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Effects of Metformin and Vitamin D on Clinical Outcome in Cholangiocarcinoma Patients

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Keywords
Biliary tract cancer · Cholangiocarcinoma · Gallbladder cancer · Metfromin · Vitamin D · Chemotherapy · Disease-free survival

Abstract
Background and Aims: In the last few years, there has been increasing interest in non-cancer medications and their potential anti-cancer activity. Data are not available in cholangiocarcinoma (CCA) patients. The aim of this study is to fill this gap by investigating the potential impact in terms of clinical outcome of the common non-cancer medications. ***Methods:*** All consecutive patients with CCAs were retrospectively identified from 7 Italian medical institutions. We investigated the role of intake of vitamin D, aspirin, metformin, statins, and diuretics. ***Results:*** A total of 537 patients with CCAs were identified; 197 patients undergoing surgery were evaluated for disease-free survival (DFS), and 509 patients with an advanced stage were evaluated for overall survival (OS). A longer DFS was found in patients with intake of vitamin D versus never users (HR 0.55, 95% CI 0.32–0.92, p = 0.02). In an advanced stage an association with OS was found in patients with intake of metformin versus never users (HR 0.70, 95% CI 0.52–0.93, p = 0.0162), and in patients who have started taking metformin after chemotherapy versus before chemotherapy and never users (HR 0.44, 95% CI 0.26–0.73, p = 0.0016). ***Conclusions:*** Our results highlighted that vitamin D intake improves DFS in patients undergoing surgery. Metformin intake after starting chemotherapy can improve the clinical outcome in advanced disease. These results could open up new therapeutic strategies in cholangiocarcinoma patients. We are planning to undertake a prospective study to validate these data.

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Introduction

Cholangiocarcinomas (CCAs) are a heterogeneous group of malignancies involving the biliary tree [1]. CCAs have always been regarded as "rare" tumors, but their incidence has steadily increased worldwide over the last decades, with different rates depending on the geographical area, and today it represents the second most common hepatic malignancy after hepatocellular carcinoma (HCC) [2]. The treatment of CCAs represents a challenge in the oncologic field, since their onset is frequently at an advanced stage, when the available systemic therapies with gemcitabine and cisplatin regimens are of limited effectiveness, with a median overall survival (OS) of less than 1 year [3]. Consequently, there is a critical need for treatments that improve CCA survival.

In the last few years there has been increasing interest in non-cancer medications and their potential anti-cancer activity, in terms of prevention and treatment. In the literature there is solid evidence regarding aspirin and metformin in the prevention setting. A recent comprehensive meta-analysis highlighted the inverse association between aspirin use and the risk of colorectum, esophagus, and stomach cancers; moreover, aspirin also seems to provide a protective effect on hepatobiliary and pancreas cancer risk [4–8]. Chronic metformin intake was shown to be associated with an overall reduction of cancer risk, and a putative effect in reducing liver, colorectal, pancreatic, stomach, and esophageal cancers, while no clear role in reducing breast, prostate, lung, and ovarian cancer risk was found [9–13]. More studies are needed to define the lowest effective doses, the ages of use, and the optimal treatment durations in order to select the subpopulation for which the benefits of chemoprevention outweigh the risks of side effects.

Data on cancer treatment are controversial. Several clinical studies have suggested the anti-cancer potential of aspirin, including anti-metastasis, anti-angiogenesis, and tumor microenvironment modulation, and the mechanisms seem to be both cyclooxygenase (COX) 2 dependent and independent [14–19]. In particular, the regular use of aspirin after diagnosis of mutated-PIK3CA colorectal cancer was associated with a longer survival [20]. In a recent study, Casadei Gardini et al. [21] highlighted a lower response to sorafenib in patients who developed HCC whilst undergoing chronic therapy with metformin.

Available data concerning vitamin D, statins, and diuretics are scarce and inconsistent. Although accumulating results from preclinical and early clinical trials strongly suggested that vitamin D deficiency increases cancer risk, thus supporting its chemopreventive role [22], the only randomized control trial in humans so far gave negative results [23]. Statins have been shown to reduce cancer-specific mortality and the recurrence/progression rates, predominantly in breast cancer [24, 25], but the level of evidence is weak, and randomized control trials are still lacking.

The potential anti-tumoral activity of common non-cancer medications in CCAs remains an open issue. On the one hand, in a recent meta-analysis chronic aspirin intake was significantly associated with a decreased risk of CCA [26] and encouraging preclinical data suggested an inhibitory effect of metformin on CCA cell migration and invasion [27]. On the other hand, solid clinical data about the potential anti-cancer activity of common non-cancer medications are lacking in this setting. The aim of this study is to fill this gap by investigating the potential impact in terms of clinical outcome of the common non-cancer medications, including aspirin, metformin, diuretics, vitamin D, and statins.

Table 1. Characteristics of patients undergoing surgery

Parameters	*N* (%)
Gender	
Male	102 (51.7)
Female	95 (48.3)
Primary tumor site	
iCCA	48 (24.4)
eCCA	118 (59.9)
GBC	31 (15.7)
Stage	
I	16 (8.1)
II	69 (35.0)
III	112 (56.9)
Adjuvant therapy	
Yes	66 (33.5)
No	131 (66.5)
Metformin users	13 (6.6)
Metformin non-users	184 (93.4)
Aspirin users	33 (16.7)
Aspirin non-users	164 (83.3)
Statin users	17 (8.6)
Statin non-users	180 (91.4)
Diuretics users	72 (36.5)
Diuretics non-users	125 (63.5)
Vitamin D users	11 (5.6)
Vitamin D non-users	180 (91.4)
No data	6 (3.0)

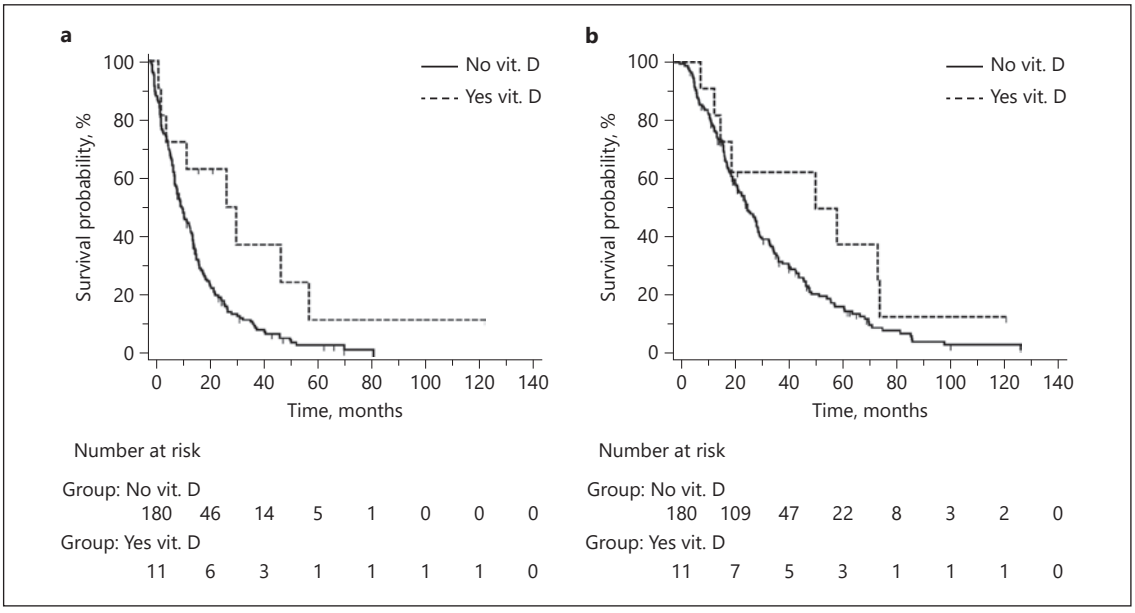

Fig. 1. DFS (**a**) and OS (**b**) in relation to vitamin D intake.

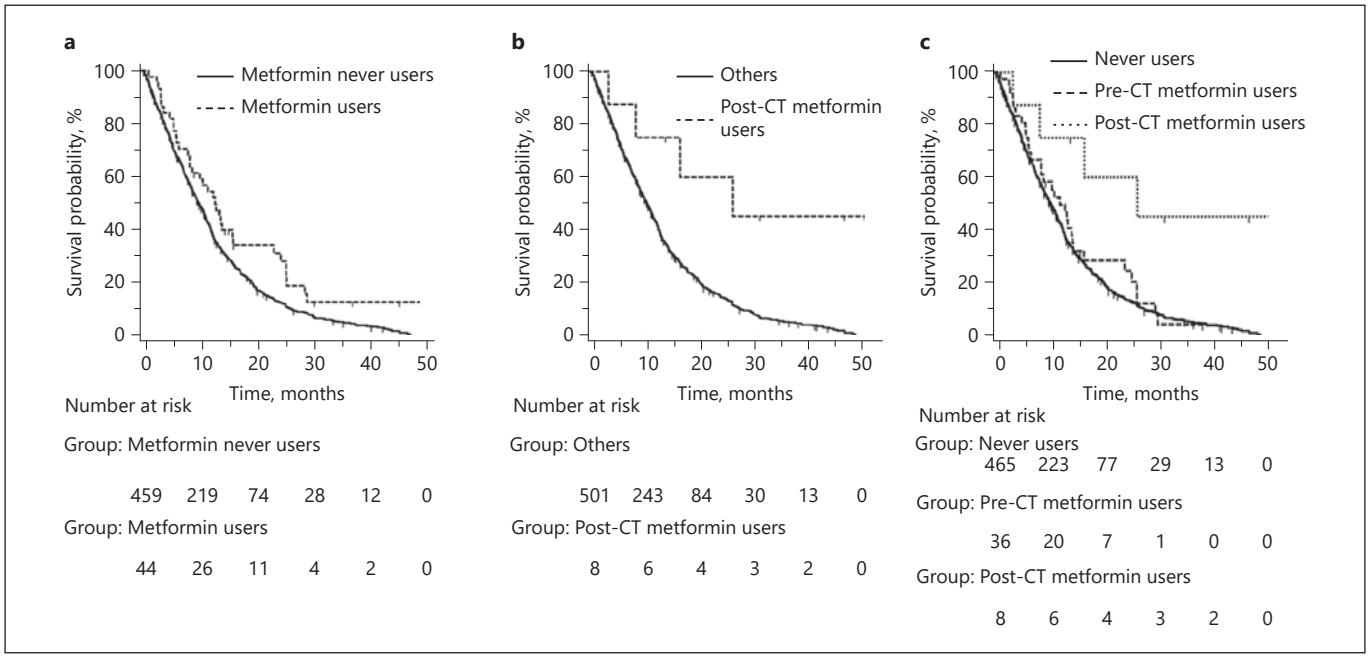

Fig. 2. OS in relation to metformin intake versus never users (**a**), post-chemotherapy (CT) metformin users versus others (**b**), and never users versus pre-CT metformin users versus post-CT metformin users (**c**).

Patients and Methods

All consecutive patients with CCAs treated with first-line chemotherapy from January 1, 2005 to April 1, 2020 were retrospectively identified from 7 Italian medical institutions (University of Turin, Turin; Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Meldola; Azienda Ospedaliero-Universitaria Pisana, Pisa; Istituto Tumori "Giovanni Paolo II," Bari;

Table 2. Univariate analysis of DFS and OS in patients undergoing surgery

	DFS		OS	
	HR (95% CI)	*p* value	HR (95% CI)	*p* value
Aspirin users				
No	1		1	
Yes	0.99 (0.67–1.45)	0.95	0.89 (0.60–1.33)	0.60
Others	1		1	
Aspirin starting after surgery	1.27 (0.69–2.32)	0.43	0.88 (0.51–1.51)	0.65
Diuretics users				
No	1		1	
Yes	0.91 (0.67–1.23	0.55	1.04 (0.75–1.43)	0.81
Others	1		1	
Diuretics starting after surgery	0.14 (0.77–1.68)	0.50	1.03 (0.70–1.52)	0.84
Metformin users				
No	1			
Yes	1.08 (0.57–2.04)	0.80	0.71 (0.39–1.30)	0.27
Others	1		1	
Metformin starting after surgery	1.21 (0.50–2.96)	0.66	0.76 (0.35–1.64)	0.48
Statin users				
No	1			
Yes	1.03 (0.59–1.80)	0.90	0.90 (0.49–1.68)	0.75
Others	1			
Statins starting after surgery	0.54 (0.21–1.37)	0.20	0.50 (0.16–1.57)	0.23
Vitamin D users				
No				
Yes	0.55 (0.32–0.92)	**0.02**	0.64 (0.36–1.13)	0.12
Others				
Vitamin D starting after surgery	0.69 (0.38–1.24)	0.22	0.87 (0.45–1.70)	0.69

Bold *p* values are significant.

Candiolo Cancer, Candiolo; Mauriziano Hospital, Turin; Azienda Sanitaria Locale di Biella, Ponderano). This study was conducted using the medical records databases at the same centers.

We investigated the role of intake of vitamin D, aspirin, metformin, statins, and diuretics. We divided the population into patients who started the drugs before and after surgery or starting first-line chemotherapy in an early CCA and advanced CCA setting, respectively. The primary objective was to examine the association between drug intake and disease-free survival (DFS) in patients with localized CCAs undergoing surgery, and OS in patients with unresectable locally advanced or metastatic CCAs treated with first-line chemotherapy.

Statistical Analysis

DFS was defined as the time interval between surgery and the day of relapse (local and/or distant). Patients alive at the time of database lock or dead from other causes were censored as appropriate. Progression-free survival (PFS) was defined as the time interval between the day of start of treatment and the day of documented disease progression, last follow-up visit if there was no progression, or the day of death. OS was defined as the time interval between the first day of chemotherapy and the day of death or last follow-up visit. Patients alive at the time of database lock or dead from other causes were censored as appropriate. DFS and OS were estimated by the Kaplan-Meier method and curves were compared by log-rank test. The MedCalc® package (version 16.8.4) was used for statistical analysis.

Table 3. Characteristics of advanced patients treated with chemotherapy

Parameters	*N* (%)
Gender	
Male	266 (52.3)
Female	243 (47.7)
Primary tumor site	
iCCA	245 (48.1)
eCCA	146 (28.7)
GBC	118 (23.2)
Platinum plus gemcitabine chemotherapy	288 (44.8)
Other treatment	221 (55.2)
ECOG	
0	279 (54.8)
1	178 (35.0)
2	52 (10.2)
Metformin users	44 (8.6)
Metformin non-users	459 (90.2)
No data	6 (1.2)
Aspirin users	73 (14.3)
Aspirin non-users	436 (85.7)
Statin users	43 (8.4)
Statin non-users	460 (90.4)
No data	6 (1.2)
Diuretics users	215 (42.2)
Diuretics non-users	284 (55.8)
No data	10 (2.0)
Vitamin D users	14 (2.7)
Vitamin D non-users	478 (93.9)
No data	17 (3.4)

Results

A total of 537 patients with CCAs were identified; 197 patients undergoing surgery were evaluated for DFS, and 509 patients (in 167 of whom the disease had relapsed from prior surgery) were evaluated for OS.

Drug Intake and Clinical Outcome in Patients Undergoing Surgery

The main characteristics of the patients are reported in Table 1. A longer DFS was found in patients with intake of vitamin D versus never users (HR 0.55, 95% CI 0.32–0.92, $p = 0.02$; Fig. 1a), whereas a trend was found in terms of OS (HR 0.64, 95% CI 0.36–1.13, $p = 0.12$; Fig. 1b). Use of Vitamin D was associated with a median DFS of 31.6 months (95% CI 4.4–57.9), compared to 12.2 months (95% CI 10.0–81.2) for patients who did not take vitamin D. The median OS was 51.9 months (95% CI 14.8–75.5) in ever-users of vitamin D versus 26.9 months (95% CI 23.3–30.7) in patients who were not.

No association was found in terms of DFS and OS in patients starting vitamin D after surgery versus never users and those starting before surgery (HR 0.69, 95% CI 0.38–1.24, $p = 0.22$; HR 0.87, 95% CI 0.45–1.70, $p = 0.69$, respectively). No other associations were found for other drugs (Table 2). After correction for stage and primary tumor site, the association of Vitamin D with DFS remained significant (HR 0.39, 95% CI 0.19–0.81, $p = 0.012$).

Drug Intake and Clinical Outcome in Advanced Settings

The main characteristics of patients are reported in Table 3. An association with OS was found in patients with intake of metformin versus never users (13.2 vs. 10.3 months; HR 0.70, 95% CI 0.52–0.93, $p = 0.0162$; Fig. 2a), and in patients who commenced intake of metformin after chemotherapy versus before chemotherapy and never users (25.9 vs. 10.6 months; HR 0.44, 95% CI 0.26–0.73, $p = 0.0016$; Fig. 2b; Table 4). Interestingly, we found no difference when we evaluated never-users versus patients who started taking metformin before chemotherapy. Conversely, patient intake of metformin starting after chemotherapy was associated with a very long survival (10.4, 12.0, and 25.9 months, respectively; Fig. 2c).

An association with PFS was found in patients who commenced intake of statins after chemotherapy versus before chemotherapy plus never users (8.0 vs. 4.7 months; HR 0.43, 95% CI 0.21–0.88, $p = 0.0162$). A trend was found in terms of OS (HR 0.50, 95% CI 0.22–1.13, $p = 0.09$).

After correction for baseline ECOG PS (Eastern Cooperative Oncology Group performance status), carbohydrate antigen 19-9, carcinoembryonic antigen, platinum plus gemcitabine therapy versus other therapy, and primary tumor site, data for metformin remained positive for OS only when we categorized patients who started metformin after chemotherapy versus others (HR 0.31, 95% CI 0.10–0.98, $p = 0.0467$). Conversely, metformin intake (before and after starting chemotherapy) versus never users lost its significance (HR 0.71, 95% CI 0.49–1.05, $p = 0.08$). A similar OS outcome was obtained for statins after correction for the same variables (HR 0.31, 95% CI 0.07–1.29, $p = 0.11$).

Table 4. Univariate analysis of PFS and OS in advanced patients treated with chemotherapy

	PFS		OS	
	HR (95% CI)	*p* value	HR (95% CI)	*p* value
Aspirin users				
No	1		1	
Yes	1.01 (0.77–1.33)	0.91	0.86 (0.67–1.11)	0.25
Others	1		1	
Aspirin starting after surgery	0.97 (0.53–1.76))	0.93	0.71 (0.42–1.21)	0.21
Diuretics users				
No	1		1	
Yes	0.98 (0.81–1.19)	0.88	1.03 (0.85–1.24)	0.72
Others	1		1	0.65
Diuretics starting after surgery	0.99 (0.80–1.22)	0.93	1.04 (0.85–1.28)	
Metformin users				
No	1		1	
Yes	1.15 (0.81–1.62)	0.39	0.70 (0.52–0.93)	**0.0162**
Others	1		1	
Metformin starting after surgery	1.01 (0.47–2.16)	0.96	0.44 (0.26–0.73)	**0.0016**
Statin users				
No	1		1	
Yes	0.82 (0.59–1.14)	0.24	0.78 (0.56–1.07)	0.12
Others	1		1	
Statins starting after surgery	0.43 (0.21–0.88)	**0.02**	0.50 (0.22–1.13)	0.09
Vitamin D users				
No			1	
Yes	1.12 (0.57–2.18)	0.72	1.44 (0.75–2.74)	0.26
Others			1	
Vitamin D starting after surgery	1.25 (0.57–2.73)	0.56	1.52 (0.72–3.20)	0.26

Bold *p* values are significant.

Discussion

Our study highlighted that vitamin D intake improves DFS in patients undergoing surgery. Metformin intake after starting chemotherapy can improve clinical outcomes in advanced disease. These results could open up new therapeutic strategies in CCA patients. The differential results between early and advanced stages are not easy to interpret, but probably vitamin D exerts greater inhibition of cell growth, while metformin has more intrinsic anti-tumor activity. Specific translational studies of this will be necessary to understand the difference between these results. To the best of our knowledge, this is the first study that highlights an association between chronic vitamin D intake and an improved DFS in patients with CCA undergoing surgery.

Several preclinical studies have demonstrated the anticancer activity of vitamin D in these tumors. In two different studies, Chiang et al. [28, 29] demonstrated the activity of vitamin D in CCA cell lines and a xenograft animal model. In the first study [28], dietary supplementation was determined in CCA cell line inhibitions in a rat model. Vitamin D supplementation modulated the expression of several genes, in particular lipocalin 2 (LCN2). These results were confirmed in the second study where they demonstrated that vitamin D represses SNU308 cell growth and in a xenograft animal experiment [29]. This study highlighted that LCN2 was repressed by vitamin D. The authors concluded that LCN2 could be a new therapeutic target for patients with CCA. Recently, the oncogenic role of LCN2 was described in several cancers, with higher LCN2 expression in cancerous cells compared to

non-cancerous ones [30]. Seubwai et al. [31] demonstrated that vitamin D receptor expression increased during CCA development. Furthermore, when they treated CCA cell lines with high receptor expression with vitamin D, it inhibited cell growth in a dose-dependent manner. MART-10 is a newly synthesized 1α,25$(OH)_2$D3 analog [32] with potent inhibition of cell growth that has been highlighted in CCA cell lines [33]. These preclinical data reinforce the role of vitamin D treatment in CCA. A previous study showed a decreased risk of CCA in a population chronically treated with metformin for type-2 diabetes mellitus [34].

Our study is the first in the literature that highlights an association between metformin intake and improved OS in patients with advanced CCA treated with chemotherapy. In particular, our study showed that this association seems to be more pronounced in those patients who started taking metformin after starting chemotherapy. These findings potentially provides the rationale for future study of combinations between this drug and chemotherapy agents. Several studies have demonstrated that metformin is a potent anti-proliferative agent in human CCA cells. It is known that metformin can downregulate the mammalian target of rapamycin (mTOR) directly by activation of the AMP-activated protein kinase (AMPK) [35]. Saengboonmee et al. [36] demonstrated that low doses of metformin significantly inhibited tissue invasion by CCA cells. Metformin activated AMPK by phosphorylation together with suppression of nuclear translocation of signal transducer and activator of transcription 3 (STAT3) and nuclear factor-kappa B (NF-κB). As demonstrated in our previous paper on metformin in HCC patients [37], STAT3 could play a primary role in this mechanism. In fact, patients taking metformin chronically showed hyperexpression of STAT-3 which determines sorafenib resistance.

Aspirin has been proposed as a treatment to reduce cancer mortality. Platelets can help cancer cells to get rid of immune cells and facilitate the arrest of cancer cells in the endothelium, thereby accelerating the process of metastasis. Aspirin appears to reduce the risk of metastasis by inhibiting the expression of COX-1 at the platelet level [38]. This mechanism reduces the growth factors involved in the epithelial-mesenchymal transition of circulating tumor cells. Recently, a large, registry-based study highlighted a reduced risk of death for post-diagnostic aspirin use in CCA patients [39]. In our multi-institutional retrospective advanced CCA case series, we could not replicate the prognostic association of post-diagnostic use of aspirin. In addition, this lack of association extended to patients receiving aspirin before diagnosis, and was not dependent on the type of first treatment received (surgery or chemotherapy). While the association between aspirin use and a decreased risk of CCAs is well demonstrated [40–42], the prognostic role of aspirin after diagnosis needs further evaluation, even more so in the light of our negative data.

In conclusion, our results highlighted that vitamin D intake improves DFS in patients undergoing surgery. Metformin intake after starting chemotherapy can improve the clinical outcome in advanced disease. These results could open up new therapeutic strategies in cholangiocarcinoma patients. We are planning to undertake a prospective study to validate these data.

Statement of Ethics

All procedures performed were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. For this type of study, formal consent is not required. The study protocol was reviewed and approved by the local Area Vasta Emilia Nord Ethics committee (Protocol number 183/2019) and was conducted according to the ethical guidelines of the 1975 Declaration of Helsinki.

Conflict of Interest Statement

The authors have no conflicts of interest to disclose.

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Author Contributions

All authors were responsible for the conception, data collection and processing, literature review, analysis and interpretation, writing, and critical review.

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