association between aspirin or NSAID use and overall survival and multivariable models were built to adjust for sex, age, histological type, location of the tumor, comorbidity, grade, stage, surgery, chemotherapy and radiotherapy. It was possible to adjust for the presence of the following comorbidities: lung diseases, other types of cancer, cardiovascular diseases, hypertension, cerebrovascular accidents, digestive diseases and diabetes. We adjusted for these comorbidities by grouping the comorbidities in none or at least 1 comorbidity. Furthermore, the survival analysis was stratified for postdiagnosis aspirin users in prediagnosis and postdiagnosis, only postdiagnosis users and a combined group.

RESULTS

In this observational study 560 patients diagnosed with esophageal cancer from 1998 to 2010 were included with a follow-up until December 2011. The patient and tumor

characteristics of this cohort are shown in Table 1. Median age at diagnosis was 66 years (interquartile range 23-97). Overall, 76% of the patients were males (n=423) and 24% were females (n=137). In total, 59% of the patients were diagnosed with adenocarcinoma (n=330) and 33% of the patients were diagnosed with squamous cell carcinoma (n=190). From all patients 157 patients (28%) were non-users of any dispensed aspirin and NSAIDs. In total, 204 patients (36%) and 89 (16%) used NSAIDs or aspirin both prediagnosis and postdiagnosis, 94 patients (17%) and 16 patients (3%) used NSAIDs or aspirin only postdiagnosis, respectively.

Table 2 shows the association between patient and tumor characteristics and aspirin or NSAID use. Patients who used aspirin only postdiagnosis were younger compared to prediagnosis and postdiagnosis users and none users (p=0.005). Half of the only postdiagnosis aspirin users were diagnosed with early stage tumors (stage I and II), in contrast, only 12% and 32% of the pre and postdiagnosis aspirin users and none users were diagnosed with stage I or II, respectively. Furthermore, none of the only postdiagnosis aspirin users were diagnosed with stage IV, whereas pre and postdiagnosis aspirin users and none users were more frequently diagnosed with stage IV tumors (25% and 45% respectively).

Pre and postdiagnosis NSAIDs users were younger compared to only postdiagnosis NSAIDs users and none users (p=0.005). Pre and postdiagnosis NSAIDs users were also more frequently diagnosed with early stage tumors (29%) than only postdiagnosis NSAIDs users (22%) and none users (12%). Only postdiagnosis NSAIDs users had less stage IV tumors at diagnosis (25%) than pre and postdiagnosis NSAIDs users (34%) and none users (45%). No differences were observed in the distribution of the sex of the patients and the grade of the tumors between the different subgroups (p>0.05).

Survival analysis

Table 3 shows the time-dependent (overall) survival analysis for non-users and users of aspirin. Median follow-up time was 0.83 years (range 0 – 13.83); with a median follow-up for deceased patients of 0.55 years (range 0 – 10.54) and 3.30 years (range 1.0 – 13.80) for patients still alive at the end of follow-up. Prediagnosis and postdiagnosis use of aspirin was associated with a significant survival gain namely in crude analyses (RR 0.55 (95%CI 0.41-0.74; p<0.001) and in multivariable analyses with a RR of 0.44 (95% CI: 0.31 – 0.61; P<0.001) after adjusting for the above mentioned confounders. Furthermore, only postdiagnosis aspirin use was associated with a significant reduction of the overall mortality rate ratio RR 0.15 (95% CI: 0.07 – 0.32; P<0.001). After adjusting for sex, age, grade, stage, histological type, location of the tumor, treatment and comorbidities the multivariable RR was 0.29 (95% CI: 0.12 – 0.70; P=0.006). Any postdiagnosis aspirin use did also result in a significant better survival outcome with a crude RR of 0.45 (95%CI 0.34-0.60; p<0.001) and an adjusted RR of 0.42 (95%CI: 0.30 – 0.57; P<0.001). Frequent postdiagnosis users of more than 30 dispensings showed a crude RR of 0.56 (95%CI 0.39-0.82; p=0.003) and a similar adjusted RR of 0.49 (95%CI: 0.33-0.73; p<0.001), while frequent users of more than 45 dispensings showed a stronger association with an adjusted RR of 0.36 (95%CI: 0.21-0.62; p<0.001 (crude RR 0.43 (95%CI 0.25-0.73; p=0.002))).

Table 2: Differences in patient and tumour characteristics between none-user, prediagnosis & postdiagnosis and only postdiagnosis users of aspirin or NSAIDs

Aspirin use	Non-users N (%)*	Pre and post-diagnosis N (%)*	Only post-diagnosis N (%)*	P-value
Sex				
Male	115 (73.2)	72 (80.9)	11 (68.8)	0.3
Female	42 (26.8)	17 (19.1)	5 (31.2)	
Age				
<60	40 (25.5)	11 (12.4)	6 (37.5)	0.005
60-70	45 (28.7)	20 (22.5)	5 (31.2)	
70-80	42 (26.8)	42 (47.2)	5 (31.2)	
80+	30 (19.1)	16 (18.0)	0 (0.0)	
Grade				
I	7 (4.5)	5 (5.6)	2 (12.5)	0.7
II	38 (24.2)	26 (29.2)	4 (25.0)	
III	65 (41.4)	30 (33.7)	6 (37.5)	
Unknown	47 (29.9)	28 (31.5)	4 (25.0)	
Stage				
I	7 (4.5)	8 (9.0)	2 (12.5)	<0.001
II	12 (7.6)	20 (22.5)	6 (37.5)	
III	26 (16.6)	13 (14.6)	4 (25.0)	
IV	71 (45.2)	22 (24.7)	0 (0.0)	
Unknown	41 (26.1)	26 (29.2)	4 (25.0)	
Comorbidities				
At least one	109 (69.4)	132 (64.7)	62 (66.0)	0.6
Other cancer	22 (14.0)	31 (15.2)	15 (16.0)	0.9
Lung diseases	17 (10.8)	25 (12.3)	13 (13.8)	0.8
Digestive diseases	12 (7.6)	21 (10.3)	3 (3.2)	0.1
Hyper-tension	28 (17.8)	43 (21.1)	15 (16.0)	0.5
CVA	9 (5.7)	4 (2.0)	3 (3.2)	0.2
CVD	32 (20.4)	44 (21.6)	17 (18.1)	0.8
Diabetes	15 (9.6)	17 (8.3)	6 (6.4)	0.7

NSAIDs use	Non-users N (%)*	Pre and post-diagnosis N (%)*	Only post-diagnosis N (%)*	P-value
Sex				
Male	115 (73.2)	156 (76.5)	69 (76.4)	0.7
Female	42 (26.8)	48 (23.5)	25 (26.6)	
Age				
<60	40 (25.5)	78 (38.2)	29 (30.9)	0.005
60-70	45 (28.7)	70 (34.3)	33 (35.1)	
70-80	42 (26.8)	43 (21.1)	21 (22.3)	
80+	30 (19.1)	13 (6.4)	11 (11.7)	
Grade				
I	7 (4.5)	12 (5.9)	2 (2.1)	0.5
II	38 (24.2)	53 (26.0)	29 (30.9)	
III	65 (41.4)	83 (40.7)	31 (33.0)	
Unknown	47 (29.9)	56 (27.5)	32 (34.0)	
Stage				
I	7 (4.5)	24 (11.8)	4 (4.3)	<0.001
II	12 (7.6)	34 (16.7)	17 (18.1)	
III	26 (16.6)	40 (19.6)	21 (22.3)	
IV	71 (45.2)	70 (34.3)	23 (24.5)	
Unknown	41 (26.1)	36 (17.6)	29 (30.9)	
Comorbidities				
At least one	109 (69.4)	132 (64.7)	62 (66.0)	0.6
Other cancer	22 (14.0)	31 (15.2)	15 (16.0)	0.9
Lung diseases	17 (10.8)	25 (12.3)	13 (13.8)	0.8
Digestive diseases	12 (7.6)	21 (10.3)	3 (3.2)	0.1
Hyper-tension	28 (17.8)	43 (21.1)	15 (16.0)	0.5
CVA	9 (5.7)	4 (2.0)	3 (3.2)	0.2
CVD	32 (20.4)	44 (21.6)	17 (18.1)	0.8
Diabetes	15 (9.6)	17 (8.3)	6 (6.4)	0.7

Abbreviations: CVA = cerebrovascular accident, CVD = cardiovascular disease,
**Data represented here are column percentages within the subgroups.*

Frequent users of more than 20 dispensings solely postdiagnosis (irrespective of the number prediagnosis) showed a lower crude (RR 0.23 (95%CI 0.11-0.50; p<0.001) and adjusted RR of 0.25 (95%CI: 0.11-0.54; p<0.001), although the number of users (n=14) was

Table 3: Time-dependent overall survival analysis (crude RR and adjusted RR) for non-users and users of aspirin or NSAIDs

		N	E**	Crude RR	p-value	Adjusted RR*	p-value
Aspirin pre and postdiagnosis	Non-user	157	129	Reference	<0.001	Reference	<0.001
	User	89	67	0.55 (0.41 – 0.74)		0.44 (0.31 – 0.61)	
Aspirin only postdiagnosis	Non-user	157	129	Reference	<0.001	Reference	0.006
	User	16	7	0.15 (0.07 – 0.32)		0.29 (0.12 – 0.70)	
Aspirin postdiagnosis ǂ	Non-user	157	129	Reference	<0.001	Reference	<0.001
	User	105	74	0.45 (0.34 – 0.60)		0.42 (0.30 – 0.57)	
	Non-user	215	171	Reference	0.003	Reference	<0.001
	Frequent user (>30)	47	32	0.56 (0.39-0.82)		0.49 (0.33-0.73)	
	Non-user	237	188	Reference	0.002	Reference	<0.001
	Frequent user (>45)	25	15	0.43 (0.25-0.73)		0.36 (0.21-0.62)	
	Non-user	248	196	Reference	<0.001	Reference	<0.001
	Frequent user post-diagnosis (>20)	14	7	0.23 (0.11-0.50)		0.25 (0.11-0.54)	
		N	**E****	**Crude RR**	**p-value**	**Adjusted RR***	**p-value**
NSAID pre and postdiagnosis	Non-user	157	129	Reference	<0.001	Reference	0.02
	User	204	146	0.45 (0.36 – 0.57)		0.72 (0.55 – 0.95)	
NSAID only postdiagnosis	Non-user	157	129	Reference	0.05	Reference	0.2
	User	94	76	0.75 (0.57 – 1.00)		0.81 (0.59 – 1.11)	
NSAID postdiagnosis ǂ	Non-user	157	129	Reference	<0.001	Reference	0.2
	User	298	222	0.61 (0.49 – 0.76)		0.84 (0.66 – 1.07)	

*ǂ = Pre and postdiagnosis and only postdiagnosis users combined. **E = number of events, RR = Rate Ratio*
** = Adjusted for sex, age, grade, stage, morphology, histological type, location of the tumour, treatment and comorbidities*

low in this group. Figure 1 shows the survival curve of postdiagnosis aspirin users and none users.

Figure 2 shows the stratified analysis of postdiagnosis aspirin use. Stratified analysis showed the point estimate of aspirin use is lower for females adjusted RR 0.24 (95% CI: 0.10 – 0.55; P<0.001) than males 0.55 (95% CI: 0.37 – 0.80; P=0.002). The estimate of aspirin users compared with non-users when having early stage tumors were lower adjusted RR 0.33 (95% CI: 0.15 – 0.74; P=0.007) than in patients with late stage tumors RR 0.56 (95% CI: 0.35 – 0.89; P=0.01). Moreover, the point estimate in patients with squamous cell carcinoma adjusted RR 0.34 (95% CI: 0.18 – 0.63; P<0.001) was lower than in patients with adenocarcinoma RR 0.43 (95% CI: 0.28 – 0.65; P<0.001). Furthermore, the point estimates of aspirin use were lower in patients who underwent surgery (RR 0.40 (95%CI 0.20-0.79) versus RR 0.45 (95%CI 0.31-0.66) in patients who did not undergo surgery), chemotherapy (RR 0.38 (95%CI 0.17-0.86) versus RR 0.43 (95%CI 0.30-0.62) in patients who were not treated with no chemotherapy) or radiotherapy (RR 0.39 (95%CI 0.26-0.58) versus RR 0.47 (95%CI 0.27-0.84) in patients who were not treated with radiotherapy than in untreated patients.

Figure 1: Overall survival curve for postdiagnosis use or non-use of aspirin in patients with oesophageal cancer (pre and postdiagnosis users and only postdiagnosis users combined).

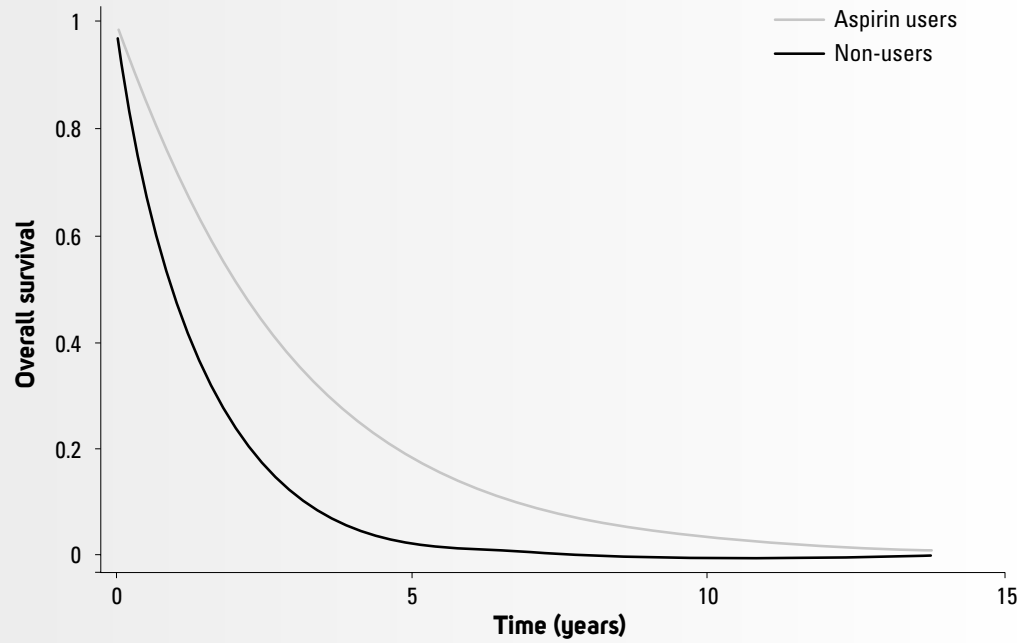


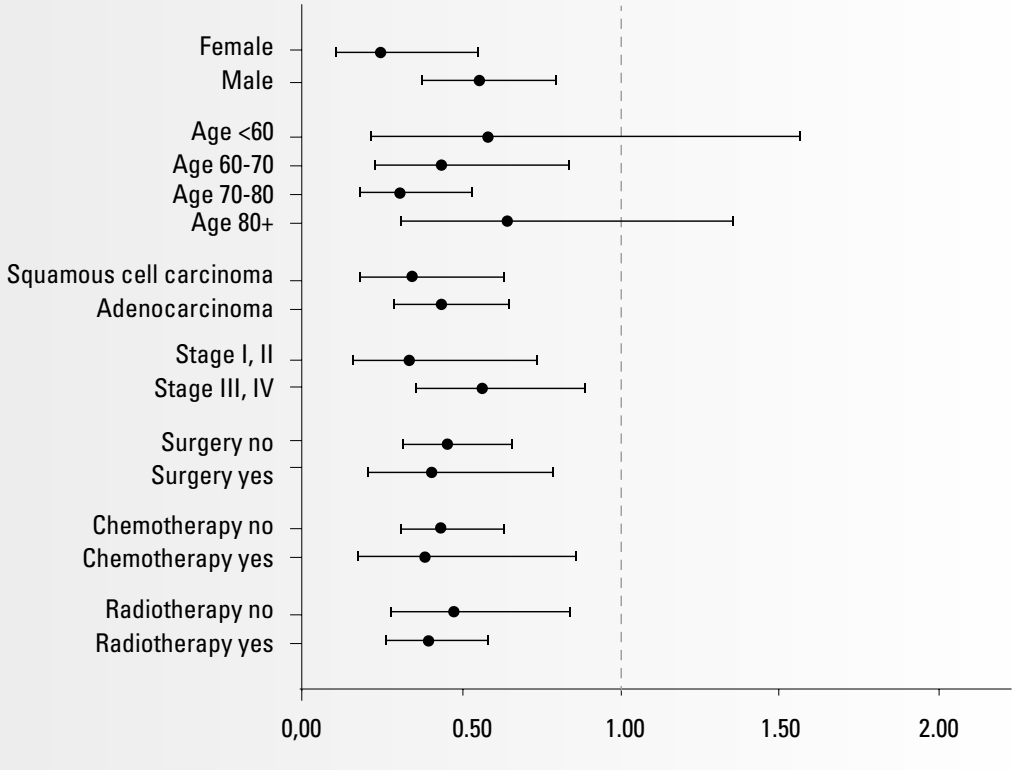
Table 3 also shows the time-dependent survival analysis for non-users and users of NSAIDs. Prediagnosis and postdiagnosis use of NSAIDs did result a significant survival gain with a RR of 0.45 (95% CI: 0.36 – 0.57; P<0.001). After adjusting for possible confounders the adjusted mortality rate ratio for prediagnosis and postdiagnosis NSAIDs use was significant 0.72 (95%CI: 0.55 – 0.95; P=0.02). Only postdiagnosis NSAIDs use however was not associated with a better survival outcome in the adjusted analyses with a RR of 0.81 (95% CI: 0.59 – 1.11; P=0.2). Any postdiagnosis NSAIDs was associated with overall survival in the crude analyses (RR 0.61 (95%CI 0.49-0.78; p<0.001), however did not result in a significant survival gain in the multivariable analyses with an adjusted RR of 0.84 (95%CI: 0.66 – 1.07; P=0.2).

DISCUSSION

This study shows an possible association between postdiagnosis aspirin use and overall survival in patients with esophageal cancer. Our results are the first to suggest an association of aspirin use after diagnosis and survival in esophageal cancer patients. Studies so far focused on the effect of aspirin and NSAIDs use on the risk of developing esophageal cancer. Also, the effect seems to be aspirin-specific; the postdiagnosis use of NSAIDs had no significant effect on outcome. Furthermore, the stratified analysis shows an association of survival with aspirin use consistent amongst different subgroups, including gender, age and tumor cell type.

Although our results suggest a consistent survival effect of aspirin use in patients with adenocarcinoma and squamous cell carcinoma, the effect might differ between patients

Figure 2: Stratified analysis for postdiagnosis aspirin use (pre and postdiagnosis users and only postdiagnosis users combined).



with tumors of a different tumor cell type. It is not clear which mechanisms cause this difference in effect of low-dose aspirin use. Furthermore, the survival effect of aspirin use was stronger in only postdiagnosis aspirin users than in 'prediagnosis and postdiagnosis' aspirin users. It is plausible that tumors who developed in presence of low plasma levels of aspirin are also not oppressed by low dose postdiagnosis aspirin use. The effect might differ between patients with an early stage tumor RR 0.33 (95% CI: 0.15 – 0.74; P=0. 007) than for late stage tumors. This might be explained by the assumed predominant anti-cancer effect of aspirin. Because the effect is also seen in the late stage tumors, the idea that the effect of aspirin is multifactorial is confirmed.[19]

The biological mechanisms involved in the anti-cancer effect of aspirin are not (yet) fully understood, but recent evidence points out a role of platelets. Aspirin inhibits COX1 expression in platelets which disrupts platelet activation and the subsequent secretion of α-granules containing TGF-β and PDGF. These growth factors are involved in the epithelial-mesenchymal transition (EMT) of circulating tumor cells; thus aspirin might diminish the metastatic potential.[20, 21] Furthermore, aspirin use might also induce COX-independent effects on platelets, for example by the acetylation of proteins and metabolites and these other mechanisms could be involved in the chemopreventive effect of aspirin.[16]

Recently, several molecular epidemiological studies have been performed to identify the subset of patients with colorectal cancer who will benefit from aspirin by chemoprevention or as adjuvant therapy and showed in two studies that the observed significant survival gain of postdiagnosis aspirin use was present in mutated-PIK3CA tumors, but not in PIK3CA wild-type tumors.[22, 23] However, PGTS2-specific inhibition by rofecoxib did not improve the relapse-free survival in PIK3CA-mutated tumors (p=0.66).[23] In contrast, the analysis of 999 colorectal tumor blocks resected from 2002-2008 in the Netherlands showed no association of survival benefit of postdiagnosis use of aspirin with tumors with mutated-PIK3CA and COX2-expression. The survival benefit of low dose postdiagnosis aspirin use did depend on the presence of another biomarker; patients with tumors with HLA Class I antigen expression showed a significant survival gain, but patients with tumors without HLA Class I antigen expression did not.[19]

The value of PIK3CA-mutation status and COX2-expression levels as biomarkers in colon cancer remains inconclusive.[19, 22] Also, the mutation frequency of PIK3CA in esophageal squamous cell carcinomas and adenocarcinomas is lower, namely 4.5%[24] respectively 6.0%[25] than the reported mutation rates of 11-17%[19, 22, 26, 27] in the tumor specimens of colorectal carcinomas. Therefore, future molecular pathological epidemiological studies should focus on a combination of potential biomarkers to examine the therapeutic effect of postdiagnosis aspirin use in patients with esophageal cancer.

The present observational study has several limitations. First, as baseline characteristics (which are associated with survival) of non-users and aspirin users differ, the survival effect of aspirin could also partially be caused by healthy-user bias. As shown in table 1, the users are younger and have a lower stage of disease at the moment of diagnosis which are associated with survival, however they are also more often diagnosed with comorbidities. We adjusted for these factors in the multivariable analyses, but residual confounding may be present and as a result of the lower number of users it remains questionable if we adjusted sufficiently, especially in the group of 'only post-diagnosis' users. However, the survival analysis of the 'prediagnosis and postdiagnosis' also resulted in a strong survival effect with an adjusted RR of 0.44 (95% CI: 0.31 – 0.61). The analyses of frequent users showed a stronger association in frequent users, although only present in the users with a high number of dispensings. A second limitation of the present study is the low number of 'only postdiagnosis' aspirin users in the patient cohort which might influence the results. Larger studies with a higher number of postdiagnosis aspirin users are needed to confirm the results. A third limitation of the study could be "over the counter-use" of aspirin. However, low dose aspirin is mainly available on dispensing in the Netherlands, but we cannot rule out the possibility of over the counter use of (higher) aspirin doses which are available as we did not have information regarding the use of aspirin or NSAIDs by patients at home. Lastly, aspirin is usually prescribed by the means of cardiovascular chemoprevention, thus confounding by indication could be a problem. It is however not likely that the indicators of an elevated risk of cardiovascular disease provide user patients with a beneficial survival prognosis with respect to non-user patients. Also, the survival effect of aspirin use cannot only be

explained by a reduction of cardiac events; in the meta-analysis of six primary prevention trials and 16 secondary prevention trials by Baigent C. et al. the survival gain of aspirin use was lower than the survival gain observed in the present study and showed a pooled gain (for primary and secondary prevention of vascular disease) of approximately 5%.[28] One of the major strengths of our study was the use of a database of dispensed medication, by which we avoided recall-bias.

Our study suggests that patients with esophageal cancer might benefit more from postdiagnosis aspirin use than patients with colon cancer. These results are in line with previous data of Rothwell's study of the long-term risk of cancer-related death in daily aspirin users; the 20-year cancer-related mortality was lower for patients with esophageal cancer 0.42 (95% CI: 0.25 – 0.71) than for patients with colon cancer 0.60 (95%CI: 0.45 – 0.81).[29]

In the future, a randomized clinical trial is needed to verify our epidemiological observations of the benefits of postdiagnosis aspirin use in patients with esophageal cancer. Furthermore, it is important to identify the subgroups in which the benefits of low-dose postdiagnosis aspirin might outweigh the risks of severe adverse effects like gastrointestinal bleeding. The identification of biomarkers could predict in which patients low-dose aspirin has a significant survival effect. Because aspirin is already a well-tested and cheap drug, it could have a beneficial clinical impact when introduced as an adjuvant therapy in patients with esophageal cancer.

Supplementary Table 1: Aspirin and NSAIDs dispensed by the out-patient pharmacy in the selected patients with oesophageal cancer

Name	Code	Number of dispensings
Acetylsalicyzuur	B01AC06 (30/80 mg)*	4835
Acetylsalicylzuur	NO2BA01 (300/500 mg)	7
Indometacine	M01AB01	69
Sulindac	M01AB02	25
Diclofenac	M01AB05	1676
Biofenac	M01AB16	8
Arthrotec	M01AB55	212
Piroxicam	M01AC01	86
Meloxicam	M01AC06	289
Ibuprofen	M01AE01	704
Naproxen	M01AE02	917
Ketoprofen	M01AE03	2
Surgam	M01AE11	1
Seractil	M01AE14	3
Celebrex	M01AH01	119
Vioxx	M01AH02	191
Bextra	M01AH03	5
Arcoxia	M01AH05	87
Nabumeton	M01AX01	57
Carbasal	NO2BA15	31
ACCod	NO2BA51	18
Total number of dispensings		9342

**= 98.1% of the dispensings were 80 mg, 1.9% 30 mg*

Reference List

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011;61(2):69-90.
2. Dubecz A, Gall I, Solymosi N et al. Temporal trends in long-term survival and cure rates in esophageal cancer: a SEER database analysis. J Thorac Oncol 2012;7(2):443-447.
3. Langley RE, Burdett S, Tierney JF, Cafferty F, Parmar MK, Venning G. Aspirin and cancer: has aspirin been overlooked as an adjuvant therapy? Br J Cancer 2011;105(8):1107-1113.
4. Algra AM, Rothwell PM. Effects of regular aspirin on long-term cancer incidence and metastasis: a systematic comparison of evidence from observational studies versus randomised trials. Lancet Oncol 2012;13(5):518-527.
5. Din FV, Theodoratou E, Farrington SM et al. Effect of aspirin and NSAIDs on risk and survival from colorectal cancer. Gut 2010;59(12):1670-1679.
6. Huang TB, Yan Y, Guo ZF et al. Aspirin use and the risk of prostate cancer: a meta-analysis of 24 epidemiologic studies. Int Urol Nephrol 2014;46(9):1715-1728.
7. Jayaprakash V, Menezes RJ, Javle MM et al. Regular aspirin use and esophageal cancer risk. Int J Cancer 2006;119(1):202-207.
8. Sadeghi S, Bain CJ, Pandeya N, Webb PM, Green AC, Whiteman DC. Aspirin, nonsteroidal anti-inflammatory drugs, and the risks of cancers of the esophagus. Cancer Epidemiol Biomarkers Prev 2008;17(5):1169-1178.
9. Abnet CC, Freedman ND, Kamangar F, Leitzmann MF, Hollenbeck AR, Schatzkin A. Non-steroidal anti-inflammatory drugs and risk of gastric and oesophageal adenocarcinomas: results from a cohort study and a meta-analysis. Br J Cancer 2009;100(3):551-557.
10. Corley DA, Kerlikowske K, Verma R, Buffler P. Protective association of aspirin/NSAIDs and esophageal cancer: a systematic review and meta-analysis. Gastroenterology 2003;124(1):47-56.
11. Bastiaannet E, Sampieri K, Dekkers OM et al. Use of aspirin postdiagnosis improves survival for colon cancer patients. Br J Cancer 2012;106(9):1564-1570.
12. Bruno A, Dovizio M, Tacconelli S, Patrignani P. Mechanisms of the antitumoural effects of aspirin in the gastrointestinal tract. Best Pract Res Clin Gastroenterol 2012;26(4):e1-e13.
13. Zimmermann KC, Sarbia M, Weber AA, Borchard F, Gabbert HE, Schror K. Cyclooxygenase-2 expression in human esophageal carcinoma. Cancer Res 1999;59(1):198-204.
14. Buskens CJ, van Rees BP, Sivula A et al. Prognostic significance of elevated cyclooxygenase 2 expression in patients with adenocarcinoma of the esophagus. Gastroenterology 2002;122(7):1800-1807.
15. Zhang L, Wu YD, Li P et al. Effects of cyclooxygenase-2 on human esophageal squamous cell carcinoma. World J Gastroenterol 2011;17(41):4572-4580.
16. Dovizio M, Bruno A, Tacconelli S, Patrignani P. Mode of action of aspirin as a chemopreventive agent. Recent Results Cancer Res 2013;191:39-65.
17. Liu JF, Jamieson GG, Wu TC, Zhu GJ, Drew PA. A preliminary study on the postoperative survival of patients given aspirin after resection for squamous cell carcinoma of the esophagus or adenocarcinoma of the cardia. Ann Surg Oncol 2009;16(5):1397-1402.
18. van Herk-Sukel MP, van de Poll-Franse LV, Lemmens VE et al. New opportunities for drug outcomes research in cancer patients: the linkage of the Eindhoven Cancer Registry and the PHARMO Record Linkage System. Eur J Cancer 2010;46(2):395-404.
19. Reimers MS, Bastiaannet E, Langley RE et al. Expression of HLA class I antigen, aspirin use, and survival after a diagnosis of colon cancer. JAMA Intern Med 2014;174(5):732-739.
20. Labelle M, Begum S, Hynes RO. Direct signaling between platelets and cancer cells induces an epithelial-mesenchymal-like transition and promotes metastasis. Cancer Cell 2011;20(5):576-590.
21. Lou XL, Deng J, Deng H et al. Aspirin inhibit platelet-induced epithelial-to-mesenchymal transition of circulating tumor cells (Review). Biomed Rep 2014;2(3):331-334.
22. Liao X, Lochhead P, Nishihara R et al. Aspirin use, tumor PIK3CA mutation, and colorectal-cancer survival. N Engl J Med 2012;367(17):1596-1606.
23. Domingo E, Church DN, Sieber O et al. Evaluation of PIK3CA mutation as a predictor of benefit from nonsteroidal anti-inflammatory drug therapy in colorectal cancer. J Clin Oncol 2013;31(34):4297-4305.
24. Song Y, Li L, Ou Y et al. Identification of genomic alterations in oesophageal squamous cell cancer. Nature 2014;509(7498):91-95.
25. Phillips WA, Russell SE, Ciavarella ML et al. Mutation analysis of PIK3CA and PIK3CB in esophageal cancer and Barrett's esophagus. Int J Cancer 2006;118(10):2644-2646.
26. Nishihara R, Lochhead P, Kuchiba A et al. Aspirin use and risk of colorectal cancer according to BRAF mutation status. JAMA 2013;309(24):2563-2571.
27. Rosty C, Young JP, Walsh MD et al. PIK3CA activating mutation in colorectal carcinoma: associations with molecular features and survival. PLoS One 2013;8(6):e65479.
28. Baigent C, Blackwell L, Collins R et al. Aspirin in the primary and secondary prevention of vascular disease: collaborative meta-analysis of individual participant data from randomised trials. Lancet 2009;373(9678):1849-1860.
29. Rothwell PM, Fowkes FG, Belch JF, Ogawa H, Warlow CP, Meade TW. Effect of daily aspirin on long-term risk of death due to cancer: analysis of individual patient data from randomised trials. Lancet 2011;377(9759):31-41.

CHAPTER 3

EFFECT OF LOW-DOSE ASPIRIN USE ON SURVIVAL OF PATIENTS WITH GASTROINTESTINAL MALIGNANCIES; AN OBSERVATIONAL STUDY

Martine A. Frouws, Esther Bastiaannet, Ruth E. Langley, John W.K. Chia, Myrthe P.P. van Herk-Sukel, Valery E.P.P. Lemmens PhD, Hein Putter, Henk H. Hartgrink, Bert A. Bonsing, Cornelis J.H. Van de Velde, Johanneke E.A. Portielje, Gerrit-Jan Liefers

British Journal of Cancer 2017 Jan;116(3):405-413

Abstract

Background

Previous studies suggested a relationship between aspirin use and mortality reduction. The mechanism for the effect of aspirin on cancer outcomes remains unclear. The aim of this study was to evaluate aspirin use and survival in patients with gastrointestinal tract cancer.

Methods

Patients with gastrointestinal tract cancer diagnosed between 1998-2011 were included. The population-based Eindhoven Cancer Registry was linked to drug dispensing data from the PHARMO Database Network. The association between aspirin use after diagnosis and overall survival was analysed using Cox regression models.

Results

In total, 13,715 patients were diagnosed with gastrointestinal cancer. A total of 1008 patients were identified as aspirin users, and 8278 patients were identified as nonusers. The adjusted hazard ratio for aspirin users versus nonusers was 0.52 (95% CI 0.44-0.63). A significant association between aspirin use and survival was observed for patients with oesophageal, hepatobiliary and colorectal cancer.

Conclusions

Post-diagnosis use of aspirin in patients with gastrointestinal tract malignancies is associated with increased survival in cancers with different sites of origin and biology. This adds weight to the hypothesis that the anti-cancer effects of aspirin are not tumour-site specific and may be modulated through the tumour micro-environment.

Introduction

The incidence of cancer is increasing, particularly in low-and medium resource countries; by the end of 2015 there were an estimated 15·2 million new cases globally with a predicted increase to 21.6 million by 2030.[1] The cost of healthcare is also increasing, and there is a real need for reasonably priced, widely available therapeutics to improve cancer outcomes. Although, the US Food and Drug Administration has approved a higher percentage of oncology drugs since 2008, many of these are expensive targeted agents with approvals based on surrogate endpoints, and infrequently improve overall survival.[2] Aspirin (acetylsalicylic acid) was originally synthesized and used as an analgesic in 1897, with the antiplatelet functions of low-dose aspirin subsequently discovered in the early 1970's. This latter discovery led to many large randomised controlled trials delineating the role of aspirin in the treatment and prevention of vascular disease. Retrospective long-term analyses of cancer outcomes in these randomised trials have revealed two interesting phenomena. Firstly, there was a 24% reduction in cancer incidence in patients allocated to aspirin, and this effect was seen across tumour types but was most marked in tumours arising from the gastrointestinal tract. Secondly, if cancers did develop they were less likely to have metastasised at presentation or subsequently if the patient received aspirin.[3-6]

Much of the work to date relating to aspirin and cancer has focussed on colorectal cancer. In particular there have been several epidemiological studies showing a reduction in cancer mortality and improved overall survival for patients taking aspirin after a diagnosis of colorectal cancer. [7-11] This has led to several ongoing adjuvant studies in colorectal cancer; the Add-Aspirin trial,[12] Adjuvant Aspirin for Colon Cancer (NCT02467582), the ALASCCA trial (NCT02647099), the ASCOLT trial (NCT00565708), and the Aspirin trial (NCT02301286). In addition, two other randomised controlled trials have focussed on primary prevention, and after long-term follow up showed a beneficial effect on primary prevention in both hereditary and sporadic colorectal cancer.[5,13] Also, a meta-analysis of four other randomised controlled trials showed an absolute risk reduction of 6·7% for the recurrence of adenoma's in patients with a history of these lesions.[14]

The mechanism(s) underlying the beneficial effects of aspirin on cancer outcomes remains unclear. Several different potential biomarkers have been investigated, but due to the multiple potential cellular pathways and conflicting results of previous studies, the mechanism of action remains unknown, though platelets may play a central role.[15] The aim of this study was to provide epidemiological evidence and further mechanistic insights on the potential beneficial effects of aspirin use after diagnosis of cancer that arises from any part of the gastrointestinal tract. Because many studies have tried to differentiate effects of aspirin use both before and after diagnosis, an additional analysis was performed including the patients that use aspirin both pre- and postdiagnosis.

Materials and Methods

Study population

Data from the Eindhoven Cancer Registry was used to identify patients diagnosed with cancer of the gastrointestinal tract between January 1998 and December 2011 in the south of the Netherlands. This area is served by ten hospitals, covers a demographic region of approximately 1·5 million Dutch citizens and is part of the nationwide Netherlands Comprehensive Cancer Organisation. The Eindhoven Cancer Registry is linked to the municipal population registry, which records the vital status (alive/dead) of all inhabitants. Patients are informed about the registration and registered except patients who objected to be registered. The Netherlands Cancer Registry is obliged to work according to the law about protection of privacy data; informed consent of the patients for this specific study was not applicable. Patient selection and data cleaning was performed by the Eindhoven Cancer Registry. Follow up for this project was until 31 December 2012.

The PHARMO Database Network is a population-based network which combines data from different healthcare settings in the Netherlands. For this study the out-patient pharmacy database was used, which contains drug-dispensing records from all community pharmacies. Drugs are coded using the Anatomical Therapeutic Chemical classification (www.whocc.no/atc_ddd_index) and the records include information on the type of product, date prescribed, dose and regimen, quantity, and route of administration. The PHARMO database was linked to the Eindhoven Cancer Registry and thus allows drug use by cancer patients to be analysed.[16] From this linked database, prescriptions for aspirin (only the ones that were actually dispensed) were selected.

Definition of users and nonusers

For this study, patients older than 18 years who used aspirin after a diagnosis of a gastrointestinal cancer were selected. The gastrointestinal tumours were coded according to the International Classification of Disease 10 [ICD-10] C15-C26. This comprises cancer from the following sites: oesophagus, stomach, small intestine, colon, recto-sigmoid and rectum, anus, liver and intra hepatic bile ducts, gallbladder and extra hepatic bile ducts, pancreas, and a group 'gastrointestinal tumours not otherwise specified (nos)'.

Patients who used aspirin before diagnosis were excluded from the analyses. Aspirin users (ATC codes: B01AC06, B01AC08, B01AC56, N02BA01, N02BA15, N02BA51, N02BA65) were defined as those prescribed aspirin for at least 30 days. Nonusers were defined as patients who received for less than 30 days or never used aspirin. Time after diagnoses was defined in periods of use and no use by analysing each single prescription during follow up. Periods of less than 14 days in between two prescriptions were considered consecutive. Follow up started 14 days after diagnosis because there was no information about in-hospital use of medication. Immortal time bias is avoided by analysing prescriptions as a time varying covariate, in periods of use and no use.[17,18]

Statistical analysis

Information from the ECR contained information about the presence or absence of the following comorbidities at cancer diagnosis: lung disease, cardiovascular disease, diabetes and disorders of the gastrointestinal tract, urinary tract, nervous system, musculoskeletal system, and a group of other comorbidities. Comorbidity was analysed as 0, 1 or ≥2 comorbidities. A Chi-square test was used to assess baseline characteristics for categorical values.

Survival analysis were performed with the Simon-Makuch method, an alternative for Kaplan Meier and with the ability to process time-varying covariates in survival curves.[19] A Cox proportional hazards model was used with aspirin use as a time-varying covariate, as described by Stricker et al.[18] Schoenfeld residuals were tested to verify the assumption of proportional hazards. Follow up duration (survival) was recorded in months from diagnosis (t=0). Multivariable survival models were built with the following covariates: age at diagnosis (continuous), sex, stage of cancer (categorical), number of comorbidities (0, 1 or ≥2), treatment (surgery yes/no, radiotherapy yes/no, and chemotherapy yes/no). Missing/unknown values were included in the multivariable model as missing indicator. Analysis were performed using Stata statistical software version 12. Statistical tests were two-sided and considered significant at the $p<0.05$ level.

Relative survival rates were used to take into account the risk of dying from causes other than the disease of interest. The excess mortality reflects the difference between the overall survival of patients and the survival that would be expected in the absence of cancer. The excess mortality was calculated as the ratio of the observed (all-cause) survival proportion to the expected survival proportion.[20] National life tables were used to estimate background mortality (expected survival) according to sex, year of age and incidence year. Relative Excess Risks were estimated using a flexible parametric model, implemented in the Stata command stpm2.[21]

Different parts of the gastrointestinal tract were analysed separately if there were as at least ten aspirin users (therefore small bowel, anal cancer and gastrointestinal tumours NOS were not considered separately). Histological subtypes (adenocarcinoma and squamous cell carcinomas) were also analysed separately in groups with at least ten aspirin users. Statistical interaction for this subgroup was tested by including an interaction term in the model of aspirin use and histological subtype and significance was assessed using the Wald test. A sensitivity analysis was performed by repeating the analysis and excluding patients with stage IV disease and separately repeating the analysis and excluding the first year of follow up from the analysis. The main analysis and all subgroup analysis were pre-planned.

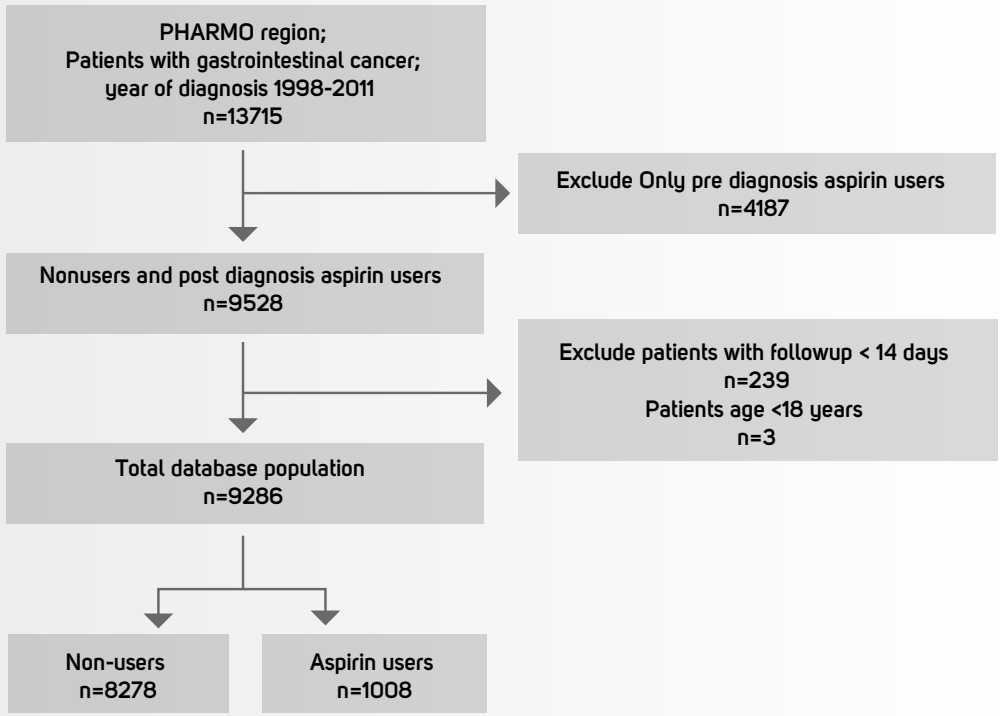
Pre- and postdiagnosis use of aspirin

For the analysis in patients that use both aspirin before and after diagnosis, the groups were selected with the same method as described in the 'definition of users and nonusers' heading. The only difference was that patients who started aspirin use before diagnosis and continued this after diagnosis were selected for the group of users of aspirin. Patients using aspirin only after diagnosis were excluded for this analysis. The statistical analysis was also equal to the analysis described above, where aspirin use was analysed as time varying covariate, and the same factors were used for the multivariable analysis.

Results

In total 13,715 patients were identified with a cancer of the gastrointestinal tract diagnosed between January 1998 and December 2011. The following were excluded from the analysis (CONSORT diagram Figure 1): 4,187 patients who were using aspirin prior to diagnosis, 239 patients with follow up of less than 14 days and three patients below the age of 18 years. Thus 9,286 patients were included in the survival analysis, of which 8,278 patients (person years: 4,375) did not use aspirin and 1,008 (person years: 2,150) used aspirin after diagnosis. In total, 5,138 events (deaths) were recorded. Table 1 shows the characteristics of this population.

Figure 1: Flowchart of patients selected for analysis



The majority of patients were diagnosed with colon cancer (43%), rectal cancer (25%) and oesophageal cancer (10%). Median age at diagnosis was 68 years (IQR 59-76) in the aspirin group and 69 (IQR 61-74) in the nonusers group. Aspirin users were less often female and more frequently diagnosed with stage I and II disease compared to nonusers. In the nonusers group, 26% of patients had stage IV disease compared to 9% in the aspirin users group. Median survival for all patients was 48 months.

Figure 2 shows survival curves for users of aspirin after diagnosis vs nonusers. In the group of aspirin users, 65% (95% CI 59%-71%) of patients was alive after five years, in contrast to nonusers, where 45% (95% CI 44%-46%) of patients was alive after five years.

A Cox proportional hazard model was used with use of aspirin as a time varying covariate. The proportional hazard assumption was fulfilled. For all patients with

Table 1: Characteristics of the cohort

	All patients	%	No Aspirin use	%	Aspirin Postdiagnosis	%	P-value
Total	9286	100	8278	89	1008	11	
Location tumour							
Oesophageal cancer	946	10.2	886	10.7	60	6	
Gastric cancer	750	8.1	700	8.5	50	5	
Small intestine cancer	97	1	88	1.1	9	0.9	
Colon cancer	3977	42.8	3434	41.5	543	53.9	<0.001
Rectal cancer	2358	25.4	2069	25	289	28.7	
Anal cancer	67	0.7	60	0.7	7	0.7	
Hepatobiliary cancer	385	4.2	360	4.4	25	2.5	
Pancreatic cancer	692	7.5	667	8.1	25	2.5	
Cancer of the gastro-intestinal tract nos[a]	14	0.2	14	0.2	0	0.0	
Sex							
Male	5140	55.4	4517	54.6	623	61.8	<0.001
Female	4146	44.7	3761	45.4	385	38.2	
Age Mean (SD). Median (IQR)[b]	67.1 (11)	68 (60-75)	66.7 (12)	68 (59-76)	67.7 (9.9)	69 (61-74)	
18-60 years	2420	26.1	2219	26.8	201	19.9	
60-69 years	2763	29.8	2437	29.4	326	32.3	<0.001
70-79 years	2831	30.5	2464	29.8	367	36.4	
80 years and older	1272	13.7	1158	14	114	11.3	
Stage							
0	204	2.2	176	2.1	28	2.8	
I	1496	16.1	1258	15.2	238	23.6	
II	2222	23.9	1900	23	322	31.9	<0.001
III	2058	22.2	1788	21.6	270	26.8	
IV	2249	24.2	2162	26.1	87	8.6	
Unknown	1057	11.4	994	12	63	6.3	
Surgery							
No	2693	29	2603	31.4	90	8.9	<0.001
Yes	6593	71	5675	68.6	918	91.1	
Chemotherapy							
No	6544	70.5	5798	70	746	74.1	0.009
Yes	2742	29.5	2480	30	262	26	
Radiotherapy							
No	7042	75.8	6291	76	751	74.5	0,3
Yes	2244	24.2	1987	24	257	25.5	

	All patients	%	No Aspirin use	%	Aspirin Postdiagnosis	%	P-value
Comorbidities							
None	3383	36.43	3056	36.92	327	32.44	0.05
One	2664	28.69	2359	28.5	305	30.26	
Two or more	2295	24.71	2027	24.49	268	26.63	
Unknown	944	10.17	836	10.1	108	10.69	
Morphology							
Adenocarcinoma	8343	89.84	7378	89.13	965	95.73	<0.001
Squamous cell carcinoma	298	3.21	280	3.38	18	1.79	
Epithelial	140	1.51	135	1.63	5	0.5	
Gastro Intestinal Stromal Tumour	58	0.62	50	0.6	8	0.79	
Other (not specified)	447	4.81	435	5.25	12	1.19	
Months survival. median (IQR)	48	(15.4-95.4)	24	(7.5-58.6)	89.4	(54.8-132.6)	

[a] nos: not otherwise specified
[b] SD: Standard Deviation. IQR: InterQuartile Range

gastrointestinal cancer, aspirin use was associated with a significant reduction in overall mortality, hazard ratio (HR) 0.57 (95% confidence interval (CI) 0.48-0.69) (Table 2). Adjusted for age at diagnosis, sex, stage of cancer, number of comorbidities, treatment (surgery, radiotherapy, and chemotherapy) the multivariable HR was 0.52 (95% CI 0.44-0.63).

Figure 2: Survival comparison for aspirin users versus non-users with Simon Makuch method

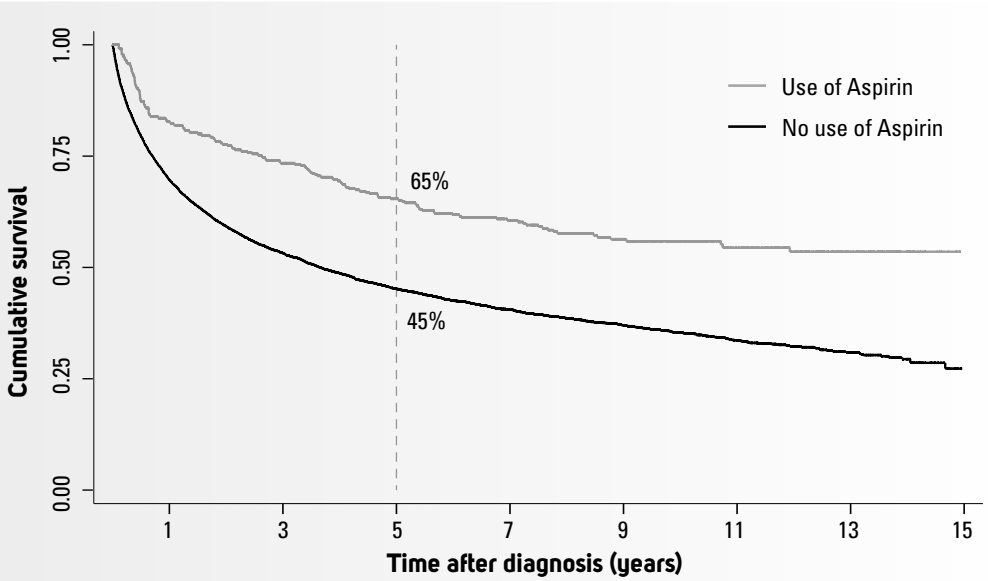


Figure 3: Overall survival analysis for aspirin users vs nonusers stratified according to tumour type

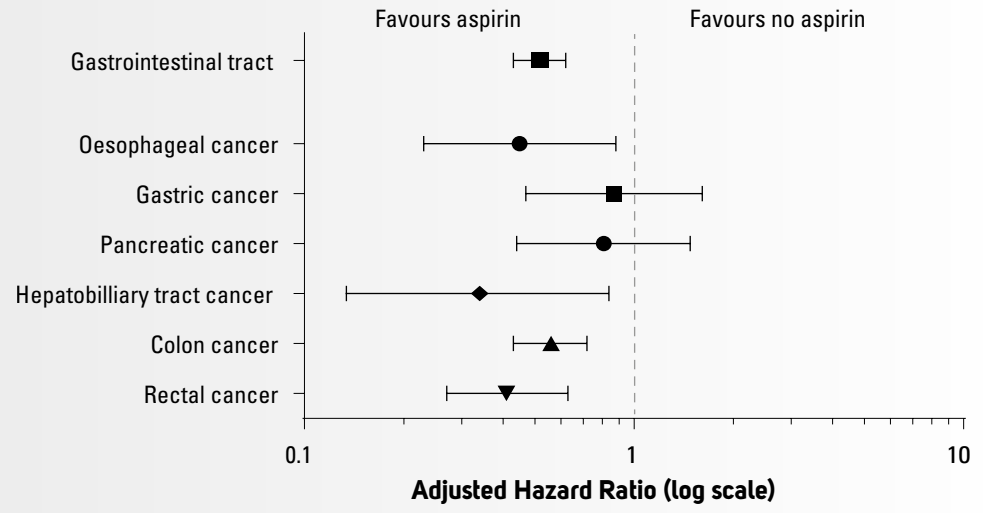
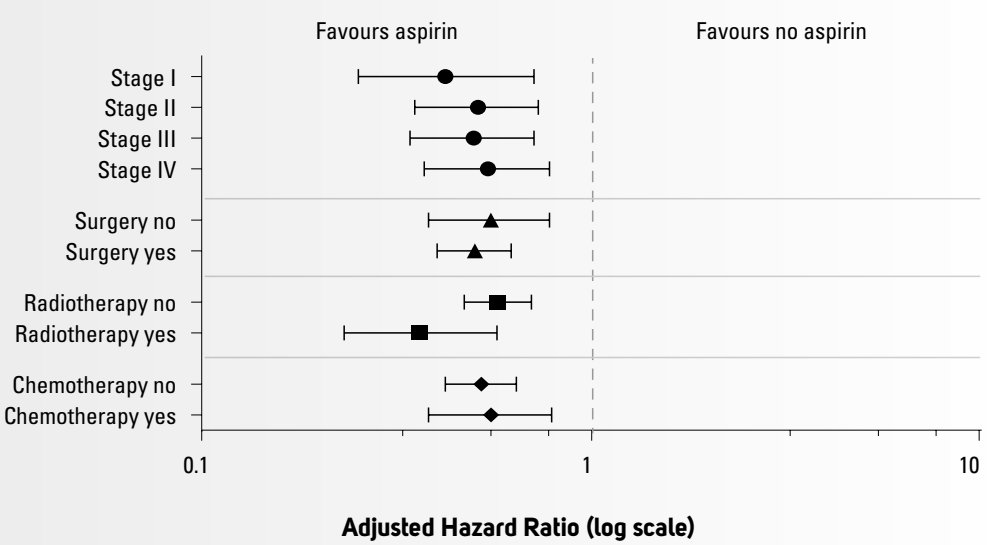


Figure 4: Forest plot of adjusted overall survival analysis in patients with gastrointestinal malignancies grouped according to aspirin users versus nonusers and stratified for stage and treatment



Stratification according to tumour type is shown in Table 2 and Figure 3. In patients with oesophageal, colon, rectal, and hepatobiliary tract cancer a significant association was found between the use of aspirin after diagnosis and overall survival. For patients with gastric and pancreatic cancer using aspirin, there was no statistically significant increase in survival. A survival benefit with aspirin was seen regardless of the stage of cancer at presentation and after all primary treatment modalities including chemotherapy, radiotherapy or surgery (Figure 4).

Table 2: Time dependent survival analysis (overall survival) stratified according to tumour type

				OVERALL SURVIVAL						RELATIVE SURVIVAL			
		No. at risk	No. events	Crude Hazard Ratio	95% C.I.	P-value	Adjusted Hazard Ratio[a]	95% C.I.	P-value	Crude Relative Excess Risk	95% C.I.	Adjusted Relative Excess Risk[a]	95% C.I.
Aspirin-users versus non-users (n=9286)													
Nonusers		8278	4776	1 (Reference)			1 (Reference)						
Aspirin Users		1008	362	**0.57**	**0.48-0.69**	**<0.001**	0.52	0.44-0.63	**<0.001**	**0.41**	**0.30-0.56**	**0.44**	**0.33-0.58**
Aspirin-users versus non-users per tumour type													
Oesophageal cancer (n=946)	Nonusers	886	722	1 (Reference)			1 (Reference)						
	Aspirin Users	60	30	**0.42**	**0.22 - 0.81**	**0.01**	**0.45**	**0.23 - 0.88**	**0.02**	**0.34**	**0.15 - 0.77**	**0.31**	**0.14 - 0.70**
Gastric cancer (n=750)	Nonusers	700	555	1 (Reference)			1 (Reference)						
	Aspirin Users	50	30	0.75	0.41 - 1.36	0.34	0.87	0.47 - 1.61	0.66	0.50	0.19 - 1.29	0.70	0.29 - 1.70
Pancreatic cancer (n=692)	Nonusers	667	631	1 (Reference)			1 (Reference)						
	Aspirin Users	25	21	1.06	0.58 - 1.93	0.84	0.81	0.44 - 1.48	0.49	1.03	0.56 - 1.89	0.77	0.42- 1.41
Hepatobiliary cancer (n=385)	Nonusers	360	311	1 (Reference)			1 (Reference)						
	Aspirin Users	25	10	**0.39**	**0.16- 0.95**	**0.04**	**0.34**	**0.14 - 0.84**	**0.02**	0.34	0.18 - 1.06	**0.35**	**0.13–0.95**
Colon cancer (n=3977)	Nonusers	3434	1587	1 (Reference)			1 (Reference)						
	Aspirin Users	543	178	**0.62**	**0.48 - 0.80**	**<0.001**	**0.56**	**0.43 - 0.72**	**<0.001**	**0.41**	**0.23 - 0.72**	**0.44**	**0.27 - 0.72**
Rectal cancer (n=2358)	Nonusers	2069	891	1 (Reference)			1 (Reference)						
	Aspirin Users	289	85	**0.51**	**0.33-0.77**	**0.001**	**0.41**	**0.27-0.63**	**<0.001**	**0.26**	**0.09 - 0.78**	**0.25**	**0.09 - 0.68**

[a] Adjusted for Stage, Sex, Age at diagnosis, Surgery, Radiotherapy, Chemotherapy, Comorbidities

Significant numbers are printed in bold

Table 2 additionally shows the Relative Survival estimates which are a good estimation of the cancer specific survival.[22] Equal to the overall survival rates, the observed Relative Excess Risks were significant in patients with oesophageal cancer, hepatobiliary cancer, colon and rectal cancer.

Squamous cell cancers accounted for 3% of the total cohort of which, 81% (n=242) arose from the oesophagus and 18% (n=53) were anal cancers. Patients with adenocarcinoma of the oesophagus who used aspirin had an adjusted HR 0.24 (95% CI 0.10-0.59) for overall survival, while those with a squamous cell carcinoma of the oesophagus had a HR for overall survival of 1.02 (95% CI 0.37-2.83) for aspirin users compared to nonusers. The test for heterogeneity of the effect of aspirin in patients with oesophageal squamous cell carcinoma vs patients with oesophageal adenocarcinoma was significant (P for interaction=0·01)

In 72% of prescriptions, 2,435 in total, the dose was reported. Of all prescribed dosages, 98% were 100 mg daily or lower. It was therefore not possible to analyse a dose-effect relationship, because only 31 prescriptions were for higher dose aspirin.

The sensitivity analysis with the exclusion of the first year follow up showed a similar effect, with an unadjusted HR of 0.56 (95% C.I. 0.45-0.69) and adjusted HR 0.49 (0.39-0.61). The sensitivity analysis or stage I-III showed an adjusted HR 0.49 (95% CI 0.39-0.61), consistent with the stratified analysis by stage in figure 4.

The analysis in the patients that use aspirin both pre and postdiagnosis can be found in table 3. Supplementary table 1 and supplementary figure 1 show the PRISMA flow chart for this cohort and the patient characteristics.

Discussion

Aspirin use after diagnosis of a gastrointestinal malignancy is associated with significantly lower mortality rates and this effect remains after adjusting for potential cofounders. It was most marked for tumours arising from the oesophagus, colon, rectum, and hepatobilliary tract. This large cohort study of almost 9 300 patients is the first observational cohort study evaluating the association of aspirin and survival in various gastrointestinal malignancies. The statistically significant effect on survival seen in patients with tumours of the oesophagus, colon and rectum is consistent with data from other published studies.[7,8,10,11,23] The effect in the tumour types was also present in patients that used aspirin both pre- and postdiagnosis.

In a recent prospective cohort study Cao et al.,[24] found that the reduced overall reduced cancer risk associated with the use of aspirin was primarily owing to gastrointestinal tract cancers. Additionally, in a meta-analysis of randomised trials evaluating aspirin for the prevention of cardiovascular disease, Rothwell et al.[3] showed a reduced risk of cancer-specific death with aspirin (HR of 0.79 (95% CI 0.68-0.92)) in all types of cancer. Stratified for tumour location, the largest benefit was found in patients with gastrointestinal tumours, with no significant heterogeneity between different gastrointestinal cancers. Consistent with our study they also showed that patients with adenocarcinomas were most likely to benefit from aspirin HR 0.70 (95% CI 0.54-0.91). However, in contrast, patients in our study only started aspirin after diagnosis of cancer

Table 3: Time dependent survival analysis (overall survival) stratified according to tumour type with prediagnosis aspirin users

				overall survival					
		No. at risk	No. events	Crude Hazard Ratio	95% C.I.	P-value	Adjusted Hazard Ratio	95% C.I.	P-value
Aspirin-users versus non-users (n=12109)									
Nonusers		8366	4913	1 (Reference)			1 (Reference)		
Pre and postdiag-nosis aspirin users[b]		2736	1647	0.69	0.64 - 0.75	**<0.001**	0.61	0.57-0.66	**<0.001**
Aspirin-users versus non-users per tumour type									
Oesophageal cancer (n=1180)	Nonusers	894	741	1 (Reference)			1 (Reference)		
	Aspirin Users[b]	286	229	**0.64**	**0.52-0.79**	**<0.001**	0.61	**0.49-0.76**	**<0.001**
Gastric cancer (n=933)	Nonusers	714	574	1 (Reference)			1 (Reference)		
	Aspirin Users[b]	219	184	0.90	0.72-1.13	0.37	0.85	0.67-1.07	0.17
Pancreatic cancer (n=876)	Nonusers	681	648	1 (Reference)			1 (Reference)		
	Aspirin Users[b]	195	183	**0.68**	**0.54-0.84**	**<0.001**	0.67	**0.53-0.84**	**0.001**
Hepatobiliary cancer (n=477)	Nonusers	364	317	1 (Reference)			1 (Reference)		
	Aspirin Users[b]	113	101	0.81	0.61-1.08	0.16	0.69	**0.51-0.93**	**0.02**
Colon cancer (n=4730)	Nonusers	3469	1642	1 (Reference)			1 (Reference)		
	Aspirin Users[b]	1261	612	**0.67**	**0.59-0.76**	**<0.001**	0.55	**0.48-0.63**	**<0.001**
Rectal cancer (n=2687)	Nonusers	2080	910	1 (Reference)			1 (Reference)		
	Aspirin Users[b]	607	306	**0.78**	**0.65-0.94**	**0.008**	0.63	**0.52-0.75**	**<0.001**

[a] Adjusted for Stage, Sex, Age at diagnosis, Surgery, Radiotherapy, Chemotherapy, Comorbidities
[b] Only patients using aspirin both pre and postdiagnosis were analysed
Significant numbers are printed in bold

which is most relevant when considering recommendations for subsequent management after a cancer diagnosis. In our study 11% of patients started using aspirin after diagnosis, which is also consistent with previous studies in cancer patients.[25]

A strength of our study is that the data is derived from linked cancer registry and pharmacy data, eliminating both recall and information bias. Though we cannot verify that patients actually ingested the aspirin, the prescriptions registered by the PHARMO institute are actually handed out to the patients by the pharmacy and this therefore adds weight to the definition of user. Additionally, immortal time bias and misclassification of exposure in follow up is avoided by the use of a Cox proportional hazards model with time varying covariate.[17] With this technique, accurate risk estimates are provided as each individual prescription is analysed.[18]

Moreover, the exclusion of patients already using aspirin before diagnosis and the determination of patient characteristics at diagnosis (t=0) mimics the use of aspirin as adjuvant therapy. In our study, patients are identified at diagnosis but before they are exposed to the treatment of interest and differentiated into groups of users and nonusers. This 'new-user design' eliminates important biases associated with observational studies.[26] Additionally, it has been suggested that for measuring the side effects of drugs, which the effect of aspirin on cancer could theoretically be considered, observational data could in some cases be considered non-inferior to results from randomised controlled trials.[27]

Our study has limitations. First, since exposure to aspirin depends on a clinician's decision to prescribe aspirin to a certain patient, it is prone to confounding by selective prescribing. For instance, oncologists may withhold aspirin treatment (as secondary prevention for cardiovascular disease) in patients diagnosed with incurable (stage IV) cancer because of the poor prognosis. Thus patients with a particularly poor prognosis may end up in the nonuser group. This reverse causation was addressed by the pre-planned sensitivity analysis excluding the first year of follow-up, which restricted the study population to patients alive at one year after diagnosis. By introducing this one year exposure lag, any undiagnosed recurrence at baseline or early recurrence would have been likely to become apparent and therefore baseline prognosis between the two groups is believed to be more similar.[28]

Second, proven cardiovascular disease is the main indication for low-dose aspirin in the Netherlands. This could imply that patients prescribed aspirin have a worse life expectancy at baseline because of lifestyle factors and risks associated with both cardiovascular disease and cancer development. Considering the absence of information on cancer specific survival and cause of death in our study, hypothetically part of the overall survival gain we observed could be explained by the prevention of cardiovascular mortality. However, in a large meta-analysis of individual participant data, the reduction in vascular specific mortality from aspirin was only 9%, HR 0·91.[29] Therefore a reduction in cardiovascular mortality could only partly explain the reduction in mortality we observed. Several of the studies evaluating the effect of aspirin use after a diagnosis of colorectal cancer have shown a significant reduction in colorectal cancer specific mortality.[7, 10]

Third, over the counter aspirin use was not included. However, prescription data can give valid estimations of association even though available over the counter.[30] No data was available to adjust for lifestyle factors, health related behaviour and mutational status.

Lastly, table 1 shows that the groups aspirin users and non-users are different with respect to baseline characteristics. This is to a large extent the result of the size of the cohort. After adjustment for these factors the association between aspirin use and

survival remained significant. Nevertheless, the confounding by indication as described remains, and therefore randomised controlled trials remain inevitable before aspirin can be used as regular anti-cancer treatment.

The mechanism responsible for the effect of aspirin on cancer remains unknown. Aspirin (acetylsalicylic acid) reduces prostanoid generation by irreversible inhibition of platelet COX-1 (cyclooxygenase-1) and COX-2 isozymes. Activated platelets release several growth factors which impact on tumour progression and metastasis.[31] Maximum platelet inactivation by COX-1 is thought to be obtained by low-dose aspirin (75-100 mg daily) and over 95% of platelet activity is inhibited for up to 24h.[32] A number of potential biomarkers have been identified as predictors of response to aspirin in terms of cancer outcomes. Chan et al. reported that the effect of high dose (325mg) aspirin after a colorectal cancer diagnosis was predominantly in patients with COX-2 (also called PTGS2) overexpression.[33] However, to achieve constant inhibition of COX in tissues, the administered daily dose of aspirin would have to be higher than 2000 mg.[34] In some studies mutations in phosphatidylinositol 3-kinase (PIK3CA) have been associated with aspirin response, however in a previous study we did not find this association but showed that the effect of aspirin was associated with tumours that expressed Human Leukocyte Antigen (HLA) class1 molecules.[9, 35]

Our observation that aspirin use is similarly associated with good prognosis in various tumour types with clearly different biology makes a non-specific mode of action plausible. It is possible that aspirin executes its effect by inhibiting platelet aggregation around circulating epithelial tumour cells, irrespective of organ site which then facilitates immune clearance. The coming years will hopefully provide answers. Several randomised clinical trials have commenced in the past years. (NCT02647099,[12], NCT02467582, NCT02301286, NCT00565708) Many of these trials are united in the 'Aspirin Trialist Collaborative Group' and will pool results regarding clinical outcome and expression of biomarkers.

Conclusion

Aspirin use after diagnosis of gastrointestinal malignancies is associated with improved overall survival. This observation makes a non-specific mode of action for aspirin on cancer plausible. These results offer direction towards future studies, both in terms of new randomised controlled trials as well as further studies to identify biomarkers that predict response to aspirin.

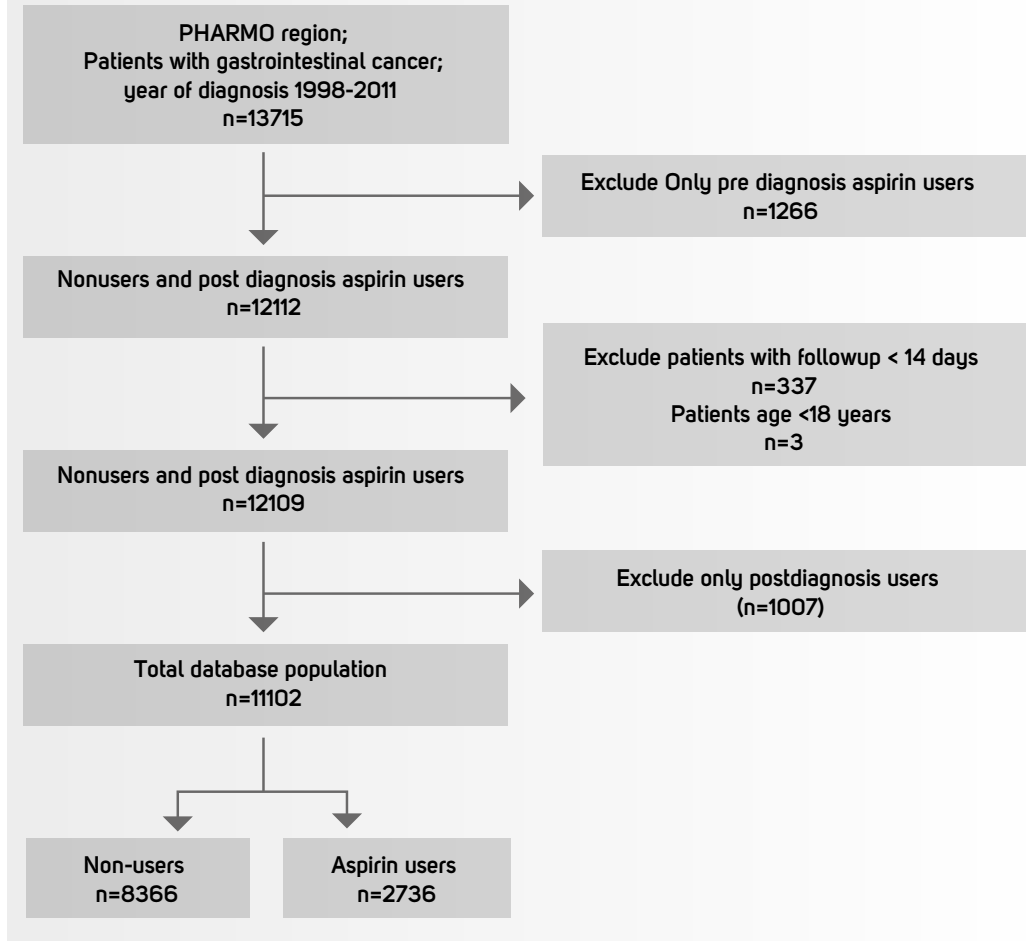
Statement

The authors declare they received no support for this work, financially or in kind from any third party, company or organisation whose finances or reputation may be affected by the publication of the work. The authors declare that no recent, existing or planned employment relationship or consultancy (whether paid or unpaid) any of the authors has with an organisation whose finances or reputation may be affected by the publication of the work. The authors declare any direct financial interest any of the authors or their spouses, parents or children has (personal shareholdings, consultancies, patents or patent applications) whose value could be affected by the publication.

Myrthe P.P. van Herk-Sukel is an employee of the PHARMO Institute for Drug Outcomes Research. This independent research institute performs financially supported studies for government and related healthcare authorities and several pharmaceutical companies. However, this study was not financially supported by a pharmaceutical company.

Ruth E. Langley has received financial support through grants from Cancer Research UK and the National Institute for Health Research (NIHR) Health Technology Assessment (HTA) as Chief Investigator of the Add-Aspirin Trial, has received compensation from Bayer and Aspirin Foundation for service on scientific advisory boards, and has received a supply of aspirin and placebo from Bayer Pharmaceuticals AG for the Add-Aspirin Trial.

Supplementary figure 1



Reference list

1. Sullivan R, Alatise OI, Anderson BO, et al. Global cancer surgery: delivering safe, affordable, and timely cancer surgery. The Lancet Oncology 2015; 16(11): 1193-224.
2. Kim C, Prasad V. Cancer Drugs Approved on the Basis of a Surrogate End Point and Subsequent Overall Survival: An Analysis of 5 Years of US Food and Drug Administration Approvals. JAMA Intern Med 2015: 1-2.
3. Rothwell PM, Fowkes FG, Belch JF, Ogawa H, Warlow CP, Meade TW. Effect of daily aspirin on long-term risk of death due to cancer: analysis of individual patient data from randomised trials. Lancet 2011; 377(9759): 31-41.
4. Algra AM, Rothwell PM. Effects of regular aspirin on long-term cancer incidence and metastasis: a systematic comparison of evidence from observational studies versus randomised trials. Lancet Oncol 2012.
5. Cook NR, Lee IM, Zhang SM, Moorthy MV, Buring JE. Alternate-day, low-dose aspirin and cancer risk: long-term observational follow-up of a randomized trial. Ann Intern Med 2013; 159(2): 77-85.
6. Rothwell PM, Price JF, Fowkes FG, et al. Short-term effects of daily aspirin on cancer incidence, mortality, and non-vascular death: analysis of the time course of risks and benefits in 51 randomised controlled trials. Lancet 2012.
7. Chia WK, Ali R, Toh HC. Aspirin as adjuvant therapy for colorectal cancer--reinterpreting paradigms. Nat Rev Clin Oncol 2012; 9(10): 561-70.
8. Jacobs EJ, Newton CC, Gapstur SM, Thun MJ. Daily aspirin use and cancer mortality in a large US cohort. J Natl Cancer Inst 2012; 104(16): 1208-17.
9. Paleari L, Puntoni M, Clavarezza M, DeCensi M, Cuzick J, DeCensi A. PIK3CA Mutation, Aspirin Use after Diagnosis and Survival of Colorectal Cancer. A Systematic Review and Meta-analysis of Epidemiological Studies. Clin Oncol (R Coll Radiol) 2015.
10. Coyle C, Cafferty FH, Langley RE. Aspirin and Colorectal Cancer Prevention and Treatment: Is It for Everyone? Current Colorectal Cancer Reports 2016.
11. Elwood PC, Morgan G, Pickering JE, et al. Aspirin in the Treatment of Cancer: Reductions in Metastatic Spread and in Mortality: A Systematic Review and Meta-Analyses of Published Studies. PLoS One 2016; 11(4): e0152402.
12. Langley RE, Rothwell PM. Aspirin in gastrointestinal oncology: new data on an old friend. Curr Opin Oncol 2014; 26(4): 441-7.
13. Burn J, Gerdes AM, Macrae F, et al. Long-term effect of aspirin on cancer risk in carriers of hereditary colorectal cancer: an analysis from the CAPP2 randomised controlled trial. Lancet 2011; 378(9809): 2081-7.
14. Cole BF, Logan RF, Halabi S, et al. Aspirin for the chemoprevention of colorectal adenomas: meta-analysis of the randomized trials. J Natl Cancer Inst 2009; 101(4): 256-66.
15. Langley RE, Burdett S, Tierney JF, Cafferty F, Parmar MK, Venning G. Aspirin and cancer: has aspirin been overlooked as an adjuvant therapy? Br J Cancer 2011; 105(8): 1107-13.
16. van Herk-Sukel MP, LV vdP-F, Lemmens VE, et al. New opportunities for drug outcomes research in cancer patients: the linkage of the Eindhoven Cancer Registry and the PHARMO Record Linkage System. Eur J Cancer 2010; 46(2): 395-404.
17. Suissa S, Azoulay L. Metformin and the risk of cancer: time-related biases in observational studies. Diabetes Care 2012; 35(12): 2665-73.
18. Stricker BH, Stijnen T. Analysis of individual drug use as a time-varying determinant of exposure in prospective population-based cohort studies. Eur J Epidemiol 2010; 25(4): 245-51.
19. Simon R, Makuch RW. A non-parametric graphical representation of the relationship between survival and the occurrence of an event: application to responder versus non-responder bias. Stat Med 1984; 3(1): 35-44.
20. Dickman PW, Adami HO. Interpreting trends in cancer patient survival. J Intern Med 2006; 260(2): 103-17.
21. Lambert PC, Royston P. Further development of flexible parametric models for survival analysis. Stata Journal 2009; 9(2): 265.
22. Sarfati D, Blakely T, Pearce N. Measuring cancer survival in populations: relative survival vs cancer-specific survival. Int J Epidemiol 2010; 39(2): 598-610.
23. Macfarlane TV, Murchie P, Watson MC. Aspirin and other non-steroidal anti-inflammatory drug prescriptions and survival after the diagnosis of head and neck and oesophageal cancer. Cancer Epidemiol 2015; 39(6): 1015-22.
24. Cao Y, Nishihara R, Wu K, et al. Population-wide Impact of Long-term Use of Aspirin and the Risk for Cancer. JAMA Oncol 2016.
25. McCowan C, Munro AJ, Donnan PT, Steele RJ. Use of aspirin post-diagnosis in a cohort of patients with colorectal cancer and its association with all-cause and colorectal cancer specific mortality. Eur J Cancer 2013; 49(5): 1049-57.
26. Ray WA. Evaluating medication effects outside of clinical trials: new-user designs. Am J Epidemiol 2003; 158(9): 915-20.
27. Vandenbroucke JP. When are observational studies as credible as randomised trials? Lancet 2004; 363(9422): 1728-31.
28. Chubak J, Boudreau DM, Wirtz HS, McKnight B, Weiss NS. Threats to validity of nonrandomized studies of postdiagnosis exposures on cancer recurrence and survival. J Natl Cancer Inst 2013; 105(19): 1456-62.
29. Baigent C, Blackwell L, Collins R, et al. Aspirin in the primary and secondary prevention of vascular disease: collaborative meta-analysis of individual participant data from randomised trials. Lancet 2009; 373(9678): 1849-60.
30. Yood MU, Campbell UB, Rothman KJ, et al. Using prescription claims data for drugs available over-the-counter (OTC). Pharmacoepidemiol Drug Saf 2007; 16(9): 961-8.
31. Bruno A, Dovizio M, Tacconelli S, Patrignani P. Mechanisms of the antitumoural effects of aspirin in the gastrointestinal tract. Best Pract Res Clin Gastroenterol 2012; 26(4): e1-e13.
32. Patrignani P, Tacconelli S, Piazuelo E, et al. Reappraisal of the clinical pharmacology of low-dose aspirin by comparing novel direct and traditional indirect biomarkers of drug action. J Thromb Haemost 2014; 12(8): 1320-30.
33. Chan AT, Ogino S, Fuchs CS. Aspirin use and survival after diagnosis of colorectal cancer. JAMA 2009; 302(6): 649-58.
34. Thun MJ, Jacobs EJ, Patrono C. The role of aspirin in cancer prevention. Nat Rev Clin Oncol 2012; 9(5): 259-67.
35. Reimers MS, Bastiaannet E, Langley RE, et al. Expression of HLA Class I Antigen, Aspirin Use, and Survival After a Diagnosis of Colon Cancer. JAMA Intern Med 2014.

CHAPTER 4

THE INFLUENCE OF BRAF AND KRAS MUTATION STATUS ON THE ASSOCIATION BETWEEN ASPIRIN USE AND SURVIVAL AFTER COLON CANCER DIAGNOSIS

Martine A. Frouws, Marlies S. Reimers, Marloes Swets, Esther Bastiaannet, Bianca Prinse, Ronald van Eijk, Valery E.P.P. Lemmens, Myrthe P.P. van Herk-Sukel, Tom van Wezel, Peter J.K. Kuppen, Hans Morreau, Cornelis J.H. van de Velde, Gerrit-Jan Liefers

PLoS One. 2017 Jan 26;12(1):e0170775

Abstract

Background

Use of aspirin after diagnosis of colon cancer has been associated with improved survival. Identification of cancer subtypes that respond to aspirin treatment may help develop personalized treatment regimens. The aim of this study was to investigate the influence of *BRAF* and *KRAS* mutation status on the association between aspirin use and overall survival after colon cancer diagnosis.

Methods

A random selection of 599 patients with colon cancer were analyzed, selected from the Eindhoven Cancer Registry, and *BRAF* and *KRAS* mutation status was determined. Data on aspirin use (80 mg) were obtained from the PHARMO Database Network. Parametric survival models with exponential (Poisson) distribution were used.

Results

Aspirin use after colon cancer diagnosis was associated with improved overall survival in wild-type *BRAF* tumors, adjusted rate ratio (RR) of 0.60 (95% CI 0.44 -0.83). In contrast, aspirin use in *BRAF* mutated tumors was not associated with an improved survival (RR 1.11, 95% CI 0.57-2.16). *P-value* for interaction was non-significant. *KRAS* mutational status did not differentiate in the association between aspirin use and survival.

Conclusion

Low-dose aspirin use after colon cancer diagnosis was associated with improved survival in *BRAF* wild-type tumors only. However, the large confidence interval of the rate ratio for the use of aspirin in patients with *BRAF* mutation does not rule out a possible benefit. These results preclude *BRAF* and *KRAS* mutation status to be used as a marker for individualized treatment with aspirin, if aspirin becomes regular adjuvant treatment for colon cancer patients in the future.

Introduction

A significant body of proof has already demonstrated that aspirin has anticancer effects in colorectal cancer (CRC)[1-5]. Randomized controlled trials investigating the cardiovascular benefits of aspirin have shown a significant reduction of CRC risk and mortality[1,6,7]. In patients with a history of colorectal adenomas, aspirin has been proven effective in the prevention of these lesions [8]. The most recent meta-analysis of observational studies by Elwood et al. found a 25% reduction in colorectal cancer-related deaths and a 20% overall mortality reduction[4]. Altogether, these publications have led to several ongoing randomized controlled trials studying the effect of aspirin on cancer mortality which are currently being conducted globally: the Add-Aspirin trial[9], Adjuvant Aspirin for Colon Cancer (NCT02467582), the ALASCCA trial (NCT02647099), the ASCOLT trial (NCT00565708), and the Aspirin trial (NCT02301286).

If the survival benefits are so obvious, why not prescribe aspirin to all colorectal cancer patients? Because of the side-effects, the use of aspirin is not without risk: common adverse effects are upper gastrointestinal symptoms, and increased bleeding tendency which can cause epistaxis, gastrointestinal bleeding or purpura[5]. Low-dose aspirin, indicated for secondary cardiovascular risk management, roughly doubles the incidence of gastric bleeding. One or two patients in every thousand are likely to have a gastric bleed each year. The bleeding risk increases with age and in patients 80 years and older, this may even be seven per 1000 people per year[10]. Identifying which patients may benefit from aspirin treatment may help develop effective personalized treatment regimens, thereby reducing overtreatment and negative side effects associated with aspirin. Several biomarkers have been suggested to be differentiating in the association between aspirin and improved cancer survival, however results are very heterogeneous[5]. Despite promising data, the clinical use of any biomarker in general practice is lacking, and currently only *KRAS*, *BRAF* and microsatellite instability are currently used in the diagnosis and treatment of colorectal cancer[11].

Mutated *BRAF* and *KRAS* oncogenes, both members of the Mitogen Activated Protein Kinase (MAPK) pathway, are respectively observed in approximately 10-20% and 35-42% of the sporadic colorectal cancers [1-13]. Mutated *BRAF* and *KRAS* have been shown to influence MAPK signaling, resulting in upregulation of Prostaglandin-endoperoxide synthase 2 (PTGS2, also known as COX-2)[14]. *BRAF* mutations are associated with the presence of high microsatellite instability, the molecular hallmark of Lynch syndrome[15]. Evidence from the CAPP2 trial demonstrated that individuals with Lynch syndrome could be recommended to consider taking daily low-dose aspirin[16]. With this link and the known crosstalk between the phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA) pathway and MAPK pathway, the assessment of *BRAF* and *KRAS* mutational status as molecular biomarker for the survival benefit associated with the use of aspirin could be a next step to unravel the biological effect of aspirin in colon cancer[17].

Therefore, the aim of this study was to investigate the association of low-dose aspirin use after colon cancer diagnosis and survival of patients according to *BRAF* and *KRAS* mutation status.

Material and Methods

Study Cohort

Data on low dose aspirin use (80-100 mg), derived from the PHARMO Database Network (PHARMO, Netherlands), were linked to the Eindhoven Cancer Registry (ECR). The validity of the linkage of these cohorts was described previously[18]. The ECR serves about 1.5 million inhabitants in the southern region of the Netherlands and is part of the nationwide Comprehensive Cancer Organisation (IKNL). The PHARMO Database Network is a population-based network and combines data from different healthcare settings in the Netherlands. The Outpatient Pharmacy Database was used for this study, which comprises drug dispensing records from all community pharmacies. The records in this database contain information on the type of product, date prescribed, dose and regimen, quantity, and route of administration. Drugs are coded using the Anatomical Therapeutic Chemical classification[19]. The Comprehensive Cancer Organisation is obliged to work according to the law on data protection; informed consent of the patients for this specific study was not applicable.

As previously published, aspirin initiated or continued after diagnosis was associated with improved survival for patients with colon cancer, but not for patients with rectal cancer, in our cohort[20]. Therefore, only patients with colon cancer were included in this study.

The vital status of patients (alive/dead) was established from medical records or through linkage of cancer registry data with the municipal population registries. As information on hospital dispensing was not available, follow-up started 14 days after diagnosis of colorectal cancer (T0), and was continued until last contact date (January 2012), date of loss to follow-up, or date of death - whichever occurred first.

Patients who only used aspirin before diagnosis were also excluded (n=40, see Fig 1). Non-users were classified as those who never had a dispensing for aspirin or had a dispensing for less than 14 days after diagnosis of colon cancer. Users were defined as those who had been given a dispensing of aspirin for 14 days or more after a colon cancer diagnosis. The median duration of one dispensing was 30 days and the mean dispensing number was 12 (range 1- 220).

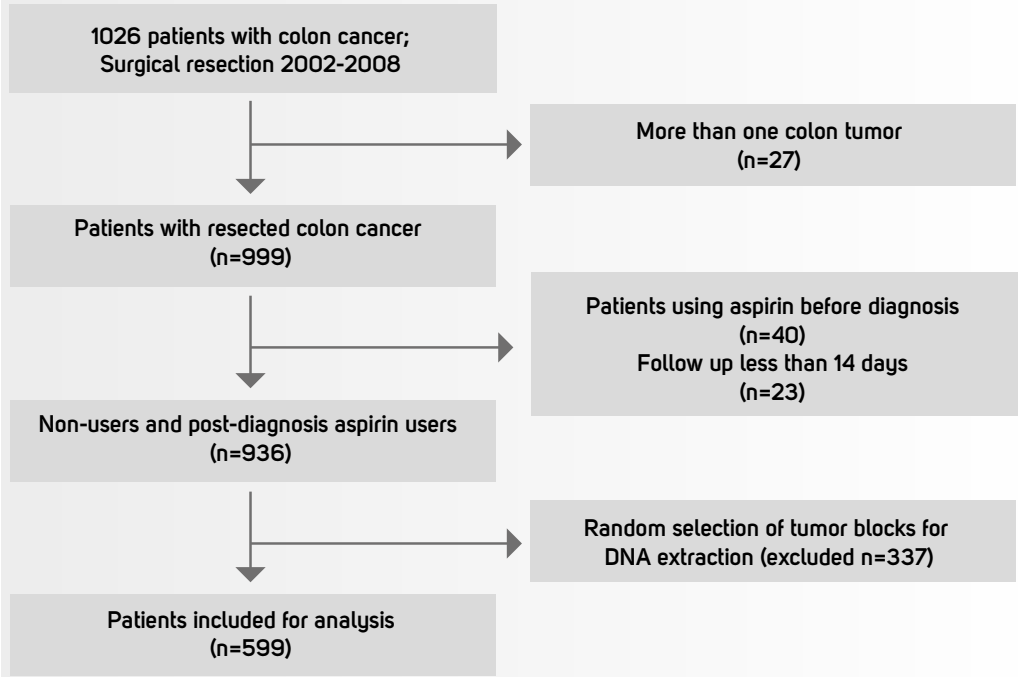
BRAF and KRAS tumor mutation analyses

The ECR-PHARMO cohort, as previously published by Bastiaannet et al, contained 3,586 patients[20]. Of this cohort, Formalin-Fixed Paraffin-Embedded (FFPE) tumor tissues were retrieved of 1,026 colon cancer patients who underwent a surgical resection between 2002 and 2008[21]. Twenty-seven patients with more than one colon tumor at the time of diagnosis were excluded from this cohort (Fig 1). Additionally, 63 patients were excluded because they used aspirin before diagnosis or with a follow up less than 14 days. Of these patients, 599 patients were randomly selected with a ratio 1:2 for aspirin user: non-user, as was previously described[22].

No significant demographic differences were calculated between the total cohort (n=999) and the randomly selected patients (n=599)[22].

Of the included patients (n=599), tumor areas on hematoxylin and eosin (H&E) stained tumor sections were marked by an experienced pathologist/researcher.

Figure 1: Flowchart of patients selected for analysis



Guided by the H&E-stained slides, 1-2 punches with a diameter of 2.0 mm diameter and variable length were taken from the tumor focus, followed by DNA extraction as described by de Jong et al[21]. For determination of KRAS and BRAF mutations status, hydrolysis probes assays were performed for the major known mutations (hotspots) in codon 600 for BRAF, c.1799T>A; p.V600E and codon 12 and 13 for KRAS; c.34G>A; p.G12S, c.34G>C; p.G12R, c.34G>T; p.G12C, c.35G>A; p.G12D, c.35G>C; p.G12A, c.35G>T; p.G12V, c.38G>A; p.G13D and c.37G>T; p.G13C, as previously described[21]. Hydrolysis probe assays were analyzed using qPCR analysis software (CFX manager version 3/0, Bio-Rad). Mutation detection was performed by two independent observers (M.R. and R.E.). All primers used for the assays were previously described[23].

Statistics

Statistical analyses were performed using the statistical packages SPSS (version 20.0 for Windows, IBM SPSS statistics) and Stata (version 12 for windows, StataCorp LP). Statistical tests were two-sided and considered significant at a *p*-value below 0.05.

A parametric survival model with an exponential (Poisson) distribution was used, with the use of aspirin as time varying covariate. This method prevents the introduction of time-related biases[24]. Non-users were defined from T0 until date of death or end of follow-up. Patients were considered aspirin users from the moment of first prescription, mimicking an intention-to-treat analysis. In order to investigate differential associations of aspirin use with overall survival by tumor molecular subtype, stratified analyses were performed for *BRAF* wild-type / *BRAF* mutation and *KRAS* wild-type / *KRAS* mutation,

followed by an interaction analysis. The interaction analysis was performed by including a cross product of *BRAF* mutation status in the survival analysis and the use of aspirin and significance was assessed with the Wald test.

Adjustments for potential confounders were made for sex, age (groups), stage (pathological stage and clinical stage if pathological stage was unknown), adjuvant chemotherapy (yes/no), co-morbidity (yes/no) and tumor grade.

Survival curves were calculated according to the Simon-Makuch method, an alternative for Kaplan Meier, with the ability to include time-varying covariates[25].

A subgroup analysis was performed by excluding patients with stage IV disease.

Results

Aspirin use, survival and tumor BRAF mutation status

Fig 1 shows the flowchart of the study population eligible for analysis. In this cohort, 29.9% (179/599) of the patients were defined as aspirin users. Of the 179 patients who used aspirin after diagnosis, 27 patients started using aspirin after diagnosis and 155 used already aspirin at diagnosis. In total, 267 deaths were recorded before January 2012.

DNA was extracted from FFPE tumor tissues and *BRAF* mutation status (wild-type/mutation) was successfully established in 98% of the samples. A BRAF mutation was found in 17% (102/599) and a *KRAS* mutation was observed in 35% of colon tumors (212/599), in accordance with previous studies[11-13,26,27].

Table 1: Baseline Characteristics of the cohort

		All patients		Non-users		Aspirin users	
		n	%	n	%	n	%
	Total	599	100	420	100	179	100
Sex	Male	327	54.6	215	51.2	112	62.6
	Female	272	45.4	205	48.8	67	37.4
Age category	<65	189	31.6	158	37.6	31	17.3
	66-74	189	31.6	118	28.1	71	39.7
	75 and older	221	36.9	144	34.3	77	43.0
Year of diagnose	2002-2004	300	50.1	208	49.5	92	51.4
	2005-2007	299	49.9	212	50.5	87	48.6
Disease stage	I	95	15.9	57	13.6	38	21.2
	II	237	39.7	166	39.7	71	39.7
	III	176	29.5	121	28.9	55	30.7
	IV	89	14.9	74	17.7	15	8.4
Comorbidity	No	209	34.9	176	41.9	33	18.4
	Yes	342	57.1	202	48.1	140	78.2
	Missing	48	8	42	10	6	3.4
BRAF mutation analysis	Wild-type	497	83	347	82.6	150	83.8
	Mutation	102	17	73	17.4	29	16.2
KRAS mutation analysis	Wild-type	387	64.6	274	65.2	113	63.1
	Mutation	212	35.4	146	34.8	66	36.9

Table 2: Rate Ratio for Death (Time-Dependent Analysis Overall Survival), According to Tumor *BRAF* and *KRAS* mutation status, and use or no use of aspirin after Diagnosis

				Univariate		Multivariate	
		n	Events	RR (95%CI)	P-value	RR[a] (95%CI)	P-value
Overall		*599*					
	No aspirin use	420	199	*1.00 (reference)*	**0.03**	*1.00 (reference)*	**0.003**
	Aspirin use	179	68	**0.73 (0.56-0.97)**		**0.64 (0.48-0.86)**	
BRAF mutation status							
Wild-type		*497*					
	No aspirin use	347	159	*1.00 (reference)*		*1.00 (reference)*	**0.002**
	Aspirin use	150	55	0.74 (0.54-1.00)[b]	0.05	**0.60 (0.44-0.83)**	
Mutation		*102*					
	No aspirin use	73	40	*1.00 (reference)*	0.34	*1.00 (reference)*	0.77
	Aspirin use	29	13	0.74 (0.39-1.38)[b]		1.11 (0.57-2.16)	
KRAS mutation status							
Wild-type		*387*					
	No aspirin use	274	130	*1.00 (reference)*	0.11	*1.00 (reference)*	**0.03**
	Aspirin use	113	43	0.75 (0.53-1.06)		**0.67 (0.47-0.97)**	
Mutation		*212*					
	No aspirin use	146	69	*1.00 (reference)*	0.14	*1.00 (reference)*	**0.03**
	Aspirin use	66	25	0.71 (0.45-1.11)		**0.56 (0.34-0.93)**	

Significant values are printed in bold
[a] *Adjusted for age, comorbidity, grade, stage and chemotherapy*
[b] *P-value for interaction=0.99*

Table 1 summarizes the clinical characteristics of the patients included in the analysis. Both age and frequency of comorbidities were found to be higher in the group of aspirin users compared to non-users. Lower disease stage and male sex were more often observed in aspirin users compared to non-users.

More detailed patient characteristics according to aspirin use and *BRAF* and *KRAS* mutation status are shown in S1 Table. Aspirin use was equally distributed: 29% in patients with wild-type *BRAF* tumors, 27% in patients with mutated *BRAF* tumors, and 29% in patients with *KRAS* wild-type and 31% in patients with mutated *KRAS* tumors.

As shown in Table 2 and Fig 2 aspirin use after diagnosis was associated with an improved overall survival in the total cohort (n=599) (crude Rate Ratio (RR) 0.73, 95% Confidence Interval (CI) 0.56-0.97, adjusted RR 0.64 (95% CI 0.48-0.86)). Fig 3 shows the survival curves for these patients.

For patients with a BRAF wild-type tumor, aspirin use after diagnosis showed a RR for overall survival of 0.74 (95% CI 0.54-1.00), and when adjusted for potential confounders this effect was more pronounced with an adjusted RR of 0.60 (95% CI 0.44-0.83, p=0.002, Fig 3).

S1 Table: Baseline Characteristics of the Colon Cancer Patients according to BRAF and KRAS mutation status and aspirin use

		BRAF mutation analysis									
		BRAF wild-type					BRAF mutation				
		n=497					n=102				
		Nonuser		Aspirin user			Nonuser		Aspirin user		
		n	%	n	%	p-value*	n	%	n	%	p-value*
	Total	347	79.9	150	29.0		73	73.4	29	26.6	
Sex	Male	189	54.5	93	62.0	0.12	26	35.6	19	65.5	**0.01**
	Female	158	45.5	57	38.0		47	64.4	10	34.5	
Age category	<65	138	39.8	29	19.3	**<0.001**	20	27.4	2	6.9	**0.04**
	66-74	97	28.0	57	38.0		21	28.8	14	48.3	
	75 and older	112	32.3	64	42.7		32	43.8	13	44.8	
Year of diagnose	2002-2004	170	49.0	79	52.7	0.45	38	52.1	13	44.8	0.51
	2005-2007	177	51.0	71	47.3		35	47.9	16	55.2	
Disease stage	I	49	14.1	30	20.0	0.68	8	11.0	8	27.6	**0.03**
	II	133	38.3	55	36.7		33	45.2	16	55.2	
	III	99	28.5	50	33.3		22	30.1	5	17.2	
	IV	64	18.4	15	10.0		10	13.7	0	0.0	
		2	0.6	0	0.0		0	0.0	0	0.0	
Comorbidity	No	150	43.2	29	19.3	**<0.001**	26	36.6	4	13.8	**0.01**
	Yes	161	46.4	115	76.7		41	56.2	25	86.2	
	Missing	36	10.4	6	4.0		6	8.2	0	0.0	

		KRAS mutation analysis									
		KRAS wild-type					KRAS mutation				
		n=387					n=212				
		Nonuser		Aspirin user			Nonuser		Aspirin user		
		n	%	n	%	p-value*	n	%	n	%	p-value*
	Total	274	70.8	113	29.2		146	68.9	66	31.1	
Sex	Male	138	50.4	63	55.8	0.34	77	52.7	49	74.2	**0.03**
	Female	136	49.6	50	44.2		69	47.3	17	25.8	
Age category	<65	103	37.6	15	13.3	**<0.001**	55	37.7	16	24.2	0.16
	66-74	78	28.5	50	44.2		40	27.4	21	31.8	
	75 and older	93	33.9	48	42.5		51	34.9	29	43.9	
Year of diagnose	2002-2004	141	51.5	59	52.2		67	45.9	33	50.0	0.58
	2005-2007	133	48.5	54	47.8	0.89	79	54.1	33	50.0	
Disease stage	I	38	13.9	25	22.1	0.19	19	13.0	13	19.7	**0.02**
	II	113	41.2	48	42.5		53	36.3	23	34.8	
	III	77	28.1	28	24.8		44	30.1	27	40.9	
	IV	44	16.1	12	10.6		30	20.5	3	4.5	
		2	0.7	0	0		0	0.0	0	0.0	
Comorbidity	No	114	41.6	19	16.8	**<0.001**	62	42.5	14	21.2	**<0.001**
	Yes	135	49.3	90	79.6		67	45.9	50	75.8	
	Missing	25	9.1	4	3.5		17	11.6	2	3.0	

Significant values are printed in bold
** p-value for aspirin users vs nonusers*

For patients with *BRAF* mutated tumors, aspirin use after diagnosis was not associated with an improved survival (adjusted RR 0.74, 95% CI 0.39-1.38, p=0.34). The Wald test showed a P for interaction of 0.99, which suggests that the difference found between the group of patients with a *BRAF* wild-type or mutation is based on chance.

For patients with a *KRAS* mutated tumor and patients with a KRAS wild-type tumor, aspirin use after diagnosis was associated with an improved overall survival in the multivariate analysis (*KRAS* wild-type RR 0.68 (0.67 95%CI 0.47-0.97) and *KRAS* mutant RR 0.56 (95% CI 0.34-0.93)), (Table 2, Fig 3).

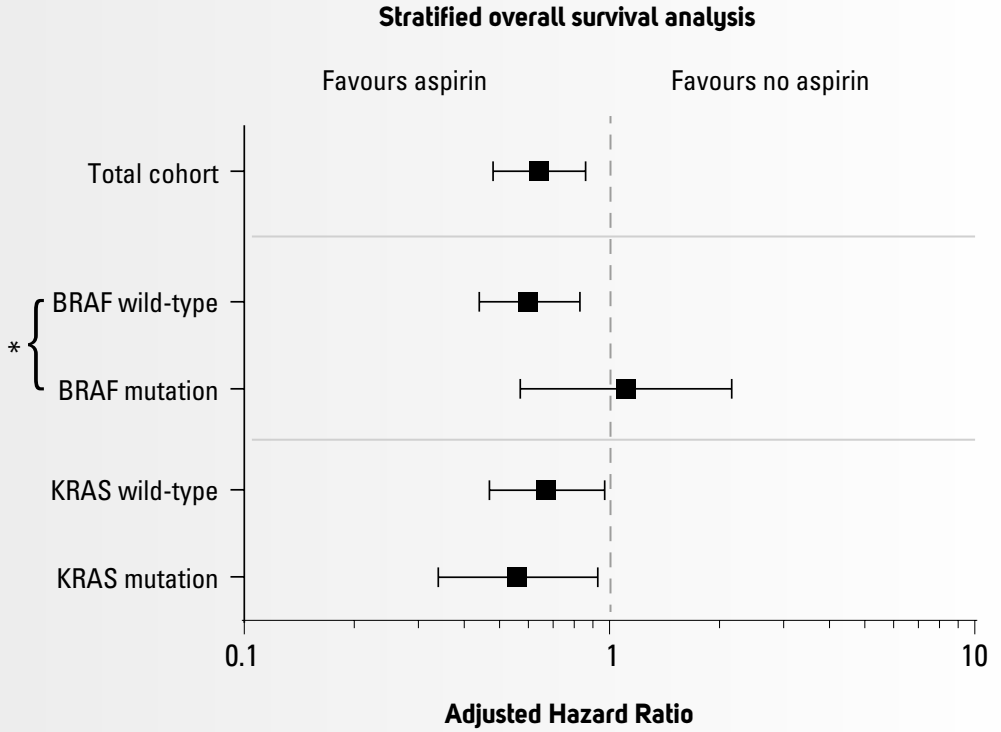
The results from the subgroup analysis that excluded patients with stage IV can be found in S2 table.

Discussion

Overview of Findings

Increasing attention is paid to a personalized treatment approach, by stratifying patients into subgroups based on biomarkers. This study investigated whether the survival benefit observed in patients with colon cancer using aspirin could be associated with *BRAF* or *KRAS* mutational status. This study found that *BRAF* mutation status and *KRAS* mutation status were not distinctive in the association between low-dose aspirin use and a survival benefit in patients with colon cancer. In the multivariate analysis, patients with wild-type *BRAF* tumors, aspirin use after diagnosis was associated with a significantly better outcome. However, the crude hazard ratios in both groups (*BRAF* wild-type and mutation) are equal and the P-value for interaction was non-significant. Because no statistical interaction was observed, the distinctiveness of *BRAF* mutational status on the association between aspirin use and survival in the multivariate analysis could very well be based

Figure 2: Overall survival analysis for patients using aspirin versus patients not using aspirin, grouped according to mutation status



* Non significant interaction test

on chance. Therefore, it could not be concluded from this study that patients with *BRAF* mutated tumors should be withheld from using aspirin. The subgroup analysis in patients with stage I-III colon cancer showed a reduced effect size. However, due to limitations in power, no firm conclusions can be drawn from the results of this subgroup analysis.

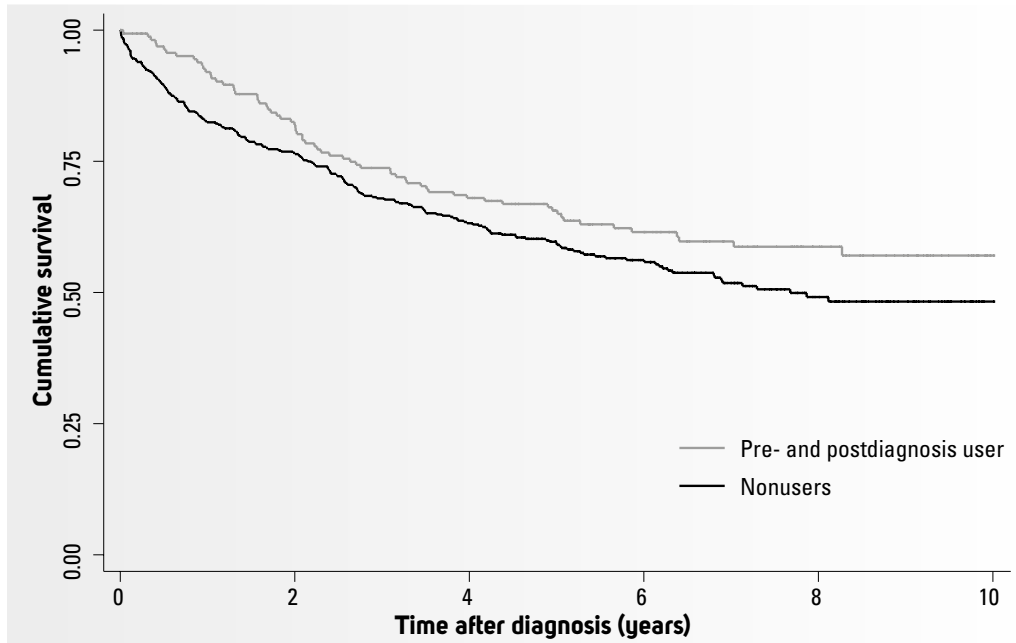
Comparison with Other Studies

Nishihara et al[26] previously studied the effect of *BRAF* mutational status on colorectal cancer incidence and survival in patients using aspirin. *BRAF* mutational status showed to be of influence on the incidence of colorectal cancer. *BRAF* wild-type was associated with a lower risk of colorectal cancer, multivariable hazard ratio; 0.73 (95% CI 0.64-0.83) whereas *BRAF* mutated tumors did not show a reduced risk of colorectal cancer (HR 1.03, 95% CI 0.76-1.38). A survival analysis in this study was performed as an exploratory analysis, and in both subgroups (*BRAF* mutation and wild-type tumors) no association between the use of aspirin and improved survival was found, in line with our study.

Strengths and limitations

Our study has several strengths. To the best of our knowledge, no studies have assessed the association between *KRAS*, aspirin and survival in patients with (colon)

Figure 3: Survival curves for aspirin users versus non-users according to the Simon-Makuch method



cancer. Information regarding aspirin use and dose was derived from prescriptions rather than patient recall, resulting in a precise definition of regular aspirin use. By using a time-varying covariate for the use of aspirin, the risk of non-differential misclassification is reduced[24]. Lastly, a robust and reliable method was used to determine *BRAF* and *KRAS* mutational status, resulting in a 98% successful determination of mutational status and therefore a relatively large cohort.

However, several limitations must be acknowledged. First, over-the-counter aspirin use and adherence was unknown and could be a potential source of bias. Nevertheless, it has been shown that pharmacy data can give valid estimates, despite over-the-counter availability of aspirin[28]. It seems unlikely that a large fraction of patients bought aspirin over-the-counter: low-dose aspirin is only indicated for secondary cardiovascular prevention in the Netherlands and therefore this should always be made available through a doctor's prescription. The main reason for over-the-counter purchase of aspirin is its use as analgesic, however low-dose aspirin does not suffice as analgesic. Moreover, the possible benefits of aspirin as treatment for cancer were not widely known during the analysis period.

Second, this is a retrospective study in which patients were not randomized. Even after adjustment for potential confounders, residual confounding may still be present. Confounding by indication could, in general, have resulted in overestimation of the results. For cancer patients to be prescribed aspirin, patients should have a cancer prognosis which outweighs the risk of cardiovascular disease. Patients to whom this does not apply should, in theory, not be prescribed aspirin. These patients are then assigned into the non-user group which could have resulted in an overestimation of the association between aspirin use and survival. The variation in length of use of aspirin and

S2 table: Rate ratio for death (time-dependent analysis overall survival), according to tumor braf and kras mutation status, and use or no use of aspirin after diagnosis, patients with stage iv disease excluded

				Univariate		Multivariate	
		n	Events	RR (95%CI)	P-value	RR[a] (95%CI)	P-value
Overall		*510*					
	No aspirin use	213	133	*1.00 (reference)*	0.32	*1.00 (reference)*	**0.02**
	Aspirin use	109	55	0.85 (0.62-1.17)		**0.67 (0.50-0.95)**	
BRAF mutation status							
Wild-type		*418*					
	No aspirin use	283	103	*1.00 (reference)*	0.31	*1.00 (reference)*	**0.02**
	Aspirin use	135	42	0.83 (0.58-1.19)		**0.63 (0.44-0.92)**	
Mutation		*92*					
	No aspirin use	63	30	*1.00 (reference)*	0.89	*1.00 (reference)*	0.65
	Aspirin use	29	13	0.95 (0.50-1.83)		1.17 (0.59-2.33)	
KRAS mutation status							
Wild-type		*331*					
	No aspirin use	230	90	*1.00 (reference)*	0.31	*1.00 (reference)*	**0.04**
	Aspirin use	101	33	0.81 (0.55-1.21)		**0.65 (0.43-0.98)**	
Mutation		*179*					
	No aspirin use	116	43	*1.00 (reference)*	0.78	*1.00 (reference)*	0.31
	Aspirin use	63	22	0.93 (0.56-1.55)		0.75 (0.44-0.1.30)	

Significant values are printed in bold
[a] *Adjusted for age, comorbidity, grade, stage and chemotherapy*

the moment patients start using aspirin makes it difficult to conclude any causality from this study, only associations were observed. Therefore, the current ongoing randomised controlled trials are highly warranted. However, this is a limitation of all retrospective studies.

Third, no information regarding disease-specific survival was available in this study. However, a large meta-analysis of individual patient data found that the benefit of patients using aspirin as secondary prevention for cardiovascular disease is only 0.91 (95% CI 0.82-1.00)[29]. This can therefore not fully explain the observed overall survival benefit for the aspirin users in the current study.

To the best of our knowledge, this study is one of the largest cohorts analyzing the association between the use of aspirin, overall survival in colon cancer patients and mutational status of BRAF and KRAS, however numbers were too small for any additional subgroup analyses.

Clinical implications

Precision medicine has gained more attention over the last couple of years and multiple publications were dedicated to the discovery and development of clinical prognostic and predictive biomarkers[11]. Nevertheless, conflicting results have been

observed for every previous appointed biomarker regarding the association between aspirin use and survival. Proposed biomarkers associated with aspirin use and survival are COX-2, HLA class I, PIK3CA mutation status and several specific genetic profiles[22,30-32]. Mutations in *BRAF* and *KRAS*, acting in the *RAS-RAF-MAPK* kinase cascade and mutated PIK3CA, acting in *PI3K-PTEN-AKT* signaling pathway, are known for their contribution to the development of CRC and are associated with cancer prognosis[11,33]. The strong survival benefit in patients with a PIK3CA mutation can only partly explain the effect of aspirin found in the general cancer population. The magnitude of the clinical benefit as found in CRC cohorts, cannot be explained by patients with a PIK3CA mutation solely, because of the low mutation frequency (15%). Therefore, additional biological processes must be responsible for the effect of aspirin on survival.

In this study we were focusing on the *RAS-RAF-MAPK* cascade, known for the crosstalk with the PIK3CA pathway, in relation to aspirin use[17]. No differentiating effect of aspirin use in *BRAF* or *KRAS* mutated tumors could be detected. It could be (cautiously) concluded that biomarkers from the *RAS-RAF-MAPK* cascade and an activated PI3K-PTEN-AKT signaling pathway may not be able to fully unravel the complexity and versatility of the aspirin effect on cancer. Therefore, the evidence points more towards a generalized, systemic effect[5,34].

One suggested hypothesis is the role of aspirin as thrombocyte aggregation inhibitor[34]. By inhibiting the aggregation of thrombocytes, which naturally shape around circulating tumor cells, the immune system is able to detect and subsequently clear tumor cells from the circulation. Another hypothesis could be found in the anti-inflammatory effects of aspirin[35]. In the past years, several publications focused on the identification of subtypes of colorectal cancer, highlighting the heterogeneity of the disease and aiming to improve optimal allocation of treatment modalities[11,36,37]. Linnekamp et al advocate that the development of new agents should take place in a disease sub-type-specific fashion and in that manner generate more effective therapies[36]. These subtypes could also be the key to personalized treatment with aspirin. With this information and growing consensus on these subtypes, new research could focus on the effect of aspirin in (inflammatory) specific subtypes.

Acknowledgements

The authors acknowledge colleagues of the laboratory of the department of Surgery of the Leiden University Medical Center, with special thanks to Ronald L.P. van Vlierberghe.

Reference list

1. Rothwell PM, Wilson M, Elwin CE, et al. Long-term effect of aspirin on colorectal cancer incidence and mortality: 20-year follow-up of five randomised trials. *Lancet* 2010; **376**(9754): 1741-50.
2. Rothwell PM, Fowkes FG, Belch JF, Ogawa H, Warlow CP, Meade TW. Effect of daily aspirin on long-term risk of death due to cancer: analysis of individual patient data from randomised trials. *Lancet* 2011; **377**(9759): 31-41.
3. Rothwell PM, Wilson M, Price JF, Belch JF, Meade TW, Mehta Z. Effect of daily aspirin on risk of cancer metastasis: a study of incident cancers during randomised controlled trials. *Lancet* 2012; **379**(9826): 1591-601.
4. Elwood PC, Morgan G, Pickering JE, et al. Aspirin in the Treatment of Cancer: Reductions in Metastatic Spread and in Mortality: A Systematic Review and Meta-Analyses of Published Studies. *PLoS One* 2016; **11**(4): e0152402.
5. Coyle C, Cafferty FH, Langley RE. Aspirin and Colorectal Cancer Prevention and Treatment: Is It for Everyone? *Current Colorectal Cancer Reports* 2016.
6. *Algra AM, Rothwell PM. Effects of regular aspirin on long-term cancer incidence and metastasis: a systematic comparison of evidence from observational studies versus randomised trials. Lancet Oncol* 2012; **13**(5): 518-27.
7. Rothwell PM, Price JF, Fowkes FG, et al. Short-term effects of daily aspirin on cancer incidence, mortality, and non-vascular death: analysis of the time course of risks and benefits in 51 randomised controlled trials. *Lancet* 2012.
8. *Cole BF, Logan RF, Halabi S, et al. Aspirin for the chemoprevention of colorectal adenomas: meta-analysis of the randomized trials. J Natl Cancer Inst* 2009; **101**(4): 256-66.
9. Coyle C, Cafferty FH, Rowley S, et al. ADD-ASPIRIN: A phase III, double-blind, placebo controlled, randomised trial assessing the effects of aspirin on disease recurrence and survival after primary therapy in common non-metastatic solid tumours. *Contemp Clin Trials* 2016; **51**: 56-64.
10. Elwood PC, Gallagher AM, Duthie GG, Mur LA, Morgan G. Aspirin, salicylates, and cancer. *Lancet* 2009; **373**(9671): 1301-9.
11. Reimers MS, Zeestraten EC, Kuppen PJ, Liefers GJ, van de Velde CJ. Biomarkers in precision therapy in colorectal cancer. *Gastroenterol Rep (Oxf)* 2013; 1(3): 166-83.
12. Lao VV, Grady WM. Epigenetics and colorectal cancer. *Nat Rev Gastroenterol Hepatol* 2011; **8**(12): 686-700.
13. Ogino S, Chan AT, Fuchs CS, Giovannucci E. Molecular pathological epidemiology of colorectal neoplasia: an emerging transdisciplinary and interdisciplinary field. *Gut* 2011; **60**(3): 397-411.
14. Wagner EF, Nebreda AR. Signal integration by JNK and p38 MAPK pathways in cancer development. *Nat Rev Cancer* 2009; **9**(8): 537-49.
15. van Lier MG, Leenen CH, Wagner A, et al. Yield of routine molecular analyses in colorectal cancer patients </=70 years to detect underlying Lynch syndrome. *J Pathol* 2012; **226**(5): 764-74.
16. Burn J, Mathers J, Bishop DT. Lynch syndrome: history, causes, diagnosis, treatment and prevention (CAPP2 trial). *Dig Dis* 2012; **30 Suppl 2**: 39-47.
17. Barault L, Veyrie N, Jooste V, et al. Mutations in the RAS-MAPK, PI(3)K (phosphatidylinositol-3-OH kinase) signaling network correlate with poor survival in a population-based series of colon cancers. *Int J Cancer* 2008; **122**(10): 2255-9.
18. van Herk-Sukel MP, LV vdP-F, Lemmens VE, et al. New opportunities for drug outcomes research in cancer patients: the linkage of the Eindhoven Cancer Registry and the PHARMO Record Linkage System. *Eur J Cancer* 2010; **46**(2): 395-404.
19. 2015 WCCfDSM. ATC classification index with DDDs 2015. Oslo, Norway, 2014; 2015.
20. Bastiaannet E, Sampieri K, Dekkers OM, et al. Use of aspirin postdiagnosis improves survival for colon cancer patients. *Br J Cancer* 2012; **106**(9): 1564-70.
21. Eijk van R, Stevens L, Morreau H, van WT. Assessment of a fully automated high-throughput DNA extraction method from formalin-fixed, paraffin-embedded tissue for KRAS, and BRAF somatic mutation analysis. *Exp Mol Pathol* 2013; 94(1): 121-5.
22. Reimers MS, Bastiaannet E, Langley RE, et al. Expression of HLA Class I Antigen, Aspirin Use, and Survival After a Diagnosis of Colon Cancer. *JAMA Intern Med* 2014.
23. *van Eijk R, Licht J, Schrumpf M, et al. Rapid KRAS, EGFR, BRAF and PIK3CA mutation analysis of fine needle aspirates from non-small-cell lung cancer using allele-specific qPCR. PLoS One 2011;* **6**(3): e17791.
24. Stricker BH, Stijnen T. Analysis of individual drug use as a time-varying determinant of exposure in prospective population-based cohort studies. *Eur J Epidemiol* 2010; **25**(4): 245-51.
25. Simon R, Makuch RW. A non-parametric graphical representation of the relationship between survival and the occurrence of an event: application to responder versus non-responder bias. *Stat Med* 1984; **3**(1): 35-44.
26. Nishihara R, Lochhead P, Kuchiba A, et al. Aspirin use and risk of colorectal cancer according to BRAF mutation status. *JAMA* 2013; **309**(24): 2563-71.
27. Ogino S, Nosho K, Kirkner GJ, et al. CpG island methylator phenotype, microsatellite instability, BRAF mutation and clinical outcome in colon cancer. *Gut* 2009; **58**(1): 90-6.
28. Yood MU, Campbell UB, Rothman KJ, et al. Using prescription claims data for drugs available over-the-counter (OTC). *Pharmacoepidemiol Drug Saf* 2007; **16**(9): 961-8.
29. Baigent C, Blackwell L, Collins R, et al. Aspirin in the primary and secondary prevention of vascular disease: collaborative meta-analysis of individual participant data from randomised trials. *Lancet* 2009; **373**(9678): 1849-60.
30. Paleari L, Puntoni M, Clavarezza M, DeCensi M, Cuzick J, DeCensi A. PIK3CA Mutation, Aspirin Use after Diagnosis and Survival of Colorectal Cancer. A Systematic Review and Meta-analysis of Epidemiological Studies. *Clin Oncol (R Coll Radiol)* 2016; **28**(5): 317-26.
31. Chan AT, Ogino S, Fuchs CS. Aspirin use and survival after diagnosis of colorectal cancer. *JAMA* 2009; **302**(6): 649-58.
32. Nan H, Hutter CM, Lin Y, et al. Association of aspirin and NSAID use with risk of colorectal cancer according to genetic variants. *JAMA* 2015; **313**(11): 1133-42.
33. Sinicrope FA, Okamoto K, Kasi PM, Kawakami H. Molecular Biomarkers in the Personalized Treatment of Colorectal Cancer. *Clin Gastroenterol Hepatol* 2016; **14**(5): 651-8.
34. Guillem-Llobat P, Dovizio M, Bruno A, et al. Aspirin prevents colorectal cancer metastasis in mice by splitting the crosstalk between platelets and tumor cells. *Oncotarget* 2016.
35. *Chia WK, Ali R, Toh HC. Aspirin as adjuvant therapy for colorectal cancer--reinterpreting paradigms. Nat Rev Clin Oncol* 2012; **9**(10): 561-70.
36. Linnekamp JF, Wang X, Medema JP, Vermeulen L. Colorectal cancer heterogeneity and targeted therapy: a case for molecular disease subtypes. *Cancer Res* 2015; **75**(2): 245-9.
37. Guinney J, Dienstmann R, Wang X, et al. The consensus molecular subtypes of colorectal cancer. *Nat Med* 2015; **21**(11): 1350-6.

CHAPTER 5

NO ASSOCIATION BETWEEN METFORMIN USE AND SURVIVAL IN PATIENTS WITH PANCREATIC CANCER: AN OBSERVATIONAL COHORT STUDY

Martine A. Frouws, Babs G. Sibinga Mulder, Esther Bastiaannet, Marjolein M.J. Zanders, Myrthe P.P. van Herk-Sukel, Eleonora M. de Leede, Bert A. Bonsing, J.Sven.D. Mieog, Cornelis J.H. Van de Velde, Gerrit-Jan Liefers

Medicine (Baltimore) 2017 Mar;96(10):e6229

Abstract

Objective

Several studies have suggested an association between use of metformin and an increased overall survival in patients diagnosed with pancreatic cancer, however with several important methodological limitations. The aim of the study was to assess the association between overall survival, pancreatic cancer and metformin use.

Material and Methods

A retrospective cohort study of 1111 patients with pancreatic cancer was conducted using data from the Netherlands Comprehensive Cancer Organization (1998-2011). Data were linked to the PHARMO Database Network containing drug-dispensing records from community pharmacies. Patients were classified as metformin user or sulfonylurea derivatives user from the moment of first dispensing until the end of follow up. The difference in overall survival between metformin users and nonusers was assessed, and additionally between metformin users and sulfonylurea derivatives users. Univariable and multivariable parametric survival models were used and use of metformin and sulfonylurea derivatives was included as time varying covariates.

Results

Of the 1111 patients, 91 patients were excluded due to differences in morphology, 48 patients because of using merely metformin before diagnosis, and 57 metformin-users ever used contemporary sulfonylurea derivatives and were therefore excluded. Lastly, eight patients with a survival of zero months were excluded. This resulted in 907 patients for the analysis. Overall, 77 users of metformin, 43 users of sulfonylurea derivatives, and 787 nonusers were identified. The adjusted rate ratio for overall survival for metformin users vs nonusers was 0.86 (95% CI 0.66-1.11; P=0.25). The difference in overall survival between metformin users and sulfonylurea derivatives users showed an adjusted rate ratio of 0.90 (95%CI 0.59-1.40; P=0.67).

Conclusions

No association was found between overall survival, pancreatic cancer and metformin use. This was in concordance with two recently published randomized controlled trials. Future research should focus on the use of adjuvant metformin in other cancer types and the development or repurposing of other drugs for pancreatic cancer.

Introduction

Pancreatic cancer is the eighth most common cause of cancer deaths in the world.[1] Only 10-20% of patients with pancreatic cancer qualify for surgery and the prognosis of this disease is poor; median survival for patients undergoing surgery ranges from 20 to 23 months.[2,3] For patients receiving solely chemotherapy, survival approximately ranges from 3 to 11 months.[1,4] With this limited prognosis there is a high and urgent need for new therapies to improve outcome.

Smoking, obesity, and type 2 diabetes are considered to be important risk factors for the development of pancreatic cancer.[5,6] Metformin is the first line treatment for patients with type 2 diabetes and is therefore the most prescribed oral glucose-lowering drug (OGLD). The decision to prescribe metformin depends on patient characteristics: metformin use is contraindicated in patients with renal failure, cardiac dysfunction and hepatic insufficiency.[7] Metformin is a biguanide antihyperglycemic agent and has three working mechanisms: it decreases the hepatic glucose production by inhibition of gluconeogenesis and glycogenolysis in muscles, it subsequently improves peripheral insulin sensitivity, and reduces glucose absorption.[7-9] Mouse models suggest that metformin could inhibit the proliferation of xenografted human pancreatic cancer cells within 30 days, but other studies point towards a systemic effect of metformin on cancer by improving the metabolic profile of patients, rather than a direct effect on tumor cells.[9-12]

Recent epidemiologic cohort studies in patients with type 2 diabetes have suggested that patients using metformin have a decreased risk of developing cancer and, possibly, a reduced cancer mortality.[13-16]

Several meta-analysis pointed out that the reduced cancer incidence was not present in all types of cancer; use of metformin seems to be associated with a reduced risk of developing cancer in patients with pancreatic, colorectal, and hepatocellular cancer, with conflicting results for breast cancer, and no association is seen in patients with lung and prostate cancer.[17-21] Preceding epidemiologic studies assessing the effect of metformin on the risk of cancer and survival may have been subject to several time-related biases, e.g. misclassifying exposure to metformin, which could have inflated the estimates. Three studies avoiding these biases have found no effect of metformin on cancer incidence.22 What additionally complicates observational studies on this subject, is that patients using metformin often have other comorbidities supplementary to type 2 diabetes, compared to non-users. Alternative treatment for type 2 diabetes are sulfonylurea derivatives users (SD), which have been used previously as a comparator group in addition to non-users.[15]

The aim of this study was to assess the association between the use of metformin and overall survival in patients with pancreatic cancer with the use of appropriate methodology, a pitfall of the previous studies. Patients using metformin were compared to non-users and additionally to patients using sulfonylurea derivatives.

Methods

Data

Data from the Eindhoven Cancer Registry (ECR) were linked on patient level to the PHARMO Database Network covering a demographic region in the South-Eastern part of the Netherlands of approximately 1.5 million inhabitants.[23] The ECR is maintained by the Netherlands Comprehensive Cancer Organisation (IKNL) and registers newly diagnosed cancer patients from ten different hospitals located in this region. Patients are informed about this registration and are registered except patients who objected to be registered. The Netherlands Cancer Registry is obliged to work according to the law about protection of privacy data and all procedures to privacy of doctors and patients is fixed in regulations. An independent Committee of Privacy reassures that the Netherlands Cancer Registry is compliant to these regulations. Therefore informed consent of the patients for this specific study was not applicable.

The PHARMO Database Network is a population-based network of healthcare databases and combines data from different healthcare settings in the Netherlands. For this study, the Outpatient Pharmacy Database is used containing drug-dispensing records from community pharmacies. All dispensed drugs are coded according to the Anatomical Therapeutic Chemical (ATC) classification (www.whocc.no/atc_ddd_index), and the records include information on type of product, date, dosage, and quantity.

Study population

All patients diagnosed with a malignancy of the pancreas (classified according to the International Statistical Classification of Diseases and Related Health Problems - 10th revision (ICD-10) code C-25), between 1998 and 2011 were selected from the ECR-PHARMO cohort. To reduce confounding by indication, metformin-users were, in addition to non-users, compared to sulfonylurea derivatives users-users. Excluded were malignancies with the following morphology; squamous cell carcinoma, epithelial carcinoma, cystic/mucinous/serous carcinoma or gastro-intestinal stromal tumor (GIST) because of the differences in disease course. The in- and excluded morphology codes can be found in supplementary table 1.

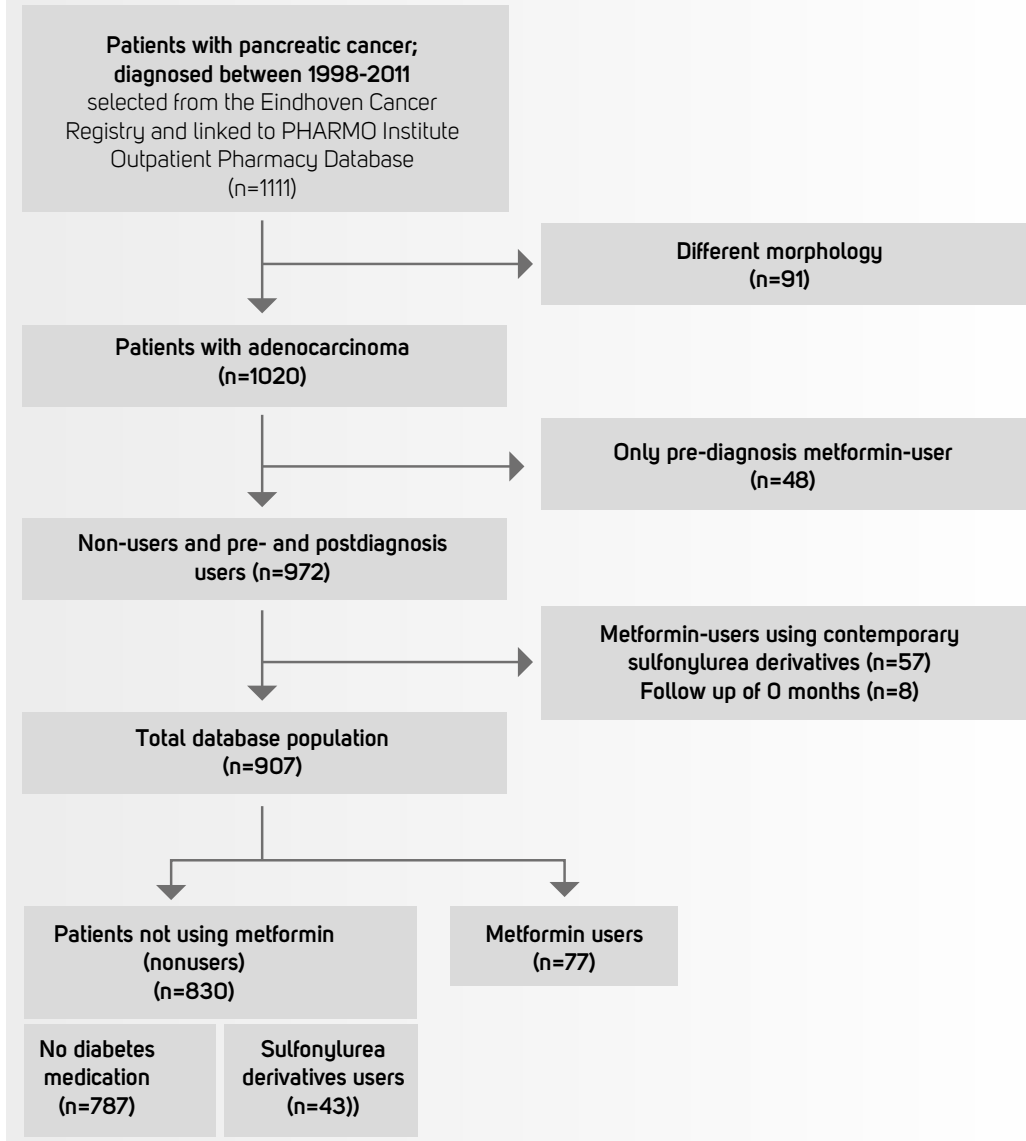
Exposure

Patients using metformin (ATC-code: A10BA02) or sulfonylurea derivatives users (ATC-code: A10BB) for at least 30 days were defined as users. Users were defined as patients using metformin before and after diagnosis, or only after diagnosis, and not using contemporary sulfonylurea derivatives users. Patients who used metformin solely before diagnosis were excluded from this analysis. Non-users were defined as patients who never used metformin or sulfonylurea derivatives. sulfonylurea derivatives-users were defined as patients who used solely sulfonylurea derivatives.

Analysis

The period of metformin or sulfonylurea derivatives use was defined from the first dispensing of metformin or sulfonylurea derivatives to the end of the follow up period. Follow up time was determined from date of diagnosis (T0) until death or end of the study

Figure 1: Flowchart of patients selected for analysis



period at 31 December 2012. Cancer registry data were linked to municipal population registries to obtain vital status. To determine time-dependent exposure, patients were defined as non-users from T0 to the date of first metformin or sulfonylurea derivatives use. Differences in patient characteristics between metformin-users and non-users, and between metformin-users and sulfonylurea derivatives were analyzed using the independent samples t-test and chi square test.

A Kaplan Meier survival curve was constructed to compare overall survival between patients using metformin, sulfonylurea derivatives and nonusers. A parametric survival model with exponential (Poisson) distribution was used to model the effect of metformin

Table 1: Characteristics of the cohort

		Overall		Non-user*		Metformin -users		*P-value*†		Metformin users		Sulfonylurea derivative users		*P-value*‡	
		n	%	n	%	n	%			n	%	n	%		
Total		907		830		77				77		43			
Sex	Male	486	54	442	53	44	57	0.55		44	57	30	70	0.18	
	Female	421	46	388	47	33	43			33	43	13	30		
Age at diagnosis (years; mean, SD)		69 (±11)		69 (±11)		70 (±10)		0.32		70 (±10)		71 (±10)		0.75	
Year of diagnosis	1998-2003	242	27	238	29	4	5	**<0.001**		4	5	25	58	**<0.001**	
	2004-2007	287	32	262	31	25	33			25	33	12	28		
	2008-2011	377	41	329	40	48	62			48	62	6	14		
Number of comorbidities	0	226	25	226	27	0	0	**<0.001**		0	0	6	14	**0.003**	
	1	245	27	225	27	20	26			20	26	15	35		
	≥2	321	35	274	33	47	61			47	61	18	42		
	Unknown	115	13	105	13	10	13			10	13	4	9		
Diabetes	yes	155	17	103	12	52	68	**<0.001**		52	68	24	56	0.17	
TNM stage	1	36	4	31	4	5	7	0.73		5	7	1	2	0.56	
	2	90	10	81	10	9	12			9	12	4	9		
	3	62	7	59	7	3	4			3	4	3	7		
	4	312	34	286	34	26	34			26	34	11	26		
	Unknown	407	45	373	45	34	44			34	44	24	56		
Treatment	Surgery (yes)	98	11	86	10	12	16	0.18		12	16	5	12	0.79	
	Radiotherapy (yes)	38	4	33	4	5	7	0.36		5	7	4	9	0.72	
	Chemotherapy (yes)	210	23	190	23	20	26	0.57		20	26	8	19	0.50	
Insulin use ever		115	13	87	11	28	36	**<0.001**		28	36	14	33	0.70	

**Non-users include sulfonylurea derivatives-user*
†P-value for non-users vs metformin users
‡P-value for metformin vs sulfonylurea derivatives users
Significant differences are printed in bold

use on overall survival, where death of any cause was coded as event. Metformin use and sulfonylurea derivatives use were included as time varying covariate in the model.

Overall survival between metformin-users and non-users was assessed. Adjustments were made for age, number of comorbidities (0, 1 or ≥2, excluding diabetes mellitus), TNM stage (categorical), year of diagnosis (1998-2003, 2004-2007, 2008-2011) surgery (yes/no), chemotherapy (yes/no), and radiotherapy (yes/no). Information about comorbidities was available for lung disease, cardiovascular disease, diabetes and disorders of the gastrointestinal tract, urinary tract, nervous system, musculoskeletal system, and a group of other comorbidities. Unknown values were taken into account as categorical variables in the multivariable model. The difference between overall survival in metformin-users and sulfonylurea derivatives-users was analyzed with the same model that was used for the analysis of metformin vs non-users. The results of the model should be interpreted as a favorable association with survival when the result shows a rate ratio smaller than 1 in relation to the comparison group.

All analysis were performed using Stata version 12 statistical software. Statistical tests were two-sided and considered significant at the P<0.05 level.

Results

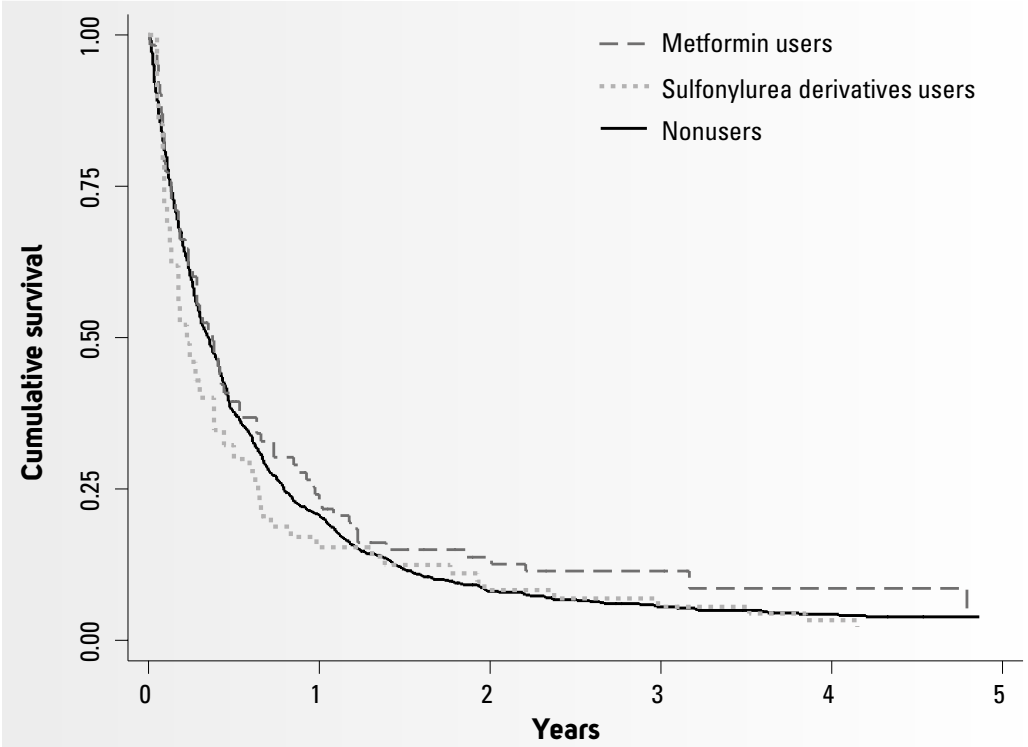
In total, 1,111 patients with pancreatic cancer were diagnosed in the period 1998-2011; 91 patients were excluded due to morphology (as described above), and 48 patients using merely metformin before diagnosis were excluded (Figure 1, flow chart of the study population). Overall, 57 metformin-users ever used contemporary sulfonylurea derivatives, and were therefore excluded. Eight patients with a survival of zero months were excluded from the analysis. This resulted in a study population of 907 patients, of which 77 patients used metformin and 43 patients who used sulfonylurea derivatives as drug for diabetes type 2.

In total 863 events were reported. In the metformin group, 64 patients deceased during study period, and in the sulfonylurea derivatives-users 41 patients deceased.

Table 1 shows the characteristics of this population. There were no significant differences between the groups concerning TNM stage and treatment (chemotherapy, radiotherapy and surgery). Metformin-users had more additional comorbidities (P<0.001) compared to both non-users and sulfonylurea derivatives-users. Incidence of diabetes did not differ between the group of metformin-users and sulfonylurea derivatives-users. Contemporary insulin use was 36% for metformin-users vs 33% among patients using sulfonylurea derivatives (P=0.70). Patients who used metformin were diagnosed in more recent years than sulfonylurea derivatives-users or non-users (P<0.001). Finally, median survival of metformin-users was 5.7 months (Inter Quartile Range (IQR) 2.2-14.7), sulfonylurea derivatives-users had a median survival of 6.0 (IQR 1.6-21.2) months, while non-users had a median survival of 4.0 months (IQR 1.5-9.2).

Table 2 shows the analysis of overall survival difference between metformin-users and non-users. For all patients with pancreatic cancer, metformin-use was associated with an improved overall survival compared to patients not using metformin, rate ratio (RR) 0.76 (95% CI 0.59-0.98; P=0.04; Table 2). This association was no longer significant after adjusting for age, number of comorbidities, stage, year of diagnosis, surgery, chemotherapy, and

Figure 2: Kaplan–Meier estimation of survival curves in patients with pancreatic cancer grouped according to medication use



radiotherapy. Multivariable RR for metformin-users compared to non-users was 0.86 (95%CI 0.66-1.12; P=0.26).

Overall survival was additionally assessed between the following three groups: patients not using OGLD, metformin-users, and sulfonylurea derivatives-users (Figure 2, Table 2). Overall survival for metformin-users versus non-users was RR 0.74 (95%CI 0.57-0.95; P=0.02). After adjusting for potential confounders, the RR for overall survival of metformin-users versus non-users was 0.85 (95% CI 0.65-1.11; P=0.23). Overall survival for sulfonylurea derivatives-users compared to non-users was RR 0.60 (95%CI 0.44-0.82; P=0.001), and multivariable analysis showed a RR 0.82 (95%CI 0.59-1.13; P=0.23).

Lastly, table 2 shows the comparison of metformin-users versus sulfonylurea derivatives-users. Sulfonylurea derivatives use was not associated with overall survival compared to metformin-users, RR 0.78 (95%CI 0.53-1.15; P=0.21), and adjusted RR was 0.86 (95%CI 0.50-1.46; P=0.57).

Discussion

This retrospective, observational cohort study showed no association between the use of metformin and overall survival in patients with pancreatic cancer.

These results are in concordance with the results of two retrospective cohort studies. These studies found no association between the use of metformin and survival in patients with (advanced) pancreatic cancer, however these studies were only done in respectively 44 and 516 patients with type 2 diabetes mellitus.[24,25] Similarly, two recently published randomized controlled trials – carried out in 121 patients in the Netherlands and 60 patients in Italy - also showed no effect of metformin on survival.[26,27] Despite differences in design, such as cancer stage, chemotherapy regime, blinding, and use of placebo, both trials show a consistent no effect of metformin on survival in patients with advanced pancreatic cancer.

The results of the randomized controlled trials and our study are in conflict with numerous other observational studies describing a beneficial effect of metformin, not only in pancreatic cancer.[15,19,28-32] Mortality decrease for patients with pancreatic cancer using metformin was consistently 27-40% in previous studies, however these cohorts were smaller than the cohort that was analyzed in this study, with respectively 302, 764 and 349 patients that were analyzed.[28-30] This discrepancy could, be partly explained by the difference in methodology.[8] Suissa et al. studied the effect of time-related biases in observational drug studies of metformin on cancer.[22] Authors state that using time varying techniques prevents misclassification of metformin exposure. There have been several publications claiming to account for these biases. These studies could not demonstrate any association between metformin and cancer incidence or the observed incidence reduction was considerably smaller than previous results.[22,33,34] Another explanation could be the high number of patients with irresectable pancreatic cancer (89%) in our study, whereas other studies found a survival benefit in patients with resectable pancreatic cancer.[29,32]

In addition, differences in patient characteristics could partly explain the results of our study. Patients using sulfonylurea derivatives had less comorbidities than metformin-users.

Table 2: Time-dependent survival analysis

	n	RR (95 % C.I.)	*P-value*	Adjusted RR* (95 % C.I.)	*P-value*
Metformin-users versus non-users					
Non-users†	830	1 (Reference)		1 (Reference)	
Metformin-users	77	0.76 (0.59-0.98)	**0.04**	0.86 (0.66-1.12)	0.26
Non-users versus metformin users and sulfonylurea derivatives users					
Non-users ‡	787	1 (Reference)		1 (Reference)	
Metformin-users	77	0.74 (0.57-0.95)	**0.02**	0.85 (0.65-1.11)	0.23
Sulfonylurea derivatives-users	43	0.60 (0.44-0.82)	**0.001**	0.82 (0.59-1.13)	0.23
Metformin-users versus sulfonylurea derivatives users					
Metformin-users	77	1 (Reference)		1 (Reference)	
Sulfonylurea derivatives-users	43	0.78 (0.53-1.15)	0.21	0.86 (0.50-1.46)	0.57

**Adjusted for age, number of comorbidities, stage, year of diagnosis and therapy (surgery, radiotherapy and chemotherapy)*
†Non-users including sulfonylurea derivatives-users
‡Nonusers do not include sulfonylurea derivatives-users
RR: Rate Ratio
Significant differences are printed in bold

Before 2006, the guidelines recommended prescription of metformin for patients with a BMI higher than 27.[35,36] It could therefore be possible that a majority of the patients that were prescribed metformin (35%) before 2006 were overweight. Obese patients have a worse prognosis and overall survival, partly due to a higher risk surgical risk.[37]
This observation could also partly explain the observed trend towards a better survival for patients using sulfonylurea derivatives, however no information on BMI was available in the current dataset.

For the direct action of metformin on cancer cells the effective drug concentrations achieved in neoplastic tissue are crucial.[38] It is possible that the required concentration in the target tissue is not attained with the current dose of metformin. Due to the retrospective nature of this study, no information is available about sufficient concentrations of the effect on tumor cells.[38]The Dutch randomized controlled trial has also addressed this aspect, where an effect on survival was seen in a subset of patients reaching adequate insulin level decrease.[26]

Our study has some notable strengths. First, this is one of the largest cohorts so far to analyze the association between the use of metformin and survival in patients with pancreatic cancer. Second, our data is linked through two validated databases - ECR and PHARMO - preventing both recall and information bias. Third, this is the first observational study about the association between the use of metformin and overall survival in patients with pancreatic cancer, taking into account the time between the beginning of the follow up and the first drug prescription, which prevents time-related biases. Not including the time varying covariate in our model also revealed a highly significant survival benefit for metformin-users. Additionally, metformin-users were compared to sulfonylurea derivative users, a group of patients with a more similar baseline prognosis. Lastly, previous articles focused on the effect of metformin on overall survival limited to patients with pancreatic cancer and type 2 diabetes, whereas this study included all patients with pancreatic cancer.

A limitation to this observational study could be the small number of patients who received OGLD in a large cohort, which also complicates a subgroup analysis, e.g. in patients who underwent a resection. Only 43 patients were using solely sulfonylurea derivatives. Due to this small number of users, no robust statements can be made. However, due to the wide interest in a growing field, this remains a relevant study, complementing the existing evidence. Moreover, because of the retrospective nature of the study, the amount of information available is limited. There is no detailed information about the population such as smoking, BMI, glycemic control or cause of death. It could hypothetically be possible that the non-significant relative risk reduction of 15% found in adjusted analysis was not significant due to a lack of power. However, in the randomized controlled trials recently published, no effect of metformin on survival of patients with pancreatic cancer could be proven, and these trials were of course designed with a power calculation.

This observational study contributes to the mounting evidence against an association between improved survival in patients with pancreatic cancer using metformin.[24,26,27,39] These findings could discourage new trials to be designed for metformin as adjuvant therapy in pancreatic cancer, as this disease is generally discovered in an advanced stage were the anti-tumor effect of metformin will not be able to sufficiently inhibit tumor growth.[40] However, this study does not exclude the opportunity that metformin could be a valuable adjuvant therapy in other cancer types or only in patients with resectable, early-stage pancreatic cancer. Nowadays new oncology drugs are very expensive and drug repurposing is an attractive strategy to offer more effective options for patients with cancer.[41]

Acknowledgements

Conflicts of interest

M.H.S. is an employee of the PHARMO Institute for Drug Outcomes Research. This independent research institute performs financially supported studies for government and related health care authorities and for pharmaceutical companies. However, this study was not financially supported by a pharmaceutical company.

No potential conflicts of interest relevant to this article were reported.

Supplementary table 1: included and excluded morphology codes (ICD-10)

Included	Excluded
8000	8070
8140	8010
8150	8011
8151	8012
8154	8013
8201	8021
8240	8041
8246	8440
8260	8453
8310	8470
8001	8471
8500	8480
8521	8481
8550	8490
8560	8936
8574	
8720	
8803	

Reference list

1. Ferlay J, Steliarova-Foucher E, Lortet-Tieulent J, et al. Reprint of: Cancer incidence and mortality patterns in Europe: Estimates for 40 countries in 2012. Eur J Cancer 2015; 51(9): 1201-2.
2. Zill OA, Greene C, Sebisanovic D, et al. Cell-Free DNA Next-Generation Sequencing in Pancreatobiliary Carcinomas. Cancer Discov 2015; 5(10): 1040-8.
3. Malik NK, May KS, Chandrasekhar R, et al. Treatment of locally advanced unresectable pancreatic cancer: a 10-year experience. J Gastrointest Oncol 2012; 3(4): 326-34.
4. National Comprehensive Cancer Network. Guidelines Pancreatic Adenocarcinoma. Version 2.2015. Guideline 2015; 2015.
5. Everhart J, Wright D. Diabetes mellitus as a risk factor for pancreatic cancer. A meta-analysis. JAMA 1995; 273(20): 1605-9.
6. Chari ST, Leibson CL, Rabe KG, et al. Pancreatic cancer-associated diabetes mellitus: prevalence and temporal association with diagnosis of cancer. Gastroenterology 2008; 134(1): 95-101.
7. Song R. Mechanism of Metformin: A Tale of Two Sites. Diabetes Care 2016; 39(2): 187-9.
8. Pollak MN. Investigating metformin for cancer prevention and treatment: the end of the beginning. Cancer Discov 2012; 2(9): 778-90.
9. Pernicova I, Korbonits M. Metformin--mode of action and clinical implications for diabetes and cancer. Nat Rev Endocrinol 2014; 10(3): 143-56.
10. Martin M, Marais R. Metformin: a diabetes drug for cancer, or a cancer drug for diabetics? J Clin Oncol 2012; 30(21): 2698-700.
11. Kisfalvi K, Eibl G, Sinnett-Smith J, Rozengurt E. Metformin disrupts crosstalk between G protein-coupled receptor and insulin receptor signaling systems and inhibits pancreatic cancer growth. Cancer Res 2009; 69(16): 6539-45.
12. Cifarelli V, Lashinger LM, Devlin KL, et al. Metformin and Rapamycin Reduce Pancreatic Cancer Growth in Obese Prediabetic Mice by Distinct MicroRNA-Regulated Mechanisms. Diabetes 2015; 64(5): 1632-42.
13. Libby G, Donnelly LA, Donnan PT, Alessi DR, Morris AD, Evans JM. New users of metformin are at low risk of incident cancer: a cohort study among people with type 2 diabetes. Diabetes Care 2009; 32(9): 1620-5.
14. Landman GW, Kleefstra N, van Hateren KJ, Groenier KH, Gans RO, Bilo HJ. Metformin associated with lower cancer mortality in type 2 diabetes: ZODIAC-16. Diabetes Care 2010; 33(2): 322-6.
15. Currie CJ, Poole CD, Jenkins-Jones S, Gale EA, Johnson JA, Morgan CL. Mortality after incident cancer in people with and without type 2 diabetes: impact of metformin on survival. Diabetes Care 2012; 35(2): 299-304.
16. Wu L, Zhu J, Prokop LJ, Murad MH. Pharmacologic Therapy of Diabetes and Overall Cancer Risk and Mortality: A Meta-Analysis of 265 Studies. Sci Rep 2015; 5: 10147.
17. Noto H, Goto A, Tsujimoto T, Noda M. Cancer risk in diabetic patients treated with metformin: a systematic review and meta-analysis. PLoS One 2012; 7(3): e33411.
18. Soranna D, Scotti L, Zambon A, et al. Cancer risk associated with use of metformin and sulfonylurea in type 2 diabetes: a meta-analysis. Oncologist 2012; 17(6): 813-22.
19. Ye XF, Wang J, Shi WT, He J. Relationship between aspirin use after diagnosis of colorectal cancer and patient survival: a meta-analysis of observational studies. Br J Cancer 2014; 111(11): 2172-9.
20. Lee MS, Hsu CC, Wahlqvist ML, Tsai HN, Chang YH, Huang YC. Type 2 diabetes increases and metformin reduces total, colorectal, liver and pancreatic cancer incidences in Taiwanese: a representative population prospective cohort study of 800,000 individuals. BMC Cancer 2011; 11: 20.
21. Zhang P, Li H, Tan X, Chen L, Wang S. Association of metformin use with cancer incidence and mortality: a meta-analysis. Cancer Epidemiol 2013; 37(3): 207-18.
22. Suissa S, Azoulay L. Metformin and the risk of cancer: time-related biases in observational studies. Diabetes Care 2012; 35(12): 2665-73.
23. van Herk-Sukel MP, van de Poll-Franse LV, Lemmens VE, et al. New opportunities for drug outcomes research in cancer patients: the linkage of the Eindhoven Cancer Registry and the PHARMO Record Linkage System. Eur J Cancer 2010; 46(2): 395-404.
24. Hwang AL, Haynes K, Hwang WT, Yang YX. Metformin and survival in pancreatic cancer: a retrospective cohort study. Pancreas 2013; 42(7): 1054-9.
25. Ambe CM, Mahipal A, Fulp J, Chen L, Malafa MP. Effect of Metformin Use on Survival in Resectable Pancreatic Cancer: A Single-Institution Experience and Review of the Literature. PLoS One 2016; 11(3): e0151632.
26. Kordes S, Pollak MN, Zwinderman AH, et al. Metformin in patients with advanced pancreatic cancer: a double-blind, randomised, placebo-controlled phase 2 trial. Lancet Oncol 2015.
27. Reni M, Dugnani E, Cereda S, et al. (Ir)relevance of metformin treatment in patients with metastatic pancreatic cancer: an open-label, randomized phase 2 trial. Clin Cancer Res 2015.
28. Sadeghi N, Abbruzzese JL, Yeung SC, Hassan M, Li D. Metformin use is associated with better survival of diabetic patients with pancreatic cancer. Clin Cancer Res 2012; 18(10): 2905-12.
29. Jo A, Kim Y, Kang S, Kim M, Ko M. The Effect Of Metformin Use And Mortality Among Those With Pancreatic Cancer And Type 2 Diabetes Mellitus: Findings From A Nationwide Population Retrospective Cohort Study. Value Health 2015; 18(7): A439.
30. Choi Y, Kim TY, Oh DY, et al. The Impact of Diabetes Mellitus and Metformin Treatment on Survival of Patients with Advanced Pancreatic Cancer Undergoing Chemotherapy. Cancer Res Treat 2015.
31. Franciosi M, Lucisano G, Lapice E, Strippoli GF, Pellegrini F, Nicolucci A. Metformin therapy and risk of cancer in patients with type 2 diabetes: systematic review. PLoS One 2013; 8(8): e71583.
32. Kozak MM, Anderson EM, von Eyben R, et al. Statin and Metformin Use Prolongs Survival in Patients With Resectable Pancreatic Cancer. Pancreas 2016; 45(1): 64-70.
33. Kowall B, Stang A, Rathmann W, Kostev K. No reduced risk of overall, colorectal, lung, breast, and prostate cancer with metformin therapy in diabetic patients: database analyses from Germany and the UK. Pharmacoepidemiology and Drug Safety 2015; 24(8): 865-74.
34. Gandini S, Puntoni M, Heckman-Stoddard BM, et al. Metformin and Cancer Risk and Mortality: A Systematic Review and Meta-analysis Taking into Account Biases and Confounders. Cancer Prevention Research 2014; 7(9): 867-85.
35. Bouma M, Rutten GE, de Grauw WJ, Wiersma T, Goudswaard AN. [Summary of the practice guideline 'Diabetes mellitus type 2' (second revision) from the Dutch College of General Practitioners]. Ned Tijdschr Geneeskd 2006; 150(41): 2251-6.
36. Wiersma TJ, Heine RJ, Rutten GE. [Summary of the practice guideline 'Diabetes mellitus type 2' (first revision) of the Dutch College of General Practitioners]. Ned Tijdschr Geneeskd 1999; 143(33): 1688-91.
37. Noun R, Riachy E, Ghorra C, et al. The impact of obesity on surgical outcome after pancreaticoduodenectomy. JOP 2008; 9(4): 468-76.
38. Christensen MM, Brasch-Andersen C, Green H, et al. The pharmacogenetics of metformin and its impact on plasma metformin steady-state levels and glycosylated hemoglobin A1c. Pharmacogenet Genomics 2011; 21(12): 837-50.
39. Suissa S, Azoulay L. Metformin and cancer: mounting evidence against an association. Diabetes Care 2014; 37(7): 1786-8.
40. Yang YX, Rustgi AK. Impact of Metformin on Advanced Pancreatic Cancer Survival: Too Little, Too Late? Clin Cancer Res 2015.
41. Bertolini F, Sukhatme VP, Bouche G. Drug repurposing in oncology-patient and health systems opportunities. Nat Rev Clin Oncol 2015; 12(12): 732-42.

CHAPTER 6

THE DIFFERENCE IN ASSOCIATION BETWEEN ASPIRIN USE AND OTHER THROMBOCYTE AGGREGATION INHIBITORS AND SURVIVAL IN PATIENTS WITH COLORECTAL CANCER

Martine A. Frouws, Eva Rademaker, Esther Bastiaannet, Myrthe P.P. van Herk-Sukel,
Valery E.P.P. Lemmens, Cornelis J.H. Van de Velde, Johanneke E.A. Portielje, Gerrit-Jan Liefers

European Journal of Cancer 2017 Mar 25;77:24-30

Abstract

Background

Several studies have suggested that the association between aspirin and improved cancer survival is mediated through the mechanism of aspirin as a thrombocyte aggregation inhibitors (TAI).The aim of this study was to provide epidemiological evidence for this mechanism assessing the association between overall survival and the use of aspirin and non-aspirin TAI in patients with colorectal cancer.

Methods

In this observational study, data from the Netherlands Comprehensive Cancer Organization was linked to PHARMO Database Network. Patients using aspirin or aspirin in combination with non-aspirin TAI (dual-users) were selected and compared to non-users. The association between overall survival and the use of (non-) aspirin TAI was analysed using Cox regression models with the use of (non-)aspirin TAI as time-varying covariate.

Results

In total, 9,196 patients were identified with colorectal cancer and 1,766 patients used TAI after diagnosis. Non-aspirin TAI were mostly clopidogrel and dipyridamole. Aspirin use was associated with a significant increased overall survival, Hazard Ratio (HR) 0.41 (95% C.I. 0.37-0.47) and the use of non-aspirin TAI was not associated with survival of HR 0.92 (95% C.I. 0.70-1.22). Dual-users did not have an improved overall survival when compared to patients using solely aspirin.

Conclusions

Aspirin use after diagnosis of colorectal cancer was associated with significantly lower mortality rates and this effect remained significant after adjusting for potential confounders. No additional survival benefit was observed in patients using both aspirin and another TAI.

Introduction

Evidence before this study

There is growing evidence that aspirin use after diagnosis could reduce metastatic spread and increase the survival of patients with colorectal cancer. A recent systematic review and meta-analyses of Elwood et al. showed a reduction of approximately 25% in colorectal cancer specific mortality (HR 0.71, CI 0.58-0.87) and 20% in all-cause mortality (HR 0.80, CI 0.70-0.92).[1] The mechanism of action of aspirin in colorectal cancer specific mortality was previously thought to be a result of the prevention of colonic adenomas and, subsequently, less cancer development from these adenomas. However, meta-analyses of large secondary cardiovascular prevention trials show a larger effect on colorectal cancer specific mortality than would be expected if only adenomas were prevented.[2]

Randomised controlled trials are eagerly awaited to provide a decisive answer on the effect of aspirin as adjuvant therapy for cancer: ASCOLT trial (NCT00565708), Add-Aspirin trial (ISRCTN74358648), Aspirin trial (NCT02301286).

Biological mechanism of aspirin

The current described mechanisms of action of aspirin on cancer are inhibition of tumour growth and angiogenesis, delay of metastatic spread, abrogation of invasiveness, improvement of cellular apoptosis and enhancements of DNA mismatch repair.[1] Previous studies attempted to unravel the mechanism of action of aspirin with the identification of a specific biomarker, which could concurrently be used to predict the effectiveness of aspirin as adjuvant therapy. PIK3CA mutation status, HLA class I antigen expression and COX-2 overexpression have been suggested to play a role in this mechanism, however study results are heterogeneous.[3]

Thrombocytes and cancer

Thrombocytes become activated and aggregated by cancer cells via various mediators, such as direct cell-cell contact, coagulant disturbances and soluble mediators (Thromboxane A_2).[4] Thrombocyte membranes consist of adhesion molecules promoting adhesion, for example to other thrombocytes and the vascular wall. Thrombocyte activation induced by cancer cells promotes several steps in cancer progression, such as cancer metastasis, tumour proliferation and angiogenesis. In this manner, cancer cell-bound thrombocytes form a cloak around the cancer cells and protect the cancer cells from immune surveillance, including cytolysis by natural killer cells.4 Although the pathogenesis is not clear, thrombocytosis in colorectal cancer patients has been observed to be associated with a poor cancer prognosis.[5] This could suggest that inhibiting the aggregation of thrombocytes could be a new therapeutic target for cancer therapy.

The effect of aspirin on cancer mortality has also been suggested to be mediated through the ability of aspirin to inhibit thrombocyte aggregation.[4,6,7] The aim of this study was to provide epidemiological evidence for the hypothesized thrombocyte-mediated mechanism of aspirin through studying other Thrombocyte Aggregation Inhibitors (TAI).

Patients and methods

Study population

Data were obtained from the Netherlands Comprehensive Cancer Organization (IKNL) and linked on a patient level to the PHARMO Database Network, covering a demographic region in the South-eastern part of the Netherlands of approximately 1,5 million inhabitants (formerly known as the Eindhoven Cancer Registry, ECR). By connecting drug dispensing records from the PHARMO Database Network to individual cancer survival data from IKNL, allows drug use to be analysed per (cancer) patient. The PHARMO Database Network is population-based and combines data from healthcare settings in the Netherlands. For this study, data from the out-patient pharmacy database was used. The construct and validity of the IKNL-PHARMO cohort have been described elsewhere.[8] Patients with colorectal cancer older than 18 years, diagnosed between January 1998 and December 2011 were included. Patients were informed about the registration and registered, unless they objected to be registered, and therefore informed consent for this study was not applicable. The vital status (dead/alive) of patients was obtained by the municipal population registry and was linked to IKNL. Follow-up of this study was until 31 December 2012.

The PHARMO database comprises GP or specialist dispensed healthcare products dispensed by the out-patient pharmacy. The dispensing records include information on type of product, date, strength, dosage regimen and quantity. Drug dispensing is coded according to the WHO Anatomical Therapeutic Chemical (ATC) Classification System (WHO Anatomical Therapeutic Chemical Classification (www.whocc.no/atc_ddd_index). From this linked database, prescriptions for dispensed TAI were selected. The ATC-codes for used for the selection of users can be found in Table A.1.

Definition of user

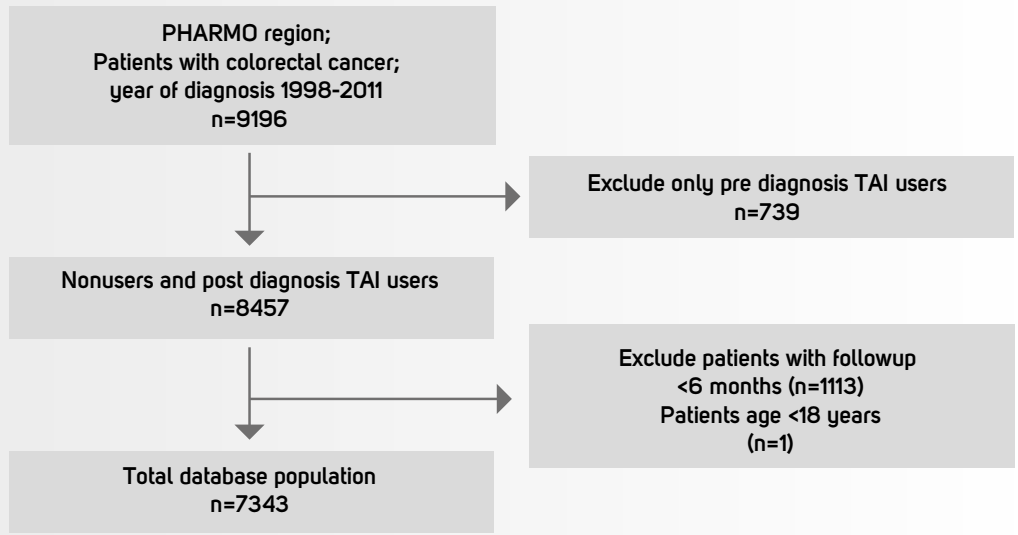
Four groups of patients were selected from the IKNL-PHARMO cohort: non-users, aspirin users, TAI users and dual users (figure 1).

Non-users were defined as patients who never used a TAI or were dispensed a TAI for less than 30 days. Patients who used any TAI (this includes aspirin) solely before diagnosis, patients younger than 18 years, and patients whose follow-up was less than six months, were excluded from the analysis.

Patients who used a TAI, were selected and defined as users under the condition that they used medication for at least 30 days. Patients who used both aspirin and a non-aspirin TAI were defined as dual-users.

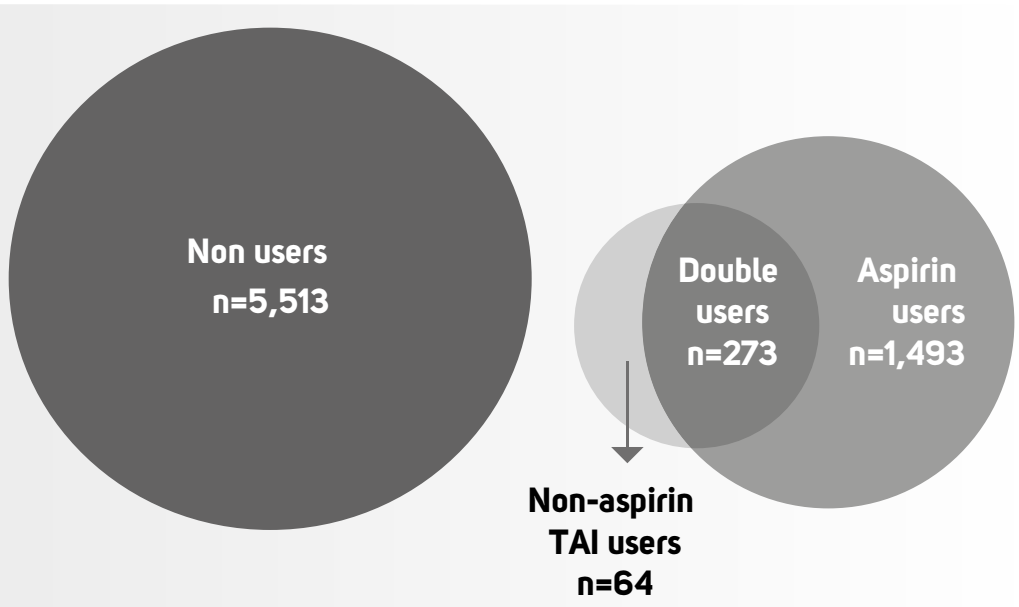
Patients who used solely a non-aspirin TAI were excluded from the analysis. The reason for this is because, according to the guidelines, a non-aspirin TAI always has to be prescribed in combination with aspirin.[9,10] A possible reason for patients not to use aspirin in combination with a non-aspirin TAI are because they are possibly intolerant for aspirin and are therefore prescribed solely a non-aspirin TAI. This is confirmed by the observation that this group is relatively small (n=64) and therefore, they were excluded from the analysis.

Figure 1: Flow chart of study population with the excluded number and reasons of exclusion of patients



Follow-up time of patients was divided into periods of use and non-use of TAI. Follow-up started six months after diagnosis (t0) and duration (survival) was recorded in months. A lag period of six months was used, after which patients were categorized patients into: groups of non-users, solely aspirin users, solely non-aspirin TAI users and dual-users (figure 2).[11,12]

Figure 2: Overview of study groups included in the study. Patients using solely non-aspirin Thrombocyte Aggregation Inhibitors (TAI, n=64) were excluded from the analysis.



Statistical analysis

The time of use of aspirin or a non-aspirin TAI was analysed as time-varying covariate (TVC) in periods of use and no use, to avoid immortal time bias.[11,13] First, a model was built where aspirin and non-aspirin TAI were used as TVC, in the total cohort. Additionally, two models were built with the selected groups, where patients using aspirin versus non-users were analysed and subsequently dual-users versus non-users. The association between the use of aspirin and overall survival was analysed using multivariable time-dependent Cox Proportional Hazard models. The models contained time-varying duration of aspirin and or non-aspirin TAI exposure. The proportional hazard assumption was tested with Schoenfeld residuals.

Comorbidities were registered by IKNL as follows: lung disease, cardiovascular disease, diabetes, disorders of the gastrointestinal tract, urinary tract, nervous system, musculoskeletal system, and a group of 'other comorbidities'. Comorbidities were analysed as 0, 1 or ≥2 comorbidities at the moment of cancer diagnosis.

Multivariable survival models were built with the following covariates: age at diagnosis (continuous), sex, stage of cancer according to TNM staging (categorical)[14], number of comorbidities (categorical, 0, 1, ≥2), and treatment: surgery (yes/no), radiotherapy (yes/no) and chemotherapy (yes/no).

Missing values were included in the multivariable model as missing indicator. Analyses were performed using Stata statistical software version 12 and statistical tests were two-sided and considered significant if $p<0.05$.

Results

In total, 9,196 patients were identified with colorectal cancer, diagnosed between January 1998 and December 2011. The following patients were excluded from the analysis: 739 pre-diagnosis TAI users, 1,113 patients with a follow-up less than six months and 1 patient below the age of 18 years. (flow chart, figure 1) Total follow-up time was 31757.93 years and in total, 2,785 deaths (30%) were recorded.

Figure 2 shows that 7,279 patients were included in the analysis, of which 5,513 (76%) patients were classified as non-users, 1,493 (21%) as solely aspirin users, and 273 (4%) as dual-users (patients using both aspirin and a non-aspirin TAI). In the group where patients used both aspirin and another TAI, 50% of patients used aspirin in combination with dipyridamole, 39% used aspirin in combination with clopidogrel, 9.2% used aspirin in combination with both clopidogrel and dipyridamole and 1.5% used aspirin in combination with clopidogrel and ticlopidine.

Table 1 shows the characteristics of the patient population. There were more male patients observed in the user groups: 53% (2,893 of 5,513) of all patients in the non-user group were male versus 64% (962 of 1,493) in the solely aspirin user group and 69% (188 of 273) in the dual-user group. Dual-users received less chemotherapy and radiotherapy compared to non-users and aspirin users. Additionally, 52% (781 of 1,493) solely aspirin users and 58% (159 of 273) dual-users had two or more comorbidities, in contrast with the non-user group where 40% (2,198 of 5,513) of the patients had no comorbidities. Non-users were generally younger compared to aspirin users and dual-users.

Table 1: Baseline characteristics of the population under study, devided into three groups; non-users, solely aspirin users and dual users

			Userstatus defined in 6 months after diagnosis					
	All patients n=7279		Non-user n=5513		Solely aspirin user n=1493		Dual user n=273	
	n	%	n	%	n	%	n	%
Age								
< 60	1617	22	1471	27	122	8	24	9
60-69	2153	30	1717	31	376	25	60	22
70-79	2475	34	1678	30	672	45	125	46
≥ 80	1034	14	647	12	323	22	64	23
Sex								
Male	4043	56	2893	53	962	64	188	69
Female	3236	45	2620	48	531	36	85	31
Location of the tumour								
Colon	4596	63	3406	62	995	67	195	71
Rectum	2683	37	2107	38	498	33	78	29
Stage								
0	246	3	173	3	61	4	12	4
I	1521	21	1124	20	332	22	65	24
II	2247	31	1678	30	470	32	99	36
III	1956	27	1502	27	397	27	57	21
IV	1061	15	852	16	183	12	26	10
Missing	248	3	184	3	50	3	14	5
Surgery								
No	531	7	402	7	109	7	20	7
Yes	6748	93	5111	93	1384	93	253	93
Chemotherapy								
No	5100	70	3752	68	1126	75	222	81
Yes	2179	30	1761	32	367	25	51	19
Radiotherapy								
No	5355	74	3991	72	1145	77	219	80
Yes	1924	26	1522	28	348	23	54	20
Amount of comorbidities								
0	2412	33	2198	40	192	13	22	8
1	2064	28	1581	29	412	28	71	26
2 or more	2142	29	1202	22	781	52	159	58
Missing	661	9	532	10	108	7	21	8

The overall survival model (n=7,279) with the use of aspirin and the use of non-aspirin TAI, both analysed as TVC, showed a hazard ratio for the use of aspirin of 0.52 (95% C.I. 0.46-0.58) and for the use of non-aspirin TAI of 0.93 (95% C.I. 0.71-1.23). The multivariable model, where adjustments were made for age at diagnosis, sex, stage of cancer, number of comorbidities and treatment, showed a hazard ratio for aspirin use of 0.41 (95% C.I. 0.37-0.47) and for the use of non-aspirin TAI of 0.92 (95% C.I. 0.70-1.22). The proportional hazard assumption was fulfilled.

The results of the survival analysis in groups of users are shown in table 2. For the analysis in the group of patients using only aspirin versus non-users, aspirin use was associated with a significant reduction in mortality (HR 0.53, 95% C.I. 0.47-0.60). When adjusted for possible confounders, the multivariable hazard ratio was 0.42 (95% C.I. 0.37-0.48). In the group of dual-users, the use of aspirin was also associated with a significant reduction in mortality (HR 0.50, 95% C.I. 0.41-0.62). The adjusted analysis in this group showed a hazard ratio of 0.43 (95% C.I. 0.35-0.52).

Table 2: Time dependent survival analysis with Cox Proportional Hazards model for the association between the use of thrombocyte aggregation inhibitors and survival, analysed into two groups; aspirin users versus non-users in patients using solely aspirin and dual users versus non-users in patients using aspirin and another TAI.

				Univariate		Multivariate	
		n	Events	HR (95%CI)	P-value	HR* (95%CI)	P-value
Aspirin users versus non-users (in patients using solely aspirin)		*7006*					
	No aspirin use	5513	2037	1.00 (reference)		1.00 (reference)	
	Aspirin use	1493	651	**0.53 (0.47-0.60)**	**< 0.001**	**0.42 (0.37-0.48)**	**< 0.001**
Dual users versus non-users (patients using aspirin and another TAI)		*5786*					
	No aspirin use	5513	2037	1.00 (reference)		1.00 (reference)	
	Aspirin use	273	97	**0.50 (0.41-0.62)**	**< 0.001**	**0.43 (0.35-0.52)**	**< 0.001**

** Adjusted for sex, age at incidence, stage, surgery (yes/no), chemotherapy (yes/no), radiotherapy (yes/no), amount of comorbidities*
Abbreviations: HR 'Hazard Ratio'
Significant values are printed in bold

Discussion

Aspirin use after diagnosis of colorectal cancer was associated with a significantly lower mortality rate and this effect remained significant after adjusting for potential confounders. No additional survival benefit was observed in patients using both aspirin and a non-aspirin TAI. In the model in which both aspirin and non-aspirin TAI were assessed, no additional survival benefit was found for the use of a non-aspirin TAI.

The association between aspirin use and overall survival has been reported in many publications.[1,3] The results of this study are in line with the previously published studies. The association between overall survival and aspirin use was not the goal of this current study, but in order to make the comparison with other TAI's this was an analysis which had to be performed.

There are two possible explanations for our results:

First, it could be possible that low-dose aspirin (75 mg) sufficiently inhibits the aggregation of thrombocytes to accomplish the hypothesised anti-cancer mechanism. According to this mechanism, because of the inhibition of aggregation of thrombocytes, circulating tumour cells are no longer surrounded by thrombocytes, facilitating detection by the immune system, and subsequently cleared from the blood stream.[2,6,15,16] Aspirin inhibits the production of Prostaglandin E2, thereby preventing direct contact between thrombocytes and tumour cells.[17] The natural production of tumour growth factor β (TFB-β) is inhibited, and the subsequent signalling of nuclear factor κB in circulating tumour cells. As a result of this inhibition the epithelial-mesenchymal transition in circulating tumour cells is prevented and the metastatic potential of circulating tumour cells is reduced.[17-19] Aspirin has a permanent anti-thrombotic effect via acetylation of COX-1 in megakaryocytes in the bone-marrow limiting de novo protein synthesis of thrombocytes.[20] It could therefore be possible that other TAI may not have been able to additionally inhibit thrombocyte aggregation and therefore unable to additionally prevent metastases.

On the other hand, these non-aspirin TAI (dipyridamole and clopidogrel) do have hypothesized anti-cancer mechanisms.

Clopidogrel causes a permanent modification of the ADP-receptors on thrombocytes.[21] Through repeated daily dosing, this modification causes cumulative inhibition of ADP-induced thrombocyte aggregation. One of the molecular mechanisms that facilitates the interaction between thrombocytes and cancer is through the release of ADP. ADP acts via its $P2Y_1$ and $P2Y_{12}$ receptors on thrombocytes and ADP depletion has been associated with a reduction in metastasis.[7,22]

Dipyridamole is a phosphodiesterase inhibitor, which causes an increase of intracellular cyclic AMP (cAMP) via several working mechanisms.[23,24] This increase in cAMP leads to reversible thrombocyte aggregation inhibition. Down-regulation of cAMP signalling enhances colorectal cancer cell proliferation, which is driven by Prostaglandin E2 (PGE2).[25]

A second explanation for our results might be that that the effect of aspirin on cancer survival is not restricted to thrombocyte aggregation only, but an additional mechanism unique for aspirin could be responsible for the observed survival benefit.

One previous study by Hicks *et al* assessed the association between the use of clopidogrel and survival of patients with colorectal cancer.[26] This study found no association between post-diagnosis clopidogrel use and cancer-specific mortality (adjusted HR 0.96, 95% C.I. 0.76-1.22) and an increased rate of all-cause mortality (adjusted HR 1.31, 95% C.I. 1.13-1.55) for patients using clopidogrel. The authors attribute this increased mortality to confounding by indication, due to a higher cardiovascular mortality in the group of patients with colorectal cancer using clopidogrel. This is an interesting observation, since this cardiovascular mortality is also present in the group of patients using aspirin. Despite a higher cardiovascular mortality, significant survival benefits are observed in numerous observational studies assessing the effect of aspirin on overall survival.[1] A very early study from 1988 randomised 144 colon cancer patients between chemotherapy and chemotherapy plus mopidamol, a derivative of dipyridamole,

and found no survival benefit.[27] Two years later, another study found no effect on survival for patients with advanced colon cancer taking dipyridamole.[7] Rothwell et al. performed a meta-analysis of individual patient data pooling large randomised controlled trials where TAI were investigated as potential cardiovascular prevention medication and found no association between non-aspirin TAI and risk of cancer death.[28]

Our study cannot state firm conclusions with regard to our primary hypothesis but does provide interesting insights in the mechanisms associated with TAI and cancer survival.

Strengths and limitations

To our knowledge, this is the first study to evaluate the effect of non-aspirin TAI on cancer survival. Another strength of this study is that the data are population-based with a long follow-up period, which resulted in a large cohort. Our data were derived from the national cancer registry and dispensing data of pharmacies, eliminating both recall and information bias.

The lag period of six months resulted in an absolute lower number of patients because of the exclusion of patients with a follow-up duration of less than six months. However, this reduced the immortal time bias associated with this type of studies, improving the quality of the results.[12,13] Despite our multivariable model, the probability of residual confounding remains. Although PHARMO only registers prescriptions that were actually dispensed by the pharmacies, it was not registered if patients actually took the medication. Lastly, confounding by indication could have overestimated the mortality benefit found for patients using aspirin. Data are not randomised, and the decision of the treating physician to prescribe aspirin to patients cannot be taken into account. Patients with a poor prognosis may not be have been treated with aspirin and, as a result, were counted in the non-user group (healthy user bias).

Regarding the subject of confounding by indication, little is known about the influence of the comorbidity of patients using aspirin on the observed survival benefit in the numerous studies that have been published until now.[1] Aspirin is currently prescribed as secondary cardiovascular prevention drug.[29] Therefore, all patients using aspirin must have had a primary cardiovascular event. It is known that cancer survival rates for patients with cardiovascular disease are lower than for patients without cardiovascular disease.[30,31] Furthermore, patients with cancer and additional comorbidities also have a lower overall survival.[32] With this knowledge, it is even more surprising that in the current studies aspirin use is associated with a survival benefit for cancer patients. Because this is an unsolvable puzzle with the current study designs, the ongoing randomised clinical trials are of utmost importance to determine what the effect of aspirin as adjuvant therapy will be for cancer survival.

Future research

Based on the results of this current study, no evidence could be provided for an additional survival benefit of a non-aspirin TAI in addition to the use of aspirin for patients with colorectal cancer. In 2012 the guidelines have changed and the use of solely clopidogrel as therapy for patients that experienced a TIA became indicated.[33] In the future, when these guidelines have been in use for several years, it will be possible to analyse patients that used exclusively non-aspirin TAI. Further research is encouraged to reveal the interaction between thrombocytes and cancer. It is important to focus on novel thrombocyte-targeted anticancer therapies.[34,35]

Conflicts of interest statement:

Myrthe P.P. van Herk-Sukel is an employee of the PHARMO Institute for Drug Outcomes Research. This independent research institute performs financially supported studies for government and related healthcare authorities and several pharmaceutical companies. However, this study was not financially supported by a pharmaceutical company.

The other authors declare no conflicts of interest.

Acknowledgments:

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. No assistance in writing for this manuscript.

Table A.1: Overview of ATC-codes used for patient selection from PHARMO database, these results were combined with data from the Eindhoven Cancer Registry

Aspirin	Non-aspirin TAI's	
ATC-code	*ATC-code*	*Name*
B01AC06	B01AC04	Clopidogrel
B01AC08	B01AC05	Ticlopidine
B01AC56	B01AC07	Dipyridamole
N02BA01	B01AC22	Prasugrel
N02BA15	B01AC24	Ticagrelor
N02BA51		
N02BA65		

References

1. Elwood PC, Morgan G, Pickering JE, et al. Aspirin in the Treatment of Cancer: Reductions in Metastatic Spread and in Mortality: A Systematic Review and Meta-Analyses of Published Studies. PLoS One 2016; 11(4): e0152402.
2. Rothwell PM, Wilson M, Elwin C-E, et al. Long-term effect of aspirin on colorectal cancer incidence and mortality: 20-year follow-up of five randomised trials. The Lancet 2010; 376(9754): 1741-50.
3. Coyle C, Cafferty FH, Langley RE. Aspirin and Colorectal Cancer Prevention and Treatment: Is It for Everyone? Current Colorectal Cancer Reports 2016.
4. Li N. Platelets in cancer metastasis: To help the "villain" to do evil. International journal of cancer 2016; 138(9): 2078-87.
5. Monreal M, Fernandez-Llamazares J, Pinol M, et al. Platelet count and survival in patients with colorectal cancer--a preliminary study. Thrombosis and haemostasis 1998; 79(5): 916-8.
6. Rothwell PM, Wilson M, Price JF, Belch JFF, Meade TW, Mehta Z. Effect of daily aspirin on risk of cancer metastasis: a study of incident cancers during randomised controlled trials. The Lancet 2012; 379(9826): 1591-601.
7. Bambace NM, Holmes CE. The platelet contribution to cancer progression. J Thromb Haemost 2011; 9(2): 237-49.
8. van Herk-Sukel MP, LV vdP-F, Lemmens VE, et al. New opportunities for drug outcomes research in cancer patients: the linkage of the Eindhoven Cancer Registry and the PHARMO Record Linkage System. Eur J Cancer 2010; 46(2): 395-404.
9. Levine GN, Bates ER, Bittl JA, et al. 2016 ACC/AHA Guideline Focused Update on Duration of Dual Antiplatelet Therapy in Patients With Coronary Artery Disease: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. Circulation 2016.
10. NHG-standaard Cardiovasculair risicomanagement (Eerste herziening) Huisarts Wet 2012; 55(1): 14-28.
11. Suissa S, Azoulay L. Metformin and the risk of cancer: time-related biases in observational studies. Diabetes Care 2012; 35(12): 2665-73.
12. Zhou Z, Rahme E, Abrahamowicz M, Pilote L. Survival bias associated with time-to-treatment initiation in drug effectiveness evaluation: a comparison of methods. Am J Epidemiol 2005; 162(10): 1016-23.
13. Stricker BH, Stijnen T. Analysis of individual drug use as a time-varying determinant of exposure in prospective population-based cohort studies. Eur J Epidemiol 2010; 25(4): 245-51.
14. Sobin LH, Fleming ID. TNM Classification of Malignant Tumors, fifth edition (1997). Union Internationale Contre le Cancer and the American Joint Committee on Cancer. Cancer 1997; 80(9): 1803-4.
15. Placke T, Orgel M, Schaller M, et al. Platelet-derived MHC class I confers a pseudonormal phenotype to cancer cells that subverts the antitumor reactivity of natural killer immune cells. Cancer research 2012; 72(2): 440-8.
16. Thun MJ, Jacobs EJ, Patrono C. The role of aspirin in cancer prevention. Nat Rev Clin Oncol 2012; 9(5): 259-67.
17. Boutaud O, Sosa IR, Amin T, et al. Inhibition of the Biosynthesis of Prostaglandin E2 By Low-Dose Aspirin: Implications for Adenocarcinoma Metastasis. Cancer Prev Res (Phila) 2016; 9(11): 855-65.
18. Reimers MS, Bastiaannet E, Langley RE, et al. Expression of HLA Class I Antigen, Aspirin Use, and Survival After a Diagnosis of Colon Cancer. JAMA Intern Med 2014.
19. Labelle M, Begum S, Hynes RO. Direct signaling between platelets and cancer cells induces an epithelial-mesenchymal-like transition and promotes metastasis. Cancer Cell 2011; 20(5): 576-90.
20. Patrono C. Low-dose aspirin in primary prevention: cardioprotection, chemoprevention, both, or neither? Eur Heart J 2013; 34(44): 3403-11.
21. Patrono C, Coller B, FitzGerald GA, Hirsh J, Roth G. Platelet-active drugs: The relationships among dose, effectiveness, and side effects : the seventh accp conference on antithrombotic and thrombolytic therapy. Chest 2004; 126(3_suppl): 234S-64S.
22. Dorsam RT, Kunapuli SP. Central role of the P2Y12 receptor in platelet activation. The Journal of clinical investigation 2004; 113(3): 340-5.
23. Kim HH, Liao JK. Translational therapeutics of dipyridamole. Arteriosclerosis, thrombosis, and vascular biology 2008; 28(3): s39-42.
24. Kapil N, Datta YH, Alakbarova N, et al. Antiplatelet and Anticoagulant Therapies for Prevention of Ischemic Stroke. Clinical and applied thrombosis/hemostasis : official journal of the International Academy of Clinical and Applied Thrombosis/Hemostasis 2016.
25. Loffler I, Grun M, Bohmer FD, Rubio I. Role of cAMP in the promotion of colorectal cancer cell growth by prostaglandin E2. BMC cancer 2008; 8: 380.
26. Hicks BM, Murray LJ, Hughes C, Cardwell CR. Clopidogrel use and cancer-specific mortality: a population-based cohort study of colorectal, breast and prostate cancer patients. Pharmacoepidemiol Drug Saf 2015; 24(8): 830-40.
27. Zacharski LR, Moritz TE, Baczek LA, et al. Effect of mopidamol on survival in carcinoma of the lung and colon: final report of Veterans Administration Cooperative Study No. 188. J Natl Cancer Inst 1988; 80(2): 90-7.
28. Rothwell PM, Fowkes FG, Belch JF, Ogawa H, Warlow CP, Meade TW. Effect of daily aspirin on long-term risk of death due to cancer: analysis of individual patient data from randomised trials. Lancet 2011; 377(9759): 31-41.
29. Mora S, Manson JE. Aspirin for Primary Prevention of Atherosclerotic Cardiovascular Disease: Advances in Diagnosis and Treatment. JAMA Intern Med 2016; 176(8): 1195-204.
30. Janssen-Heijnen ML, Szerencsi K, van de Schans SA, Maas HA, Widdershoven JW, Coebergh JW. Cancer patients with cardiovascular disease have survival rates comparable to cancer patients within the age-cohort of 10 years older without cardiovascular morbidity. Crit Rev Oncol Hematol 2010; 76(3): 196-207.
31. Janssen-Heijnen ML, Houterman S, Lemmens VE, Louwman MW, Maas HA, Coebergh JW. Prognostic impact of increasing age and co-morbidity in cancer patients: a population-based approach. Crit Rev Oncol Hematol 2005; 55(3): 231-40.
32. Bayliss E, Reifler L, Zeng C, McQuillan D, Ellis J, Steiner J. Competing risks of cancer mortality and cardiovascular events in individuals with multimorbidity. Journal of Comorbidity 2014; 4(1): 29-36.
33. Zuurbier SM, Vermeer SE, Hilkens PH, Algra A, Roos YB. [Secondary prevention with clopidogrel after TIA or stroke]. Ned Tijdschr Geneeskd 2013; 157(25): A5836.
34. Nash GF, Turner LF, Scully MF, Kakkar AK. Platelets and cancer. The Lancet Oncology 2002; 3(7): 425-30.
35. Guillem-Llobat P, Dovizio M, Bruno A, et al. Aspirin prevents colorectal cancer metastasis in mice by splitting the crosstalk between platelets and tumor cells. Oncotarget 2016.

CHAPTER 7

THE MORTALITY REDUCING EFFECT OF ASPIRIN IN COLORECTAL CANCER PATIENTS: INTERPRETING THE EVIDENCE

Martine A. Frouws, Myrthe P.P. van Herk-Sukel, Huub A. Maas, Cornelis J.H. Van de Velde, Johanneke E.A. Portielje, Gerrit-Jan Liefers, Esther Bastiaannet

Cancer Treatment Reviews 2017 Feb 20;55:120-127

Abstract

In 1971 the first study appeared that suggested a relationship between aspirin and cancer. Currently publications on the subject of aspirin and cancer are numerous, with both a beneficial effect of aspirin on cancer incidence and a beneficial effect on cancer survival. This review focusses on the relation between the use of aspirin and improved survival in colorectal cancer patients. Various study designs have been used, with the main part being observational studies and post-hoc meta-analyses of cancer outcomes in cardiovascular prevention trials. The results of these studies are unambiguously pointing towards an effect of aspirin on colorectal cancer survival, and several randomised controlled trials are currently ongoing. Some clinicians feel that the current evidence is conclusive and that the time has come for aspirin to be prescribed as adjuvant therapy. However, until this review, not much attention has been paid to the specific types of bias associated with these studies. One of these biases is confounding by indication, because aspirin is indicated for patients as secondary prevention for cardiovascular disease. This review aims to provide perspective on these biases and provide tools for the interpretation of the current evidence. Albeit promising, the current evidence is not sufficient to already prescribe aspirin as adjuvant therapy for colorectal cancer.

Introduction

Aspirin, originating from the bark of a willow, was already used by Hippocrates in 400 B.C. It was patented as an analgesic in 1897, but the analgesic working mechanism was not unravelled until 1970.[1] In the years thereafter, this appeared not to be the only ability of this medicine, as in the 1970's aspirin became regular treatment for secondary cardiovascular disease prevention. More recently, the possible anti-cancer effect of acetylsalicylic acid has gained much attention, with the most elaborative studies performed in patients with colorectal cancer (CRC).[2] A reduced cancer incidence as well as a reduced cancer mortality of aspirin users has been observed frequently.[3]

Cancer is still one of the main causes of premature death worldwide.[4] Cancer is one of the most expensive diseases for health care systems around the world with global spending on cancer drugs alone of more than $100 billion in 2014.[4] Hence, new and cheap cancer drugs that are globally available are urgently needed, and hopefully aspirin can become an additional therapeutic option in the spectrum of cancer treatment.

Albeit the promising results so far, before aspirin can be implemented as regular treatment option in cancer, randomised controlled trials (RCT's) have to be awaited. Several RCT's are currently ongoing to provide the world with a decisive answer on the mortality reducing effect of aspirin on colorectal cancer, but it will take another few years before the results of these studies will be published (table 1). Meanwhile, 16 observational studies, 4 meta-analysis and numerous reviews have been published on the subject, all pointing to beneficial effects of aspirin on survival of CRC patients.[5-25]

Cardiovascular disease is more prevalent in the group of patients using aspirin and the impact of this has been disregarded until now. The present review aims to deliver a critical appraisal of the available evidence with special focus on possible sources of bias in the current observational studies.

Also, data from the RCT's studying cancer outcomes in cardiovascular prevention trials have to deal with complex relations between cardiovascular morbidity, CRC cancer and survival. Previous studies mainly focused on the mortality reducing effect of aspirin in CRC patients, and therefore this will be the scope of this review. This review will provide a framework for the epidemiological challenges associated with the interpretation of observational studies on aspirin effects.

Table 1: Overview of current ongoing trials for the effect of aspirin use after diagnosis in patients with colorectal cancer

Trial name and registration number	Type of cancer	Patients (n)	Inclusion criteria	Randomised treatment (incl treatment duration and dose)	Primary end-point	Participating countries	Start recruitment	Expected finished recruitment
Add-aspirin trial* (ISRCTN74358648)	Colorectal, stomach, oesophagus, prostate and breast cancer	2600 colorectal (total: 9920)	Stage II and III adenocarcinoma	100 mg, 300 mg of daily aspirin or placebo, during 5 years	5 yr DFS	UK	2016	2026
ALASCCA trial (NCT02647099)	Colorectal cancer	3900	Stage II and III PIK3CA mutated patients	160 mg of daily aspirin or placebo during 3 years	3 year time to recurrence	Sweden, Norway	2016	2021
ASCOLT trial (NCT00565708)	Colorectal cancer	1200	Dukes B or C	300 mg of daily aspirin or placebo during 3 years	3 yr DFS	Singapore, Australia, India, China, Hong-Kong, South-Korea, Malaysia, Taiwan, Saudi-Arabia, Indonesia and the Philippines	2012	2022
ASPIRIN trial (NCT02301286)	Colon cancer	1588	Stage II or III adenocarcinoma	100 mg of daily aspirin or placebo during 5 years	5 yr OS	Netherlands, Belgium, Portugal	2015	2022
SAKK 41/13 (NCT02467582)	Colorectal cancer	896	Stage II and III adenocarcinoma, PIK3CA mutated patients	300 mg of daily aspirin or placebo during 3 years	3 yr DFS	Switzerland, Hungary	2015	2018

** Only the specifications of the colorectal arm of the Add-aspirin trial are provided in this overview*
Abbreviations:
DFS: Disease-Free Survival
OS: Overall Survival
PIK3CA: phosphatidylinositol 3-kinase

Current evidence from observational studies

Four meta-analyses have addressed the effect of aspirin on survival in CRC.[5-8] The most recent and comprehensive meta-analysis of Elwood et al provides a complete overview of all relevant studies.7 In this meta-analysis, sixteen observational studies were identified. A reduction in colorectal deaths of about 25%, and a reduction of approximately 20% in all-cause mortality was demonstrated. The pooled CRC specific mortality rate was 0.71 (Hazard Ratio (HR) 95% Confidence Interval (CI) 0.58-0.87) and the pooled overall mortality rate was 0.80 (95% CI 0.70-0.92). The included studies all had different study designs and methods of analysis, which resulted in a high p-value for heterogeneity (p-value= 0.0005, all studies). Table 2 provides an overview of the included studies.

Two large cohort studies from the United States that evaluated the effect on mortality in CRC with self-reported aspirin use were not included in the meta-analysis.[17,26] Both studies reported a mortality reduction for patients with CRC, although Zell et al reported that this was only the case for patients using aspirin for more than five years (overall mortality, HR 0.53 (95% CI 0.34-0.83) CRC-specific mortality 0.33 (95% CI 0.18-0.63)). The study only had data on aspirin use prediagnosis and was therefore was not included in the meta-analysis of Elwood *et al*.

In the current observational evidence on the effect of aspirin on cancer survival, unfortunately, information on adverse events is scarce. In the general population, aspirin doubles the incidence of gastrointestinal bleeding with one or two persons in every thousand may suffer from gastric bleeding each year.[27] The only colorectal study where gastrointestinal bleeding was taken into account, was a study on the primary prevention of CRC cancer in aspirin users in the Women's Health Study. This study could not prove a health benefit for the overall population of women when the negative effects of gastro-intestinal bleeding were taken into account.[28] Only in the subgroup of women older than 65 years, the authors suggested a net benefit.[28]

Several important and informative reviews have been published on the subject of aspirin and cancer mortality, some of which will be discussed here. The first review on the subject of Langley *et al.* in 2011 pleaded that an increased risk: benefit ratio for the use of aspirin as adjuvant therapy should be accepted compared to the risk: benefit ratio for primary cancer prevention. More toxicities may be acceptable in the prevention of cancer metastases than in primary prevention of cancer.[29] Another overview provided by Chia *et al.* offered a very informative framework for the understanding of the anti-cancer activity of aspirin with regard to the pathogenesis of CRC.[30] The third review focussed on the identification of specific biomarkers for the personalized treatment with aspirin, to prevent unnecessary side effects. This review concluded that probably no single biomarker is able to identify individuals that will or will not benefit from aspirin and that multiple biomarkers should be investigated in the future.[3] The focus in these previous reviews was at the molecular background of patients who benefit from aspirin treatment, aiming for better understanding of the aetiology of the effect of aspirin on cancer survival. This current review aims to provide insights in the biases associated with the present available observational studies and will focus more on the epidemiological aspects, an element of these studies that got little attention until now.

Table 2: Overview of observational studies assessing the effect of aspirin on survival in patients with colorectal cancer, adapted from Elwood et al.[7]

Authors	Year	Analysis of drug exposure (time-varying yes/no)	Moment of assessment of use	Results	Overall survival (OS)	CRC-spec survival	Source	Design	Duration of follow-up
					Deaths/nonusers Deaths/aspirin users	Deaths/nonusers Deaths/aspirin users			
Bains et al.[9]	2016	Yes	Postdiagnosis	HR CRC-spec: 1.00 (0.87-1.14) HR OS: 1.06 (0.96-1.18)	7218/17060 290/1711	7218/17060 549/1711	Cancer Registry of Norway, The Norwegian Prescription Database	Cohort of cancer patients	Median FU: 3 years
Bastiaannet et al.[10]	2012	Yes	At diagnosis and postdiagnosis	**RR OS: 0.77 (0.63-0.95)***	610/1176 114/275	No information	Eindhoven Cancer Registry-PHARMO Drug Outcome Institute	Cohort of cancer patients	Median FU: 3.5 years (0–12)
Cardwell et al.[11]	2014	Yes	Postdiagnosis	HR CRC-spec: 0.99 (0.86-1.15) HR OS: 1.06 (0.94-1.19)	1514 (nonusers) 700 (users)	1577/4794 CRC patients 7530 controls	Linkages between the National Cancer Data Repository, UK Clinical Practice	Nested case-control analysis from a cohort of 4794 cancer	Mean FU: 7.2 years (range 1-13.8)
Chan et al.[12]	2009	No	Pre- and postdiagnosis	**HR CRC-spec: 0.53 (0.33-0.86)** **HR OS: 0.68 (0.51-0.92)**	287/1279 193/549	141/1279 81/549	US Nurses and Health Professionals Cohorts	Cohort of cancer patients	Median FU: 11.8 years
Coghill et al.[13]	2011	No	Only prediagnosis use	**HR CRC-spec 0.76 (0.61-0.94)**	No information	252/889 146/652	Seattle Colon Cancer Family Registry	Cohort of cancer patients	Mean FU: 8 years
Din et al.[14]	2010	No information	Only prediagnosis use	HR CRC-spec:1.03 (0.80-1.31) HR OS: 1.12 (0.90-1.39)	459/1588 124/350	400/1588 94/350	Study of Colorectal Cancer Scottland, SOCCS	Population-based case-control study in cancer patients	Median FU: 4.7 years (IQR 2.97-5.74)
Domingo et al.[15]	2013	No	At diagnosis and postdiagnosis	HR DFS: 0.86 (0.55–1.35) HR OS: 0.88 (0.53-1.47)	22/125 174/771		Series of patients from the VICTOR trial	Cohort study in trial cohort of cancer patients	Median FU 61.5 months (IQR 49.9-69.8)
Goh et al.[16]	2014	No information	Pre- and postdiagnosis	**HR DFS: 0.38 (0.17-0.84)*** HR CRCspec: 0.71 (0.43-1.16)*	No information	160/634 21/92	Series of patients	Cohort study of cancer patients	FU 'long term'
Jacobs et al.[17]	2012	No information	No information	**HR CRC-spec: 0.63 (0.46-0.88)**	No information	116 deaths 67 deaths	CPS-II Nutrition Cohort	Cohort study of cancer patients	FU 6 years (1997-2003)
Liao et al.[18]	2012	No	Pre- and postdiagnosis	HR CRC-spec: 0.83 (0.61–1.23) HR OS: 0.87 (0.71-1.06)	240/561 155/403	122/561 68/403	Nurses Health Study and Health Professionals Cohorts	Cohort study of cancer patients	Median FU: 153 months (IQR 104-195)
McCowan et al.[19]	2012	Yes	Pre- and postdiagnosis	**HR CRC-spec: 0.58 (0.45-0.75)** **HR OS: HR 0.67 (0.57-0.79)**	1101/1650 153/350	601/1650 56/350	Cancer Registry records in Tayside region, Schotland	Cohort study of cancer patients	Median FU 2.80 years (IQR 0.63-6.21)
Ng et al.[20] (same cohort and results as Fuchs et al.[25])	2015	No information	Postdiagnosis	HR DFS: 0.68 (0.42-1.11) HR OS: 0.63 (0.35-1.12)	156/724 14/75	214/724 19/75	Series of trial patients from CALGB 89803	Cohort study in trial cohort	Median FU: 6.5 years
Reimers et al.[21]	2014	Yes	At diagnosis and postdiagnosis	**HR OS: 0.64 (0.49-0.83)**	396/817 69/182		Eindhoven Cancer Registry-PHARMO Drug Outcome Institute	Cohort study	No information
Sun et al.[22]	2013	No information	No information	HR CRC-spec: 0.77 (0.52-1.14)	931 events total	No information	US Nurses and Health Professionals cohorts	Cohort study of cancer patients	Total FU: 28 years
Walker et al.[23]	2012	Yes	Pre- and postdiagnosis	HR OS: 0.99 (0.84-1.16)	4400/11325 958/2619	No information	UK General Practice Research Database	Cohort study of cancer patients	Median FU: 1.7 years
Zanders et al.[24]	2015	Yes	Postdiagnosis	HR OS: 0.98 (0.93–1.03)	296/778	No information	Eindhoven Cancer Registry-PHARMO Drug Outcome Institute	Cohort study of cancer patients	Median FI: 1.5 years (IQR0.2–3.4)

IQR: Interquartile range; DFS Disease Free Survival; CRC-spec: Colorectal Cancer Specific; CRC Colorectal Cancer
** HR only for postdiagnosis use*

Methodology and bias

The overall goal of an epidemiological study is accuracy and precision in estimating the value of the parameter of interest, i.e. a measurement without bias. With the increased availability of population-based drug use information, the methods of analysis are increasingly important as are the consequences of biased analysis.

Nearly all types of bias can be categorised as either selection bias, misclassification bias or confounding:

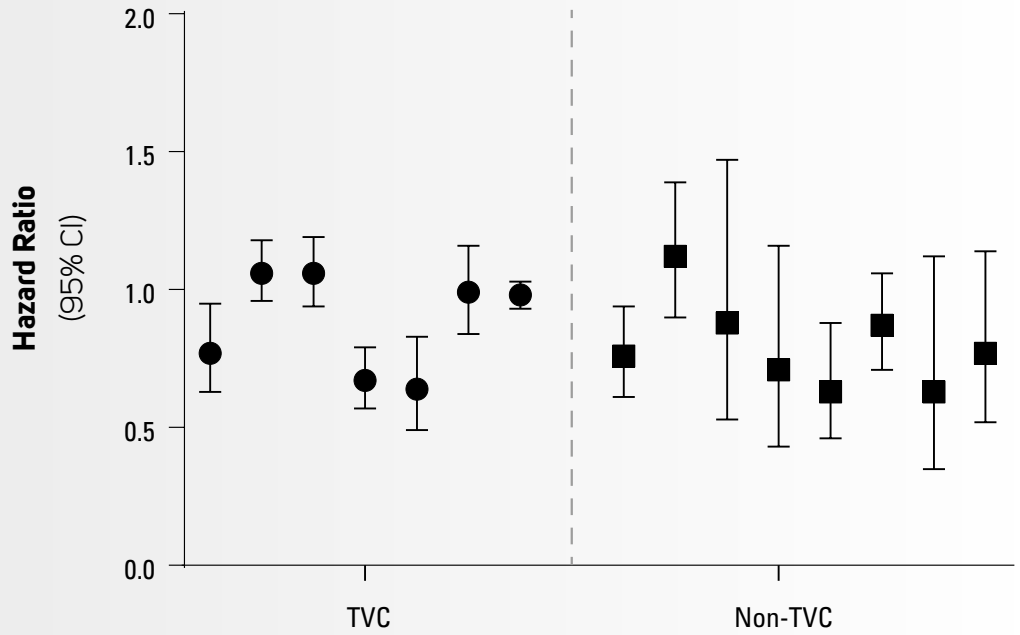
1) Selection bias entails the selective recruitment into the study of subjects that are not representative of the exposure or outcome pattern in the source population.
2) Misclassification bias arises by incorrect information about either exposure or outcome or covariates for the study participants.
3) Confounding is a bias in estimating an epidemiologic measure of effect resulting from an imbalance of other determinants of disease (or their proxies) in the compared groups.

Immortal time bias

One important pitfall for observational studies is immortal time bias.[31-33] Immortal time bias (also called survivor bias) has been described as a span of time in the follow-up period of a cohort during which the outcome under study could not have occurred, because subjects should be alive for the event to have occurred.[33] Immortal time bias is a form of information bias.[33]

First, immortal time bias could have occurred in the method of how the use of aspirin was analysed. Person-time prior to the prescription should be analysed as unexposed (by the use of a time-varying covariate) and this will result in valid and precise risk estimates.[32] Not using a time-varying covariate results in misclassification of drug exposure time. In studies where large prescription databases are used, immortal time bias is usually avoidable, but the risk of immortal time bias should be taken into account early in the designing of the study.[33] This will be more problematic in studies where patients are defined as users by means of questionnaires at one (or several) time-points. When not accounting for this type of bias, this can cause an illusory strengthening of the protective effect of medication.[31,33,34] Several publications found that when time-varying covariates are used properly they can even result in no effects of exposure.[35] The study of Assayag et al found no association between aspirin mortality in patients with prostate cancer when using proper analysis techniques.[35,36] In contrast, a large US cohort found only significant results when aspirin use was analysed as a time-varying covariate.[17] The impact of these differences of analyses have been displayed in figure 1, where the hazard ratios of the observational studies of table 2 are plotted and grouped according to whether or not a time-varying analysis was used. Van Walraven *et al*. demonstrated that appropriate time-dependent methods were used in only 40% of cohort studies published in prominent medical journals.[37] Subsequently, Austin *et al* quantified the impact of immortal time bias in drug exposure studies and found that the *estimated* treatment effect varied from 4% to 27% mortality reduction in these studies when the time-varying nature of the treatment was ignored, when there was no *actual* treatment effect.[34]

Figure 1: Observational studies grouped according to the use of a time-varying covariate (TVC)



Protopathic bias

Another form of bias that is likely to occur in drug exposure studies is reverse causation, also referred to as protopathic bias.[38] This type of bias appears when the outcome leads to changes in exposure, e.g. if cancer recurrence causes early symptoms. As a result, patients may use pain medication for early symptoms of disease and therefore, pain medication may *appear* to be associated with increased cancer recurrence.[39,40] It is unlikely that this type of bias may have influenced the results of the observational studies of aspirin and CRC mortality because aspirin is hardly used as an analgesic these days. Even more, the use of aspirin as analgesic would dilute the association between improved survival and the use of aspirin and not cause reverse causation. Lastly, aspirin in low-dose is not indicated as an analgesic.

Confounding by indication

Confounding by indication (indication bias), is an important cause of bias in non-randomised studies, and present in several forms in the observational studies assessing the association between aspirin use and cancer survival.[41] Confounding by indication occurs when the clinical indication for selecting specific treatment is also related to the outcome. When studying the effect of aspirin specific colorectal cancer survival, this is not applicable, but when studying aspirin use and the relation with overall survival this is could have influenced results.[42,43]

Several studies have suggested a difference in the association with survival with regard to the moment of starting aspirin. In general, most studies distinguish two groups of users; patients that use aspirin at the moment of diagnosis and continue after diagnosis (pre-and

postdiagnosis use), and patients starting aspirin after being diagnosed with cancer (solely postdiagnosis use). In studies that assessed both pre- and postdiagnosis use and also solely postdiagnosis use, the effect was more pronounced in patients using aspirin only after diagnosis, which was confirmed by the meta-analysis of Ye *et al.*[6]

The moment of selection of users may introduce bias. Bias arises when patients are selected to be users only in the period after diagnosis of colorectal cancer. Commencing the use of aspirin after diagnosis implies that patients are considered fit enough for the prevention of cardiovascular disease according to their (cancer) prognosis (healthy user bias). As a result, the patients with the worst prognosis will end up in the group of nonusers and the survival benefit will appear to be (falsely) larger. An additional factor complicating factor here is that patients with worse cardiovascular disease warrant treatment with oral anticoagulation therapy (mostly coumarine derivatives). Aspirin is not supposed to be prescribed in combination with oral anticoagulation therapy because of the high bleeding risk, this has only been found appropriate in patients with mechanical heart valves. [44-46] On the contrary, one advantage for the assessment of aspirin use commenced after diagnosis, is the 'new-user design'. Because patients are not yet differentiated into groups of users and nonusers at the moment of CRC diagnosis, this implies that the groups of users and nonusers are equally comparable at the moment of diagnosis with a similar prognosis.

Regarding this observation, a study with data from the Swedish Cancer Register demonstrated that aspirin use in the year prior to diagnosis had a beneficial effect on tumor stage in patients with CRC with lower invasion depth of the primary tumor (T-stage) and less distant metastasis (M-stage) but not on nodal status.[47] According to the authors this could partially account for the survival benefit found in patients using aspirin. As tumours are diagnosed in a lower stage, it could be that this can partially explain the observed survival benefit for patients that use aspirin both pre- and postdiagnosis. No difference was found between users of aspirin and non-users with regard to nodal status. This is an intriguing observation which the authors attribute to the antiplatelet properties of aspirin. Because platelets are not existent in the lymphatic systemic and therefore no effect of aspirin can be found in the nodal status.

Another hypothetical type of confounding by indication may result from earlier detection of tumours due to aspirin use, when aspirin induces early symptoms such as rectal bleeding or bleeding from polyps. This could however not been demonstrated by Rothwell, who studied time from randomisation to cancer incidence.[48]

Lastly, it could also be possible that patients who develop a tumour and already use aspirin are less sensitive for aspirin treatment, because the tumour developed in an environment where aspirin was already present. This was not found confirmed by the observations in an in vivo study, where mice were treated prediagnosis with aspirin and after tumour growth exposed to additionally postdiagnosis aspirin, versus mice that were only postdiagnosis exposed. Both tumours were reduced in size with the use of aspirin after tumor diagnosis.[49] Additionally, the meta-analysis of Elwood et al. did not detect a difference between the use of aspirin pre- and postdiagnosis and the use of aspirin solely postdiagnosis.[7] Table 2 shows the timing of assessment of use of all current observational studies.

The effect of (cardiovascular) morbidity on cancer survival

The second form in which confounding by indication is also related to the indication for which aspirin has been prescribed to these patients. In the current guidelines, aspirin is solely indicated as medication for secondary prevention of CVD.[50] Up until this point in time, the net value of aspirin as primary prevention for CVD could not be proven.[50-52] This makes it reasonable to assume that all patients taking low-dose aspirin in observational studies should have a history of CVD.[3,50] Despite (at least) one additional comorbidity (CVD) compared to other patients with cancer, patients taking aspirin seem to have a survival benefit over patients not taking aspirin. This paragraph will enlighten on the magnitude of the effect of CVD in patients with cancer on survival.

There are several mutual risk factors for both CVD and cancer, suggesting a shared biology: inflammation, oxidative stress, reactive oxygen species, hormones, and other metabolic reactions.[53] Obesity, diabetes mellitus, hypertension, hyperlipidaemia, diet, and physical activity are further overlapping, life-style related risk factors in CVD and cancer development.

The effect of CVD and the effect on the outcome of patients with cancer has been studied extensively. Patients with both CVD and cancer have a higher chance of overall *and* cancer specific mortality; 1.2 to 4.8 fold higher five-year mortality rates have been observed in patients with both cancer and CVD compared to cancer patients without comorbidity.[54-56] In patients with cardiovascular diseases survival rates are comparable to patients with cancer within the age-cohort of 10 years older without cardiovascular disease.[57] One study found that patients with previous cardiovascular condition have a HR of 1.66 (95% CI 1.20-2.31) for cancer specific mortality and this risk increases when patients have both previous CVD and another comorbidity.[58] Patients with serious comorbid conditions and stage I cancer have similar survival rates compared to patients with no comorbidity and stage II cancer.[55] Thirty-four percent of the mortality in the first year of follow-up for patients with a Charlson Comorbidity Index of four has been observed to be accountable for the interaction between comorbidity and cancer.[56]

That at least some part of the observed survival benefit can be attributed to confounding by indication is further supported by both short-term and long-term observed survival benefits of aspirin use. A study by van Erning et al. has provided insight in the long-term causes of death in patients with CRC in a Dutch population-based cohort.[59] This study showed that the risk of dying from cancer diminishes with each additional survived year after diagnosis. The risk even decreases below 5% risk of dying from CRC five years after diagnosis.[59] After these five years, there are still several studies showing a survival benefit for patients taking aspirin, suggesting that this benefit can hardly be from dying of CRC.[17,26,60]

Cancer specific mortality may be less likely when subjects are at risk of dying from another cause first, in this case cardiovascular disease.[39] Additionally, if patients have very serious cardiovascular disease they are likely to die before they are able to develop a malignancy.[34] On the contrary, patients with cardiovascular disease also experience more risk to die from colorectal cancer itself because of overall worse condition.[58]

The risk profile and unfavourable prognosis in patients with CVD is more emphasized in older patients. Colorectal cancer is associated with an increased comorbidity burden in

older patients when compared to the general population of elderly, severe comorbidity is subsequently associated with an increased overall mortality, HR 1.41 (95% CI 1.14-1.73).[61] Older patients have an average of three comorbidities in addition to cancer.[62] The proportion of patients with comorbidities increases with age; in an unselected cancer population in the Netherlands, 53% of patients aged 60-74 years have at least one comorbidity, up to 63% for patients 75 years and older with CVD being the most common comorbidity.[63] Treatment of cancer patients with comorbidity tends to be different compared to patients with no comorbidity. Patients with comorbidity are less likely to receive surgery, chemotherapy and radiotherapy compared to patients without comorbidity.[55] The underlying reason for the apparent under treatment is not clear from literature, but greater toxicity risk, poorer clinical quality, patient preferences, or poor adherence have been suggested as optional causes.[55] Lastly, older patients and patients with comorbidity seem to receive less adjuvant chemotherapy which, at baseline, also worsens their prognosis.[64] Additional to the increased risk of (cancer specific) mortality and reduced likelihood to receive treatment in patients with cardiovascular disease, complications of cancer treatment form an extra risk. Risk factors for CVD also predict cardiotoxicity from cancer therapy, e.g. for patients treated with trastuzumab, and this can consequently cause reduced treatment adherence leading to an additional worse survival.[53,61]

Current evidence from Randomised Controlled Trials

Rothwell et al. published a series of meta-analyses assessing the effect of aspirin from individual patient data from RCT's, originally designed for primary or secondary prevention of vascular events.[48,60,65,66] Additional data were collected with information on cancer incidence, cancer metastasis and cancer specific death for these meta-analyses.

The first meta-analysis (n=14,033) in 2010 showed a 40-50% reduction in 20-year risk of death due to CRC in patients using low-dose aspirin.[60] The effect of aspirin on cancer-specific mortality increased with treatment duration, with the largest effect observed in patients with gastrointestinal tumours, more specific in CRC.[48] In several subgroups an even greater mortality reduction was found, such as patients with a tumour of the proximal colon and in patients taking aspirin in low dose.[60,65]

Despite the fact that the effect on cancer death was greater after additional years of follow-up, there was also a reduction in mortality in some cancer types *already* after 2-3 years since randomisation. This appeared to be too fast to effect carcinogenesis or early cancer growth and therefore the risk of metastasis was assessed in a next study.[66] In this study, the risk of metastasis in patients diagnosed with CRC appeared to be lower when patients took aspirin (HR 0.26 (95% CI 0.11-0.57). The reduced risk of death in patients with CRC was greatest in patients without metastasis at diagnosis, compared to patients with metastasis at diagnosis.[66] Additionally, it was observed that patients who continued aspirin after diagnosis of (localized) cancer have a lower chance of developing metastasis.[66] The authors concluded that the early effects on cancer death can probably be contributed to reductions in cancer metastasis.[66]

The United States Preventive Services Task Force (USPSTF) has carried out a meta-analysis of the RCT's for primary cardiovascular prevention, analysing the effect on (colorectal) cancer outcomes. The study found a RR of 0.67 (95%CI 0.52-0.86) on CRC cancer death for patients using long-term aspirin.[67]

One additional study with randomised data is the Women's Health Study. This study randomised between alternate-day use of aspirin 100 mg versus placebo in healthy women, with a median treatment duration of 9 years. When the trial period was finished, post-trial aspirin use was additionally registered by means of annual questionnaires. After a follow-up of 12 years, a difference was found in the number of patients diagnosed with a metastatic adenocarcinoma (in favour of patients taking aspirin) but only in the group of patients who took additional aspirin in the period *after* the randomised treatment was finished. This effect could have been also the result of immortal time bias. No difference in cancer death was observed.[68] In the Women's Health Study no reduction in incidence of major CVD or CVD death was observed, correlating with the results of the study of Rothwell *et al.*[65,68]

The studies of Rothwell and his colleagues have made a valuable contribution in addition to the field of observational studies. What might have influenced the results of the above described observational studies, is confounding by indication, and this is largely avoided with the design of these meta-analyses. Some limitations remain, since most patients in the studies that were included in the meta-analysis (even in the primary prevention trials) have at least some risk factors for CVD and these risk factors overlap with the risk factors for cancer.[53] The trials were not designed to assess cancer outcomes, and it could be possible that not all death causes were recorded thoroughly, compared to when this was planned prior to the study, and the registration of cancer specific details may be less accurate. Time from randomisation in the original trials was analysed until date of death, which is different from most observational studies, where time from diagnosis until date of death was analysed. This complicates the comparison between the observational studies and the RCT's, because it is unknown if the actual time from diagnosis to death in the RCT's is also improved for patients using aspirin.

One last study of the group of Rothwell *et al* analysed if the post-hoc analysis of the cardiovascular prevention trials were comparable to observational studies. This study showed that the effects found in observational studies with a rigour definition of exposure are consistent with the results in RCT's. Sensitivity was particularly dependent on the appropriate and detailed recording and analysis of the use of aspirin.[69]

Despite the fact that the research group of Rothwell *et al*. concluded that the results of the observational studies are comparable with the results of the post-hoc results of the RCT's, several important biases could have influenced the results of the observational studies. By raising the awareness about these biases, the interpretation of the current evidence may improve.

RCT's of aspirin and CRC

In the field of CRC, there have been two RCT's specifically designed to assess the effect of aspirin on cancer outcome which have been already completed.

The first RCT, performed in 1982, assessed the effect of aspirin in patients with CRC, although with a very small number of patients (n=66) and the treatment period was only two years.[70] The results did not show a significant difference between patients taking aspirin and placebo.

The CAPP2 trial assessed the effectivity of 600 mg aspirin on cancer incidence in patients with Lynch syndrome and showed a significant reduction in time to first colorectal cancer. A significant association was observed only in patients who had taken the intervention for more than two years analysed in the per-protocol analysis, HR 0.41 (0.18-0.78). At present, the CAPP3 trial is ongoing to assess the most optimal dosage of aspirin as chemoprevention for patients diagnosed with Lynch syndrome. Rates of death were similar in any-dose aspirin versus the placebo groups.[71]

Currently, several RCT's are ongoing, designed to study the effect of adjuvant aspirin treatment on (cancer) survival in patients with colorectal cancer. An overview of these studies is provided in table 1. Two trials recruit only patients with a phosphatidylinositol 3-kinase (PIK3CA) mutation and randomise between aspirin and placebo, ALASCCA in Sweden (NCT02647099) and the SAKK 41/13 trial in Switzerland (NCT02467582)). These trials have been designed mainly based on the results of the study of Liao *et al*. where a hazard ratio of 0.18 (95% CI 0.06-0.61) was found in patients with a PIK3CA mutation.[18] After this first publication several other studies have been published on this topic, suggesting a relation between the effect of aspirin and a PIK3CA mutation on cancer survival.[8,15,72]

All trials are united in the Aspirin Trialist Collaborative Group and have plans for elaborative meta-analysis once all trials have been published individually. This will provide answers for urgent questions regarding dose, tumour location, treatment duration and racial differences.[73]

Future perspectives

Future observational studies should focus on proper methodology. Therefore we plead for (pharmaco)-epidemiologists to always be involved in studies analysing population based prescription data. Futhermore, an evolving and promising topic is Molecular Pathological Epidemiology (MPE).[74] MPE is an integrative transdisciplinary science. Because molecular pathology tests are increasingly becoming routine clinical practice, pathology tests may be utilized in population-based studies. Therefore this could provide a solid base for future studies, and MPE should be used to expand current knowledge on the aetiology of the effect of aspirin.'

Discussion

With this review we point out that the results of the current studies are promising, especially considering the results of the observational studies complemented with the results of the randomised data of Rothwell *et al*. and the USPSTF meta-analysis.

The hypothesis that death from multiple causes is prevented in a population with high-risk of several causes of death, combined with the more favourable mortality estimates that tend to be found by routinely collected health data compared to RCT's, indicate that the effect that the results from the ongoing randomised trials shall be lower than the results of the observational studies so far.[75] As described in this review, the risk of (cancer) mortality in patients with cardiovascular disease is higher than in patients without cardiovascular disease.[3] This implies that the potential benefit from aspirin in the overall cancer population will also be lower.[75] The combination of the increased risk of (cancer-specific) death and the demonstration of the more marked effect of aspirin in older patients, suggests that the observed survival benefit of cancer patients taking aspirin is because multiple death causes are prevented. Aspirin has also been shown to be associated with a reduced cancer risk in patients with Lynch syndrome and it has been suggested that patients at highest risk of CRC are most likely to gain from chemoprevention.[3,76] This has also been observed by the United Stated Preventive Services Task Force (USPSTF); the higher the risk for CRC, the higher the mortality benefit.[67] Because of this expectation, the ongoing Aspirin trial is powered conservatively (HR of 0.75), allowing to still detect a significant difference between the group of patients using aspirin versus patients randomised to a placebo.

Some clinicians in current practice plead already for the regular use of aspirin based on the results of the current studies. However, with this review we aimed to demonstrate that with the many caveats left, this is a bridge too far. In the past decade there has been many media attention for aspirin as possible anti-cancer therapy. Patients and clinicians lean towards settling for the current evidence. Recruitment of the current RCT's is lacking behind since some patients find the evidence too convincing and use aspirin regardless of the RCT's. The meta-analysis of Elwood et al endorses this statement.[7] Despite the current existing limitations, the present evidence is promising, since multiple meta-analysis and pre-clinical studies show an effect of aspirin on cancer.[7,30,77]

The results of the ongoing randomised trials will determine the effect of aspirin on survival in cancer patients. Before these trials have been finished, studies should focus on the working mechanism of aspirin in relation to cancer.

Conclusion

Current observational studies assessing the association between the use of aspirin and survival show mutually comparable results, but could have been subject to multiple forms of bias.41 The present, abundant number of observational studies and pooled trial data from the RCT's of Rothwell *et al.* combined with the numerous and promising pre-clinical studies make it highly likely that the ongoing RCT's will result in a survival benefit for colorectal cancer patients.

Conflicts of interest

Myrthe P.P. van Herk-Sukel is an employee of the PHARMO Institute for Drug Outcomes Research. This independent research institute performs financially supported studies for government and related healthcare authorities and several pharmaceutical companies. However, this study was not financially supported by a pharmaceutical company.

The remaining authors declare no competing financial interests.

All authors approved the final version of this manuscript.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgements

The authors would like to thank M. Swets (Leiden University Medical Center) for preliminary data analysis and R.E. Langley (University College London) for reviewing an early draft of the manuscript.

Reference list

1. Tsoucalas G, Karamanou M, Androutsos G. Travelling through Time with Aspirin, a Healing Companion. European Journal of Inflammation 2011; 9(1): 13-6.
2. Henschke UK, Luande GJ, Choppala JD. Aspirin for reducing cancer metastases? J Natl Med Assoc 1977; 69(8): 581-4.
3. Coyle C, Cafferty FH, Langley RE. Aspirin and Colorectal Cancer Prevention and Treatment: Is It for Everyone? Current Colorectal Cancer Reports 2016.
4. Sullivan R, Alatise OI, Anderson BO, et al. Global cancer surgery: delivering safe, affordable, and timely cancer surgery. The Lancet Oncology 2015; 16(11): 1193-224.
5. Li P, Wu H, Zhang H, et al. Aspirin use after diagnosis but not prediagnosis improves established colorectal cancer survival: a meta-analysis. Gut 2015; 64(9): 1419-25.
6. Ye XF, Wang J, Shi WT, He J. Relationship between aspirin use after diagnosis of colorectal cancer and patient survival: a meta-analysis of observational studies. Br J Cancer 2014; 111(11): 2172-9.
7. Elwood PC, Morgan G, Pickering JE, et al. Aspirin in the Treatment of Cancer: Reductions in Metastatic Spread and in Mortality: A Systematic Review and Meta-Analyses of Published Studies. PLoS One 2016; 11(4): e0152402.
8. Paleari L, Puntoni M, Clavarezza M, DeCensi M, Cuzick J, DeCensi A. PIK3CA Mutation, Aspirin Use after Diagnosis and Survival of Colorectal Cancer. A Systematic Review and Meta-analysis of Epidemiological Studies. Clin Oncol (R Coll Radiol) 2015.
9. Bains SJ, Mahic M, Myklebust TA, et al. Aspirin As Secondary Prevention in Patients With Colorectal Cancer: An Unselected Population-Based Study. J Clin Oncol 2016; 34(21): 2501-8.
10. Bastiaannet E, Sampieri K, Dekkers OM, et al. Use of Aspirin postdiagnosis improves survival for colon cancer patients. Br J Cancer 2012.
11. Cardwell CR, Kunzmann AT, Cantwell MM, et al. Low-dose aspirin use after diagnosis of colorectal cancer does not increase survival: a case-control analysis of a population-based cohort. Gastroenterology 2014; 146(3): 700-8 e2.
12. Chan AT, Ogino S, Fuchs CS. Aspirin use and survival after diagnosis of colorectal cancer. JAMA 2009; 302(6): 649-58.
13. Coghill AE, Newcomb PA, Campbell PT, et al. Prediagnostic non-steroidal anti-inflammatory drug use and survival after diagnosis of colorectal cancer. Gut 2011; 60(4): 491-8.
14. Din FV, Theodoratou E, Farrington SM, et al. Effect of aspirin and NSAIDs on risk and survival from colorectal cancer. Gut 2010; 59(12): 1670-9.
15. Domingo E, Church DN, Sieber O, et al. Evaluation of PIK3CA mutation as a predictor of benefit from nonsteroidal anti-inflammatory drug therapy in colorectal cancer. J Clin Oncol 2013; 31(34): 4297-305.
16. Goh CH, Leong WQ, Chew MH, et al. Post-operative aspirin use and colorectal cancer-specific survival in patients with stage I-III colorectal cancer. Anticancer Res 2014; 34(12): 7407-14.
17. Jacobs EJ, Newton CC, Gapstur SM, Thun MJ. Daily aspirin use and cancer mortality in a large US cohort. J Natl Cancer Inst 2012; 104(16): 1208-17.
18. Liao X, Lochhead P, Nishihara R, et al. Aspirin use, tumor PIK3CA mutation, and colorectal-cancer survival. N Engl J Med 2012; 367(17): 1596-606.
19. McCowan C, Munro AJ, Donnan PT, Steele RJ. Use of aspirin post-diagnosis in a cohort of patients with colorectal cancer and its association with all-cause and colorectal cancer specific mortality. Eur J Cancer 2013; 49(5): 1049-57.
20. Ng K, Meyerhardt JA, Chan AT, et al. Aspirin and COX-2 inhibitor use in patients with stage III colon cancer. J Natl Cancer Inst 2015; 107(1): 345.
21. Reimers MS, Bastiaannet E, Langley RE, et al. Expression of HLA class I antigen, aspirin use, and survival after a diagnosis of colon cancer. JAMA Intern Med 2014; 174(5): 732-9.
22. Sun R, Nishihara R, Qian ZR, Chan AT, Ogino S. Aspirin and Colorectal Cancer Incidence and Mortality by CTNNB1 Expression: A Molecular Pathological Epidemiology (MPE) Study. Cancer Epidemiology Biomarkers & Prevention 2013; 22: 472-3.
23. Walker AJ, Grainge MJ, Card TR. Aspirin and other non-steroidal anti-inflammatory drug use and colorectal cancer survival: a cohort study. Br J Cancer 2012; 107(9): 1602-7.
24. Zanders MM, van Herk-Sukel MP, Vissers PA, Herings RM, Haak HR, van de Poll-Franse LV. Are metformin, statin and aspirin use still associated with overall mortality among colorectal cancer patients with diabetes if adjusted for one another? Br J Cancer 2015; 113(3): 403-10.
25. Fuchs C, Meyerhardt JA, Heseltine DL, et al. Influence of regular aspirin use on survival for patients with stage III colon cancer: Findings from Intergroup trial CALGB 89803 [Abstract]. Journal of Clinical Oncology 2005; 23(16S (June 1 supplement)).
26. Zell JA, Ziogas A, Bernstein L, et al. Nonsteroidal anti-inflammatory drugs: effects on mortality after colorectal cancer diagnosis. Cancer 2009; 115(24): 5662-71.
27. Elwood PC, Gallagher AM, Duthie GG, Mur LA, Morgan G. Aspirin, salicylates, and cancer. Lancet 2009; 373(9671): 1301-9.
28. van Kruijsdijk RC, Visseren FL, Ridker PM, et al. Individualised prediction of alternate-day aspirin treatment effects on the combined risk of cancer, cardiovascular disease and gastrointestinal bleeding in healthy women. Heart 2015; 101(5): 369-76.

29. Langley RE, Burdett S, Tierney JF, Cafferty F, Parmar MK, Venning G. Aspirin and cancer: has aspirin been overlooked as an adjuvant therapy? Br J Cancer 2011; 105(8): 1107-13.
30. Chia WK, Ali R, Toh HC. Aspirin as adjuvant therapy for colorectal cancer--reinterpreting paradigms. Nat Rev Clin Oncol 2012; 9(10): 561-70.
31. Targownik LE, Suissa S. Understanding and Avoiding Immortal-Time Bias in Gastrointestinal Observational Research. Am J Gastroenterol 2015; 110(12): 1647-50.
32. Stricker BH, Stijnen T. Analysis of individual drug use as a time-varying determinant of exposure in prospective population-based cohort studies. Eur J Epidemiol 2010; 25(4): 245-51.
33. Suissa S. Immortal time bias in pharmaco-epidemiology. Am J Epidemiol 2008; 167(4): 492-9.
34. Austin PC, Mamdani MM, van Walraven C, Tu JV. Quantifying the impact of survivor treatment bias in observational studies. J Eval Clin Pract 2006; 12(6): 601-12.
35. Suissa S, Azoulay L. Metformin and the risk of cancer: time-related biases in observational studies. Diabetes Care 2012; 35(12): 2665-73.
36. Assayag J, Pollak MN, Azoulay L. The use of aspirin and the risk of mortality in patients with prostate cancer. J Urol 2015; 193(4): 1220-5.
37. van Walraven C, Davis D, Forster AJ, Wells GA. Time-dependent bias was common in survival analyses published in leading clinical journals. J Clin Epidemiol 2004; 57(7): 672-82.
38. The European Network of Centres for Pharmacoepidemiology and Pharmacovigilance (ENCePP). Guide on Methodological Standards in Pharmacoepidemiology. 2010.
39. Wu JW, Boudreau DM, Park Y, Simonds NI, Freedman AN. Commonly used diabetes and cardiovascular medications and cancer recurrence and cancer-specific mortality: a review of the literature. Expert Opin Drug Saf 2014; 13(8): 1071-99.
40. Chubak J, Boudreau DM, Wirtz HS, McKnight B, Weiss NS. Threats to validity of nonrandomized studies of postdiagnosis exposures on cancer recurrence and survival. J Natl Cancer Inst 2013; 105(19): 1456-62.
41. Csizmadi I, Collet J-P. Bias and Confounding in Pharmacoepidemiology. Textbook of Pharmacoepidemiology: John Wiley & Sons, Ltd,. 2006: 261-75.
42. Kyriacou DN, Lewis RJ. Confounding by Indication in Clinical Research. JAMA 2016; 316(17): 1818-9.
43. Vandenbroucke JP. When are observational studies as credible as randomised trials? Lancet 2004; 363(9422): 1728-31.
44. Larson RJ, Fisher ES. Should aspirin be continued in patients started on warfarin? J Gen Intern Med 2004; 19(8): 879-86.
45. Dentali F, Douketis JD, Lim W, Crowther M. Combined aspirin-oral anticoagulant therapy compared with oral anticoagulant therapy alone among patients at risk for cardiovascular disease: a meta-analysis of randomized trials. Arch Intern Med 2007; 167(2): 117-24.
46. Hansen ML, Sorensen R, Clausen MT, et al. Risk of bleeding with single, dual, or triple therapy with warfarin, aspirin, and clopidogrel in patients with atrial fibrillation. Arch Intern Med 2010; 170(16): 1433-41.
47. Jonsson F, Yin L, Lundholm C, Smedby KE, Czene K, Pawitan Y. Low-dose aspirin use and cancer characteristics: a population-based cohort study. Br J Cancer 2013; 109(7): 1921-5.
48. Rothwell PM, Fowkes FG, Belch JF, Ogawa H, Warlow CP, Meade TW. Effect of daily aspirin on long-term risk of death due to cancer: analysis of individual patient data from randomised trials. Lancet 2011; 377(9759): 31-41.
49. Maity G, De A, Das A, Banerjee S, Sarkar S, Banerjee SK. Aspirin blocks growth of breast tumor cells and tumor-initiating cells and induces reprogramming factors of mesenchymal to epithelial transition. Lab Invest 2015; 95(7): 702-17.
50. Mora S, Manson JE. Aspirin for Primary Prevention of Atherosclerotic Cardiovascular Disease: Advances in Diagnosis and Treatment. JAMA Intern Med 2016; 176(8): 1195-204.
51. Baigent C, Blackwell L, Collins R, et al. Aspirin in the primary and secondary prevention of vascular disease: collaborative meta-analysis of individual participant data from randomised trials. Lancet 2009; 373(9678): 1849-60.
52. Puhan MA, Yu T, Stegeman I, Varadhan R, Singh S, Boyd CM. Benefit-harm analysis and charts for individualized and preference-sensitive prevention: example of low dose aspirin for primary prevention of cardiovascular disease and cancer. BMC Med 2015; 13: 250.
53. Koene RJ, Prizment AE, Blaes A, Konety SH. Shared Risk Factors in Cardiovascular Disease and Cancer. Circulation 2016; 133(11): 1104-14.
54. Sarfati D, Hill S, Blakely T, et al. The effect of comorbidity on the use of adjuvant chemotherapy and survival from colon cancer: a retrospective cohort study. BMC Cancer 2009; 9: 116.
55. Sogaard M, Thomsen RW, Bossen KS, Sorensen HT, Norgaard M. The impact of comorbidity on cancer survival: a review. Clin Epidemiol 2013; 5(Suppl 1): 3-29.
56. Erichsen R, Horvath-Puho E, Iversen LH, Lash TL, Sorensen HT. Does comorbidity interact with colorectal cancer to increase mortality? A nationwide population-based cohort study. Br J Cancer 2013; 109(7): 2005-13.
57. Janssen-Heijnen ML, Szerencsi K, van de Schans SA, Maas HA, Widdershoven JW, Coebergh JW. Cancer patients with cardiovascular disease have survival rates comparable to cancer patients within the age-cohort of 10 years older without cardiovascular morbidity. Crit Rev Oncol Hematol 2010; 76(3): 196-207.
58. Bayliss E, Reifler L, Zeng C, McQuillan D, Ellis J, Steiner J. Competing risks of cancer mortality and cardiovascular events in individuals with multimorbidity. Journal of Comorbidity 2014; 4(1): 29-36.
59. van Erning FN, van Steenbergen LN, Lemmens VE, et al. Conditional survival for long-term colorectal cancer survivors in the Netherlands: who do best? Eur J Cancer 2014; 50(10): 1731-9.
60. Rothwell PM, Wilson M, Elwin C-E, et al. Long-term effect of aspirin on colorectal cancer incidence and mortality: 20-year follow-up of five randomised trials. The Lancet 2010; 376(9754): 1741-50.
61. Jorgensen TL, Hallas J, Friis S, Herrstedt J. Comorbidity in elderly cancer patients in relation to overall and cancer-specific mortality. Br J Cancer 2012; 106(7): 1353-60.
62. Extermann M. Interaction between comorbidity and cancer. Cancer Control 2007; 14(1): 13-22.
63. Coebergh JW, Janssen-Heijnen ML, Post PN, Razenberg PP. Serious co-morbidity among unselected cancer patients newly diagnosed in the southeastern part of The Netherlands in 1993-1996. J Clin Epidemiol 1999; 52(12): 1131-6.
64. Janssen-Heijnen ML, Houterman S, Lemmens VE, Louwman MW, Maas HA, Coebergh JW. Prognostic impact of increasing age and co-morbidity in cancer patients: a population-based approach. Crit Rev Oncol Hematol 2005; 55(3): 231-40.
65. Rothwell PM, Price JF, Fowkes FG, et al. Short-term effects of daily aspirin on cancer incidence, mortality, and non-vascular death: analysis of the time course of risks and benefits in 51 randomised controlled trials. Lancet 2012.
66. Rothwell PM, Wilson M, Price JF, Belch JFF, Meade TW, Mehta Z. Effect of daily aspirin on risk of cancer metastasis: a study of incident cancers during randomised controlled trials. The Lancet 2012; 379(9826): 1591-601.
67. Chubak J, Whitlock EP, Williams SB, et al. Aspirin for the Prevention of Cancer Incidence and Mortality: Systematic Evidence Reviews for the U.S. Preventive Services Task Force. Ann Intern Med 2016; 164(12): 814-25.
68. Cook NR, Lee IM, Zhang SM, Moorthy MV, Buring JE. Alternate-day, low-dose aspirin and cancer risk: long-term observational follow-up of a randomized trial. Ann Intern Med 2013; 159(2): 77-85.
69. Algra AM, Rothwell PM. Effects of regular aspirin on long-term cancer incidence and metastasis: a systematic comparison of evidence from observational studies versus randomised trials. Lancet Oncol 2012.
70. Lipton A, Scialla S, Harvey H, et al. Adjuvant antiplatelet therapy with aspirin in colo-rectal cancer. J Med 1982; 13(5-6): 419-29.
71. Burn J, Mathers JC, Bishop DT. Chemoprevention in Lynch syndrome. Fam Cancer 2013; 12(4): 707-18.
72. Murphy C, Turner N, Wong HL, et al. Examining the Impact of Regular Aspirin Use and PIK3CA Mutations on Survival in Stage 2 Colon Cancer. Intern Med J 2016.
73. ATCG. Website Aspirin Trialist Collaborative Group. 2016. www.aspirinagainstcancer.org.
74. Ogino S, Nishihara R, VanderWeele TJ, et al. Review Article: The Role of Molecular Pathological Epidemiology in the Study of Neoplastic and Non-neoplastic Diseases in the Era of Precision Medicine. Epidemiology 2016; 27(4): 602-11.
75. Hemkens LG, Contopoulos-Ioannidis DG, Ioannidis JP. Agreement of treatment effects for mortality from routinely collected data and subsequent randomized trials: meta-epidemiological survey. BMJ 2016; 352: i493.
76. Cole BF, Logan RF, Halabi S, et al. Aspirin for the chemoprevention of colorectal adenomas: meta-analysis of the randomized trials. J Natl Cancer Inst 2009; 101(4): 256-66.
77. Guillem-Llobat P, Dovizio M, Bruno A, et al. Aspirin prevents colorectal cancer metastasis in mice by splitting the crosstalk between platelets and tumor cells. Oncotarget 2016.

CHAPTER 8
SUMMARY

Major advances have been made in the treatment of cancer patients over the past decades. Improvements in quality and efficiency in surgery, enhanced postoperative care and availability and improvement in radiotherapy and systemic treatments have contributed to this. Despite these developments, cancer mortality is still high. In 2014 in the United States, 25% of all cancer mortality was due to cancers of the gastrointestinal tract, and this number was 30% in the Netherlands.[1,2]

In 1970, aspirin was suggested to affect cancer outcome for the first time, and ever since, many studies have been published on this subject.[3,4] Initially, the working mechanism of aspirin was thought to be mediated through the inhibition of cyclooxygenase-2 (COX-2, also called PTGS-2) on tumour tissue. One of the first biomarker studies showed that the survival benefit was only observed in patients with COX-2 expressing tumours.5 However, only high-dose aspirin (>2,000 milligram daily) would be sufficient to achieve systemic concentrations to inhibit both COX-1 and COX-2.[6] Hence it was agreed that another working mechanism must be effective.

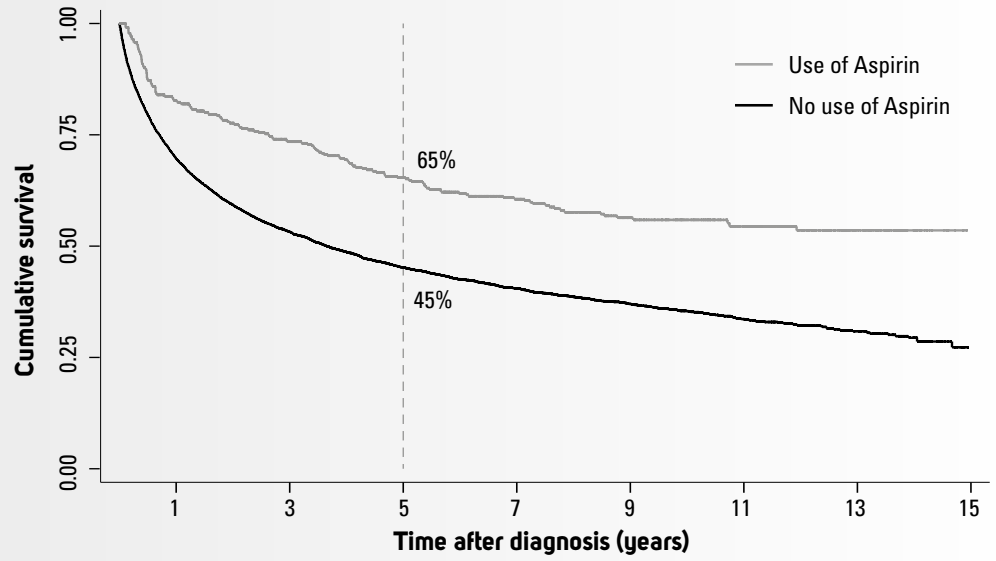
Aspirin is unique in the group of Non-Steroidal Anti-Inflammatory Drugs (NSAID's) because aspirin irreversibly inhibits the COX isozymes through selective acetylation and NSAID's block the COX-channel in a different manner.[6]Thrombocytes are particularly susceptible to the effects of, even low-dose, aspirin. Mature thrombocytes do not have a nucleus and only contain COX-1. Therefore, thrombocytes with an acetylated COX-channel are not capable of producing new COX-1 enzymes and remain inactivated until new thrombocytes are produced.[6]The effect of low-dose aspirin on cancer was therefore hypothesized to be mediated through the antiplatelet effect, the same mechanism that is responsible for its cardioprotective effect.[6]

The role of aspirin in cancer incidence and mortality has been granted top priority in a list of provocative questions in cancer epidemiology by the National Cancer Institute.7 Previous studies have mainly focussed on colorectal cancer, probably because this is the most common type of cancer of the gastrointestinal tract. Indeed, in the Netherlands, 64% of the incidence of all gastrointestinal cancers is colorectal cancer.[2]

Because a relatively low number of studies have focussed on other tumor types, this thesis started in **chapter two** with a study that evaluated the association between the use of aspirin in patients with oesophageal cancer, the second-most common cancer type (10%) of the gastrointestinal tract.[2] Data from patients from the Eindhoven Cancer Registry were linked to the drug dispensing database of PHARMO. Patients were selected and analysed according to use of aspirin and NSAID's before or after diagnosis. This study demonstrated in 560 patients that the use of aspirin was associated with improved survival in patients with oesophageal cancer with a hazard ratio (HR) of 0.42 (95% CI 0.30-0.57).

In the next study in **chapter three**, this cohort was extended to all patients with cancer of the gastrointestinal tract. The goal of this study was to evaluate whether the association between aspirin use and overall survival was tumor specific or a more generalised effect, present in all tumor types. Almost 14,000 patients were analysed. An association between the use of low-dose aspirin after diagnosis and improved survival in patients with different types of cancer of the gastrointestinal tract, adjusted HR 0.52 (95% CI 0.44-0.63) was found. After five years, 65% of patients using aspirin was alive versus 45% in

Figure 1: Survival comparison for aspirin users versus non-users with Simon Makuch method



the group of patients that did not use aspirin (figure 1). Stratified according to tumour type, the association was significant in patients with oesophageal, hepatobilliary, and colorectal cancer (figure 2). This study strengthened our hypothesis that the association between aspirin and cancer survival might be a generalized effect, mediated through thrombocytes.

Could it be that other regularly used medication is associated with a survival benefit in patients with cancer? Several previous publications showed a survival benefit for the use of metformin in patients with pancreatic cancer.[8,9]These studies had however several

Figure 2: Overall survival analysis for aspirin users vs nonusers stratified according to tumour type

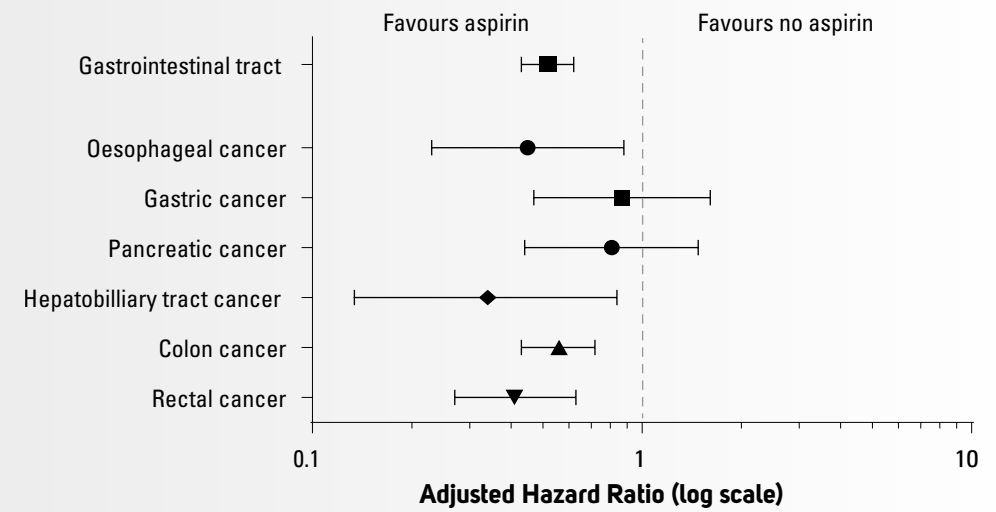
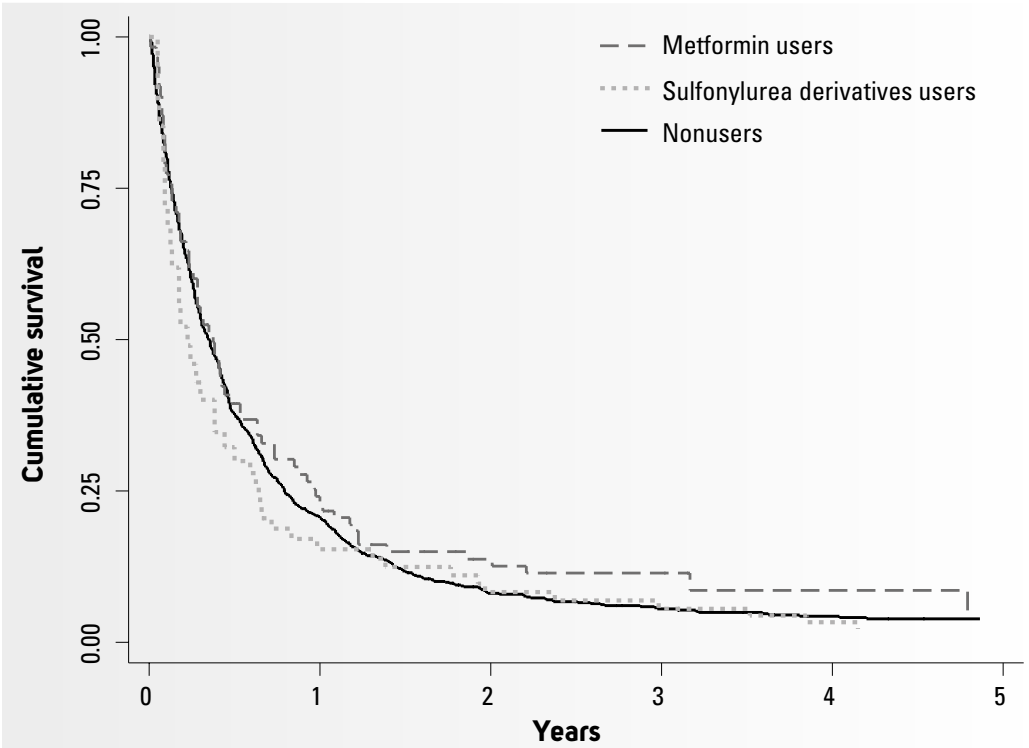


Figure 3: Kaplan–Meier estimation of survival curves in patients with pancreatic cancer grouped according to medication use



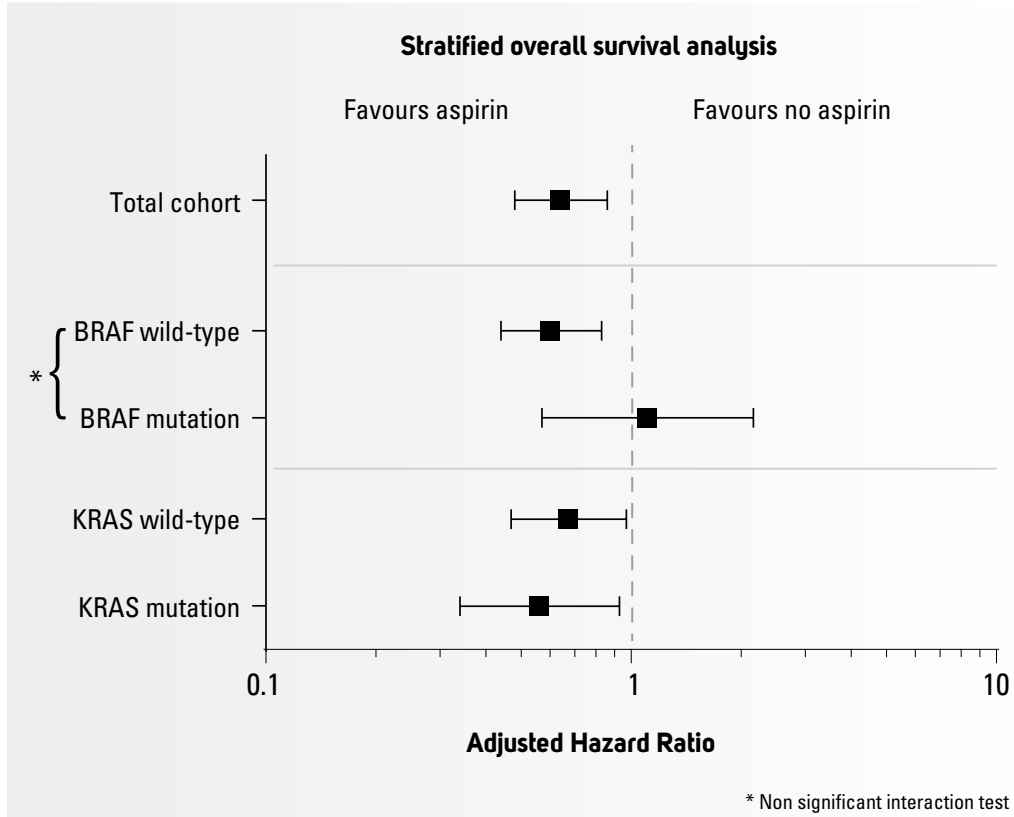
methodological limitations and the survival results may partially have been the result of immortal time bias.[10] In addition, selection bias may have been an issue, as these studies of metformin had only been done in patients with diabetes mellitus type II. **Chapter four** describes how, as a next step, we decided to analyse the association between the use of metformin after diagnosis and survival in patients with pancreatic cancer. This study was done with data from IKNL and PHARMO with the appropriate methodology and careful description of possible causes of bias. Patients using metformin, sulfonylurea derivatives, and nonusers with pancreatic cancer were compared and the association of medication with overall survival was analysed. No significant difference between survival of patients with pancreatic cancer using metformin and nonusers of metformin was observed. Additionally, the group of patients that used sulfonylurea derivatives and metformin did also not show a difference in survival (figure 3). During this study, two randomised controlled trials were published and also could not prove an effect of metformin in patients with pancreatic cancer.[11,12] It could therefore be possible that the effect of metformin in previous studies was therefore the result of improper methodology and therefore an overestimation of the effect of metformin on survival.

Many studies have focused on biomarker expression in colorectal cancer, to examine if this could provide clues about the mechanism of action of aspirin on cancer. The most frequent studied biomarker so far is PIK3CA (phosphatidylinositol-4,5-bisphosphonate 3-kinase), since Liao et al. observed a survival benefit in patients with a PIK3CA mutated

tumor in 2012.[13] However, since this first encouraging result, studies where not all straightforward, and a recent meta-analysis did not show a differentiating effect of aspirin use and PIK3CA mutations.[14] Other suggested biomarkers have been COX-2, HLA (Human Leukocyte Antigen) class I, and several specific genetic profiles, but with conflicting results.[5,15,16] In **chapter five** of this thesis, we studied if the effect of aspirin could be mediated through the mitogen activated protein kinase (*MAPK*) pathway. The influence of *BRAF* and *KRAS* mutation status in patients using aspirin with colorectal cancer was assessed. Mutated *BRAF* and *KRAS* have been shown to influence *MAPK* signalling, resulting in up regulation of *PTGS2*.[17] The results of this study could not prove a distinctive effect of these mutations in the association between the use of aspirin and survival (figure 4). This study again strengthened our hypothesis that the effect of aspirin is not mediated through specific tumour biomarkers, but is a generalised effect, mediated through thrombocytes.

The option that the effect of aspirin on cancer is mediated through thrombocytes has been suggested a few years ago, but no study so far has actually proven this mechanism. It has been proposed that circulating tumor cells in the blood stream are physiologically surrounded by thrombocytes, that guard the circulating tumor cells from detection by the immune system.[18] It has been hypothesised that aspirin, a thrombocyte

Figure 4: Overall survival analysis for patients using aspirin versus patients not using aspirin, grouped according to mutation status



* Non significant interaction test

aggregation inhibitor, causes this cloak to fall apart thereby exposing the circulating tumor cells to components of the immune system that initiate an immune response with subsequent prevention of metastasis.[18-21] According to this hypothesis, a survival benefit in patients using other thrombocyte aggregation inhibitors should also be observed. However, until the study reported in **chapter six**, this was never addressed before. We studied the association between survival and different thrombocyte aggregation inhibitors. In addition to aspirin, mainly clopidogrel and dipyridamole were studied. In the Netherlands, aspirin is the first choice thrombocyte aggregation inhibitor in patients with cardiovascular disease.[22] We compared patients using solely aspirin, versus patients using aspirin in combination with another thrombocyte aggregation inhibitor. No additional survival benefit was observed in the group of patients using two thrombocyte aggregation inhibitors. There are two possible explanations for these results. Either the effect of aspirin was sufficient for all thrombocytes lose function, and therefore, no additional survival benefit was observed from additional thrombocyte aggregation inhibitors. Alternatively, the effect of aspirin on cancer metastasis is unique, and not related to general inhibition of thrombocyte aggregation.

In the analysis of the observational studies in this thesis, we have carefully described the important methodological limitations that may complicate the interpretation of this type of data. Some doctors and patients tend to settle for the current epidemiological evidence for the anticancer effect of aspirin but treatment and effect conclusions from observational studies may be seriously flawed due to confounding by indication. It is therefore unavoidable to wait for the results of the randomised controlled trials to consider aspirin as regular treatment for cancer. In **chapter seven** we have tried to sum the current evidence and provide some perspective on the subject of bias in the current observational evidence.

CHAPTER 9
GENERAL DISCUSSION AND FUTURE PERSPECTIVES

Currently, several randomised controlled trials are ongoing in the field of aspirin and cancer. Until the results of these trials are available, aspirin should not be implemented as adjuvant therapy, as has been shown by this thesis. The studies in this thesis led to the hypothesize that aspirin might have a general effect on cancer, possibly mediated through thrombocytes. Because of the difficulties interpreting cohort studies, results from the randomised controlled trials, combined with results from pre-clinical studies, should be awaited in order to provide clarity on the anti-cancer effect of aspirin and the mechanism.

Future research in this field requires international collaboration in order to assemble sufficient numbers of patients to adequately address the important research questions in less common tumor types.

One of the limitations of some of the studies in this thesis was a low number of patients in several subgroups. One of the reasons that aspirin has been found to be beneficial in colorectal cancer, is because this is the most prevalent gastrointestinal malignancy, with a sufficiently largestudy population. For the less frequent cancer in other parts of the gastrointestinal organs, it will be more difficult to obtain evidence.

In the near future, the cohort of PHARMO and IKNL will expand to a national coverage of the linking of these databases. With this improvement it will be possible, for example, to study the cohort of patients with all gastrointestinal tumours nationwide (**chapter three**), and in that manner it will be possible to analyse the current biomarkers of these tumours. This would be a huge step towards unravelling the working mechanism of aspirin.

The Colorectal Subtyping Consortium (CRCSC) was established to explore four core subtype patterns of colorectal cancer based on gene expression and to characterise key biological features of these subtypes.[23] With these prognostic subtypes, a new taxonomy of colorectal cancer was created called Consensus Molecular Subtypes (CMS): CMS1 (MSI immune), CMS2 (canonical), CMS3 (metabolic), and CMS4 (mesenchymal). The current improvements in the subtyping of colorectal cancer should also be adapted by the current research field, abandoning the single biomarker studies. Studying the effectivity of aspirin patients with in these different colorectal cancer subtypes needs further investigation.[23]

With upcoming health care registrations, both national and international, this will hopefully stimulate more joint efforts towards combining datasets, and a low number of patients will not have to be a limitation in the future. One major remark should be placed with this, because with the upcoming large databases, hypothesis for research questions should be chosen upfront and not all study designs are suitable for every research question.

In 2015, the Aspirin Trialist Collaborative Group (ATCG) was founded, a collaboration between the current ongoing aspirin trials.[24] This collaboration already started before the trials have finished. In this way we were able to match aspects of the study designs and we were able to pool and compare the trials in the most optimal fashion. All principal investigators of the current trials assessing the effect of aspirin and cancer survival are united in this group and help each other with obstacles in the execution of the individual trials.

Another obstacle for investigator-initiated studies is the limited interest in studies where drugs are repurposed for new indications. This limited interest results in many missed, cheap opportunities for improvements of cancer care.

Aspirin has been free of patent since 1917. When prescribed and obtained through a pharmacy, the daily costs for an 80 mg tablet are only 3 cents.[25] Adding the costs for pharmacy issuing, the costs of five-year aspirin treatment are about €115 per patient. Each year, 3500 colon cancer patients will be eligible for aspirin treatment (i.e. non-metastasized patients who do not already use aspirin at diagnosis). Thus, the annual costs in the Netherlands for treating these patients with aspirin would be €400.000. Among the eligible patients, 5-year mortality is currently about 21%.[2] Assuming a 25% mortality reduction (this is the hazard ratio for which the Aspirin trial has been powered), the total prevented mortality after five years would be about 147 patients per year. Cost-effectiveness is therefore preliminarily estimated at only €2100 per averted death.

Current options to fund research are: pharmaceutical companies, government, and public funding.[26] Pharmaceutical companies are however not interested in aspirin because the patent has already expired after World War I, and therefore no high profits can be expected.[27] Government funding unfortunately has to deal with the interests of the individual researchers, and aspirin as anti-cancer drug may not be as attractive as novel targeted therapy. Nobody is going to win the Nobel Prize with aspirin anymore. Lastly, although the investigator initiated trials are relatively cheap, public funding is hindered as well. Budgets are static or shrinking and if aspirin proves to be an effective and cost-saving drug, the other studies aiming for new personalized targeted therapies may be cut-off.[26] Despite these hurdles, it is of high importance that funding becomes available for these type of investigator driven studies. Projects that aim to set the agenda for these type of studies should be greatly supported. It is time to face the common goal in research: improving worldwide sustainable cancer care, and collaboration is an unavoidable aspect in this journey.

Reference list

1. Alpers DH, Yamada T. Textbook of Gastroenterology. Chichester, West Sussex: Wiley-Blackwell; 2009.
2. www.cijfersoverkanker.nl accessed November 17 2016.
3. Elwood PC, Morgan G, Pickering JE, et al. Aspirin in the Treatment of Cancer: Reductions in Metastatic Spread and in Mortality: A Systematic Review and Meta-Analyses of Published Studies. PLoS One 2016; 11(4): e0152402.
4. Gasic GJ, Gasic TB, Murphy S. Anti-metastatic effect of aspirin. Lancet 1972; 2(7783): 932-3.
5. Chan AT, Ogino S, Fuchs CS. Aspirin use and survival after diagnosis of colorectal cancer. JAMA 2009; 302(6): 649-58.
6. Thun MJ, Jacobs EJ, Patrono C. The role of aspirin in cancer prevention. Nat Rev Clin Oncol 2012; 9(5): 259-67.
7. Lam TK, Schully SD, Rogers SD, Benkeser R, Reid B, Khoury MJ. Provocative questions in cancer epidemiology in a time of scientific innovation and budgetary constraints. Cancer Epidemiol Biomarkers Prev 2013; 22(4): 496-500.
8. Ambe CM, Mahipal A, Fulp J, Chen L, Malafa MP. Effect of Metformin Use on Survival in Resectable Pancreatic Cancer: A Single-Institution Experience and Review of the Literature. PLoS One 2016; 11(3): e0151632.
9. Hwang AL, Haynes K, Hwang WT, Yang YX. Metformin and survival in pancreatic cancer: a retrospective cohort study. Pancreas 2013; 42(7): 1054-9.
10. Suissa S, Azoulay L. Metformin and the risk of cancer: time-related biases in observational studies. Diabetes Care 2012; 35(12): 2665-73.
11. Reni M, Dugnani E, Cereda S, et al. (Ir)relevance of metformin treatment in patients with metastatic pancreatic cancer: an open-label, randomized phase 2 trial. Clin Cancer Res 2015.
12. Kordes S, Pollak MN, Zwinderman AH, et al. Metformin in patients with advanced pancreatic cancer: a double-blind, randomised, placebo-controlled phase 2 trial. Lancet Oncol 2015.
13. Liao X, Lochhead P, Nishihara R, et al. Aspirin use, tumor PIK3CA mutation, and colorectal-cancer survival. N Engl J Med 2012; 367(17): 1596-606.
14. Paleari L, Puntoni M, Clavarezza M, DeCensi M, Cuzick J, DeCensi A. PIK3CA Mutation, Aspirin Use after Diagnosis and Survival of Colorectal Cancer. A Systematic Review and Meta-analysis of Epidemiological Studies. Clin Oncol (R Coll Radiol) 2015.
15. Nan H, Hutter CM, Lin Y, et al. Association of aspirin and NSAID use with risk of colorectal cancer according to genetic variants. JAMA 2015; 313(11): 1133-42.
16. Coyle C, Cafferty FH, Langley RE. Aspirin and Colorectal Cancer Prevention and Treatment: Is It for Everyone? Current Colorectal Cancer Reports 2016.
17. Wagner EF, Nebreda AR. Signal integration by JNK and p38 MAPK pathways in cancer development. Nat Rev Cancer 2009; 9(8): 537-49.
18. Li N. Platelets in cancer metastasis: To help the "villain" to do evil. International journal of cancer 2016; 138(9): 2078-87.
19. Reimers MS, Bastiaannet E, Langley RE, et al. Expression of HLA Class I Antigen, Aspirin Use, and Survival After a Diagnosis of Colon Cancer. JAMA Intern Med 2014.
20. Rothwell PM, Wilson M, Price JF, Belch JFF, Meade TW, Mehta Z. Effect of daily aspirin on risk of cancer metastasis: a study of incident cancers during randomised controlled trials. The Lancet 2012; 379(9826): 1591-601.
21. Bambace NM, Holmes CE. The platelet contribution to cancer progression. J Thromb Haemost 2011; 9(2): 237-49.
22. NHG-standaard Cardiovasculair risicomanagement (Eerste herziening) Huisarts Wet 2012; 55(1): 14-28.
23. Guinney J, Dienstmann R, Wang X, et al. The consensus molecular subtypes of colorectal cancer. Nat Med 2015; 21(11): 1350-6.
24. ATCG. Website Aspirin Trialist Collaborative Group. 2016. www.aspirinagainstcancer.org.
25. www.medicijnkosten.nl. (accessed Accessed December 20 2016.
26. Evans RG. "Frankly, my dear, I don't give a damn". Healthc Policy 2014; 10(2): 10-8.
27. Tsoucalas G, Karamanou M, Androutsos G. Travelling through Time with Aspirin, a Healing Companion. European Journal of Inflammation 2011; 9(1): 13-6.

APPENDICES

NEDERLANDSE SAMENVATTING

De afgelopen decennia is er veel progressie geboekt in de zorg voor patiënten met kanker aan het maagdarmstelsel. De kwaliteit en effectiviteit van chirurgie hebben hieraan bijgedragen, zoals de introductie van laparoscopie en het ERAS protocol (Early Recovery After Surgery). Een toegenomen beschikbaarheid en verbeteringen in (neo-) adjuvante (radio) therapie heeft hier tevens een grote rol in gespeeld. Ondanks deze verbeteringen is de mortaliteit ten gevolge van kanker van het maagdarmstelsel nog steeds hoog. In 2014 in de Verenigde staten was 25% van alle mortaliteit aan kanker het gevolg van kanker aan het maagdarmstelsel en in Nederland was dit 30%.[1,2]

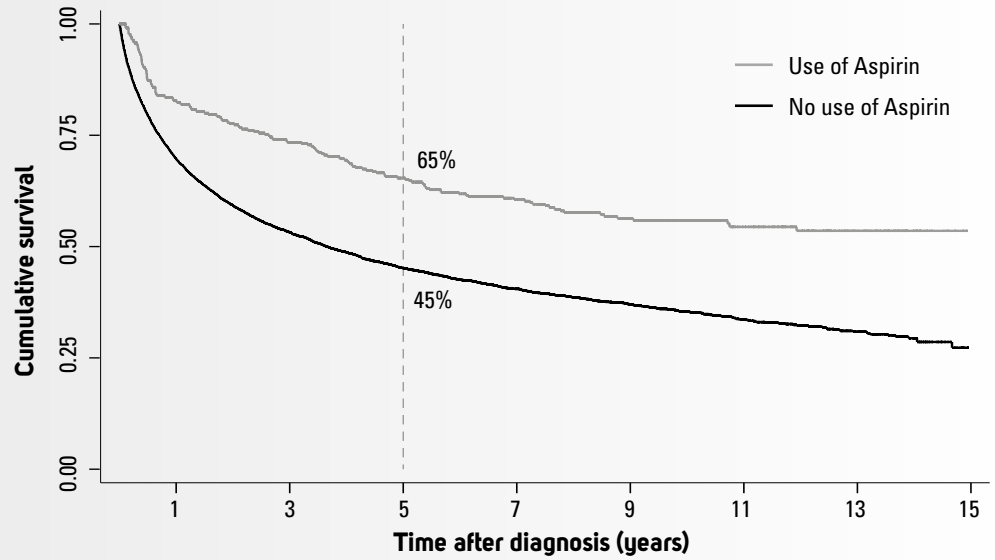
In 1970 werd voor het eerst gesuggereerd dat aspirine, oorspronkelijk gemaakt van de bast van een wilg, een gunstige invloed op de uitkomsten van patiënten met kanker zou kunnen hebben.[3,4] Sindsdien zijn er vele wetenschappelijke publicaties op dit gebied verschenen. In eerste instantie werd gedacht dat het werkingsmechanisme van aspirine berustte op de inhibitie van expressie van cyclooxygenase-2 (COX-2, ook wel PTGS-2 genoemd) in tumorweefsel. Een van de eerste studies op het gebied van biomarker expressie en aspirine liet zien dat het effect van aspirine op de overleving van patiënten alleen werd gevonden in tumoren die COX-2 tot expressie brengen.[5] Echter, een recentere publicatie liet zien dat COX-1 en COX-2 inhibitie pas afdoende zou zijn bij een dosering van meer dan 2000 milligram per dag.[6]

Aspirine is een uniek middel in de groep geneesmiddelen die NSAID's worden genoemd, de zogenaamde: 'Non-Steroidal Anti-Inflammatory Drugs'. Aspirine bezit de unieke eigenschap dat het de COX isozymen op een niet-reversibele manier kan acetyleren. Andere NSAID's blokkeren het COX-kanaal op een andere manier.[6] Trombocyten zijn bij uitstek gevoelig voor het effect van aspirine, met name in lage dosering. Dit komt doordat trombocyten geen celkern bezitten en daardoor alleen COX-1 tot expressie brengen. Hierdoor zijn bloedplaatjes met een ge-acetyleerd COX-kanaal niet in staat om nieuwe COX-1 enzymen te produceren. De bloedplaatjes blijven daardoor inactief totdat er weer nieuwe worden geproduceerd door het beenmerg. Om deze reden wordt gedacht dat het gunstige effect van aspirine op kanker afkomstig is van de trombocytenaggregatieremming. Ditzelfde werkingsmechanisme is ook verantwoordelijk is voor de effect van aspirine op patiënten met cardiovasculaire aandoeningen.[6]

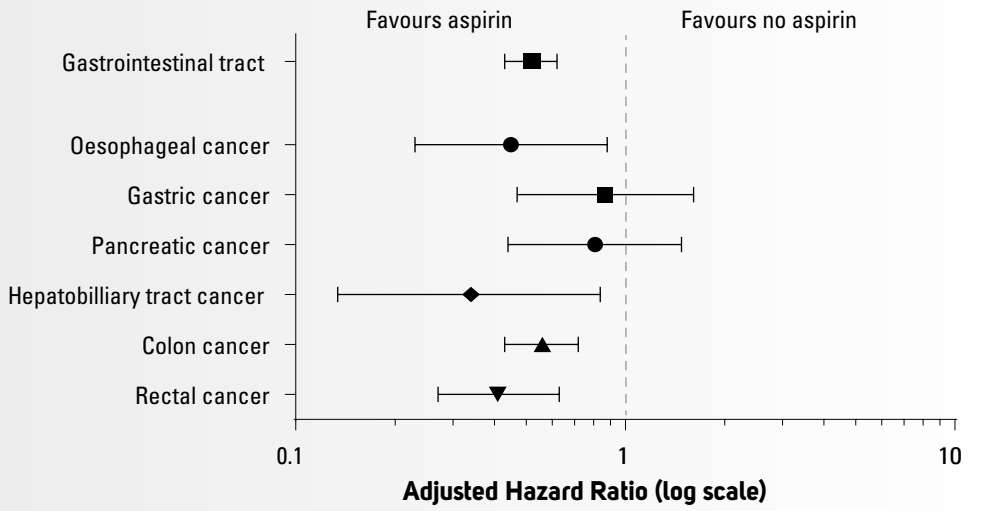
De invloed van aspirine op de behandeling van patiënten met kanker is benoemd als vraagstuk met de hoogste prioriteit door het National Cancer Institute.[7] Eerdere publicaties hebben zich met name gericht op patiënten met colorectaal carcinoom, hoogstwaarschijnlijk omdat dit de meest voorkomende tumorsoort in het maagdarmstelsel is. In Nederland is 64% van alle tumoren aan het maagdarmstelsel afkomstig van het colon en het rectum.[2] Vanwege de beperkte beschikbaarheid van studies op het gebied van andere tumoren aan het maagdarmstelsel heeft **hoofdstuk twee** van dit proefschrift zich gericht op de invloed van aspirine (en andere NSAID's) op de overleving van patiënten met een oesofagus carcinoom. Het oesofagus carcinoom is na het colorectaal carcinoom de op een-na meest voorkomende tumorsoort in het maagddarmstelsel. 10% van de gastro-intestinale maligniteiten is een oesofaguscarcinoom.[2] In deze studie werden data van het Integraal Kankercentrum Nederland (IKNL) regio Zuid, gekoppeld aan data van het PHARMO instituut, betreffende uitgegeven recepten door de apotheek aan deze patiënten. Patiënten die aspirine en/of andere NSAID's gebruikten gedurende de periode nadat zij waren gediagnosticeerd met kanker werden door middel van deze koppeling geïdentificeerd. 560 patiënten werden geanalyseerd in deze studie en er werd geobserveerd dat aspirine gebruik geassocieerd was met een significante invloed op de overleving van patiënten met een oesofagus carcinoom, hazard ratio (HR) van 0.42 (95% betrouwbaarheidsinterval (b.i.) 0.30-0.57).

In **hoofdstuk drie** werd het cohort uitgebreid naar patiënten met alle verschillende soorten gastro-intestinale maligniteiten. Het doel van deze studie was om te kijken of de associatie tussen aspirine gebruik en een gunstigere overleving ook te zien was voor alle verschillende tumorsoorten van het gastro-intestinale stelsel. In deze studie werden bijna 14.000 patiënten geanalyseerd. Er werd een associatie gevonden tussen het gebruik van lage dosis aspirine (80-100 milligram) na de diagnose kanker aan verschillende delen van het gastro-intestinale systeem en een betere overleving voor deze patiënten. De gecorrigeerde HR hiervoor was 0.52 (95% b.i. 0.44-0.63). Na vijf jaar was 65% van de patiënten die aspirine gebruikten nog in leven terwijl dit ten 45% was voor de groep die geen aspirine gebruik (figuur 1). Wanneer er per individuele tumor gekeken werd, werd een significante associatie met betere overleving gevonden in patiënten met oesofagus tumoren, hepatobiliaire tumoren en colorectale tumoren (figuur 2). Dit sterkte ons in de hypothese dat de associatie tussen aspirine gebruik en de geobserveerde betere overleving zou kunnen berusten op een meer gegeneraliseerd effect, afkomstig van de werking van aspirine als trombocytenaggregatieremmer.

Figuur 1: De overleving van aspirinegebruikers vergeleken met niet-gebruikers. Vergelijking volgens de Simon-Makuch methode.



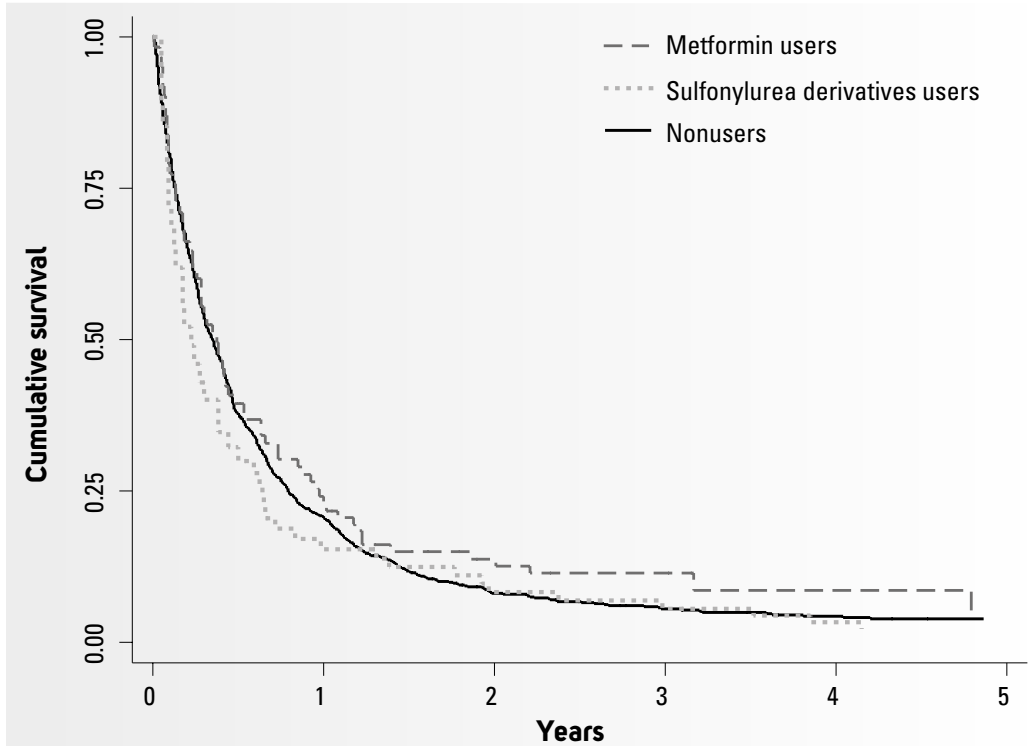
Figuur 2: Analyse van de overleving van aspirinegebruikers versus niet gebruikers, uitgesplitst per type tumor.



Zou het mogelijk kunnen zijn dat meer veelgebruikte medicijnen een overlevingsvoordeel laten zien bij patiënten met kanker? Verschillende eerdere publicaties hebben laten zien dat patiënten met een pancreastumor die metformine gebruiken, een overlevingsvoordeel hebben ten opzichte van patiënten die geen metformine gebruiken.[8, 9] De eerdere studies hebben helaas niet altijd de juiste methodologie gebruikt en daardoor zou het kunnen dat het geobserveerde overlevingsvoordeel berust op immortal time bias.[10] Daarnaast kan selectiebias een rol hebben gespeeld omdat de eerdere studies met name zijn uitgevoerd in patiënten die diabetes mellitus type II hebben. **Hoofdstuk vier** beschrijft daarom, als volgende stap, de associatie tussen uitkomsten van patiënten met een pancreascarcinoom en het gebruik van metformine. Dit werd gedaan met dezelfde dataset als boven beschreven, maar in deze studie werden patiënten die metformine en/of sulfonylureum derivaten gebruikten geselecteerd. Patiënten die metformine gebruikten, patiënten die sulfonylureum derivaten gebruikten en niet-gebruikers werden geanalyseerd en de relatie met hun overleving werd bekeken met de juiste methodologie, met een zorgvuldige beschrijving van de mogelijke vormen van bias die hiermee gepaard gaan. In deze studie bleek er geen significant overlevingsvoordeel te zijn voor patiënten die metformine slikten, niet ten opzichte van niet-gebruikers, maar ook niet ten opzichte van de sulfonylureum derivativen gebruikers (figuur 3). Tijdens het uitvoeren van deze studie zijn er in de tussentijd twee gerandomiseerd trials gepubliceerd die lieten zien dat metformine niet van invloed is op de uitkomsten van patiënten met kanker.[11,12] De resultaten van de eerdere studies zouden daardoor het gevolg kunnen zijn van bias, waardoor het effect van metformine op de uitkomsten van de patiënten in die studies is overschat.

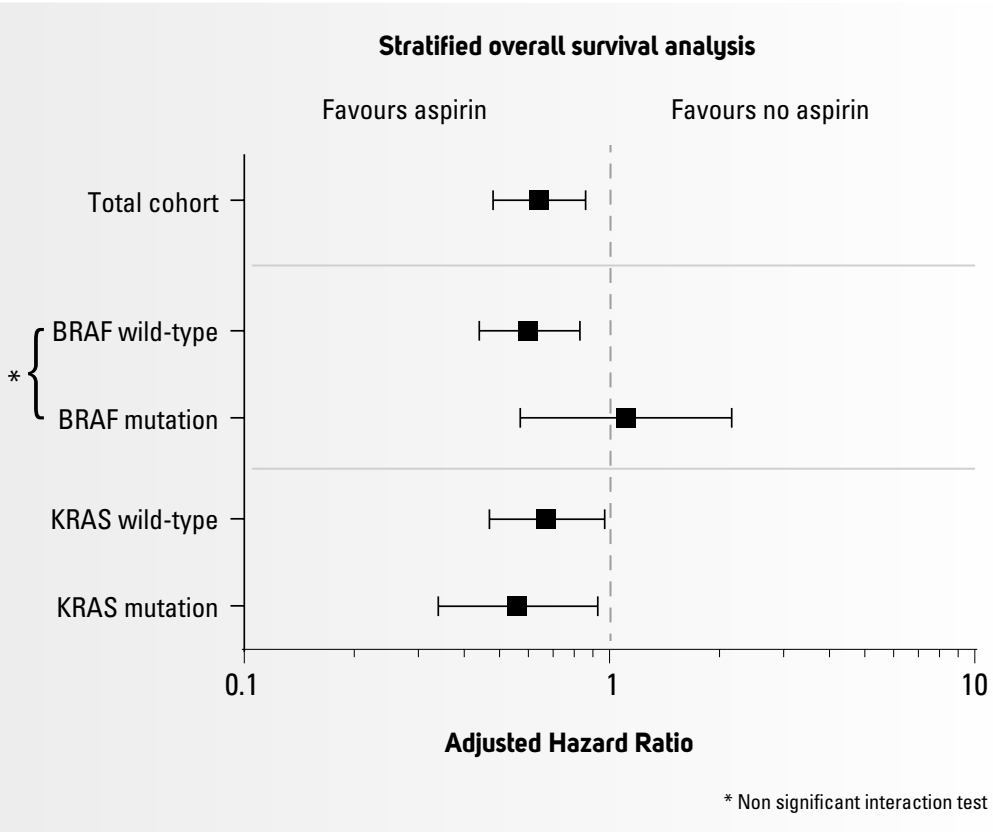
Vele eerdere studies in het verleden hebben gekeken naar het expressiepatroon van verschillende biomarkers in het colorectaal carcinoom en een relatie met aspirine

Figuur 3: Kaplan-Meier curve voor de overleving van patiënten die metformine gebruiken, patiënten die sulfonylureum derivaten gebruiken en niet-gebruikers.



gebruik. Het doel van deze studies was om meer inzicht te krijgen in het anti-tumor mechanisme van aspirine. De biomarker die het meest bestudeerd is, is PIK3CA (phosphatidylinositol-4,5-bisphosphonate 3-kinase). In 2012 werd in een eerste publicatie van Liao et al gevonden dat patiënten, die aspirine gebruiken en een PIK3CA mutatie in hun tumorweefsel hebben, een langere overleving hebben.[13] Studies die sindsdien zijn gepubliceerd op dit gebied hebben echter geen eenduidig resultaat laten zien. De meest recente meta-analyse kon niet aantonen dat het al dan niet hebben van een mutatie in een van de genen van de PIK3CA pathway een relatie heeft met het effect van aspirine op de overleving in patiënten met colorectaal carcinoom.[14] Andere biomarkers die de afgelopen jaren genoemd zijn als mogelijke verklaring voor de relatie tussen aspirinegebruik en de uitkomsten van patiënten met kanker zijn COX-2, HLA (Human Leukocyte Antigen) class I en verschillende specifieke genetische profielen.[5,15,16] In **hoofdstuk vijf** van dit proefschrift werd onderzocht of de associatie van aspirine met een gunstige overleving mogelijk een relatie heeft met veranderingen in de Mitogen Activated Protein Kinase (*MAPK*) pathway. De invloed van het al dan niet hebben van een mutatie in een van de genen in *BRAF* en *KRAS* (beiden onderdeel van de *MAPK* pathway) en de relatie met de uitkomsten van patiënten die aspirine slikken met een coloncarcinoom werd daarom onderzocht. Mutaties in *BRAF* en *KRAS* zijn van invloed op de *MAPK* signalering in cellen, wat over-expressie van COX-2 als gevolg heeft.[17] De resultaten van deze studie konden geen onderscheidend effect aantonen van het hebben van een *BRAF* of *KRAS* mutatie in de relatie tussen

Figuur 4: Analyse van de overleving van patiënten met een coloncarcinoom die wel of geen aspirine gebruiken, uitgesplitst per mutatietype.



aspirine gebruik en gunstige overleving in patiënten met een coloncarcinoom (figuur 4). Deze studie heeft ons opnieuw gesterkt in de hypothese dat het effect van aspirine niet gemedieerd is via specifieke biomarkers, maar dat er meer gedacht moet worden in de richting van een gegeneraliseerd, systemisch effect.

Tot nu wordt trombocytenaggregatie genoemd als de verklaring voor het gunstige effect van aspirine op de uitkomsten van patiënten met kanker. Dit wordt in verschillende publicaties gesuggereerd, maar is nooit aangetoond. Trombocyten vormen een fysiologische beschermende schil rondom circulerende tumorcellen in de bloedbaan. Hierdoor worden de tumorcellen door het immuunsysteem minder makkelijk herkend en kunnen ze zich nestelen in andere organen (metastasen).[18] Door aspirine, een trombocytenaggregatieremmer, valt de beschermende schil van trombocyten rondom deze circulerende tumorcellen weg en kan de tumorcel door het immuunsysteem worden opgeruimd. Hierdoor worden metastasen voorkomen en hebben patiënten met kanker betere overlevingskansen.[16,19-21] Volgens deze hypothese zou er bij patiënten die andere medicijnen gebruiken die de trombocytenaggregatie remmen ook een overlevingsvoordeel geobserveerd moeten worden. Daarom hebben we in **hoofdstuk zes** het effect van verschillende trombocytenaggregatieremmers (naast aspirine met name clopidogrel en dipyridamol) onderzocht. In Nederland wordt aspirine als eerste keus trombocytenaggregatieremmer voorgeschreven aan patiënten met cardiovasculaire aandoeningen.[22] Clopidogrel en dipyridamol worden daardoor alleen in aanvulling op behandeling met aspirine voorgeschreven. Als gevolg hiervan kon in deze studie alleen het *additionele* effect van de andere trombocytenaggregatieremmers bestudeerd worden naast aspirinebehandeling in patiënten met kanker. Er werd geen extra overlevingsvoordeel gezien bij de patiënten met kanker die naast aspirine ook een andere trombocytenaggregatieremmer gebruikten. Voor deze uitkomst hebben we twee mogelijke verklaringen. Enerzijds, zou het zo kunnen zijn dat het effect van aspirine alleen afdoende was voor de trombocyten om geen resterende functie meer te hebben. Hierdoor werd geen extra overlevingsvoordeel gezien bij patiënten die een additionele trombocytenaggregatieremmer gebruiken. Anderzijds zou het zo kunnen zijn dat de interactie tussen aspirine, trombocyten en kankercellen uniek is. Verschillende mechanismen zijn verantwoordelijk voor de trombocytenaggregatieremming in de geanalyseerde medicijnen. Hierdoor is het mogelijk dat deze middelen geen invloed hebben op de uitkomsten van patiënten met het colorectaal carcinoom.

Tijdens het schrijven van dit proefschrift hebben we ons terdege gerealiseerd dat er vele beperkingen zijn in het huidige observationele bewijs. Na bestudering van de huidige literatuur zijn wij tot de conclusie gekomen dat deze beperkingen nooit duidelijk in kaart zijn gebracht, met name het aspect van 'confounding by indication'. In **hoofdstuk zeven** zijn wij daarom ingegaan op de vormen van bias waarvoor gewaakt dient te worden in de huidige observationele studies. Tevens hebben we getracht handvaten te bieden voor de interpretatie en omvang waarmee deze vormen van bias het huidige bewijs kunnen hebben beïnvloed. Door deze kanttekeningen is het onvermijdelijk om de resultaten van de gerandomiseerde trials af te wachten die momenteel worden uitgevoerd, voordat aspirine kan worden overwogen als mogelijke aanvulling op de huidige bestaande (adjuvante) behandelingen.

Reference list

1. Alpers DH, Yamada T. Textbook of Gastroenterology. Chichester, West Sussex: Wiley-Blackwell; 2009.
2. www.cijfersoverkanker.nl accessed November 17 2016.
3. Elwood PC, Morgan G, Pickering JE, Galante J, Weightman AL, Morris D, et al. Aspirin in the Treatment of Cancer: Reductions in Metastatic Spread and in Mortality: A Systematic Review and Meta-Analyses of Published Studies. PLoS One. 2016;11:e0152402.
4. Gasic GJ, Gasic TB, Murphy S. Anti-metastatic effect of aspirin. Lancet. 1972;2:932-3.
5. Chan AT, Ogino S, Fuchs CS. Aspirin use and survival after diagnosis of colorectal cancer. JAMA. 2009;302:649-58.
6. Thun MJ, Jacobs EJ, Patrono C. The role of aspirin in cancer prevention. Nat Rev Clin Oncol. 2012;9:259-67.
7. Lam TK, Schully SD, Rogers SD, Benkeser R, Reid B, Khoury MJ. Provocative questions in cancer epidemiology in a time of scientific innovation and budgetary constraints. Cancer Epidemiol Biomarkers Prev. 2013;22:496-500.
8. Ambe CM, Mahipal A, Fulp J, Chen L, Malafa MP. Effect of Metformin Use on Survival in Resectable Pancreatic Cancer: A Single-Institution Experience and Review of the Literature. PLoS One. 2016;11:e0151632.
9. Hwang AL, Haynes K, Hwang WT, Yang YX. Metformin and survival in pancreatic cancer: a retrospective cohort study. Pancreas. 2013;42:1054-9.
10. Suissa S, Azoulay L. Metformin and the risk of cancer: time-related biases in observational studies. Diabetes Care. 2012;35:2665-73.
11. Reni M, Dugnani E, Cereda S, Belli C, Balzano G, Nicoletti R, et al. (Ir)relevance of metformin treatment in patients with metastatic pancreatic cancer: an open-label, randomized phase 2 trial. Clin Cancer Res. 2015.
12. Kordes S, Pollak MN, Zwinderman AH, Mathot RA, Weterman MJ, Beeker A, et al. Metformin in patients with advanced pancreatic cancer: a double-blind, randomised, placebo-controlled phase 2 trial. Lancet Oncol. 2015.
13. Liao X, Lochhead P, Nishihara R, Morikawa T, Kuchiba A, Yamauchi M, et al. Aspirin use, tumor PIK3CA mutation, and colorectal-cancer survival. N Engl J Med. 2012;367:1596-606.
14. Paleari L, Puntoni M, Clavarezza M, DeCensi M, Cuzick J, DeCensi A. PIK3CA Mutation, Aspirin Use after Diagnosis and Survival of Colorectal Cancer. A Systematic Review and Meta-analysis of Epidemiological Studies. Clin Oncol (R Coll Radiol). 2015.
15. Nan H, Hutter CM, Lin Y, Jacobs EJ, Ulrich CM, White E, et al. Association of aspirin and NSAID use with risk of colorectal cancer according to genetic variants. JAMA. 2015;313:1133-42.
16. Reimers MS, Bastiaannet E, Langley RE, van ER, van Vlierberghe RL, Lemmens VE, et al. Expression of HLA Class I Antigen, Aspirin Use, and Survival After a Diagnosis of Colon Cancer. JAMA Intern Med. 2014.
17. Wagner EF, Nebreda AR. Signal integration by JNK and p38 MAPK pathways in cancer development. Nat Rev Cancer. 2009;9:537-49.
18. Li D, Yeung SC, Hassan MM, Konopleva M, Abbruzzese JL. Antidiabetic therapies affect risk of pancreatic cancer. Gastroenterology. 2009;137:482-8.
19. Li N. Platelets in cancer metastasis: To help the "villain" to do evil. International journal of cancer. 2016;138:2078-87.
20. Rothwell PM, Wilson M, Price JF, Belch JFF, Meade TW, Mehta Z. Effect of daily aspirin on risk of cancer metastasis: a study of incident cancers during randomised controlled trials. The Lancet. 2012;379:1591-601.
21. Bambace NM, Holmes CE. The platelet contribution to cancer progression. J Thromb Haemost. 2011;9:237-49.
22. NHG-standaard Cardiovasculair risicomanagement (Eerste herziening) Huisarts Wet. 2012;55:14-28.

LIST OF PUBLICATIONS

2017

M.A. Frouws, H.S. Snijders, S. Malm, P.A. Neijenhuis, C.J.H. van de Velde, H.M. Kroon, *Clinical Relevance of a Grading System for Anastomotic Leakage After Low Anterior Resection: Analysis From a National Cohort Database.*
Diseases of the Colon and Rectum 2017 Jul;60(7):706-713

M.A. Frouws, E. Rademaker, E. Bastiaannet, M.P.P. van Herk-Sukel, V.E. Lemmens, C.J.H. Van de Velde, J.E.A. Portielje, G.J. Liefers, *The mechanisms of action of thrombocyte aggregation inhibitors in patients with colorectal cancer*
European Journal of Cancer 2017 Mar 25;77:24-30

M.A. Frouws, B.G. Sibinga Mulder, E. Bastiaannet, M.M.J. Zanders, M.P.P. van Herk-Sukel, E.M de Leede, B.A. Bonsing, J.S.D. Mieog, C.J.H. Van de Velde, G.J. Liefers, *No Association Between Metformin Use and Survival in Patients with Pancreatic Cancer: an Observational Cohort Study*
Medicine (Baltimore) 2017 Mar;96(10):e6229

M. A. Frouws, M.P.P. van Herk-Sukel, R.E. Langley, H. Maas, C.J.H. Van de Velde, J.E.A. Portielje, G.J. Liefers, E. Bastiaannet, *The association between aspirin and survival benefit in cancer patients: interpreting the evidence*
Cancer Treatment Reviews 2017 Feb 20;55:120-127

M.A. Frouws, M.S. Reimers, M. Swets, E. Bastiaannet, B. Prinse, R. van Eijk, V. E.P.P. Lemmens, M. P.P. van Herk-Sukel, T. van Wezel, P.J.K. Kuppen, H. Morreau, C.J.H. van de Velde, G.J. Liefers, *The influence of BRAF and KRAS mutation status on the association between aspirin use and survival after colon cancer diagnosis*
PLoS One 2017 Jan 26;12(1):e0170775

M.A. Frouws, E. Bastiaannet, R. Langley, W.K. Chia, M.P.P. van Herk-Sukel, H. Putter, H.H. Hartgrink, B.A. Bonsing, C.J.H. Van de Velde, J.E.A. Portielje, G.J. Liefers, *Effect of low-dose aspirin use on survival of patients with gastrointestinal malignancies*
British Journal of Cancer 2017 Jan;116(3):405-413.

2016

M. Swets, A. Zaalberg, A. Boot, T. van Wezel, **M.A. Frouws,** H. Gelderblom, C.J.H. van de Velde, P.J.K. Kuppen, *LINE-1 hypomethylation in association with survival in stage II colon cancer,*
International Journal of Molecular Science 2016 Dec 27;18(1)

M.A. Frouws, C.J.H. Van de Velde, *Routine prophylactic drainage in rectal surgery; closing the chapter?*
Translational Cancer Research Vol 5, Supplement 7 (December 2016)

M.A Frouws, Y.H.M. Claassen, A. Breugom, E. Bastiaannet, Mocellin S, Prof. Dr. Van de Velde, dr. Liefers, dr. Kapiteijn, *Intermittent versus continuous systemic therapy as treatment for unresectable metastatic colorectal cancer, protocol for a Cochrane review*
Published 21 December 2016

M.A. Frouws, C.J.H. van de Velde, *Het geheim van een gezonde (chirurgische) trial*
Nederlands Tijdschrift voor Heelkunde november 2016

Y.H.M. Claassen, A.J Breugom, **M.A. Frouws**, E. Bastiaannet, C.R. Sabajo, G.J. Liefers, C.J.H. van de Velde, E. Kapiteijn, *Survival between delayed versus immediate chemotherapy for asymptomatic metastatic colorectal cancer, protocol for a Cochrane review*
Published 5 October 2016

J. Staalduinen, **M.A. Frouws**, M. Reimers, E. Bastiaannet, M.P P van Herk-Sukel, V. Lemmens, Wobbe. de Steur, H. Hartgrink, C.J.H. van de Velde, GJ. Liefers, *The effect of aspirin and nonsteroidal anti-inflammatory drug use after diagnosis on survival of oesophageal cancer patients*
British Journal of Cancer;2016:114:1053–1059

2015

M.A. Frouws, E. Kapiteijn, J.E.A. Portielje, J. Pon, H. Schipper, G.J. Liefers, *De Aspirin trial: een systemische behandeling voor oudere patiënten met coloncarcinoom*
Nederlands Tijdschrift voor Oncologie 2015;12:198-200

M.A. Frouws, *Aspirine voor oudere patiënten met coloncarcinoom de 'ASPIRIN TRIAL'*
Nederlands Tijdschrift voor Geneeskunde 2015;159:A8796

CURRICULUM VITAE

Martine Aletta Frouws was born on August 21 1988 in Tilburg. She grew up in Oisterwijk (Noord-Brabant) with her father, mother, brother Dirk and sister Eveline. After graduating from the Odulphus lyceum in Tilburg in 2006, she took a gap-year to finish additional courses at the James Boswell Institute in Utrecht (math, biology, chemistry and physics) to become qualified for medical school.

In 2007, she started medical school at the Leiden University Medical Center (LUMC). She gained her first experience in research after finishing the first part of her clinical rotations. This internship was part of the *Kauwgomstudie* (gum chewing trial), a multicenter randomised clinical trial under supervision of dr. B.A. Bonsing, dr. N.J. van Leersum and drs. E.M. de Leede.

After graduating from medical school in 2014, she started her PhD research project at the department of surgery in the LUMC, which has resulted in the current thesis, under supervision of prof. dr. C.J.H. van de Velde (surgical oncologist), dr. G.J. Liefers (surgical oncologist) and prof. dr. J.E.A. Portielje (medical oncologist). Part of the PhD research project was the start-up and coordination of a large, international, multicenter, randomised controlled trial, the *Aspirin trial*. While writing her thesis Martine was a volunteer for the foundation 'Medical Business'. This foundation attempts to raise the awareness for the management and financial aspects of the health care industry. This side of health care is of special interest to her and her ambitions are to continue to be educated in this aspect of healthcare in addition to general medical training.

In January 2017 she started working at the department of surgery in the Groene Hart Hospital in Gouda under supervision of dr. R.F. Schmitz, surgical oncologist. She intends to start her surgical residency in 2018.

DANKWOORD

Dit proefschrift is tot stand gekomen met hulp van velen, die ik graag de eer zou willen doen toekomen die zij verdienen.

Allereerst de patiënten die deel nemen aan de Aspirin trial. Niet alleen voor uzelf maar ook voor de wetenschap in de toekomst is dit van onschatbare waarde.

Prof. dr. C.J.H. van de Velde, beste professor, het was een eer om deel te hebben uitgemaakt van uw onderzoeksgroep en om van uw ervaring en inzichten op het oncologisch gebied te mogen leren. U bent een inspiratie voor velen, uw werk zal niet snel worden vergeten.

Dr. G.J. Liefers, beste GJ, je bent een onvermoeibare bron van ideeën, originele invalshoeken en oplossingen en ik heb enorm veel van je geleerd.
Jouw optimisme maakt onderzoek nog leuker!

Prof. dr. J.E.A. Portielje, beste Johanneke, jouw medisch oncologische visie is in dit proefschrift van groot belang geweest. Als Principal Investigator van de Aspirin trial vorm je samen met Gerrit-Jan een ijzersterk team.

Dr. M.S. Reimers, beste Marlies, als jij niet met veel enthousiasme samen met Gerrit-Jan financiering had gezocht voor de Aspirin trial was onder andere dit proefschrift er niet geweest. Je hebt veel werk verricht dat voor mij heel waardevol is geweest.

Dr. E. Bastiaannet, beste Esther, de nauwe samenwerking en jouw epidemiologische inzichten hebben dit proefschrift tot een succes gemaakt. Zonder dat je het misschien doorhebt, ben je onmisbaar voor veel promovendi bij de heelkunde!

To all my other co-authors, thank you for sharing your valuable scientific experience, insights and ideas with me.

Dear members of the Aspirin Trialist Collaborative Group (ATCG), it was an honor to be part of the foundation of the ATCG. Your joint attitude towards sharing experience and data should be an example to all trial clinicians and the science community worldwide.

Het Datacenter Heelkunde, in het bijzonder Elma en Jeffrey, voor het faciliteren en meedenken over de Aspirin trial. Jullie zijn de stabiele factor binnen de onderzoeksgroep en hebben onmisbare ervaring bij de opzet van klinische studies.

Lieve collega's van J10, wat zijn jullie een grote inspiratiebron geweest. De steun onderling en het vele lachen hebben ervoor gezorgd dat ik deze twee jaar en acht maanden elke dag met plezier naar mijn werk ging. Annemarie en Graziella, in de jarenlange stroom van promovendi ben ik vast een van de velen die jullie als een soort moeder Theresa hebben begeleid, maar ik ben jullie hier heel dankbaar voor. Dank ook voor de geïncludeerde aspirine patiënten!

Lieve Xandra, je bent een grote steun geweest, veel dank voor al je hulp, ook in de periode dat je niet officieel meer voor professor van de Velde werkte.

Babs en Eva, jullie waren fijne stagiaires, veel dank voor jullie hulp.

Heleen, je bent een mentor voor me geweest de afgelopen jaren, heel veel dank voor al je hulp, steun en leuke momenten, je bent echt een bijzonder mens.

Al mijn nabije collega's, Anne, Ayoub, Babs, Erik, Hein, Jorinde, Mandy, Marloes D., Marloes S., Nikki, Nina, Noor B, Tessa, Willemieke, en alle andere collega-promovendi waar ik de afgelopen jaren mee heb gewerkt, dank voor alle gezelligheid, samenwerking, borrels en skireizen, we zien elkaar later in het MDO.

Dank aan al mijn lieve collega's in Gouda die mijn eerste tijd als ANIOS heel leuk hebben gemaakt. Ik realiseer me dat het warme bad waarin ik ben ontvangen heel bijzonder is.

Sonja en Anne, onze gezellige verjaardagen met avondjes stappen hebben de afgelopen jaren de nodige ontspanning gebracht.

Mijn lieve paranimfen, Yvette en Noor, dank voor het lachen en alle hulp de afgelopen jaren.

Helga, dank voor de vele uren die je hebt gestoken in de opmaak van mijn proefschrift, het wordt zeer gewaardeerd! Zonder jou was het niet zo'n prachtig proefschrift geworden.

Lieve Dirk en Eveline, al willen jullie niet graag genoemd worden, als broer en zus kunnen jullie niet worden gemist in dit dankwoord. Dirk, je inhoudelijke bijdrage aan het Engels onmisbaar geweest voor dit proefschrift. Eef, dank voor je hulp bij de organisatie rondom mijn promotie.

Papa en mama, jullie onvoorwaardelijke steun is voor mij heel belangrijk. Mama, jij zegt altijd: 'voor alles is een oplossing' en dat is de afgelopen jaren wel gebleken. Papa, samen als enige leden van de 'medische gemeenschap' binnen de familie, begrijp jij als geen ander in welke wereld ik me bevind en dat maakt onze band heel bijzonder.

Lex, niet alleen ben je mijn grote motivator geweest de afgelopen jaren, maar ook onze vele uren SPSS in de avonden hebben dit proefschrift gemaakt tot wat het nu is.
Je bent de liefste!