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Are metformin, statin and aspirin use still associated with overall mortality among colorectal cancer patients with diabetes if adjusted for one another?

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Background: Metformin, statin and aspirin use seem associated with decreased mortality in cancer patients, though, without adjusting for one another. Independent associations of these drugs with overall mortality after colorectal cancer (CRC) diagnosis within glucose-lowering drugs (GLDs) users were assessed.

Methods: Patients starting GLDs before CRC diagnosis (1998–2011) were selected from the Eindhoven Cancer Registry linked with the PHARMO Database Network. The Cox regression model, with time since CRC diagnosis, included time-dependent variables of cumulative exposure to metformin, statins and aspirin after cancer diagnosis and time-dependent ever-never terms for drug exposure.

Results: A total of 1043 patients used GLDs before CRC diagnosis; 666 (64%) used metformin, 639 (61%) used statins and 490 (47%) used aspirin after CRC diagnosis. Multivariable analyses revealed that longer cumulative exposure to metformin was not associated with overall mortality ($HR_{Cumulative\ exposure/6\ months}$ 1.02; 95% CI 0.97–1.07), whereas the favourable effect of statins increased with cumulative exposure ($HR_{Cumulative\ exposure/6\ months}$ 0.93; 95% CI 0.89–0.98). No association between aspirin use and overall mortality was seen ($HR_{Cumulative\ exposure/6\ months}$ 0.98; 95% CI 0.93–1.03).

Conclusions: No independent association between cumulative exposure to metformin, aspirin and overall mortality was found. Cumulative exposure to statins after CRC diagnosis was associated with lower overall mortality, supporting a drug effect of statins among GLDs users.

Although diabetes patients appear to have higher overall mortality after colorectal cancer (CRC), those treated with metformin, a first-line glucose-lowering drug (GLD), appear to have decreased overall mortality compared with other diabetes patients (Lee *et al*, 2011; Bo *et al*, 2012; Garrett *et al*, 2012; Spillane *et al*, 2013). In addition, other drugs, such as statins and aspirin have also been associated with decreased overall mortality in CRC patients (Chan *et al*, 2009; Bardou *et al*, 2010; Bastiaannet *et al*, 2012; Lakha *et al*, 2012; McCowan *et al*, 2013; Cardwell *et al*, 2014). These drugs are frequently prescribed to individuals with diabetes, that is, around

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50% of them use statins and 40% use aspirin according to the international literature (van Dijk *et al*, 2011; Smiechowski *et al*, 2013). As many diabetes patients use a combination of these three types of drugs, it is justified to wonder if, the suggested association between metformin and overall mortality among cancer patients is explained by the concomitant use of aspirin or statins, and vice versa (Yu *et al*, 2014). Moreover, these drugs might have synergism in their effects to improve outcomes of cancer patients, via different biological targets: metformin via the inhibition of mitochondrial activity (Pollak, 2012a,b), statins via blocking the production of mevalonate (Goldstein and Brown, 1990) and aspirins might influence cancer outcomes by inhibiting cyclooxygenase-2 dependent pathways (Chan *et al*, 2007). Therefore, the potential favourable effect of these drugs should be studied taking into account the effects of the other drugs. A few researchers have followed this approach, adjusting for the associations between mortality and other drugs, but always on the basis of dichotomized variables (Lee *et al*, 2011; Bo *et al*, 2012; Garrett *et al*, 2012; Spillane *et al*, 2013). Those studies reported low hazard ratios (HRs) for the effects of metformin (HR 0.4–0.9; Lee *et al*, 2011; Bo *et al*, 2012; Garrett *et al*, 2012; Spillane *et al*, 2013), statins (HR 0.5–0.9; Bardou *et al*, 2010; Lakha *et al*, 2012; Cardwell *et al*, 2014) or aspirin (HR 0.7–0.9; Chan *et al*, 2009; Bastiaannet *et al*, 2012; McCowan *et al*, 2013) on overall mortality in CRC patients. Unmeasured differences in prognostic factors will overestimate a potential drug effect (Zanders *et al*, 2014), because the dichotomous variable will not reveal the effect of the drug over time. This type of bias in pharmaco-epidemiology, known as confounding by indication or allocation bias, has received increasing attention in the field of diabetes and cancer and experts are debating whether the inclusion of the time-dependent cumulative exposure is the best option to prevent this bias (Suissa and Azoulay, 2012; Walker *et al*, 2013).

Thus, the primary objective of this study was to assess the independent association of metformin, statins and aspirin with overall mortality among CRC patients with diabetes. We hypothesised that overall mortality will decrease with increasing cumulative exposure to metformin, statins and aspirin independently of the effects of the other studied drugs.

MATERIALS AND METHODS

Data sources. Data were obtained from the Eindhoven Cancer Registry (ECR) linked on a patient level to the PHARMO Database Network, together the databases cover a demographic region in the Southern part of the Netherlands of approximately one million inhabitants, which is a subset of both databases. The construct and validity of the ECR-PHARMO cohort have been described elsewhere (van Herk-Sukel *et al*, 2010). The ECR, maintained by the Netherlands Comprehensive Cancer Organisation, records data on all patients newly diagnosed with cancer in the Southern part of the Netherlands, an area with 2.4 million inhabitants. The registry is notified by 6 pathology departments and 10 community hospitals. Trained registration clerks collect data on patient characteristics, cancer diagnosis, staging and initial treatment from hospital medical records. The PHARMO Database Network is a large, patient-centric data network including multiple linked observational databases designed for safety and outcomes research of drugs. For this study the out-patient pharmacy database was used, which includes data on the dispensed drug, dispensing date, amount and regimen dispensed. The duration of use was calculated by dividing the total amount dispensed by the amount used per day (both variables are registered within the PHARMO Database Network). All drugs are coded according to the Anatomical Therapeutic Chemical (ATC) classification (WHO, 2014). Both the ECR and the PHARMO Database Network are recognised as high-quality sources for epidemiological research, the linkage of correct patient records between these databases had a sensitivity of 98.3% and a positive predictive value of 98.6% (van Herk-Sukel *et al*, 2010). Together they collect information in overlapping regions in the Netherlands for a period of at least 10 years (van Herk-Sukel *et al*, 2010).

Study population. The source population included all CRC patients registered in the ECR-PHARMO cohort between 1998 and 2011 ($n = 8725$; Figure 1). From this source population, patients using any type of GLD (ATC-code: A10) before CRC diagnosis were selected ($n = 1043$). GLD use was used as a proxy for diabetes onset. In individuals who used GLD before CRC diagnosis, the use of metformin (ATC-code: A10BA02), statins (ATC-code: C10AA, C10BA and C10BX) and low-dose aspirin (ATC-code: B01AC06, B01AC08 and B01AC30; $\leqslant 100$ mg daily) was evaluated (ATC-codes see Supplement Table 1). The exact duration of GLD use before cancer diagnosis was only known for incident users (i.e., users who started with GLDs after the moment they were eligible to be followed in the database of the PHARMO Institute, $n = 607$; 58%).

Exposure and outcome. For each CRC patient, the cumulative days of metformin, statins and aspirin exposure *after* CRC diagnosis were calculated and determined from CRC diagnosis until death, leaving the ECR-PHARMO area, or end of study at 31 December 2011. The cumulative exposure variables represent the number of days of on-treatment time during follow-up, that is, if a patients stops treatment the value of the exposure variable will not increase until treatment is reinitiated. The outcome measure for the study was overall mortality, which was obtained from the municipal personal records database.

Statistical analyses. The association of metformin, statins and aspirin with overall mortality after CRC diagnosis was analysed using a time-dependent multivariable Cox proportional hazards model, which included all studied drugs. Time since the diagnosis of CRC was used as the underlying timescale in the time-dependent Cox proportional hazard model. Within this manuscript the index date refers to the start of follow-up. The use of metformin, aspirin and statins before CRC diagnosis was included as ever-never (1 *vs* 0) terms in the model, whereas the cumulative days of drug use after CRC diagnosis were included as time-dependent determinants. As this study included users with different durations of exposure, as well as non-users of the drugs,

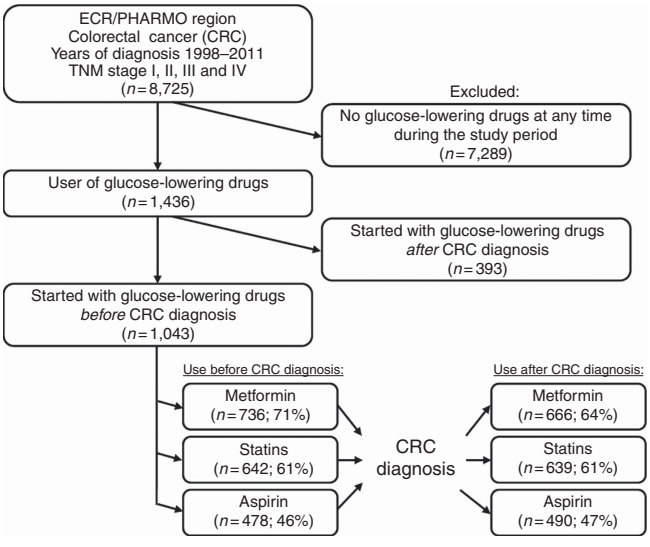

Figure 1. Flowchart of patients selected for analysis.

the inclusion of cumulative drug exposure alone might not be sufficient (Kirkwood and Sterne, 2003). The events for overall mortality for non-users (representing most of the events within the total cohort) will all be clustered at the cumulative exposure of zero months. The HR comparing 0 months of cumulative exposure with, for example, 6 months of cumulative exposure, has a great impact on modelling the overall HR for cumulative exposure. This may introduce allocation bias or confounding by indication, which we tried to avoid by including time-dependent ever-never terms for drug use after CRC diagnosis (Kirkwood and Sterne, 2003; Zanders *et al*, 2014). The change in overall mortality risk with cumulative drug exposure to either metformin, statins or aspirin was illustrated by calculating HRs at start of follow-up, and after 6, 12, 18, 24, 30 and 36 months of cumulative exposure to the drugs. The reference group for these HRs were patients with zero cumulative exposure at that specific time in follow-up.

As our cohort includes patients with diabetes, the use of other GLDs before and after cancer diagnosis needed to be adjusted for: sulfonylurea derivatives (ATC-code: A10BB), insulin (ATC-code: A10A) and other GLDs. The cumulative exposures and ever-never terms of these drugs were included in the multivariable model using a similar approach as for the other drugs. Age at CRC diagnosis, sex, calendar year of CRC diagnosis, stage of cancer, CRC subsite (proximal colon, distal colon or rectal), administration of surgery, radiotherapy and/or chemotherapy were considered potential confounders. These covariates, determined at the start of follow-up, were included in the multivariable analyses as time-fixed variables. The presence of effect modification between metformin, statin and aspirin use was evaluated by including interaction terms in our full model (cumulative exposure of studied drug × ever-never term of potential effect modifying drug). A two-sided P-value < 0.05 was considered statistically significant. Analyses were performed using SAS software (version 9.3, SAS institute, Cary, NC, USA).

Subgroup and sensitivity analyses. Cancer subsite (colon or rectal), cancer stage (I–III or IV) and cancer treatment were evaluated as effect modifiers of the studied drugs.

To determine whether our results were confounded by the duration of drug use before cancer diagnosis, users of either metformin, statins or aspirin with unknown duration were excluded in an additional analysis in which was adjusted for drug duration. As many patients in this study used diuretics, beta-blocking agents and renin-angiotensin system agents, we adjusted for the use of these drugs by including cumulative exposures and ever-never terms in another sensitivity analysis. To evaluate the association between metformin, statins, aspirin and long-term survival, index date (i.e., the start of study follow-up) was pushed to be 6 months after CRC diagnosis (thereby the patients who died or were not followed for 6 months were removed from the cohort), and the values of the exposure variables at this new index date are the days cumulated in the 6 months between CRC and the (new) index date.

As the influence of statins on mortality may be different for lipophilic and hydrophilic statins, two sensitivity analyses were included, in which respectively only lipophilic or hydrophilic statin use, was defined as statin use. Because especially the association between statins and survival might be confounded by sick-stopper (lower adherence among groups with high risks of poor outcomes) or healthy user bias (selection of more health-conscious statin users; Stavrou *et al*, 2012), which as a result might have overestimated the effect, we assessed the accuracy of our analyses further by performing three other sensitivity analyses. In the first sensitivity analysis a grace period of 3 months after each discontinuation of treatment was added, assuming that they were still on treatment for these 3 months even though there were no records of drug use in the data. In the other two sensitivity analyses restricted to patients with a follow-up of more than, respectively, 6 and 12 months, a lag of 6/12 months was included. As a result all GLD dispensings in the 6/12 months prior to the end of study or death were excluded, as GLD use in these months might reflect end of life treatment and might be influenced by sick-stopper bias.

As it could be hypothesised that only very recent drug exposure could influence mortality, an analysis was performed in which cumulative drug exposure was brought back to zero after not using the drug for 3 months. Instead of cumulative drug duration, the cumulative dose of the drug was included as exposure variable in another analysis. The HR for cumulative exposure was shown per 100 defined daily dose (DDD) instead of per 6 months of cumulative drug duration.

RESULTS

The study population consisted of 1043 patients who used GLDs before CRC diagnosis (Table 1), of whom 666 (64%) used metformin, 639 (61%) used statins and 490 (47%) used aspirin after CRC diagnosis (Figure 1, Table 1). Many patients had unknown duration of GLD use at the time of cancer diagnosis (42%), whereas 32% of the patients in the total cohort had a duration of GLD use which was $\geqslant 3$ years at that time. During a mean follow-up of 3.4 years (s.d. ± 3.0), 494 patients (47%) died before the study end, fewer deaths occurred in groups which used one of the studied drugs (Supplement Table 2).

After the diagnosis of CRC, the median duration of metformin use was 1.6 years (interquartile range (IQR) 0.5–3.3), for statins this was 2.0 years (IQR 0.6–3.9) and for aspirin this was 1.5 years (IQR 0.2–3.4). Of the total study cohort, 25% of the patients used all drugs under study, whereas 15% of the patients used none of them after CRC diagnosis (Supplement Table 2). Many CRC patients used other drugs after the diagnosis of cancer (mean follow-up 3.4 ± 3.0 years), 58% used sulfonylurea derivatives, 47% diuretics, 45% beta-blocking agents and 53% renin-angiotensin system agents. Metformin, statin and aspirin users, used significantly more beta-blocking agents and renin-angiotensin system agents compared with those not using the studied drugs (Supplement Table 2). Although the characteristics of CRC were comparable for the different drug groups according to Table 1, the proportion of statin users with rectal cancer was higher (33% *vs* 24%; $P = 0.005$; Supplement Table 2).

Full model. The multivariable time-dependent analysis seemed to suggest that ever-users of metformin had lower overall mortality compared with those never using metformin after CRC diagnosis ($HR_{Drug\ ever/never}$ 0.78; 95% CI 0.59–1.01; Table 2 and Figure 2). However, in patients using metformin after CRC diagnosis longer cumulative exposure was not associated with overall mortality ($HR_{Cumulative\ exposure\ per\ six\ months}$ 1.02; 95% CI 0.97–1.07). Furthermore, analysis revealed that overall mortality was in favour of ever-users of statins compared to those never using statins after cancer diagnosis ($HR_{Drug\ ever/never}$ 0.73; 95% CI 0.54–0.99). Importantly, cumulative exposure to statins was also associated with better overall mortality ($HR_{Cumulative\ exposure\ per\ 6\ months}$ 0.94; 95% CI 0.89–0.98). We did not observe differences between ever and never users of aspirin ($HR_{Drug\ ever/never}$ 0.96; 95% CI 0.73–1.26) nor an association between cumulative exposure and overall mortality ($HR_{Cumulative\ exposure\ per\ 6\ months}$ 0.98; 95% CI 0.94–1.03). In the full model, the cumulative exposures to other GLDs that were included did not show any association with overall mortality (Supplement Table 3). Moreover, no significant interactions were found between metformin, statins and aspirin use after CRC diagnosis.

Subgroup and sensitivity analyses. The association between metformin and overall mortality in the full model was comparable

Table 1. Baseline characteristics of the study population according to medication use after CRC diagnosis ($n = 1043$)

	Total	Metformin users	Statin users	Aspirin users
	n %	*n* %	*n* %	*n* %
Patients	1043 (100)	666 (64)	639 (61)	490 (47)
Age at CRC diagnosis (years; means (s.d.))	73.2 (±9.1)	72.3 (±8.8)	71.9 (±8.5)	73.5 (±8.8)
Male	543 (52)	366 (55)	377 (59)	284 (58)
Duration of GLD use at CRC diagnosis				
<1 year	108 (10)	74 (11)	63 (10)	50 (10)
1–3 years	168 (16)	110 (16)	103 (16)	68 (14)
⩾3 years	331 (32)	225 (34)	206 (32)	153 (31)
Unknown duration	436 (42)	257 (39)	267 (42)	219 (45)
Follow-up				
Duration of follow-up (years; mean (s.d.))	3.4 (±3.0)	3.7 (±3.0)	3.8 (±3.0)	3.9 (±3.2)
End of follow-up				
Death	494 (47)	272 (41)	223 (35)	219 (45)
Loss to follow-up	11 (1)	7 (1)	7 (1)	3 (1)
End of study (31 December 2011)	538 (52)	387 (58)	409 (64)	268 (54)
Use of the drugs under study after CRC diagnosis				
Metformin	666 (64)	666 (100)	469 (73)	336 (69)
Duration of metformin use (years; median (IQR))	1.6 (0.5–3.3)	1.6 (0.5–3.3)	1.9 (0.7–3.6)	1.9 (0.6–3.6)
Statins	639 (61)	469 (70)	639 (100)	359 (73)
Duration of statin use (years; median (IQR))	2 (0.6–3.9)	2.2 (0.8–4.1)	2 (0.6–3.9)	2.2 (0.7–4.2)
Type of statin used after CRC diagnosis				
Lipophilic	541 (52)	394 (59)	541 (85)	302 (61)
Hydrophilic	135 (13)	100 (15)	135 (21)	76 (16)
Aspirin	490 (47)	336 (51)	359 (56)	490 (100)
Duration of aspirin use (years; median (IQR))	1.5 (0.2–3.4)	1.6 (0.2–3.5)	1.7 (0.2–3.6)	1.5 (0.2–3.4)
Use of the drugs under study before CRC diagnosis				
Metformin	736 (71)	591 (89)	480 (75)	336 (69)
Unknown duration (percentage of metformin use)	158 (21)	128 (22)	103 (21)	80 (24)
Statins	642 (61)	437 (66)	556 (87)	342 (70)
Unknown duration (percentage of statin use)	182 (28)	111 (25)	164 (29)	111 (32)
Aspirin	478 (46)	305 (46)	326 (51)	386 (79)
Unknown duration (percentage of aspirin use)	189 (40)	108 (35)	131 (39)	156 (40)
Use of other frequently prescribed drugs in individuals with diabetes after CRC diagnosis[a]				
Sulfonylurea derivatives	606 (58)	439 (66)	384 (60)	301 (61)
Insulin	368 (35)	224 (34)	251 (39)	189 (39)
Other GLDs	82 (8)	65 (10)	61 (10)	49 (10)
Diuretics	491 (47)	321 (48)	325 (51)	256 (52)
Beta-blocking agents	465 (45)	334 (50)	337 (53)	276 (56)
Renin-angiotensin system agents	557 (53)	405 (61)	416 (65)	307 (63)
Type of CRC				
Proximal colon	439 (42)	267 (40)	251 (39)	193 (39)
Distal colon	295 (28)	194 (29)	176 (28)	144 (30)
Rectal	309 (30)	205 (31)	212 (33)	153 (31)
TNM stage[b]				
I	209 (20)	138 (21)	143 (22)	103 (21)
II	324 (31)	207 (31)	205 (32)	168 (34)
III	245 (23)	167 (25)	148 (23)	109 (22)
IV	189 (18)	112 (17)	100 (16)	73 (15)
Period of CRC diagnosis				
1998–2002	123 (12)	67 (10)	45 (7)	65 (13)
2003–2007	402 (39)	260 (39)	252 (39)	196 (40)
2008–2011	518 (50)	339 (51)	342 (54)	229 (47)
Treatment of CRC				
Surgery	891 (85)	580 (87)	571 (89)	430 (88)
Chemotherapy	225 (22)	157 (24)	149 (23)	92 (19)
Radiotherapy	196 (19)	134 (20)	140 (22)	99 (20)

Abbreviations: CRC = colorectal cancer; GLD = glucose-lowering drugs; IQR = interquartile range; TNM = classification of malignant tumours.
[a]Ever use of other drugs after CRC diagnosis (mean follow-up 3.4 ± 3.0 years): sulfonylurea derivatives (ATC-code: A10BB), insulin (ATC-code: A10A), other GLDs, diuretics (ATC-code: C03), beta-blocking agents (ATC-code: C07) and drugs for renin-angiotensin system (ATC-code: C09).
[b]Does not add up to total due to missing values.

with the HRs found for cumulative exposure to metformin in subgroup and sensitivity analyses (Table 2). The HR for ever-never use of metformin seemed to be lower when a lag time of 6/12 months was included or when the exposure variable was replaced by cumulative exposure in DDD.

The HR of 0.94 for the association between cumulative exposure to statins and overall mortality seemed to be even more protective among CRC patients who received chemotherapy or among CRC patients with stage IV disease ($HR_{\text{Cumulative exposure per 6 months}}$ 0.84; 95% CI 0.70–1.00 and $HR_{\text{Cumulative exposure per 6 months}}$ 0.82; 95% CI

Table 2. Multivariable Cox regression analyses of the time-dependent HR of cumulative exposure to metformin, statins and aspirin per 6 months of use after CRC diagnosis on overall mortality

		Metformin		Statins		Aspirin	
		HR_{Drug} ever/never	$HR_{Cumulative\ exposure\ per\ 6\ months}$	HR_{Drug} ever/never	$HR_{Cumulative\ exposure\ per\ 6\ months}$	HR_{Drug} ever/never	$HR_{Cumulative\ exposure\ per\ 6\ months}$
Model of exposure after CRC diagnosis	**Deaths/*n***	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**
Full model[a]	494/1043	0.78 (0.59–1.01)	1.02 (0.97–1.07)	0.73 (0.54–0.99)	0.94 (0.89–0.98)	0.96 (0.73–1.26)	0.98 (0.94–1.03)
Subgroup analyses[b]							
Colon cancer patients	348/734	0.72 (0.52–0.99)	1.01 (0.95–1.07)	0.84 (0.58–1.20)	0.93 (0.88–0.99)	0.76 (0.55–1.06)	0.98 (0.93–1.05)
Rectal cancer patients	146/309	0.71 (0.42–1.20)	1.04 (0.94–1.14)	0.61 (0.35–1.08)	0.92 (0.83–1.02)	1.49 (0.86–2.60)	1.00 (0.90–1.10)
Patients with stage I–III CRC	296/778	0.91 (0.65–1.27)	1.01 (0.96–1.07)	0.74 (0.49–1.12)	0.94 (0.89–1.00)	0.98 (0.70–1.37)	0.98 (0.93–1.03)
Patients with stage IV CRC	158/189	0.50 (0.29–0.87)	1.06 (0.88–1.26)	1.21 (0.64–2.29)	0.82 (0.67–1.00)	1.20 (0.66–2.17)	0.81 (0.67–0.97)
Patients who received chemotherapy	111/225	0.61 (0.33–1.13)	1.05 (0.90–1.22)	1.13 (0.58–2.22)	0.84 (0.70–1.00)	1.36 (0.68–2.71)	0.97 (0.81–1.16)
Sensitivity analyses[b]							
Adjustment for drug duration before CRC diagnosis[c]	302/667	0.92 (0.64–1.30)	1.01 (0.94–1.08)	0.68 (0.54–1.03)	0.92 (0.85–1.00)	0.88 (0.61–1.28)	0.96 (0.89–1.03)
Adjustment for diuretics, beta-blocking agents and renin-angiotensin system agents[d]	494/1043	0.78 (0.60–1.02)	1.02 (0.97–1.07)	0.71 (0.53–0.97)	0.93 (0.88–0.98)	0.94 (0.71–1.23)	0.97 (0.92–1.02)
Cohort entry 6 months after CRC diagnosis[e]	310/858	0.77 (0.55–1.09)	1.01 (0.96–1.07)	0.67 (0.46–0.98)	0.93 (0.88–0.98)	1.08 (0.77–1.52)	0.98 (0.93–1.04)
Defined as statin use if lipophilic statin used	494/1043	0.75 (0.57–0.97)	1.02 (0.97–1.07)	0.80 (0.59–1.08)	0.95 (0.90–1.00)	0.95 (0.73–1.25)	0.98 (0.94–1.04)
Defined as statin use if hydrophilic statin used	494/1043	0.76 (0.58–0.99)	1.00 (0.96–1.05)	0.88 (0.54–1.41)	0.92 (0.82–1.04)	0.92 (0.71–1.21)	0.97 (0.93–1.02)
After discontinuation of drug use 3 months of exposure included[f]	494/1043	0.77 (0.59–1.01)	1.02 (0.98–1.07)	0.73 (0.54–0.99)	0.94 (0.90–0.99)	0.91 (0.69–1.20)	0.98 (0.93–1.03)
Inclusion of a lag time of 6 months[g]	310/858	0.83 (0.62–1.13)	1.02 (0.96–1.08)	0.53 (0.38–0.73)	0.97 (0.91–1.03)	0.79 (0.56–1.13)	1.01 (0.95–1.07)
Inclusion of a lag time of 12 months[g]	249/795	0.94 (0.67–1.31)	1.00 (0.94–1.07)	0.46 (0.32–0.66)	0.98 (0.92–1.05)	1.06 (0.73–1.54)	0.96 (0.90–1.02)
Cumulative exposure brought back to zero after 3 months of not using the drug[h]	494/1043	0.58 (0.45–0.74)	1.05 (1.00–1.11)	0.47 (0.35–0.62)	0.99 (0.94–1.04)	0.76 (0.57–1.00)	1.02 (0.97–1.08)
Cumulative dose, HR per 100 defined daily dose[i]	494/1043	0.61 (0.48–0.77)	1.02 (0.99–1.05)	0.46 (0.35–0.59)	0.93 (0.71–1.20)	0.79 (0.60–1.04)	1.08 (0.83–1.40)

Abbreviations: CI = confidence interval; CRC = colorectal cancer; HR = hazard ratio.

[a]Full model, adjusted for use of metformin, sulfonylurea derivatives, insulin, other diabetes medication, statins and aspirin after diagnosis as time-dependent cumulative exposure and as time-dependent ever-never terms, the use of these drugs before diagnosis as a dichotomized variable, and the time-fixed variables: sex, age at CRC diagnosis, calender year of CRC diagnosis, type of CRC, stage at CRC diagnosis and administration of surgery, radiotherapy and/or chemotherapy.

[b]Multivariable subgroup or sensitivity analyses with similar variables as in the full model.

[c]Users of either metformin, statins or aspirin with unknown duration of drug use before CRC diagnosis were excluded in an additional analysis in which was adjusted for the drug duration before CRC diagnosis.

[d]The time-dependent cumulative exposure, time-dependent ever-never terms after CRC diagnosis and the dichotomized variable for drug use before CRC diagnosis for the use of diuretics, beta-blocking agents and renin-angiotensin system agents were added to the full model.

[e]Index date was pushed to be 6 months after CRC diagnosis (thereby the patients who died or were not followed for 6 months were removed from the cohort), and the values of the exposure variables at this new index date are the days cumulated in the 6 months between CRC and the (new) index date.

[f]In this sensitivity analysis a grace period of 3 months after each discontinuation of treatment was added, assuming that they were still on treatment for these 3 months even though there were no records of drug use in the data.

[g]A lag of, respectively, 6 and 12 months was included, which excluded all GLD dispensings in the 6/12 months prior to the end of study or death, as GLD use in these months might reflect end of life treatment.

[h]Cumulative drug exposure was brought back to zero after not using the drug for 3 months.

[i]Instead of cumulative drug duration, the cumulative dose of the drug was included as exposure variable. The HR for cumulative exposure was shown per 100 defined daily dose.

0.67–1.00; Table 2). In sensitivity analyses, adjusting for duration of drug use before CRC diagnosis, for important cardiovascular co-medication or taking into account the type of statin use and including a new index date which was 6 months after CRC diagnosis, the HRs were comparable with the main analyses for the association between cumulative exposure to statins and overall mortality (Table 2). In the sensitivity analysis in which 3 months of exposure was added after each discontinuation of treatment, cumulative exposure to statins was still associated with overall mortality ($HR_{Cumulative\ exposure\ per\ 6\ months}$ 0.94; 95% CI 0.90–0.99; Table 2). By including a lag time of, respectively, 6 and 12 months, the HR per 6 months of cumulative exposure to statin use moved closer to 1 ($HR_{Cumulative\ exposure\ per\ 6\ months}$ 0.97; 95% CI 0.91–1.03 and $HR_{Cumulative\ exposure\ per\ 6\ months}$ 0.98; 95% CI 0.92–1.05; Table 2). Although allowing cumulative exposure to drop back to zero after 3 months of not using the drug resulted in a HR close to 1, replacing cumulative exposure in days by cumulative exposure in dose, did not change the HR, but did broaden the confidence intervals (Table 2).

Subgroup or sensitivity analyses did not reveal different associations between aspirin use and overall mortality than was seen already in the full model (Table 2). Among CRC patients with stage IV disease (84% died) or when a lag time of 12 months was included, cumulative exposure to aspirin seemed to be associated

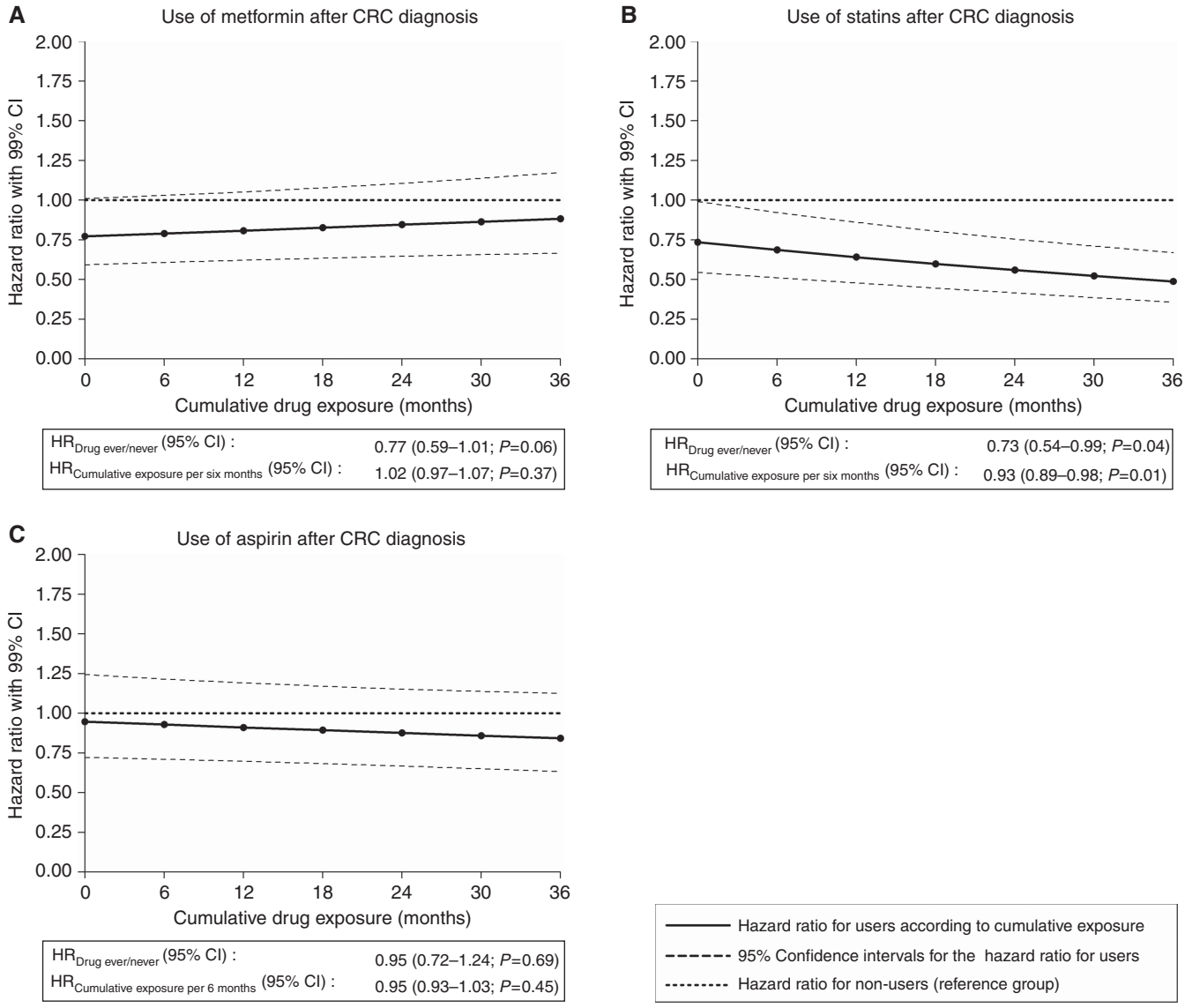

Figure 2. Hazard ratio's of overall mortality of CRC patients using metformin, statins or aspirin compared with those not using the specific drug after CRC diagnosis according to cumulative drug exposure per 6 months. **Full model, adjusted for use of metformin, sulfonylurea derivatives, insulin, other diabetes medication, statins and aspirin after diagnosis as time-dependent cumulative exposure and as time-dependent ever-never terms, the use of these drugs before diagnosis as a dichotomised variable, and the time-fixed variables: sex, age at CRC diagnosis, calender year of CRC diagnosis, type of CRC, stage at CRC diagnosis and administration of surgery, radiotherapy and/or chemotherapy.

with mortality ($HR_{Cumulative\ exposure\ per\ 6\ months}$ 0.81; 95% CI 0.67–0.97 and $HR_{Cumulative\ exposure\ per\ 6\ months}$ 0.96; 95% CI 0.90–1.02).

DISCUSSION

This population-based study revealed that among CRC patients who started using GLDs before cancer diagnosis, cumulative exposure to metformin or aspirin was not associated with overall mortality. However, longer cumulative exposure to statins was independently associated with lower overall mortality, suggesting a drug effect of statins in CRC patients with diabetes. This might imply that the survival benefit for metformin and aspirin, seen in recently reported studies (Chan *et al*, 2009; Lee *et al*, 2011; Bastiaannet *et al*, 2012; Garrett *et al*, 2012; McCowan *et al*, 2013; Spillane *et al*, 2013), may not be induced by these drugs, but the result of suboptimal methodology or confounded by prognostic differences between ever and never users of these drugs.

Although many of the earlier observational studies revealed that metformin is associated with lower overall mortality in CRC patients (Lee *et al*, 2011; Bo *et al*, 2012; Garrett *et al*, 2012; Spillane *et al*, 2013), today we understand that many of them contained time-related biases and other limitations that artificially made metformin look like a 'wonderdrug'(Renehan *et al*, 2012; Suissa and Azoulay, 2012; Walker *et al*, 2013; Suissa and Azoulay, 2014). However, many preclinical studies have revealed various mechanisms by which metformin might influence cancer progression and prognosis. The fundamental mechanism of action of metformin seems to involve inhibition of respiratory complex I, resulting in inhibition of mitochondrial oxidative phosphorylation and a reduction in ATP production (Pollak, 2012a,b). AMP kinase is activated, which downregulates cellular processes that consume energy, such as the synthesis of oncogenes like mTOR. The downregulation is hypothesised to reduce neoplastic growth (Pollak, 2012a,b). By including cumulative exposure variables for drug exposure in our analyses, which most previous studies did not, the potential biological mechanism, an increase in metformin

exposure would further reduce neoplastic growth, is incorporated in the study design. Moreover, the inclusion of a time-dependent cumulative exposure variable for drug exposure was needed as the effect of exposure depends on duration of use and timing in relationship to the event. Nevertheless, previous studies formed the driving force for the conduct of randomised metformin trials (National Cancer Institute, 2014). Similar considerations should have been made in studies on aspirin use and mortality after cancer, although some of these studies used pharmacy records, unfortunately, exposure was not analysed as continuous cumulative exposure (Chan *et al*, 2009; Bastiaannet *et al*, 2012; McCowan *et al*, 2013).

Although with the inclusion of time-dependent ever-never terms for the studied drugs in the model, the HR of the cumulative effect term seems to be not dependent on the events in the unexposed group (Colhoun *et al*, 2012), the inclusion of these terms is still subject of recent debate. Some experts in the field fear that the inclusion of both cumulative exposure and ever-never terms in a model introduces collinearity, our model has not given a sign of this. Nevertheless, per 6 months of cumulative metformin use or aspirin use the hazard rate for overall mortality in CRC patients did not change, thus in this study the use of metformin and aspirin was not associated with mortality.

Observational studies have investigated the association between statin use and outcomes among CRC patients regardless of diabetes status, but findings were inconsistent (Bardou *et al*, 2010; Lakha *et al*, 2012; Cardwell *et al*, 2014). Such discrepancies are likely a result of methodological limitations comparable with those in studies on metformin. In the current study we investigated a selection of statin users, thus comparing our results with previous studies on statin use and mortality after cancer patients might be incorrect.

Statins are drugs of prevention and sicker patients with a poorer prognosis might be more likely to discontinue preventative treatments for non-symptomatic illness (Stavrou *et al*, 2012). Because pharmacy records provide no ascertainment whether patients are compliant with their medication prescriptions, our results might be biased. Although we tried to avoid sick-stopper bias, by including a lag time which resulted in HRs closer to one, unfortunately there is no consensus on the optimal approach to avoid this bias (Glynn *et al*, 2001; Stavrou *et al*, 2012; Wang *et al*, 2013), thus this remains an important limitation of our study. Although we performed several corrections for time-exposure-related confounding risk factors, these findings do not necessarily imply a causal relationship between the use of statins and better overall mortality in CRC patients. Our analyses do not exclude that the association between the use of statins and the reduced risk of mortality in our data set are partly due to residual confounding.

Several epidemiological studies have been interested in the potential of statins as a chemo preventative, as statins may interact with various signalling pathways that are critical for CRC development, as well as progression (Goldstein and Brown, 1990; Agarwal *et al*, 1999; Katz *et al*, 2005; Kodach *et al*, 2011; Mace *et al*, 2013). The favourable effect of statins seemed to increase more clearly with cumulative drug exposure among patients who received chemotherapy. Thus, this study might support the hypothesis that statins, widely used for the treatment of hypercholesterolemia, might act as chemopreventative agent (The Long-Term Intervention with Pravastatin in Ischaemic Disease (LIPID) Study Group, 1998). Statins inhibit the conversion of HMG-CoA to the cholesterol precursor mevalonate, which is the rate-limiting step in cholesterol biosynthesis (Goldstein and Brown, 1990). Mevalonate is critical for the modification of proteins involved in cell growth, including both the RAS and RHO oncogenes (Goldstein and Brown, 1990). These potential antineoplastic benefits of statins were studied with regard to chemotherapy and radiotherapy administration, though this was only studied irrespective of diabetes status (Agarwal *et al*, 1999; Katz *et al*, 2005; Kodach *et al*, 2011; Mace *et al*, 2013). Two studies in rectal cancer patients revealed that the use of statins was associated with improved pathologic response after neoadjuvant chemoradiation (Katz *et al*, 2005; Mace *et al*, 2013). These findings were supported by cell line studies, because lovastatin augmented apoptosis induced by chemotherapeutic agents such as 5-FU and cisplatin in colon cancer cells (Agarwal *et al*, 1999; Kodach *et al*, 2011).

The number of patients included for sub-analyses was rather small and the follow-up was short. In addition, as no information was available on cause of mortality, we were not able to verify whether metformin, statins and aspirin are associated with decreased cancer-specific mortality. The protective association between statins and overall mortality in this study might be highly attributed to the decrease in cardiovascular deaths instead of cancer deaths (The Long-Term Intervention with Pravastatin in Ischaemic Disease (LIPID) Study Group, 1998). However, a recent study among CRC patients revealed that statin use after CRC diagnosis was associated with reduced CRC-specific mortality (HR 0.71; 95% CI 0.61–0.84; Cardwell *et al*, 2014). Because clinical laboratory data were only available for a subcohort, we could not include information on cholesterol levels, HbA1c values and BMI in the analyses. The influence of these metabolic factors on overall mortality in GLD users might be of interest and should be evaluated in future studies. Moreover, as the pharmacy data has only information for drugs on prescription, over-the-counter drug use of aspirin could not be captured by our data. Thus, in our study only aspirin dispensings with the indication 'platelet aggregation inhibition' and considered low-dose aspirin were included. Consequently, the misclassification of exposure due to over-the-counter low-dose aspirin use is likely to be minimal, because low-dose aspirin for this indication is only available on prescription in the Netherlands. Nevertheless, with our data we are not able to draw conclusions regarding the association between general aspirin use and mortality.

In conclusion, longer cumulative exposure to metformin or aspirin was not associated with overall mortality among CRC patients. But, longer cumulative exposure to statins after the diagnosis of CRC was associated with lower overall mortality among CRC patients starting on GLDs before cancer diagnosis. Our findings support a protective effect of statins, independent of metformin and aspirin use, in CRC patients using GLDs. As this study had an observational design our results are based on the decision of a clinician to prescribe a certain type of drugs, based on the patient characteristics together with the experience of the clinician. The findings of the current study substantiate to elucidate the association between statins and mortality after CRC diagnosis in future randomized and in-depth studies, with larger study populations. In addition these studies need to deal with the mentioned pharmaco-epidemiological challenges, sick-stopper bias and need to adjust for additional metabolic factors, such as HbA_{1c} and cholesterol levels.

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CONFLICT OF INTEREST

The authors declare no conflict of interest. Dr MPPvH-S and RMCH are employees of the PHARMO Database Network for Drug Outcomes Research. This independent research institute

performs financially supported studies for government and related healthcare authorities and several pharmaceutical companies. This study however, is not financially supported by a pharmaceutical company.

AUTHOR CONTRIBUTIONS

MMJZ contributed to the conception and design of the study, acquired and analysed the data and drafted the manuscript. MPPvH-S, PAJV and LVvdP-F contributed to the conception and design of the study and critically reviewed the manuscript. RMCH and HRH critically reviewed the manuscript.

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