**A POPULATION BASED STUDY SHOWING THE UNIQUE AND CONSISTENT CHEMOPREVENTATIVE EFFECT OF METFORMIN IN PROSTATE CANCER**

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**Abstract**

***Introduction***

Metformin, an insulin sensitizer is recommended as first-line antidiabetic therapy. There is a growing amount of evidence showing a chemopreventative effect of metformin in prostate cancer (PCa). We aimed to analyze the chemopreventative role of metformin, in conjunction with other putative chemopreventative medications in PCa, in a population-based cohort study.

***Materials & methods***

Data were incorporated from the Institute for Clinical and Evaluative Sciences to identify all diabetic men aged 66 and above with a history of a single negative prostate biopsy between 1994 and 2016. Multivariable Cox regression models with time-dependent covariates were used to assess the effect of metformin on PCa diagnosis, androgen deprivation therapy (ADT) use, as a surrogate marker for advanced disease, and on undergoing an additional prostate biopsy (PB). Aside from including other putative chemopreventative medications, all models were adjusted for age, rurality, comorbidity, and year of patient study inclusion.

***Results***

Overall, 2,332 diabetic men were included, with a mean follow-up time of up to 11.06 years (SD 6.16 years). A total of 2,036 patients (87.3%) used metformin. Metformin was associated with decreased PCa diagnosis, (HR 0.69, 95% CI 0.54-0.88, p=0.003), lower hazard of undergoing an additional PB (HR 0.64, 95% CI 0.44-0.95, p=0.03), and treated with ADT (HR 0.72, 95% CI0.54-0.96, p=0.003).

***Conclusion***

Even when analyzed with other putative chemopreventative medications, metformin appears to be associated with lower PCa diagnosis rate, receiving hormonal therapy and may even decrease the probability of undergoing a prostate biopsy. We await validation of these findings in ongoing prospective randomized trials.

**Introduction**

Chemoprevention is defined as the use of natural or synthetic agents to suppress or prevent the carcinogenic process, resulting in the prevention of or delay in the development of clinically evident cancer[1](#_ENREF_1). Chemoprevention is challenged by the difficulty of finding an effective intervention with acceptable toxicity and cost. Moreover, there is a need to identify a population of individuals at sufficiently increased risk for developing specific cancer for which chemoprevention will be appropriate.

Many of the known risk factors for prostate cancer (PCa) such as increasing age, race, and genetic factors are not modifiable, and approximately 10% of PCa risk is estimated to be genetic[2](#_ENREF_2). However, the unique features of PCa make it an attractive target for primary chemoprevention. These include its high incidence, prevalence, morbidity, and treatment-associated cost. Chemoprevention has become an important public health approach in the continuing mission to decrease diagnosis, delay progression, and lower the morbidity and burden of PCa-associated therapy[3](#_ENREF_3).

In recent years, the concept of PCa chemoprevention has been addressed by several groups including the publication of large, randomized trials. One such example is the PCa Prevention Trial [PCPT], showing that this disease may be prevented by a relatively nontoxic oral agent (finasteride, a 5alpha-reductase-inhibitor [5ARI])[4](#_ENREF_4). Other putative chemopreventative commonly studied medications include statins[5](#_ENREF_5), proton pump inhibitors (PPIs)[6](#_ENREF_6), and alpha blockers[7](#_ENREF_7). Less commonly studied chemopreventative medications with in-vitro supported evidence include dipyridamole[8](#_ENREF_8) and chloroquine[9](#_ENREF_9).

One of the more interesting studied medications with potential chemopreventative PCa effects is the anti-diabetic medication metformin. In Canada, rates of diabetes and prediabetes continue to rise at an alarming rate. Currently, one in three Canadians has either diabetes or prediabetes[10](#_ENREF_10), and approximately 5 in 10 people aged 20 years will develop diabetes in their remaining lifetime[11](#_ENREF_11). Metformin (1,1-dimethylbiguanide hydrochloride) is an insulin sensitizer and is part of the biguanide oral hypoglycemic family[12](#_ENREF_12). Treatment guidelines for diabetics, recommend metformin as the first-line therapy[13](#_ENREF_13), making it the most widely prescribed antidiabetic drug in the world resulting from its clinical effectiveness and tolerability[14](#_ENREF_14). Importantly, there is a growing amount of evidence showing an association between metformin, decreased cancer risk and improved cancer-related outcomes in general,[15](#_ENREF_15) and in PCa specifically[16](#_ENREF_16).

Data on the role of metformin, in conjunction with other putative PCa chemopreventative medications, is lacking. Additionally, to date, the role of metformin in preventing an additional prostate biopsy (PB) has not been assessed. In this population-level-based study, we aimed to investigate the effect of metformin on these PCa-associated outcomes after incorporating other medications with a putative beneficial PCa effect. We hypothesized that metformin would decrease the rate of PCa diagnosis, advanced disease, and hazard of undergoing an additional PB.

**Methods**

This study received approval by the ethics board committee of the University Health Network and the University of Toronto. The study was reported according to Strengthening the Reporting of Observational Studies in Epidemiology guidelines[17](#_ENREF_17) and Reporting of Studies Conducted Using Observational Routinely-Collected Health Data statement[18](#_ENREF_18). We used administrative data housed at the Institute for Clinical and Evaluative Sciences (ICES) to perform a retrospective population-based cohort study. In the province of Ontario, the Ontario Health Insurance Plan (OHIP) is a single government-funded health insurance system that is responsible for reimbursement of all essential medical care. This enables capture of the entire adult population and access to their anonymized data. Additionally, in Ontario, medication prescription is freely available to everyone 65 years and older through the Ontario Drug Benefit (ODB) program. Therefore, we were able to accurately capture all provided prescriptions in the analyzed population.

**Data sources**

Data were acquired from several specific datasets housed at ICES[19](#_ENREF_19) and detailed in supplemental Table 1. The retrieved data contained demographic, baseline comorbidity, medication prescription, cancer diagnosis, and vital status details. The data of each patient in each of the various datasets are linkable using a unique encoded identifier.

**Study design and participants**

Only men with medically treated diabetes with a minimum age of 66 years and with a history of one single negative transrectal ultrasound-guided prostate biopsy (TRUS-Bx) in the province of Ontario between January 1st, 1994 and September 30th, 2016 were included. Age 66 and not 65 was chosen as the cut-off, to enable a one-year look-back period, confirming that no drug prescription of any of the analyzed medications was given during a minimum period of one year. This was our best way to ensure that all men analyzed in the study were medication-naïve. For the purpose of identification of all relevant patients, OHIP billing codes for TRUS-Bx, and the specific Canadian Classification of Diagnostic, Therapeutic, and Surgical Procedures codes were used to make sure no record of PCa diagnosis, nor receipt of PCa-specific treatment existed within the three months after the biopsy. The codes used are detailed in Supplemental Table 2. Men with a history of a previous negative biopsy were chosen for two main reasons: 1) These men are at an increased risk to develop PCa as previously shown in the PLCO trial, demonstrating that men with a negative biopsy having a PCa-incidence and PCa-specific mortality rate of 2.63 2.93 fold higher than men in the general population, respectively[20](#_ENREF_20) 2) As part of a pre-screening method to include a ‘healthier’ population, seen fit to undergo a biopsy. We utilized a look-back window of a minimum of three years, from January 1991 until cohort entry (as data were not available before that), to ascertain that included men had only a single negative TRUS-Bx and were not diagnosed with PCa. The index date was defined as the date 90 days after the date of the single negative prostate biopsy (PB) to ensure no PCa diagnosis.

Patients were followed from the index date until one of four possible outcomes: 1) Death, 2) Last health services contact in Ontario, 3) Becoming OHIP ineligible, or 4) End of the study period (September 30th, 2016).

**Study outcomes**

Our primary outcome was PCa diagnosis, examined as a time to event outcome. Secondary outcomes included undergoing an additional PB, and use of androgen deprivation therapy (ADT), serving as a surrogate marker for advanced disease. Patients who were diagnosed with PCa were censored from the analysis of undergoing an additional PB.

**Study variables**

PCa diagnosis was defined as having either a record of PCa or having received PCa-specific treatment (radical prostatectomy, primary radiotherapy to the prostate with or without ADT, or primary ADT). Data on several medications with putative anti-cancer properties were acquired. These included medications for diabetes (metformin, insulin, sulfonylureas, thiazolidinediones), statins, 5ARIs, alpha-blockers, chloroquine, and dipyridamole. Of note, Glaucoma eye drops served as a negative tracer drug and were incorporated into all models. A detailed list of all medications analyzed is shown in appendix 1.

Other variables acquired included patient age (categorized as 66-69, 70-74, 75-79, 80-84, and 85 years and above), rurality index (continuous variable, with a higher number representing a more rural area)[21](#_ENREF_21), year of study inclusion (index year), and comorbidity status quantified with the Collapsed Ambulatory Diagnostic Groups (ADG) score (a continuous comorbidity variable derived from the Johns Hopkins Adjusted Clinical Groups System)[22](#_ENREF_22). The comorbidity score of each patient was captured with a three-year look-back period at study inclusion date.

**Statistical analyses**

In this study, continuous variables were described using means and standard deviations (SD); categorical variables were characterized using proportions. We assessed the association between medication exposure and PCa diagnosis, undergoing an additional PB, and ADT use. Multivariable Cox proportional hazard regression models with time-dependent exposure were used for each cause-specific hazard. To obtain information on general medication exposure, and on cumulative exposure, the exposure to each medication was specified as a time-dependent variable (ever vs. never exposure at any time point during the follow-up, and the effect of the cumulative exposure to each medication per six-months of use), which was then associated with all outcomes of interest. All models were also adjusted for a priori selected covariates, treated as time-independent variables and using the values at study onset. These included age group, and the following continuous variables with log-linear effects: rurality index (0-100), index year (1994-2016) and the ADG comorbidity score. The proportionality and log-linearity assumptions underlying the multivariable models were assessed using residual-based diagnostics, and no violations were found. All statistical tests were two-tailed, and a p-value of <0.05 was considered significant. All statistical analyses were performed using R software version 3.3.1.

**Sensitivity analyses**

To assess for potential health utilization bias, we performed a tracer analysis, assessing the effects of all medications on the occurrence of presbyopia.

**Results**

From 1994 until 2016, a total of 2,332 men 66 years or older with medically treated diabetes and with a history of a single negative PB were identified in the province of Ontario. The mean follow-up time (SD) was 11.06 years (6.16 years) and 8.48 years (5.65 years) for diabetic patients treated and not treated with metformin, respectively. Table 1 depicts basic demographic data of all diabetic patients at study inclusion stratified by metformin use. A total of 2,036 (87.3%) patients used metformin during the study period. Figure 1 depicts the mean cumulative use (in months) of all analyzed medications among the study patients, stratified by age. The figure shows that on average, the longest used medications were metformin (42.5 months), hydrophilic statins (38.9 months), hydrophobic statins (37.1 months), sulfonylurea (20.5 months), and alpha-blockers (20.4 months).

A total of 27.6% of patients were diagnosed with PCa, with 3.6% dying of PCa, and 34.9% dying of other causes, as detailed in Supplemental Figure 1. Of those diagnosed with PCa, 36.2% were either treated with active surveillance, watchful waiting or not treated at all. A total of 32.1% were treated with ADT, 18.5% received primary radiotherapy to the prostate with or without ADT, and 13.2% underwent radical prostatectomy, as detailed in Supplemental Figure 2.

The primary outcome of PCa diagnosis was assessed in the multivariable model depicted in Table 2. This table showed that increased age (80-84) compared to age 66-69 was associated with an increased hazard of being diagnosed with PCa (HR 1.60, 95% CI 1.08-2.35, p=0.02). Moreover, every six months cumulative use of pantoprazole was associated with an increased hazard of being diagnosed with PCa (HR 1.069, 95% CI 1.001-1.140, p=0.05). In contrast, any use of 5ARIs and metformin was associated with a decreased hazard of being diagnosed with PCa (HR 0.64, 95% CI 0.44-0.95, p=0.03) and (HR 0.69, 95% CI 0.54-0.88, p=0.003), respectively.

The multivariable model assessing the secondary outcome of PB is described in Table 3. This model showed that increasing age category was associated with a decreased hazard of undergoing an additional PB. Additionally, any use and every six months cumulative use of metformin was associated with a lower hazard of undergoing another PB (HR 0.62, 95% CI 0.49-0.78, p<0.0001) and (HR 0.95, 95% CI 0.91-0.99, p=0.02), respectively. In contrast, any use of alpha-blockers was associated with an increased hazard of undergoing an additional PB (HR 1.50, 95% CI 1.10-1.65, p=0.003).

The final secondary outcome of being treated with ADT was assessed in Table 4. This model revealed that increasing age category was associated with an increased hazard of being treated with ADT. In contrast, a more contemporaneous index year and any use of metformin were associated with a decreased hazard of being treated with ADT (HR 0.960, 95% CI 0.930-0.996, p=0.003), and (HR 0.72, 95% CI0.54-0.96, p=0.003), respectively.

Other medications did not seem to show a beneficial or chemopreventative effect on any of the study’s outcomes. We could not assess PCa specific death as the number of events was to small (<60 events) to conduct a multivariable analysis. No association between the tracer medication (glaucoma eye drops) and any of the study outcomes were apparent. Lastly, associations between any of the medications to the tracer outcome of presbyopia (Supplemental Table 3) were not found.

**Discussion**

In this study any use of metformin was shown to be associated with 39%, 38% and 28% decreased hazard of being diagnosed with PCa, undergoing an additional PB, and being treated with ADT. Additionally, 5ARIs was associated with a 36% decreased hazard of being diagnosed with PCa, and every six months cumulative use of pantoprazole was associated with a 6.9% increased hazard of being diagnosed with PCa. Lastly, any use of alpha-blockers was associated with a 50% increased hazard of undergoing a PB.

The validity of our analyses are supported by four distinct findings: a) The lack of associations between all analyzed medications and presbyopia; b) The lack of association between all the study’s outcomes and the tracer medication (glaucoma eye drops) c) The similar PCa diagnosis rate that was previously shown using ICES datasets, demonstrating a 23.7% PCa diagnosis rate[23](#_ENREF_23); and d) The finding that 5ARIs were associated with a decreased hazard of being diagnosed with PCa, as shown in the landmark PCPT[4](#_ENREF_4) and REDUCE[24](#_ENREF_24) trials.

The anti-neoplastic potential of metformin is manifested through several mechanisms including adenosine monophosphate-activated protein kinase (AMPK)-dependent, AMPK-independent, insulin-mediated, and antiandrogenic mechanisms[25](#_ENREF_25). Metformin is a potent activator of AMPK[15](#_ENREF_15), and when activated, AMPK inactivates the enzymes involved in adenosine triphosphate consumption[26](#_ENREF_26) required for cancer cells. Additionally, AMPK activation inhibits the mammalian target of rapamycin (mTOR) complex 1 pathway and S6K1 phosphorylation implicated in carcinogenesis[27](#_ENREF_27). Metformin may also affect autophagic cell death[28](#_ENREF_28) and have an antineoplastic effect by decreasing c-Myc oncogene levels[29](#_ENREF_29). Metformin decreases PCa cell growth by stalling them at the G1/S checkpoint in a time- and in a dose-dependent manner[30](#_ENREF_30). By decreasing the hepatic production of glucose, leading to less uptake of insulin, metformin reduces the number of insulin receptors on the cell membrane[31](#_ENREF_31). These receptors, normally triggering a cascade of downstream effects in cancer cells, including the Ras/Raf/MEK/ERK and PI3K/AKT/mTOR signaling pathways, undergo inhibition induced by metformin[31](#_ENREF_31).

In a study assessing prostate-specific antigen (PSA) levels among patients with diabetes, metformin users exhibited significantly lower PSA levels compared with non-metformin users (OR  0.790; 95% CI 0.666–0.938; p = 0.007)[32](#_ENREF_32). Other studies have shown similar metformin-induced effects on PSA levels[33](#_ENREF_33),[34](#_ENREF_34), supporting our results of metformin lowering the hazard of undergoing an additional prostate biopsy. Other noteworthy observational studies in Europe and the USA, have shown a benefit for diabetes patients taking metformin. In 2008 a large Finnish cancer registry with over 24,000 case-control pairs, PCa risk was decreased in metformin users with an odds ratio of 0.87 (95% CI 0.82-0.92)[7](#_ENREF_7). In a large Danish nested case-control study with over 12,000 PCa patients, metformin users were at decreased risk of PCa diagnosis (OR 0.84, 95% CI 0.74-0.96)[35](#_ENREF_35). These support our results of decreased PCa diagnosis rates in diabetic men using metformin. Lastly, metformin was also shown to decrease the risk of progression to advanced disease among PCa-treated patients with external radiotherapy to the prostate. Over 2,700 diabetic PCa patients were assessed showing that metformin was associated with improved recurrence-, distant metastases- and castrate-resistant PCa-free survival[36](#_ENREF_36). Data also exists on the improved PCa-specific survival in US diabetic metformin-treated patients compared to those not treated with metformin[37](#_ENREF_37).

As previously mentioned, our study’s finding that 5ARIs were associated with decreased PCa diagnosis coincides with landmark randomized trials (PCPT[4](#_ENREF_4) and REDUCE[24](#_ENREF_24)) showing a similar effect for 5ARIs. Cumulative use of pantoprazole was shown to be associated with an increased hazard of being diagnosed with PCa, coinciding with in-vitro data supporting PPIs to be associated with increased PCa diagnosis[38](#_ENREF_38), and worse PCa-associated outcomes[39](#_ENREF_39). Lastly, our study showed that alpha-blockers were associated with an increased hazard of undergoing a PB. Alpha-blockers are given for benign prostatic hyperplasia (BPH) and/or lower urinary tract symptoms, which also lead to more frequent urology follow-ups. The differential diagnosis of BPH includes latent PCa[40](#_ENREF_40), which is detected by PB, possibly explaining why these patients are at an increased probability of undergoing PBs.

Our study’s strength lies in its incorporation of ‘real-world’ clinical data with a long follow-up time. To our knowledge, this is the only study specifically assessing the role of incident use of metformin combined with other putative chemopreventative PCa medications, assessing PCa diagnosis, PB, and ADT. However, there are several noteworthy limitations. Aside from its retrospective nature and its inherent selection bias, it undoubtedly consists of inaccuracies, embedded in health administrative databases. Additionally, our data was limited to men older than 66, consisting of 20-year old data. Clinically important information including race, PSA levels, disease stage and grade, pertinent family history, and personal genetic risk factors were lacking. Diabetes was defined as medically-treated diabetes only, and for this analysis, non-medically treated diabetic patients were not included in the analyses. For some patients ADT could have been given for local disease, which was more commonly accepted in the past, due to increasing age or significant comorbidities, making it a less than ideal surrogate marker of advanced disease. Importantly, there is perceived reverse causality, meaning that patients with metabolic syndrome, who are treated with anti-diabetic medications and statins, have been shown to be at an increased risk of developing PCa[41](#_ENREF_41), at an earlier age, with more advanced disease, and with higher PSA levels[42](#_ENREF_42). This could potentially appear as if metformin is associated with an increased risk of undergoing a PB, increased hazard of being diagnosed with PCa, and being treated with ADT. However, despite these known associations between metabolic syndrome and PCa, our results showed that metformin managed to “overcome” the associations between metabolic syndrome and worse PCa outcomes, and still be associated with a decreased risk of being diagnosed with PCa. This leads us to carefully surmise that the protective associations shown in our study may be underestimated. This leads us to wonder whether metformin given preventatively in men with no diabetes, might have an even more substantial chemopreventative effect than in men with diabetes, or it might not exist at all. The ongoing randomized multicenter Canadian MAST (Metformin Active Surveillance Trial) study (NCT01864096) and the STAMPEDE trial (NCT00268476) arm K evaluating the role of metformin in PCa patients will hopefully answer these pressing questions. Lastly, the risk of unaccounted residual confounding in these analyses is always present.

**Conclusions**

When incorporating metformin with other putative chemopreventative PCa medications, it consistently showed a protective effect with a decreased rate of PCa diagnosis, decreased hazard of undergoing another PB and being treated with ADT. This is in accordance with previously published retrospective reports and will hopefully be validated in the ongoing prospective randomized trials, assessing the role of metformin in PCa.

**Abbreviations:**

5ARIs = Five alpha-reductase inhibitors

AMPK = Adenosine monophosphate-activated protein kinase

ADT = Androgen deprivation therapy

ADG = Ambulatory Diagnostic Groups

BPH = Benign prostatic hyperplasia

ICES = Institute for Clinical and Evaluative Sciences

mTOR = mammalian target of rapamycin

ODB = Ontario drug benefit

OHIP = Ontario health insurance plan

PB = Prostate biopsy

PCa = Prostate cancer

PPIs = Proton pump inhibitors

PSA = Prostate specific antigen

SD = Standard deviation

TRUS Bx = Transrectal ultrasound guided prostate biopsy

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Writing of manuscript: HG

Editing and reviewing of manuscript: FKM, AB, SA, RS, CJDW, ZK, TC, AEA, RS, OS, GSK, NF

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