Effects of Beta-Blockers on Survival in Head and Neck Cancer

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DEDICATION

This thesis is dedicated to my amazing family, girlfriend, supervisors, friends and colleagues who never stopped believing in me and supported me each step of the way.

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LIST OF ABBREVIATIONS

CVD: Cardiovascular Disease

HNC: Head and Neck Cancer

HPV: Human Papillomavirus

HR: Hazard Ratio

OR: Odds Ratio

OS: Overall Survival

SES: Socioeconomic Status

ABSTRACT

Beta-blockers are a class of drugs mainly used for the management of cardiovascular disease (CVD) and hypertension. In 2008, these drugs were discovered to be effective against infantile hemangioma, a form of vascular tumour, and have since become the first line therapy for this condition. As a result, researchers began to examine the potential link between beta-blocker use and improved cancer risk and prognosis. However, no study has examined the association between beta-blocker use and survival in head and neck cancer (HNC).

Objective: to estimate the extent to which the long-term use of beta-blockers is associated with better overall survival (OS) in a sample of Canadian HNC patients.

Methods: A total of 303 HNC patients, a sub-cohort of the HeNCe Life study Canadian site, were recruited from Hôpital Notre-Dame in Montreal, QC, Canada. We retrieved baseline and follow-up information on an array of exposures (e.g., beta-blocker use, comorbidities) from medical records using the OACIS database, which was subsequently merged with the HeNCe Life database. Survival information was retrieved from the Register of Civil Status of the Government of Quebec. Data analysis included descriptive statistics, cox proportional regression and Kaplan-Meier survival analysis.

Results: The survival profile of the beta-blocker users (mean survival time: 74.2 months, 95% CI: 60.3-88.3) did not appear to be significantly different from that of the non-users (mean survival time: 89.9 months, 95% CI: 84.5-95.1) in Kaplan-Meier survival analysis. We did not observe any significant association between long-term beta-blocker use and improvements in HNC OS in both the crude (HR: 1.46, 95% CI: 0.75-2.85) and the adjusted Cox proportional hazards model (HR: 0.82, 95% CI: 0.38-1.77).

Conclusion: Long term beta-blocker use did not appear to be associated with improved survival in HNC. More studies are required to examine the potential role of beta-blocker selectivity in affecting HNC prognosis.

RÉSUMÉ

Les bêta-bloquants sont une classe de médicaments principalement utilisés pour la prise en charge des maladies cardiovasculaires et de l'hypertension. En 2008, il a été découvert que ces médicaments sont efficaces contre l'hémangiome infantile, une forme de tumeur vasculaire, et ils sont depuis devenus la première ligne de traitement de cette maladie. En conséquence, les chercheurs ont commencé à examiner le lien potentiel entre l'utilisation des bêta-bloquants et l'amélioration du risque et du pronostic de cancer. Cependant, aucune étude n'a examiné l'association entre l'utilisation de bêta-bloquants et la survie pour le cancer de la tête et du cou (CTC).

Objectif : estimer dans quelle mesure l'utilisation à long terme des bêta-bloquants est associée à une meilleure survie globale (SG) dans un échantillon de patients canadiens atteints de CTC.

Méthodes : Un total de 303 patients atteints de CTC, une sous-cohorte du site canadien de l'étude HeNCe Life, ont été recrutés à l'Hôpital Notre-Dame de Montréal, QC, Canada. Nous avons recueilli des informations de référence et de suivi sur un éventail d'expositions (par exemple, utilisation de bêta-bloquants, comorbidités) à partir des dossiers médicaux en utilisant la base de données OACIS, qui a ensuite été fusionnée avec la base de données HeNCe Life. L'information sur la survie a été extraite du Registre de l'état civil du gouvernement du Québec. L'analyse des données comprenait des statistiques descriptives, la régression proportionnelle de Cox et l'analyse de survie de Kaplan-Meier.

Résultats : Le profil de survie des utilisateurs de bêta-bloquants (temps de survie moyen : 74,2 mois, IC 95%: 60,3-88,3) ne semblait pas significativement différent de celui des non-utilisateurs (temps de survie moyen : 89,9 mois, IC 95 %: 84,5-95,1) dans l'analyse de survie de Kaplan-Meier. Nous n'avons pas observé d'association significative entre l'utilisation de bêta-bloquants à long terme et des améliorations de la SG pour le CTC tant dans le modèle brut (HR: 1,46, IC 95%: 0,75-2,85) que dans le modèle ajusté de risques proportionnels de Cox (HR: 0,82, IC 95 %: 0,38-1,77).

Conclusion : L'utilisation de bêta-bloquants à long terme ne semble pas être associée à une amélioration de la survie pour le CTC. D'autres études sont nécessaires pour examiner le rôle potentiel de la sélectivité des bêta-bloquants dans le pronostic du CTC.

CONTRIBUTION OF AUTHORS

Letian Li, MSc candidate, designed the objectives and analytical strategy, conducted the medical records data collection, data analysis and wrote this thesis including the manuscript.

Dr. Belinda Nicolau, conceived and designed the HeNCe Life study, acquired funding, directed its implementation, quality assurance and control. She supervised all the work, helping with the interpretation of results as well as the review and editing of all chapters of this thesis.

Dr Faleh Tamimi, proposed the idea and commented on the draft of the manuscript.

Dr. Genevieve Castonguay coordinates the HeNCe Life study, reviewed and edited of all chapters of this thesis.

Dr. Claudie Laprise was involved in the editing of the manuscript (Chapter 6).

Dr. Sreenath Madathil supervised and participated in the data analysis of the study. He also contributed to the writing of the methods by producing Figures 5-1 and 5-2 and Appendices II.

Dr Martin Morris provided assistance with the literature review and produced Appendix I.

1. INTRODUCTION

Cancer in the upper aero-digestive tract, also known as head and neck cancer (HNC), consists of various cancers that originate in the oral, pharyngeal, and laryngeal regions. It represents a prominent global issue for the contemporary healthcare system. The combination of low survival rate, reduced functionality of the upper aerodigestive structures and high suicide rate associated with the disease tremendously undermines the quality of life of the patients (1-3). Despite the availability of several clinical interventions, including chemotherapy, radiotherapy and surgery, the 5-year survival rate of the disease has hardly improved and has remained low for the past few decades (1). Traditional risk factors for HNC such as smoking (4), alcohol (5) and age (6) have been shown to affect HNC survival. In addition, HPV infection is also identified as a major prognostic factor; HPV-related HNC is associated with better response to treatments and hence better HNC survival (7, 8). Other prognostic factors include cancer stage, cancer site and treatment strategy (9).

Beta-blockers are a major class of drugs for the management of cardiovascular disease (CVD) and hypertension. In 2008, it was accidentally discovered that the drug was effective in treating infantile hemangioma, a benign form of vascular tumour of infancy (10). The findings, which were later confirmed by numerous studies including observational studies and randomized controlled trials, led beta-blockers to become the first line intervention for the disease. This phenomenon sparked interest among cancer researchers in whether beta-blockers can reduce the risk of cancer and improve prognosis and survival. However, the relationship between beta-blocker use and cancer aetiology and survival including HNC remains unclear, with the exception of breast cancer, for which a definitive protective effect has been shown. If proven effective, beta-blockers can potentially become a simple and relatively safe intervention in addition to the traditional therapies.

To the best of our knowledge, there has been no previous attempt to examine the association between beta-blockers and HNC survival. Therefore, the aim of our project is to investigate this association.

2. LITERATURE REVIEW

2.1 Head and Neck Cancer

2.1.1 Definition and Epidemiology of Head and Neck Cancer

The collective term HNC refers to a group of cancers located in the upper aerodigestive tract, including the oral cavity, pharynx, and larynx (11). The specific anatomical sites of HNC include the mucous membrane of the lips, tongue, gum, floor of mouth, palate, oropharynx, hypopharynx, and nasopharynx (11) Other cancers in the head and neck region, such as brain cancer, thyroid cancer, and melanoma, are not considered as HNC. According to statistics from GLOBOCAN, there was an estimated 686,328 incident cases of HNC worldwide in 2012 (12). Among them, 330,373 cases were cancers in the oral cavity or on the lip, 156,877 cases were located in the larynx, and 229,078 cases were found in the pharynx (12). Between 1973 and 2006, tongue, oral cavity, and tonsil cancers were the most frequently diagnosed types of HNC (1). Squamous cell carcinoma accounts for 90% of all HNC cases (13).

Overall, HNC accounted for 4.8% of incident cancer cases worldwide in 2012 (12), making it the 5th most common cancer. It also represented 5.3% (1,682,513) of 5-year cancer prevalence. In terms of associated mortality, HNC is placed 7th with 4.6% (375,665) of cancer deaths (12). Less developed regions in the world accounted for most of the incidence (68.0%), mortality (76.7%), and 5-year prevalence (62.3%) of HNC (12). In all three categories, men display higher percentages than women; the incidence, mortality, and 5-year prevalence among men are 7.0%, 6.2%, and 8.3%, respectively; for women, these figures are 2.6%, 2.5%, and 2.4%, respectively (12).

In Canada, there were 4,989 incident and 15,626 prevalent HNC cases in 2012, which accounted for 2.7% and 2.8% of all cancer incidence and 5-year prevalence, respectively (12). HNC also represented 2.1% (1,494) of Canadian cancer mortalities (12). Similar to the world estimates, the three statistics were significantly higher in men compared to women. The percentages of incidence, mortality, and 5-year prevalence were respectively 3.8%, 2.9%, and 4.0% for men, and 1.7%, 1.2%, and 1.6% for women (12).

HNC is the eighth most common form of cancer among Canadian men (12). According to Statistics Canada, in Quebec, there were approximately 1,375 incident HNC cases in 2013, with an incidence rate of 17.4 per 100,000 population (14).

The impact of HNC on the patient is severe. Indeed, HNC is associated with one of the highest suicide rates among all cancers (3, 15, 16) at 50.5 per 100,000 person-years, which is significantly higher than for all cancers (33.6 per 100,000 person-years) and the general US population (12.0 per 100,000 person-years) (3). This phenomenon is linked to alterations in functionalities, such as speaking, swallowing, and chewing, of the head and neck anatomical structures caused by the disease. In addition, most patients (62%) undergoing the chemotherapy and radiotherapy treatment combination experience complications in terms of esophageal strictures, which further aggravates difficulty in swallowing (3). Alterations in head and neck functionality are consequently linked to psychological stress and lowered quality of life (3).

It is well-established that tobacco and alcohol and their joint effects account for at least 75% of HNC (17). In addition, for a subset of HNC – oro-pharyngeal cancers, human papillomavirus (HPV) infection is an important aetiological factor (18). Other risk factors include socioeconomic status (SES) (19), gender (20), diets poor in fresh fruits and vegetables (21), oral health status (22), family inheritance (23), poor oral hygiene (24) and psychosocial factors (25).

2.1.2 Survival in Head and Neck Cancer

Survival rates following a HNC diagnosis were remarkably stable during the 20th century. In the US, the 5-year survival rates were 53% between 1973 and 1988, 52.7% between 1982 and 1986, and 54.7% between 1992 and 1996 (1, 26). However, the following decade saw an improvement: the 5-year survival rate increased to 65.9% between 2002 and 2006, while the prevalence of the disease remained approximately the same (1). This improvement was seen in the great majority of HNC subtypes, with the exception of lip and laryngeal cancers. The most notable improvements were observed in tonsillar,

tongue, and oral cancers (1). Survival for these three types of cancer increased in all age groups with the exception of those 75 years and older (1).

The improvements observed in survival are linked to a variety of factors. First, the increased portion of HPV positive HNC in the last two decades in western countries could have had a direct impact on survival, as HPV positive HNCs are associated with a better prognosis (27-29). In addition, HPV-related HNC occurs more frequently in younger patients, who have better survival compared to older patients as a result of their age. Changes in treatment strategies also contributed to improved survival. The traditional treatment protocol for HNC using radiotherapy alone was gradually replaced with a combined therapy of concurrent chemotherapy and radiotherapy (1). Evidence shows that the combined treatment plan is associated with increased HNC survival compared to the traditional single therapy protocol (30-32). Improvements in screening and staging procedures could also have played a role in increasing survival rates due to lead-time bias. The introduction of fiberoptic laryngoscopy, high resolution radiology, and higher frequency of cancer screening procedures performed by dental professionals resulted in earlier diagnosis and more accurate staging (26). Evidence shows that there has been an increase in the proportion of HNC diagnosed at earlier stages (26).

Different anatomical HNC sites may display very distinctive survival profiles. In the US, the highest 5-year HNC survival rate is observed for lip cancer, and the lowest for hypopharyngeal cancer. Specifically, the anatomical sites ranked in order of descending survival rate are lip (82.6%), salivary gland (56.7%), larynx (54.8%), oral cavity (45.8%), nasopharynx (50.9%), oropharynx (41.2%), and hypopharynx (29.7%).

2.2 Beta-blockers

This section aims to provide detailed overview of beta-blockers, including their history, clinical applications, pharmacodynamic properties and mechanisms of action.

2.2.1 The History of Beta-blockers

2.2.1.1 The Invention of the Beta-blocker

The root of the beta-blocker can be traced back to more than 100 years ago, when the idea that the pharmaceutical properties of catecholamines are linked to their receptor binding selectivity was proposed (details about the beta-adrenergic receptors will be presented in section 2.2.2.1). The year 1948 saw the publication of a paper by Raymond P. Ahlquist naming and describing the different properties of alpha and beta adrenoceptors for the stimulation of responses in heart muscles (33). In 1958, the first type of beta-blocker, dichloroisoproterenol (DCI), was synthesized by Eli Lilly Laboratories, who demonstrated the ability of the compound to inhibit the actions of epinephrine and chemically confirmed the existence of the beta -adrenoceptor (34). The beta-blocker pronethalol was synthesized in 1960 by John Stephenson and demonstrated sufficient efficacy in treating angina pectoris (34). The year 1964 marked an important time point in the evolution of beta-blockers. It is the year in which propranolol was successfully synthesized by Sir James Black, who was awarded the 1988 Nobel Prize in Medicine and Physiology for this achievement. Black was influenced by Ahlquist's work on the dual receptor theory in his early years of research on angina pectoris (35). Two years after the start of the clinical trials in 1964, propranolol was launched under the trade name Inderal (35). The ground-breaking introduction of propranolol, in both oral and intravenous forms, revolutionized the treatment of cardiovascular conditions, including hypertension, arrhythmia, and hypertrophic cardiomyopathy (34). For angina pectoris in particular, the introduction of propranolol marked the first major therapeutic innovation in over a century (34).

2.2.1.2 The Evolution of Beta-blockers

The commercial success of propranolol sparked competition among pharmaceutical firms, who actively searched for new forms of beta-blockers that would offer fewer side effects and less toxicity than propranolol. Practolol, a selective beta-blocker with structural resemblance to non-selective beta-blockers (the difference between selective and non-selective beta-blockers will be explained in section 2.2.2.2), was introduced in 1970 under the brand name Eraldin (34). Although achieving commercial success, several severe side effects, such as blindness, skin rash, and conjunctiva, were identified, and the compound was withdrawn from the market in 1975 (35).

The year 1976 saw another milestone in the evolution of beta-blockers. Selective beta-blocker atenolol was launched under the trade name Tenormal as a hypertensive drug and soon established its status as one of the best-selling heart drugs (35). It was followed by the introduction of a variety of selective beta-blockers with distinctive therapeutic properties. Thus, the launch of Tenormal marked the gradual rise of the selective beta-blockers that are referred to as the second-generation beta-blockers and the demise of the non-selective first-generation beta-blocker propranolol. The development of the second generation beta-blockers established general acceptance of Ahlquist's dual receptor theory in the field of pharmacology (36). In the late 20th century, third generation beta-blockers that consist of either selective or non-selective beta-blockers with additional vasodilation properties were introduced into the market (37). Compared with the first and second generations, the third generation is associated with fewer metabolic side effects (38). In addition, the development of a fourth generation of beta-blockers that would consist of non-selective beta-blockers with greater efficacy and fewer side effects was proposed (39). Information on beta-blockers currently available on the market is listed in Table 2-1.

Table 2- 1: General Information on Beta-blockers

Agent	Trade Name	Generation	Selectivity
Propranolol	Hemangeo, Inderal LA, Inderal XL, InnoPran XL	First	Non-selective
Timolol	Blocadren, Timoptic ,Betimol, Istalol	First	Non-selective
Pindolol	Visken	First	Non-selective
Penbutolol	Levatol	First	Non-selective
Sotalol	Betapace,Sotalex, Sotacor, Sotylize	First	Non-selective
Nadolol	Corgard	First	Non-selective
Atenolol	Tenormin	Second	Selective
Acebutolol	Sectral	Second	Selective
Betaxolol	Kerlone, Betoptic S	Second	Selective
Bisoprolol	Zebeta	Second	Selective
Esmolol	Brevibloc	Second	Selective
Metoprolol	Lopressor, Toprol XL	Second	Selective
Labetalol	Trandate, Normodyne	Third	Non-selective
Carvedilol	Coreg	Third	Non-selective
Carteolol	Cartrol	Third	Non-selective
Nebivolol	Bystolic	Third	Selective
Celiprolol	Cardem, Selectol, Celipres, Celipro, Celol, Cordiax, Dilanorm	Third	Selective

2.2.2 The Pharmacodynamic Properties of Beta-blockers

2.2.2.1 Beta-adrenergic Receptors

Beta-adrenergic receptors are a critical part of the sympathetic nervous system that modulates the fight-or-flight response in the human body. These receptors belong to the family of G protein-coupled receptors (GPCRs) (40), which share a common structure. This structure consists of two domains, including a seven-transmembrane-spanning domain and an intracellular heterotrimeric G-protein complex ($G_{\alpha\beta\gamma}$) (40). Beta-adrenergic receptors respond to the binding of catecholamines such as epinephrine (adrenaline) and norepinephrine (noradrenaline) (41). The transduction of beta-signals is achieved via a conformational change of the seven-transmembrane-spanning domain, which in turn exchanges guanosine diphosphate (GDP) for guanosine triphosphate (GTP) through the action of guanine nucleotide exchange factors. The exchange subsequently dissolves the G-protein complex into activated $G_{\beta\gamma}$ and G_{α} subunits (42). The difference in the downstream effects of the signal activation is determined by the type of G_{α} subunit present in the G-protein complex. Four types of G_{α} subunits are commonly identified: $G_{\alpha.S}$, $G_{\alpha.I}$, $G_{\alpha.q}$ and $G_{\alpha.12}$ (43). Among them, $G_{\alpha.S}$ and $G_{\alpha.I}$ initiate stimulatory and inhibitory pathways, respectively (40). Both the activated $G_{\beta\gamma}$ and G_{α} subunits are capable of amplifying and propagating the signal by affecting a variety of different types of downstream effector molecules, including enzymes and ion channels. Subsequently, these effector molecules will affect the production of second messenger molecules, which are responsible for initiating a variety of different signalling pathways. For beta-adrenergic receptors, the second messenger is cyclic adenosine monophosphate (cAMP), the function of which is to activate downstream protein kinase A (PKA). Increase in cAMP level and localization of PKA assisted by A-kinase anchoring proteins (AKAPs) to specific sites will allow PKA to phosphorylate regulatory molecules in its vicinity (44). The phosphorylation elevates the activities of lusitropy and inotropy that are linked to the relaxation and contractility, respectively, of muscle cells (40).

The activities of beta-adrenergic receptors are critical for the maintenance of normal cardiac functions. It has been demonstrated that chronic exposure to a high level of

catecholamines results in the desensitization of beta-adrenergic receptors through to their degradation and a lowering of their affinity for catecholamines (45, 46). Distorted beta-adrenergic signalling is linked to cardiovascular conditions, such as heart failure, hypertension, myocardial hypertrophy, and myocardial infarction (40, 42, 47, 48). Beta-blockers are able to reverse the desensitization process of the beta-adrenergic receptors (48), thereby lowering the risk of heart disease.

2.2.2.2 Selectivity of Beta-blockers

Three subtypes of beta-adrenergic receptors have been identified: beta-1, beta-2, and beta-3. These three subtypes have different expression patterns in the human body. In the heart, the ratio between the amount of beta-1 and beta-2 adrenergic receptors expressed is approximately 3-4:1 (49). There is a very limited expression of the beta-3 adrenergic receptor in the heart (50). The specific locations where the three subtypes of beta-adrenergic receptors are expressed are listed in Table 2-2.

Table 2- 2: Location of Expression of Beta-adrenergic Receptor Subtypes

Beta-adrenergic Receptor Subtype	Location of Expression
Beta-1 Receptor	Salivary glands (51), Atrioventricular node, Sinoatrial node, and cardiac muscle cells of heart (52), juxtaglomerular cells in kidney (53), urinary bladder wall (54), cerebral cortex (55), stomach, and retina blood vessels (56)
Beta-2 Receptor	Smooth muscle cells in uterus (57), GI tract (58), bladder wall (59), seminal tract, and bronchi (60), prostate (61), lung (62), blood vessels (63), astrocytes of optic nerves (64), cerebral cortex (55), skeletal muscle (65), pancreatic islets (66), heart (49), retina blood vessels (56), liver (67), salivary glands, lacrimal glands, kidney
Beta-3 Receptor	Adipose tissue (50), gallbladder (50), colon (50), and urinary bladder (54)

Beta-1 adrenergic receptors are primarily located in the heart, and mainly responsible for regulating cardiac output (68) and renin secretion in the kidney (53). Beta-2 adrenergic receptors are located throughout the sympathetic nervous system to mediate the fight-or-flight response and have a greater affinity for epinephrine compared to norepinephrine (69). Beta-3 adrenergic receptors have a lower affinity for norepinephrine compared to

both beta-1 and beta-2 adrenergic receptors (70), and the primary function of beta-3 adrenergic receptors is to assist the process of lipolysis in adipose tissues (71).

Beta-blockers are generally classified into two categories on the basis of their selectivity for the beta-1 adrenergic receptor. Because this receptor is mostly expressed in the heart, selective beta-blockers target it to achieve higher efficacy in treating cardiovascular conditions. As a result, they are often referred to as cardio-selective beta-blockers. Non-selective beta-blockers have equal affinity for both beta-1 and beta-2 adrenergic receptors. The selectivity of beta-blockers is a relative term that reflects the pharmacodynamic property of a beta-blocker under low dosage. Thus, although selective beta-blockers demonstrate significantly higher affinity for beta-1 than for beta-2 adrenergic receptors (34), at high doses they also demonstrate an ability to target beta-2 adrenergic receptors (34).

There are several advantages of using a selective beta-blocker over a non-selective form. Aside from having greater potency to treat cardiovascular conditions, the selective beta-blocker offers greater safety for patients with bronchospastic disease compared to a non-selective one, because it does not affect the adrenergic bronchodilation process mediated by beta-2 adrenergic receptors (34). Similarly, the vasodilation process, in which beta-2 adrenergic stimulation plays a role, will also be unaffected by selective beta-blockers, preventing the risk of peripheral blood flow reduction (34). In addition, the unregulated beta-2 adrenergic receptor allows the activation of platelets caused by a sympathetic stimulus (72). These serve as important safety features for patients with asthma, drug-induced hypoglycaemia, and peripheral vascular disease (34).

However, in some scenarios, non-selective beta-blocker use can possess advantages over selective beta-blocker use. For instance, a reduced occurrence of hypokalemia is observed with non-selective beta-blocker use; this deficiency of potassium in the bloodstream is related to a surge in serum epinephrine level associated with physical stress and myocardial infarction (73). Non-selective beta-blocker use is also associated with a reduced occurrence of ventricular fibrillation and ventricular tachycardia, as these conditions are directly associated with hypokalemia (74). Non-selective beta-blocker use

may be associated with additional benefits in preventing a second heart attack and in treating noninfarctional ischemic heart disease (73).

2.2.2.3 Other Pharmacodynamic Properties of Beta-blockers

Aside from selectivity, beta-blockers also differ in other pharmacodynamic properties. Certain beta-blockers possess intrinsic sympathomimetic activity, that is, the ability to act not only as a beta-antagonist, but also simultaneously as a partial beta-agonist under certain scenarios involving a beta-adrenergic receptor (34). The display of these intrinsic sympathomimetic activity properties depends on the concentrations of both the beta-blocker and the antagonized endogenous catecholamines.

In addition, two beta-blockers, labetalol and carvedilol, can simultaneously act as antagonists for both alpha and beta-adrenergic receptors. The affinity of each beta-blocker for the alpha-adrenergic receptor is much lower compared to that for the beta-receptor; the ratio of alpha to beta-adrenergic affinity is 1:10 and 1:4 for carvedilol and labetalol, respectively (75). Nonetheless, the additional alpha-adrenergic antagonism is associated with reduced peripheral vascular resistance upon vasodilation in both beta-blockers (75).

The peripheral vasodilating property of beta-blockers is the result of different combinations of properties mentioned above. Beta-1 antagonism combined with either partial beta-2 agonism or alpha antagonism will lead to a significant peripheral vasodilating ability in a beta-blocker (76). Other mechanisms, such as blocking calcium channels seen in beta-blockers used for glaucoma and the one seen in nebivolol that involves nitric oxide, can also lead to peripheral vasodilation (76).

Another important characteristic of beta-blockers is lipid solubility. Beta-blockers that are lipid-soluble are able to cross the blood-brain barrier, which allows them to manage conditions such as migraine and anxiety. In addition, these beta-blockers have relatively shorter plasma half-lives and are degraded by hepatic metabolism (73). In comparison, water-soluble beta-blockers tend to have longer plasma half-lives and are eliminated unchanged via intestinal absorption and kidney secretion (73). However, a potential

downside to lipid-soluble beta-blockers is that they may be associated with side effects related to the central nervous system, such as hallucinations and insomnia (77).

Membrane stabilization is another pharmacodynamic property of beta-blockers. The term, with relevance to beta-blockers, refers to the effect of inhibiting the propagation of cardiac action potential. However, this effect is only observable at high concentrations of beta-blockers and therefore is not of great clinical importance (76). The pharmacodynamic properties of beta-blockers are summarized in Table 2-3 with details on the strength of each effect.

Table 2- 3: Pharmacodynamic Properties of Beta-blockers

Agent	ISA	Alpha Antagonism	Peripheral Vasodilation	Lipid Solubility	Membrane Stabilization
Propranolol	–	–	DNF	++	++
Timolol	–	–	+	+	–
Pindolol	+++	–	DNF	+	–
Penbutolol	+	–	DNF	–	–
Sotalol	–	–	–	–	–
Nadolol	–	–	DNF	–	–
Atenolol	–	–	–	–	–
Acebutolol	+	–	DNF	+	–
Betaxolol	–	–	DNF	–	–
Bisoprolol	–	–	+	+	–
Esmolol	–	–	DNF	–	–
Metoprolol	–	–	+	+	–
Labetalol	–	++	++	+	–
Carvedilol	–	+	++	+++	++
Carteolol	++	–	DNF	–	–
Nebivolol	DNF	DNF	DNF	+	–
Celiprolol	+	DNF	DNF	–	DNF

ISA: intrinsic sympathomimetic activity; "–" represents no effect, "+" indicates the presence of effect, and the number of "+" indicates the strength of the effect, "DNF" indicates that information regarding the effect was not found.

2.2.3 Clinical Uses of Beta-blockers

2.2.3.1 Management of Cardiovascular Conditions

Beta-blockers possess therapeutic effects on a large variety of conditions; the majorities are cardiovascular. Table 2-4 summarizes the conditions for which beta-blockers are used.

Table 2- 4: Indications for Beta-blocker Use

Type of Condition	Name of Condition
Cardiovascular	Angina (78), aortic dissection (79), arrhythmia (80), atrial fibrillation (81), congestive heart failure (82), hypertension (83), hypertrophic obstructive cardiomyopathy (84), long QT syndrome (85), mitral valve prolapse (86), myocardial infarction (87), postural orthostatic tachycardia syndrome (88)
Others	Anxiety (89), tremor (90), glaucoma (91), migraine (92), pheochromocytoma (93), hyperthyroidism (94), Theophylline overdose (95), Marfan syndrome (79), portal hypertension (96), hyperhidrosis (97), perioperative care (98)

2.2.3.2 Management of Infantile Hemangioma

The initial enthusiasm for studying the effect of beta-blockers on cancers stemmed from the discovery of the therapeutic property of propranolol against infantile hemangioma in 2008 (10). This common benign tumour, present in 5-10% of infants (99), is associated with excessive angiogenesis and vasculogenesis caused by an abnormal level of vascular endothelial growth factor (VEGF) expression.

The effect of beta-blockers on infantile hemangioma was accidentally discovered when a significant regression of tumours was observed in children who were simultaneously treated for congestive heart failure with propranolol. Additional trials confirmed the effect of this beta-blocker among all children with infantile hemangioma (100-102). In addition, the side effects were tolerable when appropriate clinical precautions were taken (103). As a result, beta-blockers, propranolol in particular, rapidly replaced corticosteroids as the first line therapy for infantile hemangioma. In general, patients will receive a daily dose of 2-3mg/kg over a period of six months.

Other selective and nonselective beta-blockers, including nadolol, atenolol, acebutolol, and timolol are also effective against infantile hemangioma (104-107). These beta-

blockers lack the ability to cross the blood-brain barrier and thus do not cause some of the side effects associated with propranolol, including hypoglycemia and bronchospasm.

The molecular mechanism behind the action of beta-blockers on infantile hemangioma has not been completely understood. Overall, the effect can be potentially attributed to three mechanisms, including vasoconstriction, suppression of angiogenesis, and induction of apoptosis, all via beta-adrenergic signal blockade (99). These molecular pathways are also shared by cancer progression. For vasoconstriction, the effect is ultimately caused by protein kinase G (PKG) molecules modulating vascular smooth muscles. The suppression of angiogenesis is achieved by the downregulation of VEGF, and by matrix metalloproteinase (MMP)-2 and MMP-9 expression, causing disruptions in the vascular growth signal and extracellular matrix formation. The induction of apoptosis is attributed to the inhibition of the action of Bcl-2, an anti-apoptotic protein, in capillary endothelial cells (99).

2.3 Beta-blockers and Head and Neck Cancer

2.3.1 Biological Mechanisms of Beta-adrenergic Signaling Affecting Cancer Progression

One of the most important functions of the sympathetic nervous system (SNS) is to maintain homeostasis and respond to stress, whether it is physiological, psychological, or environmental (108). Examples of stress include physical trauma, post-traumatic stress disorder, chronic insomnia, depression, marital failure, and stress-prone personality (109, 110). In response to the onset of stress, both chronic and acute, the SNS is activated, which in turn triggers the release of epinephrine and norepinephrine from chromaffin cells of the adrenal medulla and vascular neuro-muscular junctions, respectively, into the bloodstream (108). The level of these catecholamines will be monitored and regulated by the central nervous system (CNS). As a result, there will be different amounts of catecholamines released to different tissue sites (108). In the presence of a tumour, the catecholamines in the bloodstream will be released into the microenvironment that surrounds the tumour (108). The binding of the catecholamines to the different subtypes of beta-adrenergic receptors on the tumour cells will lead to the activation of the beta-

adrenergic signalling pathways, which elevate the synthesis of cAMP via the activation of the $G_{\alpha s}$ guanine nucleotide-binding protein (108). In turn, cAMP will interact with various downstream effectors to initiate different cellular responses.

One of the potential signaling pathways downstream of cAMP involves the activation of PKA, an effector of cAMP. PKA is capable of regulating a large variety of cellular functions such as metabolism, growth, differentiation, gene transcription, morphological transformation, mobilization, secretion, and neurotransmission. This wide range of activities result from the capability of PKA to phosphorylate proteins with specific motifs (108). For instance, PKA is able to regulate gene transcription by phosphorylating the ser-133 site on a cAMP response element-binding protein (CREB); this transcription factor is a critical component of the transcription machinery for cAMP response element t n(CRE) genes (111). In the same fashion, PKA activates the activating transcription factor 1 (ATF1), another transcription factor for the CRE genes (112). Overall, through its association with CREB and ATF1, PKA regulates the expression of 20% of human genes. These genes ultimately provide the PKA signaling pathway with the tools to suspend cell proliferation in favour of cell differentiation and allow PKA to dominate cellular processes (108). In addition, the stimulated expression of growth factors and cytokines allow the PKA pathway to dominate cellular processes of inflammation, invasion, and angiogenesis in tumours (108).

PKA also has the capacity to activate beta-adrenergic receptor kinase (BARK) via phosphorylation. BARK partially functions through the formation of a negative feedback loop by recruiting beta-arrestin that desensitizes beta-adrenergic receptors. Concurrently, BARK is capable of activating downstream transcription factors such as STAT3 as well as focal adhesion kinase (FAK); the latter plays a critical role in monitoring cellular trafficking and motility by manipulating the mechanical properties of the cytoskeleton (108).

Another potential signaling pathway downstream of cAMP is s the phosphorylation of Bcl-2-associated death promoter (Bad), which contributes to resistance to chemotherapy-induced apoptosis of the tumour cells. Bcl-2-associated X protein (Bax) and Bcl-2

homologous antagonist killer (Bak) exhibit the capability of perforating the outer membrane of the mitochondria, allowing entry of cytochrome C to initiate apoptosis (113). Bcl-2 and Bcl-x_L proteins are able to inhibit this process to exert their anti-apoptotic effects. The inactive dephosphorylated Bad binds to Bcl-2 and Bcl-x_L in a heterodimeric fashion and inhibit their anti-apoptotic functions (114). However, PKA-dependent phosphorylation of Bad prevents the dimerization of Bad/Bcl-2 and Bad/ Bcl-x_L, thus promoting anti-apoptotic effects and the resistance of tumour cells to programed cell death (108).

Alternatively, cAMP can activate another major effector pathway that involves exchanged protein activated by adenylyl cyclase (EPAC), a protein capable of activating downstream effector Ras1A. Ras1A, in turn, activates the signaling pathway that involves the sequential activation of B-Raf, MEK1/2, and ERK1/2. Through the phosphorylation of transcription factors such as AP1 and Ets, this signaling pathway controls several cellular processes. The major difference between the PKA and the EPAC signaling pathways is that, whereas PKA can regulate a large variety of cellular functions, the impact of EPAC signaling is more restricted to cell morphology and motility (108).

2.3.2 Association between Head and Neck Cancer and Beta-blocker Use

Despite a thorough search, we failed to identify a significant amount of information regarding the relationship between beta-blocker use and HNC in the literature. The search was performed in the database MEDLINE with the assistance of the Liaison Librarian for Life Sciences at McGill University, and the detailed search strategy is outlined in APPENDIX I.

Only a few *in vitro* and animal studies were identified. Yang et al. showed that in *in vitro* settings, propranolol was capable of reversing the surge in the levels of MMP-2, MMP-9, and VEGF caused by catecholamine signaling that are responsible for tumour progression in nasopharyngeal cancer cell lines (115). Similarly, propranolol inhibs the expression of IL-6, a cytokine known for regulating tumour cell progression and angiogenesis, in oral squamous cell carcinoma (OSCC) cells upon exposure to

physiological stress (116). This beta-blocker was also shown to inhibit OSCC cell proliferation and biopsies confirmed the presence of both beta-1 and beta-2 adrenergic receptors in all 20 OSCC tumour specimens extracted from clinical settings (116). The presence of beta-2 adrenergic receptors in OSCC was additionally confirmed by a study from Shang et al.; in addition, the study concluded that beta-2 adrenergic expression correlates with a variety of factors, including cervical lymph node metastasis, tumour size, tumour stage, and age (117). Interestingly, beta-2 adrenergic expression was absent in the normal oral mucosa cell line. Furthermore, propranolol decreases the expression of Delta Np63 alpha and VEGF and increases the expression of TAp73 and p53 family target genes in head and neck squamous cell carcinoma, resulting in the reduction of cell viability and the activation of apoptosis. In addition, when combined with cisplatin, the treatment produced synergistic effects. The combination of gamma-irradiation and propranolol demonstrated higher potency in reducing cell viability (118).

Other evidence identified in the literature offered alternative perspectives. For example, Vilardi et al. concluded that neither norepinephrine nor the antagonist propranolol was capable of altering the expression pattern of VEFG-C (a subtype of VEGF) *in vitro* OSCC cell lines (119). VEFG-C, in particular, has been linked to lymph node metastasis and poor disease prognosis in oral cancer (120).

Only a few epidemiological observational studies have examined the effect of beta-blockers on the risk of developing HNC. A case-control study by Assimes et al. conducted in Saskatchewan showed that beta-blocker use was associated with significantly lower risks of developing HNC compared to thiazide diuretic use only, when considering both any duration of use (OR: 0.58; 95%CI: 0.43-0.78) and above 7.5 years (OR: 0.48; 95%CI: 0.24-0.94) (121). The thiazide diuretic, an antihypertensive drug with null effect on the risk of any cancer, was chosen as the reference group to minimize confounding by hypertension. The study also investigated the risk of death caused by all cancers, and beta-blocker use was not associated with a significant risk reduction. Similarly, another population-based cohort study showed that beta-blocker Taiwanese users are less likely to develop HNC (HR: 0.58; 95%CI: 0.35-0.95); however, the study only examined the use of non-selective beta-blocker propranolol (122). A case-control study conducted among

non-Hispanic whites in northern California concluded that long-term atenolol users who consume additional antihypertensive drugs (drochlorothiazide-triamterene, lisinopril, and nifedipine) were not exposed to additional risk of developing on lip cancer. Indeed, when examined alone, atenolol was associated with a reduced risk of lip cancer (OR: 0.43; 95%CI: 0.19–0.98) (123).

Although to our knowledge there is no study investigating the effect of beta-blockers on HNC survival, Bravo-Calderon et al. showed that stronger expression of the beta-2 receptor is associated with better overall survival and cancer-specific survival (124).

2.3.3 Beta-blocker use and Survival in Other Cancers

Given the lack of literature with regard to HNC, we examined the evidence regarding the impact of beta-blockers on survival in other cancers.

For breast cancer, there is an overwhelming amount of evidence supporting the idea that beta-blocker intake prolongs both cancer-specific survival and overall survival (125-130). The reported reduction in breast cancer-specific mortality for beta-blocker users is as high as 81% (HR: 0.19; 95%CI: 0.06-0.60) (129). A meta-analysis of 11 papers concluded that, overall, beta-blocker use is associated with summary hazard ratios of 0.44 (95%CI: 0.26-0.73) and 0.55 (95%CI: 0.35-0.88) in terms of breast cancer-specific and overall survival, respectively; in addition, the researchers concluded that a clinical trial was warranted to study the clinical effects of beta-blocker use on breast cancer prognosis (125).

On the other hand, in addition to a small amount of papers suggesting a null effect for breast cancer survival, a study in 2013 showed that the significant reduction in breast cancer mortality associated with beta-blocker use could be explained by the simultaneous consumption of aspirin (131-133). Nevertheless, although not statistically significant, the adjusted HR suggested beneficial effects of beta-blocker use (HR: 0.83; 95%CI: 0.60-1.16).

Our literature search only located five papers discussing the link between beta-blocker use and survival rates for melanoma, and their results are mixed. Three of the papers

reported positive effects on mortality reduction from beta-blocker use (134-136), whereas the other two studies concluded that the effect was null (137, 138). A small number of studies concluded that beta-blocker use is associated with a reduced risk and progression of melanoma (134, 136, 139). However, the low number of studies limits the strength of the evidence.

Similar to melanoma, only a very small numbers of studies examined the associations of beta-blocker user with prostate, ovarian, lung, and colorectal cancer survival; there is approximately an equal amount of evidence in favour of both positive and null effects on cancer survival (140-149).

We identified two meta-analyses studying the overall effect of beta-blockers on survival for all types of cancer. Choi et al. analyzed 12 studies comprising 20,898 subjects and concluded that beta-blocker use improves both overall survival (HR: 0.79; 95 % CI 0.67–0.93) and disease-free survival (HR: 0.69; 95 % CI 0.53–0.91). In addition, results showed that the effect is strongest in patients with early stage cancers who were primarily treated with surgery (150). Meanwhile, Zhong et al. examining 20 cohort and 4 case-control studies (76,538 subjects) concluded that post-diagnostic beta-blocker use was associated with improvements in both overall survival (HR: 0.89; 95 % CI 0.81–0.98) and cancer-specific survival (HR: 0.89; 95 % CI 0.79–0.99); the same could not be said for pre-diagnostic use (151).

On the other hand, a British retrospective cohort study featuring all cancer types from 3,462 subjects concluded that beta-blocker use was associated with worse survival compared to other antihypertensive medications (HR: 1.18; 95 % CI 1.04–1.33). This result was primarily explained by poorer survival in pancreatic cancer and prostate cancer (131). Similarly, the use of atenolol among 6,528 hypertensive patients was not statistically significant associated with cancer survival, although the HR (HR: 0.71; 95 % CI 0.45–1.11) was heavily skewed toward a protective effect (152).

In conclusion, we succeeded in identifying multiple *in vitro* and animal studies associating beta-blocker use (propranolol in particular) to various biological pathways that may

ultimately lead to reduced tumour progression and improved cancer survival in HNC. In addition, we uncovered evidence confirming the expression of beta-1 and beta-2 adrenergic receptors in HNC. However, although beta-blocker use has been investigated in relation to other cancers with mainly positive and null results, our thorough literature search was unable to identify any evidence related to their effect on HNC survival. This potential effect must be investigate taking into account possible confounders. In the next section, we describe the main risk factors associated with HNC survival.

2.4 Confounders of Head and Neck Cancer Survival

2.4.1 Smoking

Smoking is a major risk factor of HNC (17, 153, 154) and its effect extends to the survival of the disease. A systemic review of 131 studies reported that between 1980 and 2014, 53.8% of HNC patients were current smokers at the time of diagnosis and 33.0% of HNC patients continued to be current smokers after the diagnosis (155). Similarly, a longitudinal study of 590 HNC patients showed that continuing smoking drastically reduced overall survival compared to never smoking (HR: 2.71; 95 % CI 1.48–4.98). Although other less intense forms of smoking behaviours, including quitting after diagnosis and quitting prior to diagnosis, showed less impact on overall survival, they were still significantly associated with lower survival rates compared with never-smokers (156). A large Irish cohort study featuring 5,652 HNC cases also concluded that continuous smoking was associated with reduced cancer-specific survival compared with never smoking (HR: 1.36; 95 % CI 1.21–1.53) (4). A large population-based Canadian study reported that between 1992 and 2005, a 10% increase in smoking rate was associated with a 31% decrease in the odds of survival (157).

2.4.2 Alcohol

Similar to smoking, alcohol consumption is a major risk factor of HNC (158-160). According to the “Global status report on alcohol and health 2014” published by the WHO, people in the WHO Region of the Americas on average consumed 8.4 litres of pure alcohol annually in 2010, compared to 6.2 litres worldwide. In the Americas region,

61.5% of the population were identified as current drinkers and 19.5% as former drinkers. Beer constituted 55.3% of total alcohol consumption in this region; in addition, male drinkers were reported to consume on average 2.3 times more alcohol compared with female drinkers.

Regarding HNC survival, a recent Japanese study featuring 427 patients concluded that moderate drinking (23-46g ethanol/day) and heavy drinking (>46g ethanol/day) were associated with reductions in both overall survival and disease-specific survival compared with no drinking (5). These results were confirmed by a multicentre Italian study including 801 subjects in which both moderate drinking (14-28g ethanol/day) and heavy drinking (>28g ethanol/day) were associated with increased all-cause mortality, with HRs of 2.83 (95 % CI 1.30–6.16) and 3.93 (95 % CI 1.79–8.63), respectively (161). Similar results were seen in a Norwegian cohort study showing that a high level of alcohol consumption was a significant predictor of HNC survival (HR: 2.24; 95 % CI 1.09–4.60) (162).

2.4.3 HPV

The human papillomavirus (HPV) is a DNA virus. Currently, 170 types of the virus have been identified, and new types are continuously being discovered (163). The average worldwide prevalence of HPV in women was estimated to be 11.7% (164). However, HPV is more prevalent in men. The prevalence in different regions of the world range from 1% to 93%, and is the highest among HIV-positive homosexual men (165). In the US, the prevalence of oral HPV in men and women are 10.1% and 3.6%, respectively (166).

Various types of HPV are strongly associated with cancers and are classified as high-risk. Among them, HPV-16 and HPV-18 are responsible for 71% of cervical cancer cases (167) and also are the most common types associated HNC. The most common form of HPV transmission is through sexual intercourse.

The identification of HPV as a major risk factor of HNC is relatively recent (18). In the US, it is estimated that 40% to 80% of oropharyngeal cancers, the anatomical site most strongly associated with HPV, are associated with infection with this virus. HPV-16 has

been identified in approximately 60% of oropharyngeal cancer cases (28). Evidence shows that HPV contributes to the development of HNC through a different mechanism compared with anogenital cancer (18).

However, contrary to expectations, HPV positivity is associated with prolonged HNC survival. A study of the correlation between the expression of p16INK4A, a marker of HPV infection, and HNC survival reported that HPV infection was associated with both reduced cancer-specific death (HR: 0.36; 95 % CI 0.20–0.64) and reduced overall death (HR: 0.44; 95 % CI 0.28–0.68) (8). A meta-analysis also concluded that HPV is associated with improved overall survival (HR: 0.42; 95 % CI 0.27–0.56). In addition, the analysis concluded that HPV positive patients respond better to both radiotherapy (HR: 4.07; 95 % CI 1.48–11.18) and concurrent chemo-radiation therapy (HR: 2.87; 95 % CI 1.29–6.41) compared with HPV negative patients (7).

2.4.4 Socioeconomic Status

Socioeconomic status (SES) is an indication of a person's economical standing in a society and is determined by the combination of many factors. Among these factors, education, occupation, and income level are most commonly used as proxy measures of a person's SES. Differences in SES are associated with increased or decreased risk of a wide variety of health conditions, including HNC, in which lower SES is associated with higher cancer risk (19).

In addition to HNC risk, lower SES measured by years of education, is also associated with poorer survival of HNC patients (168-170). High school education or less was associated with an increased mortality rate compared with higher levels of education (HR: 1.43; 95 % CI 1.03–1.99). In addition, lower education level was associated with both lower overall survival (HR: 2.42; 95 % CI 1.62–3.63) and lower HNC cancer-specific survival (HR: 2.07; 95 % CI 1.39–3.09) (171).

2.4.5 Other Factors

2.4.5.1 Sociodemographic Factors

Advanced age is a major risk factor for all cancers and has a great impact on cancer survival. Older patients, especially those 70-years-old and above had significantly higher risks of HNC mortality compared to those 50-years-old and younger (6).

Gender is another well-established risk factor for HNC and HNC survival. Males have significantly higher risk compared to females; approximately 75% of the disease occurs in males (20). This gender differences holds true for HPV related HNC; males with HPV display a better survival profile than HPV-infected females in HNC (172).

2.4.5.2 Comorbidities

A comorbidity is defined as a medical condition co-existing with the disease of interest. For overall survival, comorbidities account for approximately 18% of the mortalities in HNC (173). Comorbidities are intricately connected to HNC in various ways. For instance, patients of higher age have more comorbidities, and also a higher HNC risk and worse survival outcomes. Similarly, behaviours such as smoking and alcohol drinking may provoke comorbidities and are associated with cancer risk and prognosis. In addition, based on the type and severity of the comorbidities, clinicians will devise different treatment strategies that have distinctive effects on HNC prognosis and survival. Patients with severe comorbidities will not be eligible for surgery and will consequently suffer from poorer prognosis (174).

Myocardial infarction, angina, stroke, and heart failure, which are classified as clinical cardiovascular diseases, are associated with severe clinical consequences and thus may affect HNC survival. For example, HNC patients with severe cardiovascular comorbidities had reduced short-term mortality compared to patients without these diseases (175). Hypertension and diabetes mellitus have been also associated with HNC mortality. The former, a moderate cardiovascular condition and is a major indication for beta-blocker treatment, has been associated with an increased risk for HNC mortality (175). Diabetes mellitus, a type of metabolic disorder characterized by an abnormally high blood sugar level, is also a significant predictor of HNC survival (175).

2.4.5.3 Other Medications

In addition to beta-blockers, physicians often simultaneously prescribe to the patients other drugs for the management of CVD and hypertension. Commonly prescribed antihypertensive drugs include calcium channel blockers (CCBs), Angiotensin-converting-enzyme inhibitors (ACE inhibitors) and Angiotensin II Receptor Blockers (ARBs). The literature does not provide a solid conclusion regarding the relationship between use of CCBs and cancer risk or survival. While some have reported an association between CCBs and increased risks for breast, colon, and all cancers (176-178), other have showed no association between these drugs and overall cancer survival or risk (179, 180). It has been theorized that CCBs can potentially have negative impacts on cancer risk and survival due to their ability to interfere with the cellular signals that are crucial to apoptosis and proliferation (181). With regards to ACE inhibitors and ARBs, a systematic review from Menamin et al. examining 10 studies concluded that the two types of drugs may have positive effects on cancer outcomes (182). Prolonged survival time was observed in the cases of pancreatic cancer, non-small cell lung cancer and colon cancer (183-185). However, the small number of studies limits the strength of the evidence.

The same systematic review additionally accounted for the potential effects of statins and nonsteroidal anti-inflammatory drugs (NSAIDs). A study surveying the entire Danish population concluded that statins are associated with a significant improvement in survival for all cancers. Similarly, both pre-diagnostic and post-diagnostic uses of statins have been associated with significant reductions in breast cancer mortality, as great as 50% (186). Statins exert their effects by inhibiting the mevalonate and p-53 pathways, which are responsible for cell growth and cancer promotion, respectively (187, 188). In addition, statins affect intracellular trafficking (189). All of these characteristics give statins antitumoral properties.

Among NSAIDs, the antineoplastic properties of acetylsalicylic acid (ASA), commonly known as aspirin, have been well demonstrated, particularly for colorectal and breast cancers (190, 191). A phase III clinical trial in 2016 has demonstrated the effectiveness of aspirin in prolonging disease-free survival in colorectal, gastro-oesophageal, breast and prostate solid tumours (192). In addition, aspirin use reduces the risk of HNC by

approximately 25% (193). For colorectal and breast cancers, other types of NSAIDs are also significantly associated with reduced all-cause mortality, although the effects are smaller compared with those of aspirin (194, 195). However, the association is not apparent in other cancers. NSAIDs exert their effects on cancer by inhibiting the action of COX-2, a molecule overexpressed in cancer cells that is linked to carcinogenesis (196). In addition, for HNC, it has also been demonstrated that NSAIDs are capable of activating NAG-1, a molecule that possesses proapoptotic and anticarcinogenic properties (197).

3. Rationale

HNC stands fifth among the most prevalent cancers worldwide and seventh among the cancers with the highest mortality (12). It consists of a group of cancers of the upper aerodigestive regions that occur mostly in males (75%) (20). In addition, HNC is associated with one of the highest suicide rates among all cancers (3). The 5-year survival rate of HNC in the early 2000s was estimated to be approximately 65%, which was an notable improvement compared with statistics from the last century (1).

The beta-blocker, or beta-adrenergic blocking agent, is a type of cardiac medication widely prescribed for the management of hypertension, heart arrhythmia, angina pectoris, and myocardial infraction; its medicinal effect also extends to a large number of non-cardiovascular diseases and conditions, including glaucoma, tremor, social anxiety, and perioperative care. The invention of beta-blockers has been held as one of greatest achievements of the 20th century in the field of medicine.

In 2008, a discovery was made that beta-blockers were likely effective against infantile hemangioma, a benign vascular tumour seen in infants. Clinical studies demonstrated the effect of beta-blockers, which caused the tumours to shrink in size and change colour. As a result, the link between the consumption of beta-blockers and cancer prognosis soon became a topic that attracted the attention of many researchers.

The biological rationale behind this research interest is the involvement of the beta-adrenergic receptor in both the beta-adrenergic signalling blockade and cancer progression. The beta-adrenergic receptor belongs to the GPCRs family. Upon the binding of catecholamines, the receptor will initiate complex signalling pathways that involve effector molecules such as PKA, Bcl-2, and Ras. These molecules and their signalling pathways are crucial for regulating important cellular functions, including angiogenesis, migration, cell growth, gene expression, protein translation, and cellular survival, in a cancer cell. Thus, beta-adrenergic signalling is crucial to cancer progression. As a competitive antagonist for the beta-adrenergic receptor, the beta-blocker is able to prevent the activation of the receptor by binding to it and thus inhibiting the downstream

beta-adrenergic signals. Therefore, it is theorized that beta-blockers are capable of delaying cancer progression by preventing the stimulatory effects of the beta-adrenergic signals, thus improving cancer prognosis and survival.

To this date, many studies, including individual observational studies and meta-analyses, have examined the impact of beta-blocker consumption on disease risk, prognosis, and survival in different cancers. Although the effect of beta-blockers on cancer survival remains unclear for the majority of cancers, a significant improvement is seen in breast cancer (125). For HNC, *in vitro* studies have demonstrated the capability of beta-blockers, propranolol in particular, to regulate the expression of several key markers of cancer progression (115, 116, 118). Some studies, in addition, have confirmed the presence of beta-adrenergic receptors in clinical HNC specimens and demonstrated the correlation between a higher level of beta-adrenergic expression and a poorer HNC prognosis (116, 117).

Evidence from observational studies on this topic is scarce. Only a few studies have investigated the relationship between beta-blocker use and HNC risk and concluded that beta-blockers are associated with a reduced HNC risk (121-123). Nonetheless, no study has examined the association between beta-blocker use and HNC survival until now. Therefore, our project was designed to be the first to provide insight into the relationship between beta-blocker use and HNC survival.

4. STUDY OBJECTIVES

The aim of this study is to investigate the relationship between beta-blocker use and HNC survival. Specifically, we address the following research question: among HNC patients, to what extent is long-term beta-blocker use associated with better overall survival (OS) in a sample from Canada? Based on our knowledge of the biological mechanisms behind the potential associations between beta-blockers and HNC and the relevant literature, we hypothesize that committed beta-blocker use will prolong the OS of HNC patients.

5. METHODS

5.1 Study Design

This study adopted an inception cohort design, which is a cohort assembled at a specific time in the early developmental stage of a disease/disorder and followed thereafter. In our case, the specific time was the time of HNC diagnosis for each patient for survival analysis.

5.2 Study Location

All patients were participants of the Canadian site of the Heck and Neck Cancer (HeNCe) Life Study recruited at Hôpital Notre-Dame, a major referral hospital in Montreal (Quebec, Canada), between 2007 and 2013. All participants were local residents living within a 50km radius of the hospital.

5.3 HeNCe Life Study

The HeNCe Life study is a multi-center project that consists of independently conducted studies in Brazil, Canada, and India, led by Dr. Belinda Nicolau at the McGill University Faculty of Dentistry in Montreal. The project utilized a life course theoretical framework and a case-control design to investigate the role of environmental, behavioural, psychosocial, and biological factors at different life stages, including birth, childhood, adolescence and adulthood, in the aetiology of HNC.

A full description of the study can be found elsewhere (198). Briefly, the Canadian site of the HeNCe Life study recruited participants from four major referral hospitals in Montreal. The participants were patients with newly diagnosed and untreated histologically confirmed head and neck squamous cell carcinomas that were identified by attending the tumour board meetings in different hospitals. The present study included HeNCe Life study participants recruited at the oncology department of Hôpital Notre-Dame, the hospital providing care to the great majority of HNC cancer patients in the city and surrounding areas.

5.4 Exclusion Criteria

Since this study conducted analysis on a sub-cohort of the HeNCe Life study Canadian site, the exclusion criteria for this study is a modified version of the HeNCe Life study exclusion criteria for the Canadian site. These criteria mandate that the patient must: (i) be born in Canada; (ii) be at least 18 years of age; (iii) reside within a 50km radius of the study site (Hôpital Notre-Dame); (iv) have no previous history of cancer; (v) have no previous history of HIV infection; (vi) have no cognitive and mental disorder and give consent; (vii) speak either English or French and be capable of giving verbal responses; (viii) have oral, pharyngeal, or laryngeal cancer. Patients with cancer in the nasal cavity, salivary glands and external lips were excluded due to their different etiologies. For the current study, we added the criteria that the patient (ix) must have a complete record of his/her consultation appointments in the hospital database, OACIS. Patients with incomplete consultation records were excluded.

5.5 Data Sources

This study consists of combined data obtained from three sources: the HeNCe Life study database, the OACIS database, and the register of civil status of the Government of Quebec.

5.5.1 HeNCe Life Study Database

The HeNCe Life study database for the Canadian site was established by collecting information via a face-to-face interview and an oral specimen collection. The interviews were conducted by trained interviewers with the help of a questionnaire and a life grid.

5.5.1.1 The Questionnaire

The questionnaire was developed for all HeNCe Life study sites and adapted to the local context at each site (Canada, Brazil, India). The questions were derived from various high-impact cohort studies from the UK, including the Whitehall II study on British civil servants, British Birth Cohort (BBC) 1946, BBC 1958, and the INHANCE studies (199-

202). The questionnaire documented a broad variety of patient information and was structured into several categories, including medical information; socio-demographic information; education; occupation and employment; housing conditions and residential environment; smoking and chewing habits; drinking habits; dietary habits; oral health; family history of cancer; family environment in childhood; marriage, intimacy and life as a couple; and social support. The questionnaire was validated by extensive pilot studies at each site prior to use in the main studies.

5.5.1.2 The Life Grid

The life grid, based on work by Blane et al., documented major life circumstances (e.g. occupation, marriage, and family environment) during 3 life stages: childhood (birth-16 years old), early adulthood (17-30 years old) and late adulthood (30 years old and above) (203). It acts as a memory tool that promotes the accurate recall of past events (204).

5.5.1.3 Biological Sampling

Oral epithelial cell samples in the buccal and tonsil regions of the oral cavity were retrieved from the patients to analyze HPV and human DNA. Non-invasive brush and mouthwash protocols were used to collect the samples, which were analyzed at a laboratory located at Hôpital Notre-Dame. DNA in the samples was extracted by the method of centrifugation. PCR analyses were performed on the purified DNA, and primers were used to detect specific HPV genotypes (198).

5.5.2 OACIS Database

OACIS is an electronic clinical information system that integrates different clinical data to provide physicians with a holistic view of the patient's condition. The system was successfully adopted by MUHC and CHUM, two academic health centers established by McGill University and Université de Montréal. As Hôpital Notre-Dame is affiliated with CHUM, it uses this system. OACIS contains information on clinical testing, biological sampling, pathology and laboratory reports, medication prescriptions, and dates of hospital visits. In addition, it stores clinical reports written by clinicians for each hospital

visit, including external clinic visits, in the formats of both electronic data entry and scans of original handwritten records. These records contain baseline information on diagnosis, comorbidities, family history, and medications. It also contains treatment and follow-up information such as treatment type, treatment length, disease recurrence, primary metastasis, new secondary cancer, and new secondary metastasis. As part of my master's project, I designed a specific form which was used to extract the information from OACIS and subsequently I entered the data in the study database (please refer to section 5.7.1)

5.5.3 Register of Civil Status of the Government of Quebec

Survival information was obtained from the register of civil status of the Government of Quebec. The records were matched on the full names and the date of birth of the patients. All-cause mortalities of the subjects were collected up to May 13th, 2016, the date of termination of the study.

5.6 Data Collection and Management

5.6.1 Data Collection Procedures

To collect information from OACIS, an electronic database was created using the software "FileMaker" to systematically enter and store the collected information using checkboxes, dropdown menus, and text boxes. The information was grouped into different categories, including general information, data collection/disease details, diagnostic tests, comorbidity, family history, medication habits, systemic therapy, radiotherapy, subsequent visits, recurrence, new secondary, and comments (See APPENDIX II). The scripting capability of the software allowed information entered in the database using checkboxes, dropdown menus, and text boxes to be translated into continuous or categorical values of designated variables.

The data in the OACIS system was retrieved from computers inside Hôpital Notre-Dame. A list of eligible patients who participated in the HeNCe Life study was created as a reference file for the data collectors. The file contained the HeNCe Life study ID number,

full name, Quebec Health Insurance (RAMQ) card number, gender, preferred language, date of birth, date of interview, and age at the time of interview of the patients. In total, three people participated in the data collection process. Meetings assembling these three people, the FileMaker database developer, the coordinator and the PI of the study were held to ensure that the FileMaker fields were optimal for the project and that information was collected and entered in the same way by all involved. During data entry, the date of each entry and the initials of the data collector were recorded. Detailed baseline information on the cancer, diagnostic test results, comorbidities, family history of disease, medications, and habits was retrieved from the consultation reports in the "Transcription" section of the OACIS database. The details of diagnostic tests were retrieved from the pathology reports in the "Pathologie" section. After the conclusion of the collection process in OACIS, the data was extracted from the FileMaker database in the format of an Excel file, which was subsequently imported in statistical software Stata 13.0 for analysis. A selected subset of variables from the previously established HeNCe Life database was extracted and converted into a .dta file. The two data sets were then merged into a single .dta file using the study ID number as the matching variable, as both datasets shared this variable.

After gaining approval from the office of the Directeur de l'état civil (Director of civil status) of the Government of Quebec and supplying them with a list of patients' full names and dates of birth, an Excel file was obtained on May 13th, 2016 with data extracted from the Québec civil status register. This file contained the living status of the patients as of that day and, when applicable, the date of death. After transforming this file into the .dta format, it was merged with the previously combined master file for this study.

5.6.2 Participation Rate

During data collection for the HeNCe Life study – Canadian site, 460 patients out of all HNC cases identified in the four major referral hospitals agreed to participate in the HeNCe Life study. The participation rate was 47% (198). Out of these cases, 347 (75%) were from Hôpital Notre-Dame, and 112 (25%) were from the other three hospitals.

5.6.3 Quality Insurance and Data Management

A variety of measures were adopted to ensure high quality of the data used for analysis. All data collectors were well trained on the layout and functionalities of the OACIS database as well as the design of the FileMaker database. The data collection process was constantly monitored. Any question arising during data collection was resolved by discussion with the supervisor and senior lab members. All relevant information was collected, and additional details that occasionally could not be fitted into the designated FileMaker fields were recorded in a comment section. Multiple cycles of extensive data cleaning procedures were performed in Stata 13.0 on a single Mac computer running OS X Yosemite. This measure eliminated potential errors that could be caused by conflicting formats due to differences in platforms and systems; this type of error was discovered by the team during the data collection process: although team members followed the same procedure for entering dates, FileMaker running on Windows systems and Mac OS recorded different date formats. Data cleaning also involved fixing simple data entry errors and crosschecking different variables within the dataset. For example, exposure status was crosschecked with cardiovascular comorbidities and use of antihypertensive mediations (i.e., calcium channel blockers, ACE inhibitors, angiotensin II receptor antagonists) indicated in the consultation report. Conflicting information was checked in the OACIS database and subsequently fixed. A Do-file (command sheet for Stata) was created to document all the changes made to the original FileMaker dataset. A modified open-access FileMaker database with all the corrections made to the original file was uploaded to a secure server shared among lab members, allowing the data to be validated by others. A variable dictionary was created and uploaded along with the database. Important information such as exposure and outcome status were reviewed as least twice to ensure accuracy.

The validity of information in the HeNCe Life database was ensured by previous work from senior lab members, who performed rigorous data cleaning procedures on the database (198, 205-208). After the merge of the OACIS and HeNCe Life databases, crosschecking procedures were performed between the two datasets. Differences in information were investigated and resolved. To examine the validity of the information

collected from the OACIS database, preliminary tests were performed to analyze the overall agreement between the two datasets for variables present in both. For the majority of these variables, an overall agreement of more than 80% was achieved, suggesting good reliability.

5.7 Definition of Exposure and Outcome

Our original aim was to obtain information on prescription period and daily dose of beta-blockers for each patient and analyze the relationship between pre-diagnosis beta-blocker consumption and survival in HNC on the basis of cumulative dosage of beta-blockers. However, no accurate account of prescription period and daily dose was found in the medication prescription section of the OACIS database. As a result, after carefully studying the OACIS database and beta-blocker prescription guidelines for the treatment of cardiovascular conditions, we found reliable indicators of chronic beta-blocker use in the consultation reports of the patients (209-212). A consultation report is a structured clinical evaluation of the patient's condition written by oncologists during the first consultation appointment with the patient shortly after the diagnosis of HNC, which is the baseline of the study. The report contains a record of medications used by the patient in a section devoted to this information. According to prescription guidelines, cardiovascular conditions such as myocardial infarction, arrhythmia, angina, heart failure, and hypertension are, in general, managed by chronic commitment use of beta-blockers. Patients wishing to discontinue beta-blocker use due to side effects are required to go through a gradual withdrawal procedure of decreasing dosage under careful instruction from the physicians. In addition, patients with a history of myocardial infarctions and angina are strongly advised not to withdraw from using beta-blockers (213, 214). Based on these facts, an assumption was made that a recorded consumption of beta-blockers at baseline combined with the knowledge that the patient had cardiovascular conditions could be regarded as an indication of chronic commitment use of beta-blockers before the diagnosis of cancer. It was also assumed that the use of beta-blockers for that patient would extend into the time period after the diagnosis. Therefore, our definition of exposure was chronic use of beta-blockers. Patients with information indicating chronic commitment use of beta-blockers before the baseline were considered as exposed.

Otherwise, a patient with daily-used medication information in the initial consultation report that did not include beta-blocker use was categorized as unexposed. Because beta-blockers could also be prescribed for short-term use such as for preoperative care, anxiety, migraine and glaucoma, we classified patients with a record of short-term or temporary beta-blocker consumption as unexposed (215-218).

The fact that we could not obtain accurate information on cause of death for the participants limited the scope of our analysis to overall survival, which examines the association between an exposure and the all-cause mortalities of the subjects. In essence, this analysis considers all deaths as outcome events, regardless of their cause.

5.8 Definition of Covariates

5.8.1 Use of Other Types of Medications

Similar to how exposure status was determined for beta-blockers in our study, exposure to other medications was assessed from information in the medication section of the first consultation report since the time of diagnosis. A similar type of definition of exposure was applied to these medications as well; a patient was considered as exposed to a type of medicine if his/her record included indications of chronic commitment use of this type of medicine at and prior to the time of diagnosis, and the patient would be assumed to continue with the chronic use after the diagnosis of cancer due to his/her relevant condition. If there was no indication of such a type of medication in the consultation report, the patient was designated as unexposed. For each type of medication, a binary variable was used to represent exposure status: exposed (1) and unexposed (0). Patients taking one or more of the same kind of medication were equally considered as exposed.

5.8.1.1 Calcium Channel Blockers

Calcium channel blockers are a type of drug acting as calcium channel antagonists by blocking the entrance of calcium (Ca^{2+}) into calcium channels. They are primarily used as antihypertensive agents to reduce blood pressure and have gradually replaced beta-blockers as the first line therapy for hypertension due to their relatively stronger effects

(219-222). A beta-blocker is commonly used as a second drug to a calcium channel blocker for hypertension, as the combination has shown greater effects than each type of drug consumed alone (223). In addition, calcium channel blockers are used for other conditions, such as arrhythmia and chest pain caused by angina. Table 5-1 lists all the generic and brand names of calcium channel blockers used by subjects in this study.

Table 5- 1: Calcium Channel Blockers Used by Study Participants

Generic name	Brand name
amlodipine	Norvasc
diltiazem	Cardizem LA, Tiazac, Cardene IV
felodipine	Plendil
isradipine	Dynacirc
nifedipine	Adalat, Procardia
nicardipine	Cardene, Cardene IV
nimodipine	Nimotop
nisoldipine	Sular
verapamil	Covera-HS, Verelan PM, Calan, Cardene IV

5.8.1.2 Statins

Statins are HMG-CoA reductase inhibitors and a type of medication that manages CVD by lowering the lipid level in the blood. The action of statins is mediated by their ability to block the production of liver enzymes that produce cholesterols, as lower bloodstream cholesterol is associated with a lower incidence of ischemic cardiac events and reduced mortality rate from CVD (224). Table 5-2 lists all the generic and brand names of statins used by subjects in this study.

Table 5- 2: Statins Used by Study Participants

Generic name	Brand name
atorvastatin	Lipitor
fluvastatin	Lescol, Lescol XL
lovastatin	Mevacor, Altoprev
pravastatin	Pravachol
rosuvastatin	Crestor
simvastatin	Zocor
pitavastatin	Livalo

5.8.1.3 Angiotensin-Converting-Enzyme Inhibitors

The Angiostensin-converting-enzyme inhibitor (ACEi) is one of the four major types of medications prescribed for hypertension. This family of drugs is also capable of managing congestive heart failure. It exerts its action by tackling the angiotensin converting enzyme (ACE) that is responsible for producing angiotensin II, a chemical whose role is to contract the blood vessel and increase blood pressure. Table 5-3 lists all the generic and brand names of ACEis used by subjects in this study.

Table 5- 3: ACEis Used by Study Participants

Generic name	Brand name
benazepril	Lotensin
captopril	Capoten
enalapril	Vasotec, Epaned
fosinopril	Monopril
lisinopril	Prinivil, Zestril
moexipril	Univasc
perindopril	Aceon
quinapril	Accupril
ramipril	Altace
trandolapril	Mavik

5.8.1.4 Angiotensin II Receptor Blockers

Angiotensin II receptor blockers (ARBs), also known as angiotensin II receptor antagonists, are a type of medication that is primarily used for the treatment of hypertension, congestive heart failure, and diabetic nephropathy. Regarding hypertension, it exerts effects on blood vessels similar to that of the ACEi. However, it does not reduce the production of angiotensin II, which is responsible for vasoconstriction. Rather, it prevents the binding of angiotensin II to its receptor located on blood vessels. Their similar but slightly different mechanism of action allow ARBs to be used in conjunction with ACEis, and the combination has been suggested to offer additional benefits compared to using each type of drug alone (225). Table 5-4 lists all the generic and brand names of ARBs used by subjects in this study.

5.8.1.5 Aspirin

Aspirin is the commonly known name for acetylsalicylic acid (ASA), a type of medicine used to treat pain, fever, and inflammation. It belongs to the drug class of nonsteroidal anti-inflammatory drugs (NSAIDs). However, it has additional antiplatelet properties, as it decreases platelet aggregation. The effect of aspirin on cancer survival is a topic that has been extensively covered in the literature, and separately analyzing aspirin and other NSAIDs has become popular (190, 226). In some instances, aspirin was shown to have a different effect on cancer survival compared to other NSAIDs (227). As a result, aspirin will be considered as a separate confounder in our analysis.

Table 5- 4: ARBs Used by Study Participants

Generic name	Brand name
candesartan	Atacand
eprosartan	Teveten
irbesartan	Avapro
losartan	Cozaar
olmesartan	Benicar
telmisartan	Micardis
valsartan	Diovan

5.8.1.6 Other Nonsteroidal Anti-Inflammatory Drugs

Aside from aspirin, the study also considered the effect of other nonsteroidal anti-inflammatory drugs (NSAIDs), which function in a similar way as aspirin with the exception of the antiplatelet properties. Table 5-5 lists all the generic and brand names of NSAIDs used by subjects in this study.

5.8.2 Comorbidities

Similar to how exposure status was determined in this study, the comorbidity status of the patients was determined by examining the comorbidity section of the consultation record. The diseases and conditions analyzed in the study were all chronic ones. Patients with records of a specific disease or condition at the time of diagnosis were assigned the value of “1” for that disease or condition and were assumed to carry it all the way through their follow-up period. Otherwise, a value of “0” was assigned to that disease or condition.

Table 5- 5: NSAIDs Used by Study Participants

Generic name	Brand name
celecoxib	Celebrex
diclofenac	Cambia, Cataflam, Voltaren-XR, Zipsor, Zorvolex
diflunisal	Dolobid
etodolac	Lodine
ibuprofen	Motrin, Advil
indomethacin	Indocin
ketoprofen	Active-Ketoprofen, Orudis
ketorolac	Toradol
nabumetone	Relafen
naproxen	Aleve, Anaprox, Naprelan, Naprosyn
oxaprozin	Daypro
piroxicam	Feldene
salsalate	Disalsate, Amigesic
sulindac	Clinoril
tolmetin	Tolectin

5.8.2.1 Clinical Cardiovascular Disease

CVD is a broad term describing a large variety of diseases and conditions related to the heart and blood vessels. The commonly known ones include angina, myocardial infarction, stroke, heart failure, arrhythmia, hypertension, congenital heart disease, peripheral artery disease, and venous thrombosis (228).

In this study, we classified cardiovascular conditions on the basis of clinical symptoms and adopted the definition of clinical CVD. This collective term refers to a group of conditions that have recognizable clinical symptoms, including myocardial infarction and angina (and coronary artery disease that includes both), stroke, and heart failure (229). A binary variable was created to represent clinical CVD in this study. Patients with one or more of the cardiovascular conditions mentioned above were given a value of “1”. Patients with a clear indication of having none of the conditions mentioned above would be assigned a value of “0”. Otherwise, patients with missing information for one or more conditions and without any indication of having one of the conditions were classified as missing.

5.8.2.2 Diabetes

In this study, diabetes was represented by a single binary variable. Patients with either type 1 or 2 diabetes were assigned a value of “1”, and patients with neither were given a value of “0”.

5.8.3 BMI

Body mass index (BMI) is a value that is used to categorize a person as normal, overweight, obese or underweight. The calculation of BMI is achieved by dividing body mass in kg by the square of body height in meters. In North America, BMI≥25.0 is considered overweight, and BMI≥30.0 obese (230). In this study, BMI was used as a continuous variable. The body height used in the calculation was reported by the patient in inches or cm and was subsequently converted to a value in m. Body weight was self-reported by the patient in kg, or in pounds converted to kg. This measure was for two years prior to the diagnosis, so that it would not be affected by weight changes brought about by the cancer.

5.8.4 Cancer Stage

The clinical evaluation of a patient’s cancer involves cancer staging by oncologists. The overall stage system classifies cancer into five different categories (0-IV) and offers a general sense of the size and metastatic characteristics of the tumour. The higher the stage number, the larger the size of the tumour, and the farther it has migrated to different parts of the body. Stage 0 describes carcinoma *in situ*, the non-invasive precursor form of cancer. Stages I-III describe cancers that reach as far as lymph nodes. Stage IV is used to describe invasive cancers that have metastasized to distant parts of the body (231). For this study, the ordinal variable of cancer stage was reduced to a binary variable. A value of “0” was assigned to stages 0-II, which were labeled as “low-stage cancers”. Stages III-IV were considered as high-stage cancers and were assigned a value of “1”. Low-stage cancers were used as the reference group in the analysis.

5.8.5 Behaviours

5.8.5.1 Smoking

The variable used to capture smoking behaviour in this study, pack-years, represents the lifetime amount of tobacco products consumed. This variable was created from the comprehensive information on smoking behaviour recorded using the HeNCe Life questionnaire. The information recorded included whether the patient was an ever smoker, and details related to the consumption of three types of tobacco products: cigarettes, cigars, and pipes. For each type of product, the information collected included starting age and last age of smoking as well as brand and daily amount of tobacco product smoked. In addition, the type of cigarettes smoked (filter, non-filter, or hand-rolled) was recorded. Similarly, the distinction between units of grams and pipes was made for pipe smoking.

To estimate of the total lifetime consumption of tobacco, a standardization procedure was applied. After analyzing the tobacco content of each type of product, equivalency in tobacco content was established among 1 pack of cigarettes, 20 filtered or non-filtered cigarettes, 4 hand-rolled cigarettes, 4 cigars, and 5 pipes. All products consumed were converted to cigarettes on the basis of this ratio, and a “standardized cigarette” was designated as the unit of this estimate. In addition, the number of years that the patient had been a smoker was obtained by calculating the difference between the starting and last ages. Time periods outside of the interval designated by the starting and last ages of smoking for ever-smokers and all ages for non-smokers were assigned a value of “0” for tobacco consumption. Finally, the lifetime consumption of tobacco in pack-years was calculated by multiplying the number of packs of standardized cigarettes smoked daily and the duration of smoking in years; it was used as a continuous variable in the analyses.

5.8.5.2 Alcohol Consumption

The lifetime amount of alcohol consumed was computed using an approach similar to the one used for tobacco smoking. In the HeNCe Life questionnaire, a patient with drinking behaviour for at least one year was considered an “ever drinker”. Aside from this information, the questionnaire documented the situations in which the patient would drink, type of beverage, starting and last age of drinking, units of the beverage consumed, and the frequency of drinking. The type of beverage featured five categories: wine, beer/cider,

hard liquor, aperitif, and other types of beverages. The unit of the beverage also featured five different sizes: small glass, medium glass, big glass, half small bottle, and bottle corresponding to 50ml, 100ml, 150ml, 330ml, and 750ml, respectively. The standardization of alcohol consumption was established upon assigning a percentage of alcohol content to each type of beverage. Toddy/wine, beer, and hard liquor were assigned a 10%, 5%, and 50% of ethanol content, respectively. The daily consumption of alcohol in milliliters was calculated by multiplying the percentage of ethanol content of each type of beverage by the volume consumed and summing up the total volume of alcohol. The time interval, in number of days, that the person consumed alcohol was calculated from the starting and last ages of drinking behaviour. Time periods outside of the interval designated by the starting and last ages of drinking for ever-drinkers and all ages for non-drinkers were assigned a value of “0” for ethanol consumption. Finally, the daily volume of ethanol consumed was multiplied by the duration of alcohol drinking in days to obtain the continuous variable for lifetime amount of alcohol consumption.

5.8.6 HPV

A 4-level categorical variable was used to represent HPV status in this study, and each level denotes a different risk level of HPV infection. A value of “0” was assigned to patients with negative HPV testing results. A value of “1” was given to patients with positive results for low risk HPV strains (6,11, 40, 42, 54, 55, 61, 62, 64, 67, 69, 70, 71, 72, 81, 83, 84, 45, 52, 56, 58, 59, 68, 73, 82, 26, 53, 66, 114 and 120). A value of “2” was assigned to patients with the high-risk HPV strains with the exception of 16 (18, 31, 33, 35, 39, 51). Finally, patients with high-risk HPV strain 16 were given a value of “4”.

5.8.7 Treatment

After clinically evaluating the health condition of the patient following the cancer diagnosis, the oncologist offered three main types of treatment: chemotherapy, radiotherapy, and surgery. For most cancers, a combination of different therapies was offered to the patient. A single categorical variable was created to represent the total number of different types of treatments that a patient had received. A value of “0”, “1”, “2”

or “3” was assigned to each patient accordingly. For the analysis, the no treatment group (“0”) was used as the reference group.

5.8.8 Cancer Site

The analysis took into account three main sites of HNC: the pharynx, larynx, and oral cavity. A categorical variable was created to represent the three sites, and values of “1”, “2”, and “3” were assigned to the pharynx, larynx, and oral cavity, respectively. In the analysis using the Cox proportional hazards model, the pharynx site was used as the reference category.

5.9 Statistical Analysis

5.9.1 Descriptive Statistics

Descriptive statistics were carried out to explore the characteristics of both the exposed group and unexposed group. For categorical variables, Fisher’s exact test was used to compare the 2 groups; for continuous variables, 2-sample student *t* tests were used to compare the means.

5.9.2 Cox Proportional Hazards Model

The Cox proportional hazards model, or Cox model, is a statistical model for survival analysis. Compared to the t-test, linear and logistic regression, the method is advantageous as it has the ability to account for the effect of censoring and differences in follow-up times.

Hazard is a term describing the conditional probability of an event occurring at a particular point of time, given that the subject has survived up to this point of time. The relationship between the hazard function and the survival function is summarized by the equation below:

$$h(t) = -\frac{d\log\big(S(t)\big)}{dt} = \frac{\frac{dS(t)}{dt}}{S(t)} = \frac{f(t)}{S(t)}$$

In essence, the hazard function is the negative derivative of the log of the survival function. As a result, the hazard rate illustrates the instantaneous potential for failure events.

The semi-parametric Cox proportional hazards model was first introduced by Sir David Cox in 1972. The model is represented by the equation below:

$$h(t, X) = h_0(t) \times \exp(X\beta)$$

The component of $h_0(t)$ describes the baseline hazard at time t. $X\beta$ is a linear combination of parameters, where X represents covariates. In this sense, the Cox model shares similarities with conditional logistic regression. However, unlike the latter, the Cox model does not require an intercept term of α, because the intercept is incorporated into the baseline hazard $h_0(t)$. In addition, the Cox model estimates a hazard ratio (HR), as opposed to an odds ratio (OR) that is estimated by conditional logistic regression.

The implementation of the Cox model requires the satisfaction of several assumptions about censoring. First, the failure rate in censored subjects is equal to that in the remaining subjects. The same assumption applies to all different subgroups of the cohort. In addition, the distribution of survival times should not reflect the distribution of the censoring times, and vice-versa.

To examine the effect of beta-blocker consumption on overall survival in this study, Cox proportional hazards models were used to estimate the HR and corresponding 95% confidence intervals (95% CIs) of all-cause mortality with chronic consumption of beta-blockers. P values were calculated and those <0.05 were considered to be statistically significant. The adjusted model controlled for potential confounders measured at baseline, including age, gender, BMI, lifetime smoking, lifetime alcohol consumption, years of education, HPV infection status, use of calcium channel blocker, statin, angiotensin converting enzyme inhibitors (ACEi), Aspirin, other nonsteroidal anti-inflammatory drugs (NSAIDs), angiotensin II receptor blockers (ARB), clinical cardiovascular disease, hypertension, diabetes, cancer stage, and cancer site. In addition, the model was adjusted for the combination of treatments after diagnosis. For the survival analysis, survival time was defined as time from the date of diagnosis to the date of death, or

administrative censoring. As described before, all-cause mortality was used as the outcome. Subjects with missing living status were considered as censored, and their last dates of follow-up were used as exit dates.

5.9.3 Kaplan-Meier Survival Analysis

The Kaplan-Meier method utilizes a non-parametric estimation of the survival function based on survival time data. The method was named after Edward L. Kaplan and Paul Meier, who came up with the method separately and simultaneously (232). The method can be summarized by the following mathematical equation:

$$\hat{S}(t) = \prod_{i:\, t_i \leq t} (1 - \frac{d_i}{n_i})$$

t_i refers to the time when an event occurs (or more than one event if the events occur simultaneously). d_i refers to the number of events that have occurred at time t_i. n_i refers the number of subjects who survive (excluding censored subjects) immediately prior to the occurrence of the event/events at time t_i.

Essentially, the method estimates the survival function by dividing survival time into different risk sets. A risk set is created at the precise moment when an event (death in our case) occurs (or multiple events if all the events happen precisely at one moment in time). At each risk set, the probability of survival is calculated by dividing the number of subjects who did not experience the event by the total number of subjects in the risk set, including those who experienced the event. The cumulative probability of survival at a risk set is calculated by multiplying the probability of survival for this risk set by each of the probabilities for all of the previous risk sets.

The cumulative probability of survival at each risk set will be plotted on a graph, which is called a Kaplan-Meier curve. The horizontal axis of the graph denotes observation time, while the vertical axis denotes probability of survival. The curves are step functions; at each risk set, a new step will occur. For comparison, subjects from each category of the exposure variable will be plotted in a different curve. If two curves run parallel to each

other without any intersection, the survival profiles of the two exposure categories are said to be significantly different from each other. Two curves that intersect indicate no statistically significant difference.

5.10 Sample Size and Power Considerations

To analyze the power of the study, post-hoc power analyses were performed for both the crude and adjusted Cox proportional hazards models in Stata 14.0. Both models contained 274 subjects (30 beta-blocker users and 244 non-users) with complete information for all the covariates used in the adjusted model. For both analyses, the alpha-value (probability of type I error) was set at 5%, and the variability value at 0.5. For the crude model, the correlation value was set at 0, and at 0.6 for the adjusted model.

Figure 5- 1: Post-hoc Power Analysis for the Crude Cox Proportional Hazards Model

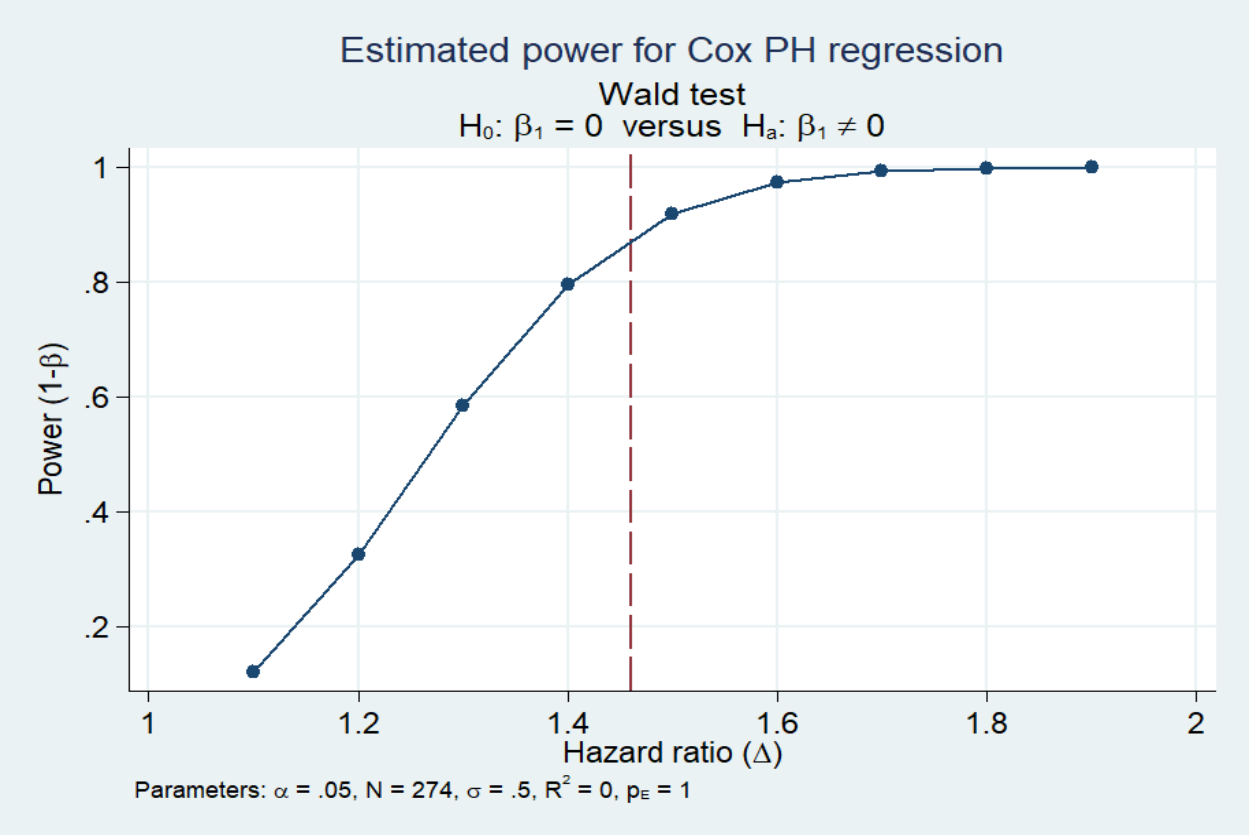
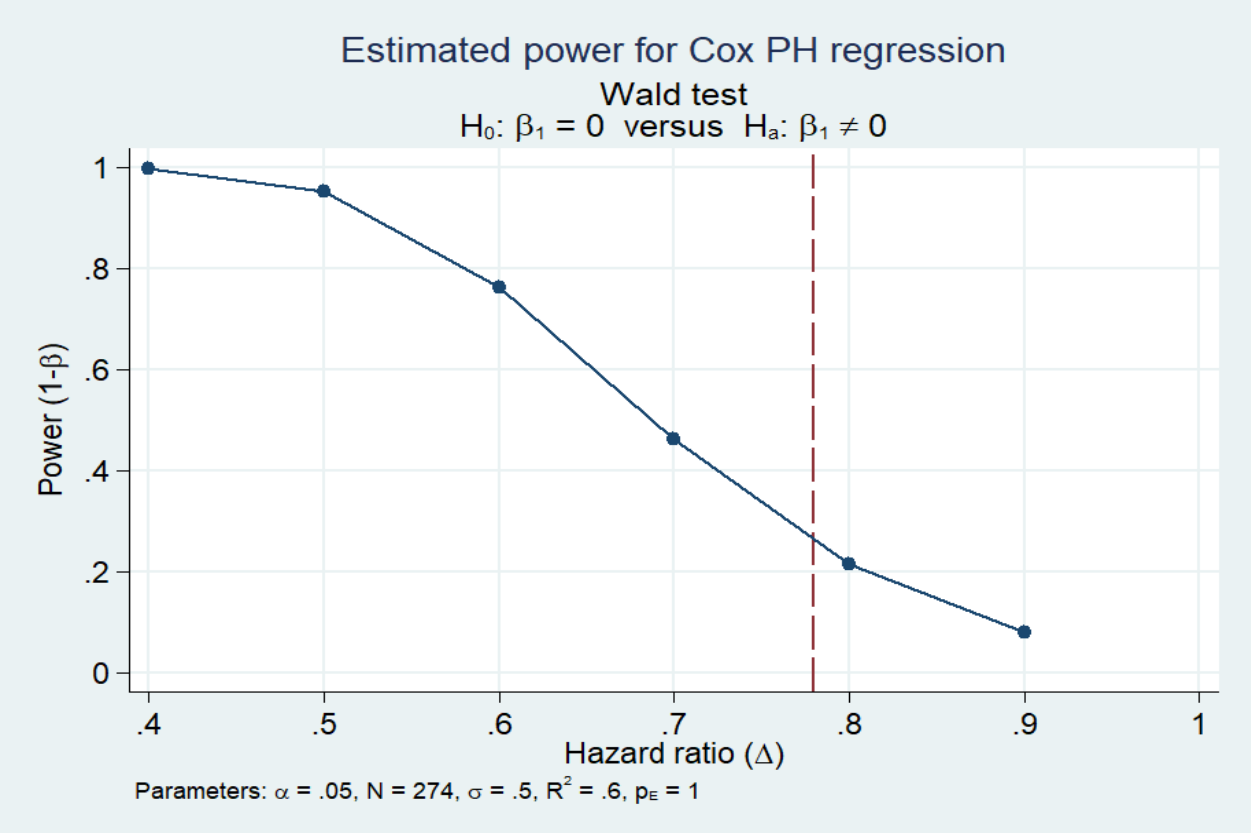


Figure 5-1 shows the result for the crude model. For the crude Cox proportional hazards model, an HR of 1.46 (indicated by the red line) was observed, which corresponded to a power of 87.9% to reject the null hypothesis, a value above the desired level of 80%. It indicated that given the sample size, the study had sufficient power to detect an effect on the hazard rate represented by an HR of 1.46 if the null hypothesis is false.

The result for the adjusted model is shown in Figure 5-2. For the adjusted Cox proportional hazards model, an HR of 0.82 (indicated by the red line) was observed, which corresponded to a power of 25.5% to reject the null hypothesis, a value below the desired level of 80%. It indicated that given the sample size, the study had insufficient power to detect an effect on the hazard rate represented by an HR of 0.82 if the null hypothesis is false.

Figure 5- 2: Post-hoc Power Analysis for the Adjusted Cox Proportional Hazards Model



6. RESULTS

The results of our study are presented in a manuscript format. At the time of completion of this thesis, the manuscript has yet to be submitted to publication.

The Association between Beta-Blockers on Survival in Head and Neck Cancer

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Key words: head and neck cancer, beta clockers, survival

Abstract

Objective: Beta-blockers, drugs commonly prescribed for the management of cardiac arrhythmias and hypertension, seem to have an anti-proliferation effect in tumors and to increase the overall survival of cancer patients in general. However, studies on head and neck cancer (HNC) are lacking. We aim to estimate the extent to which beta-blocker intake is associated with an increase in overall survival among HNC patients.

Method: Subjects (n=303) were a sub-cohort of the HeNCe Life Study – Canada. All subjects resided within 50km of Notre-Dame Hospital in Montreal, where they received treatments for HNC confirmed by histology. A database was established using the HeNCe Life database combined with comprehensive data extracted from the hospital OACIS database, documenting disease details, diagnostic tests, comorbidities, family history of cancer, medications, treatments, and recovery information. Living statuses of patients were retrieved from the death registry of the Quebec Government. Patients who had been committed beta-blocker users before the date of diagnosis for cancer were considered as exposed subjects. Data analysis included descriptive statistics, Kaplan-Meier survival analysis and Cox proportional hazards regression.

Results: Overall, after adjusting for age, gender, smoking, alcohol, human papillomavirus infection, other medications, comorbidities, cancer site, stage, and treatment, committed beta-blocker use was not associated with an increase in the overall survival of HNC patients (HR: 0.82, 95% CI: 0.38-1.77).

Conclusion: Long-term beta-blocker intake is not significantly associated with an increase in survival for HNC. Future studies are required to examine the impact of beta-blocker selectivity on HNC survival.

Introduction

Cancer of the upper aerodigestive tract region commonly referred to Head and neck cancer (HNC) include cancer of the mouth, pharynx and larynx. They are the 8th most prevalent cancer among males worldwide, and the male gender accounts for 75% of the disease prevalence (20). Globally, there are approximately 686,328 incident cases and 375,665 deaths of HNC annually (12). The average 5-year survival rate is approximately 50% (233). Moreover, the suicide rate among HNC patients is 4 times the rate in general population (3, 15, 16). All of these factors are indications of the tremendous burden on the patients and the healthcare system caused by the disease.

The association between beta-blockers, a class of drugs commonly prescribed for the management of cardiac arrhythmias and hypertension, and cancer progression has become a focus of research in recent years. Beta-adrenergic receptor are over-expressed in cancer cells and linked to tumor proliferation (234). Indeed, they have been identified on pancreatic, breast, ovarian, nasopharyngeal, and oral cancer cells (115, 235-237) and associated with metastasis, tumor size, and cancer stage (238). The ability of beta-blockers to suppress adrenergic signaling has marked these drugs as a promising therapeutic option for these cancers. Moreover, *in vitro* studies have demonstrated that tumor cell growth and invasion can be reversed by beta-blocker propranolol in nasopharyngeal and oral squamous cell carcinoma (115, 238-241). Further studies with animal models in different cancers, including HNC, have also confirmed the effect of beta-blockers on reducing cancer progression (96, 237, 242-245).

However, no clinical trial on the effect of beta-blockers has yet been completed, and the association between beta-blockers and cancer survival in epidemiological studies remains unclear. Some studies show that beta-blocker use is associated with a reduced risk of cancer and improved cancer survival, while others suggest a null association (122, 127, 130, 135, 141, 150). These divergent findings could be due to differences in study designs or in how information on beta-blocker exposure and other confounders was collected across studies.

To the best of our knowledge, no study has ever investigated the relationship between beta-blockers and survival in HNC. Therefore, the aim of this study is to estimate the extent to which beta-blocker use is associated with improved HNC overall survival (OS) in a sample of subjects living in Canada.

Method

Study population and data collection

Our inception cohort study uses data combined from three sources: the Head and Neck Cancer (HeNCe) Life Study database, the OACIS database, and the register of civil status of the Government of Quebec. The HeNCe Life Study is an international hospital-based case-control study investigating HNC aetiology in three countries: Brazil, Canada, and India. Data used to answer the research question were drawn from the Canadian site, which recruited patients from four major referral hospitals in Montreal, Quebec. Subjects recruited between 2007 and 2013 from Hôpital Notre-Dame, the hospital providing care to the great majority of HNC cancer patients in the area, were selected. All patients were adults (18 years and older) living within a 50km-range of Hôpital Notre-Dame, were born in Canada, and had no previous history of cancer or HIV. All newly diagnosed HNCs were histologically confirmed to be squamous cell carcinoma of stage I-IV in the upper aero-digestive tract region, including the pharynx, larynx, and oral cavity. We excluded patients with nasopharyngeal cancers. In addition, the study was restricted to patients with a complete record of consultation at the time of diagnosis. All patients had at least two years of follow-up time.

Prior to data collection, an informed consent was signed by each of the participants. Approvals for the study protocol were obtained from the Research Ethics Office at McGill University, Hôpital Notre-Dame and Institut National de la Recherche Scientifique (INRS) (198). Individual semi-structured interviews were conducted to collect information on sociodemographic, environmental and behavioural factors during three periods of the participants' lives (16 years, 17- 30 years and > 30 years) using the life-grid technique.

In addition, information was retrieved from the OACIS database at Hôpital Notre-Dame. The database contains baseline medical information such as diagnosis details, comorbidities, family history, and medications. It also contains treatment and follow-up information such as treatment type, treatment length, disease recurrence, primary metastasis, new secondary cancer, and new secondary metastasis.

Survival information was obtained from the register of civil status of the Government of Quebec, in Canada. The records were matched on full name and date of birth. All-cause mortalities of the subjects were collected up to May 13th, 2016, the date of termination of the study.

Beta-blocker exposure assessment

Beta-blocker exposure status was retrospectively determined at baseline by examining the consultation record at the time of diagnosis. The exposure was expressed as a binary variable on the basis of whether a beta-blocker was present in the medication section of the consultation report created by the oncologist at Hôpital Notre-Dame. The consultation reports recorded daily-used medications chronically consumed by the patients. Therefore, our definition of exposure is chronic consumption of beta-blockers. Given the knowledge that beta-blockers are chronically used for cardiovascular conditions but can be prescribed for short-term use such as for preoperative care, anxiety, and glaucoma, we omitted from the exposure group patients with records of short-term beta-blocker consumption in the prescription section of the OACIS database. In addition, exposure status was cross-checked with cardiovascular comorbidities and use of antihypertensive mediations (i.e., calcium channel blockers, ACE inhibitors, angiotensin II receptor antagonists) indicated in the consultation report.

Analysis

Fisher's exact test and *t* tests were used to compare the characteristics of the exposed and unexposed group, when appropriate. Univariate and multivariate Cox proportional hazards models were used to assess the association between beta-blockers and HNC survival by estimating hazard ratios (HR) and respective 95% confidence intervals (CI).

Survival time was defined as time from date of diagnosis to date of death, or administrative censoring. All-cause mortality was used as the outcome. Subjects with missing living status were considered as censored, and their last dates of follow-up were used as exit dates.

Covariates included age, gender, lifetime smoking, lifetime alcohol consumption, HPV infection status, statins, acetylsalicylic acid (ASA), clinical cardiovascular disease, diabetes, cancer stage, and cancer site. In addition, the model was adjusted for the total number of different types of treatments received after diagnosis (radiotherapy, chemotherapy, surgery). The analysis was performed using Stata 13.0 (Stata Corp., College Station, Texas, USA).

Results

Characteristics of the Cohort

Overall, 350 patients with newly diagnosed HNCs participating in the HeNCe Life study – Canada from Hôpital Notre-Dame were identified. After applying the exclusion criteria, the final cohort included 303 subjects. Beta-blocker consumption was identified in 31 patients (10.2%). Among them, only 1 person (3.2%) was reported to have chronically non-selective beta-blocker use. The median follow-up time was 47.6 months. In total, 81 all-cause deaths were identified. Time to death ranged from 1.8 months to 96.2 months, and the average was 25.5 months. The characteristics of the cohort are shown in Table 6-1. Compared to the non-user group, the beta-blocker users were older, and had fewer years of education. In addition, the users were more prone to have comorbidities such as clinical cardiovascular disease, hypertension, and diabetes; they were also more likely to consume statins, aspirin and ACE inhibitors. The other characteristics were similar between the two groups.

Table 6- 1: Frequency Distribution of Selected Variables According to Exposure Status

Baseline Characteristics	Use of Beta-Blockers	
	None-user n (%)	User(%)
Age, mean, ±SD	61.8 ±10.1	67.7±9.3
BMI, mean, ±SD	27.6 ±13.4	28.1 ±5.5
Missing	4 (1.5)	0 (0.0)
Smoking (Pack Years), mean, ±SD	40.9 ±47.0	41.5 ±32.8
Missing	2 (0.7)	0 (0.0)
Alcohol (L), mean, ±SD	653.2 ±1256.7	595.5 ±792.5
Missing	2 (0.7)	0 (0.0)
Education (Years), mean, ±SD	12.2 ±4.0	10.6 ±3.3
Gender		
Female	62 (23.1)	8 (25.8)
Male	206 (76.9)	23 (74.1)
HPV		
No	142 (53.0)	16 (51.6)
Low Risk	22 (8.2)	3 (9.7)
High Risk without 16	18 (6.7)	3 (16.1)
HPV 16	70 (26.1)	6 (19.4)
Missing	16 (6.0)	1 (3.2)
Calcium Channel Blocker Intake	28 (10.5)	7 (22.6)
Missing	0 (0.0)	0 (0.0)
Statin Intake	83 (31.0)	22 (71.0)
Missing	0 (0.0)	0 (0.0)
ACE Inhibitors Intake	23 (8.6)	7 (22.6)
Missing	0 (0.0)	0 (0.0)
Aspirin Intake	73 (27.2)	18 (58.1)
Missing	0 (0.0)	0 (0.0)
Other NSAIDs Intake	24 (9.0)	1 (3.2)
Missing	0 (0.0)	0 (0.0)
Angiotensin II Receptor Blocker use	30 (11.2)	6 (19.4)
Missing	0 (0.0)	0 (0.0)
Clinical Cardiovascular Disease	40 (14.9)	17 (54.8)
Missing	4 (1.5)	0 (0.0)
Hypertension	87 (32.5)	26 (80.7)
Missing	5 (1.9)	0 (0.0)
Diabetes	32 (11.9)	9 (29.0)
Missing	3 (1.1)	0 (0.0)
Cancer Stage		
0-II	97 (36.2)	13 (41.9)
III-IV	170 (63.4)	18 (58.1)
Missing	1 (0.4)	0 (0.0)
Cancer Site		
Pharynx	140 (52.2)	12 (38.7)
Larynx	86 (32.1)	13 (41.9)
Oral Cavity	42 (15.7)	6 (19.4)
Treatment		
0	8 (3.0)	1 (3.2)
1	95 (35.5)	15 (48.4)
2	127 (47.4)	10 (32.3)
3	34 (12.7)	5 (16.1)
Missing	4 (1.5)	0 (0.0)
F-up Time (Month), mean (SD)	53.4 (28.7)	49.5 (30.8)

ACE: Angiotensin converting Enzyme, NSAD: Nonsteroidal Antiinflammatory Drugs

OS by Beta-Blocker Consumption Status

The association between beta-blocker consumption and OS is presented in Table 6-2. Overall, there was little evidence of association between chronic beta-blocker consumption and improved OS in HNC patients in both the crude model (HR: 1.46, 95% CI: 0.75-2.85) and the adjusted model (HR: 0.82, 95% CI: 0.38-1.77).

Table 6- 2: Crude and Adjusted HR for HNC OS Based on Exposure Status

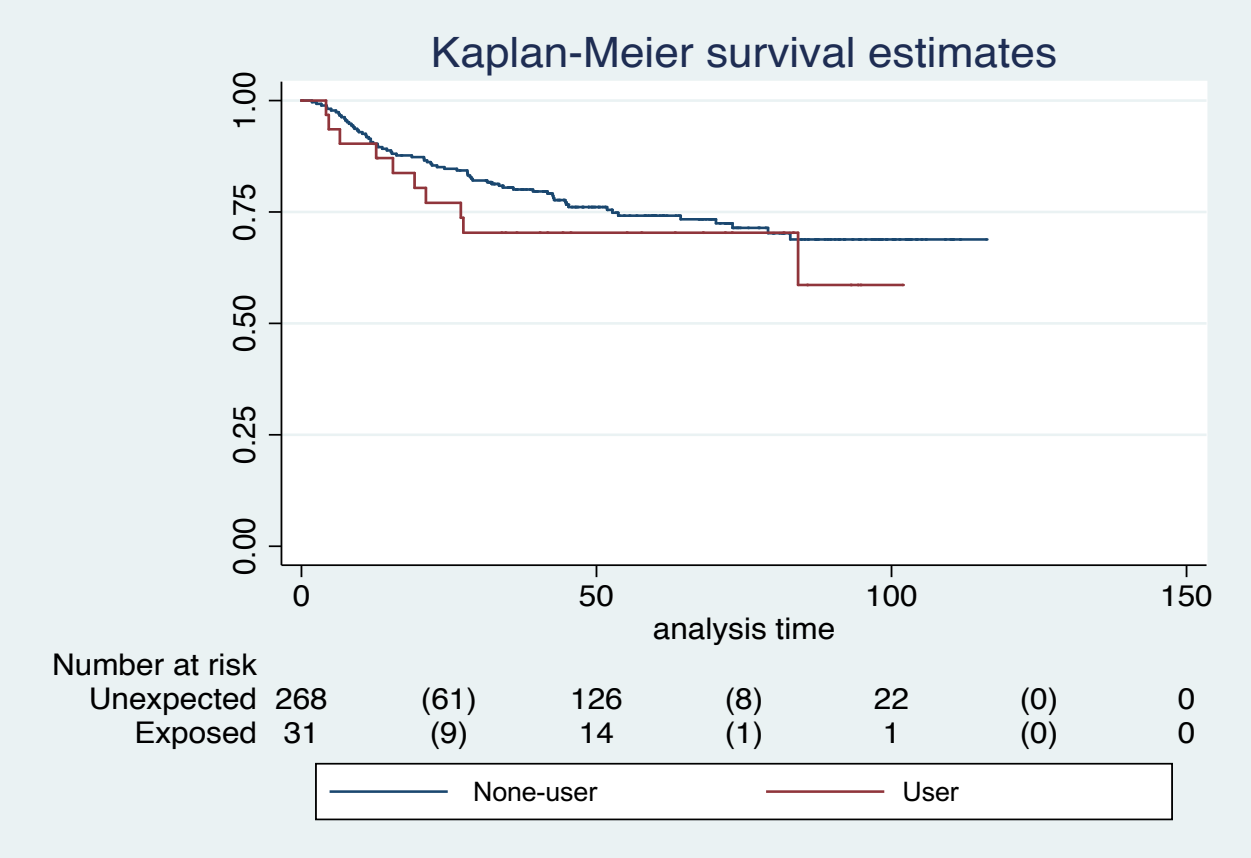
Chronic Use of Beta-blocker	Events (n=70)	Person-Months	Crude Rate (per 1000/month)	Crude HR	Adjusted HR* (95% CI)
No	60	12976.9	4.6	1.00 (Reference)	1.00 (Reference)
Yes	10	1491.6	6.7	*1.46 (0.75-2.85)	0.82 (0.38-1.77)

* Multivariate Cox proportional hazard model adjusted for age, gender, lifetime smoking, lifetime alcohol consumption, HPV infection status, use of statins, and acetylsalicylic acid (ASA), clinical cardiovascular disease, diabetes, cancer stage, cancer site and treatments.

Kaplan-Meier Survival Analysis

The result of the Kaplan-Meier Survival Analysis is presented in Figure 6-1. In the plot, the Kaplan-Meier curve for the exposed group displayed lower cumulative survival probabilities compared with the unexposed group, although no statistically significant difference was observed due to overlapping. The mean survival time for the exposed group was 74.2 months (95% CI: 60.3-88.3), which was lower than the mean survival time of 89.9 months (95% CI: 84.5-95.1) for the unexposed group.

Figure 6- 1: Kaplan-Meier Curve Based on the Exposure Status



Discussion

To our knowledge, this is the first study to investigate the impact of beta-blocker use on HNC survival, and it was limited by a number of factors. First, the large majority of beta-blockers used by the subjects were selective beta-blockers (SBB), which reflects the fact that non-selective beta-blockers (NSBB) are less commonly prescribed for cardiovascular conditions compared to SBBs, as SBB exert a more potent effect with fewer side effects. As a result, it was not possible to analyze the potentially differential effects of selective and NSBB on HNC survival. In essence, the analysis provided insights into the effects of the SBB only.

In addition, due to the lack of relevant information in the source database, we used a binary exposure variable that did not allow us to study the effects of different beta-blocker

exposure time lengths and dosages on survival. These factors can lead to residual confounding in the analysis and render it susceptible to immortal time bias.

Similarly, the lack of information on cause of death restricted our analysis to OS rather than cancer-specific survival, which prevented us from studying the direct link between beta-blocker use and HNC prognosis.

Moreover, the small sample size in combination with low exposure proportion in the sample and low number of outcomes in the exposure group lead to low statistical power. Our inability to produce a meaningful restrictive analysis on patients with cardiovascular comorbidities due to the small sample size exposed the analysis to confounding by indication, which cannot be completely eliminated by stratification.

Overall, due to these limitations, the results of the study cannot be used to draw a definitive conclusion about the relationship between beta-blocker use and HNC survival. Consequently, future efforts to examine the same topic should seek to improve on these aspects.

Regarding strengths, the HeNCe Life database contains very well documented information on lifelong consumption of tobacco products and alcohol. It allowed the study to consider the lifelong cumulative effects of these behaviours and minimize the potential for residual confounding due to these important habits.

Recently, an increasing amount of studies examining the relationship between beta-blockers and survival in other cancers have started to analyze the effects of SBBs and NSBBs separately. Some have suggested that the effect of beta-blockers on cancer survival is perhaps limited to NSBB, while others have suggested an overall null effect. Watkins et al. have shown that among epithelial ovarian cancer patients with hypertension, NSBB use drastically increased median OS compared to no use (90 months vs 34.2 months; $p<0.001$), while the large increase in survival time was not seen in SBB (38.2 months; $p=0.007$) (144). On the other hand, a large UK population-based case-control study concluded that post-diagnostic NSBB use among cardiovascular patients was not associated with improved survival in breast cancer (OR: 0.90; 95%CI: 0.69-1.17).

However, the study is potentially flawed due to the lack of adjustment for cancer stage (132). Another large UK cohort study showed that post-diagnostic NSBB use is not associated with prostate cancer survival (HR: 1.05, 95%CI: 0.72-1.53) and OS (HR: 0.94, 95%CI: 0.74-1.78) (246). These studies are a break from the earlier studies that did not account for the selectivity of beta-blockers. Out of the 12 studies in the 2014 meta-analysis by Choi et al., only 2 adjusted for selectivity of beta-blockers (150).

For HNC, we are expecting to see protective effects only from NSBBs in future studies, not from SBB. This speculation takes into account the fact that all *in vitro* and animal model *in vivo* experiments related to HNC tested the effect of beta-blockers by using NSBB propranolol (115, 238). Different types of beta-adrenergic receptors have different expression patterns in the human body. Beta-2 adrenergic receptors (ADRB2) are expressed in smooth muscle cells throughout the human sympathetic nervous system and have been shown to express in HNC cells (115, 238, 245). By contrast, beta-1 adrenergic receptors (ADRB1) are only located in smooth muscles of the heart and kidney, and we were unable to find any evidence from the literature suggesting the presence of ADRB1 in HNC cells. NSBB, such as propranolol that is capable of targeting ADRB2, have a clearly demonstrated ability to affect tumor progression in *in vitro* and animal model studies. However, all is subject to further examination, as in the past significant effects shown in animal models have been followed by insignificant results from observational studies by the same team.

7. DISCUSSION

This section aims to provide a holistic overview of the study. First, we summarize the results of this work in the context of the current literature. Subsequently, we examine the strengths and limitations of the study, as well as its contribution to knowledge.

7.1 Summary of the Findings

This study was set out to investigate the association between the use of beta blockers and HNC survival. After analyzing our data with the Cox proportional hazards model and Kaplan-Meier survival analysis, we did not find a statistically significant association between beta-blocker use and improvements in HNC OS. Neither the crude (HR: 1.46; 95%CI: 0.75-2.85) nor the adjusted (HR: 0.82; 95%CI: 0.38-1.77) cox proportional hazards models produce a hazard ratio significantly different from the null value of 1. A directional change of the point estimates of HR was observed between the two models; the crude model showed an inconclusive harmful value, whereas the adjusted model showed an inconclusive protective one. In the Kaplan-Meier survival analysis, the survival function curve of the beta-blocker users overlapped with that of the non-users; thus, there was no significant difference between the survival profiles of the two groups.

To the best of our knowledge, this is the first attempt to explore the relationship between beta-blocker use and HNC survival. However, given the relatively small sample size, our analysis cannot lead to a strong conclusion regarding the relationship investigated. In addition, almost all the beta-blocker users identified in the study were users of selective beta-blockers. As emerging studies suggest, the selectivity of beta-blockers may have a major impact on cancer survival in general. It is currently believed that non-selective beta-blockers such as propranolol, not the selective ones, have a protective effect against cancer mortality (144). This is one of the potential explanations for not observing a significant association in our study. Indeed, our results can be interpreted as addressing the impact of selective beta-blockers on HNC survival, rather than beta-blockers in general. As a result, future efforts should explore the role of beta-blocker selectivity by acquiring sufficient sample sizes for both selective and non-selective beta-blockers.

7.2 The Strengths of Our Study

Our study utilized a combination of datasets from various sources. Among them, the HeNCe Life database adopted a life course approach that documented lifelong behavioural information, such as smoking and alcohol consumption. The cumulative effects of lifetime consumption of tobacco and alcohol allowed for more precise adjustments compared to other studies that adjusted for short-term consumption after diagnosis or daily consumption. In addition, the use of the life-grid technique during the data collection for the HeNCe Life database encouraged more accurate recall from the subjects. Furthermore, extracting the same information from different sources allowed us to compare and crosscheck different datasets, which further improved the accuracy of our data. Reliability tests performed showed a high degree of agreement among different datasets. The disagreements were carefully investigated and subsequently resolved.

The combination of different datasets created a large database covering a wide range of information on all aspects of subjects' quality of life. This abundance of information allowed us to adjust for the effect of all traditional confounders of HNC survival (e.g., age, gender and HPV) as well as new ones suggested by emerging studies in the literature (e.g., statin and aspirin use). Oral HPV infection status is among the confounders included in our analysis. The high quality of HPV information collected, with analyses conducted in a world-renowned laboratory, ensured the precision and accuracy of our HPV data.

All study subjects were recruited locally in Montreal, Canada. The Canada Health Act dictates that all Canadian residents are entitled to free health care regardless of their social and financial status. Such a setting is advantageous to our analysis as it minimizes selection bias arising from barriers to care. Thus, although we had a relatively low response rates, our sample is very likely a good representation of Canadian HNC patients.

7.3 Methodological Considerations and Limitations

The methodological limitations of the study were briefly touched upon in the manuscript. This section will describe and discuss additional limitations and considerations.

7.3.1 Sample Size

Sample size is crucial to the quality of a cohort study. Unfortunately, as an exploratory study on this topic, our study suffered from a small sample size, which greatly limited the power of our statistical analysis. The initial 350 subjects extracted from the HeNCe Life database was reduced to 303 due to incomplete medical records; this number was further reduced to 274 (30 beta-blocker users and 244 non-users) due to missing information in the complete case analysis. A post-hoc power analysis for the adjusted Cox proportional hazards model concluded that the power associated with the HR produced was insufficient to reject the null hypothesis, given that the null hypothesis is false. In other words, there is a possibility that our failure to observe a significant association between beta-blocker use and improved HNC survival is due to insufficient power as a consequence of having a small sample size.

7.3.2 Selection Bias

Similar to all cohort studies, the integrity of our analysis is susceptible to selection bias. This form of bias refers to the phenomenon in which individuals, groups or data for analysis are not representative of the population because of the way they are selected. The effect of selection bias is that an association between exposure and outcome among those included in the study is different than those who were eligible for the study, that is, if the exposure, outcome or confounders factors are linked to the probability to be selected for the study, this will lead to spurious associations. In this section, we will consider a few scenarios in which selection bias could have jeopardized the integrity of our analysis.

7.3.2.1 Subject Selection Bias

Subject selection bias arises when a criterion for subject selection is a common effect of both the exposure and the outcome of a study. This type of selection bias generally poses a greater threat to retrospective cohort studies, since the investigators usually have knowledge of both the exposure and the outcome status of the subjects at the time of subject selection. Another common way for this bias to occur is when the selection is conditioned on recruiting subjects who have given consent. Subjects who have been

exposed and have experienced the outcome will generally have more incentives to participate in the study.

The subjects in our study were determined prospectively; we had no knowledge of the survival status of subjects at the time of subject selection. In addition, our sample is a sub-cohort of the HeNCe study (Canadian site), which was not originally set up to study beta-blockers. Therefore, having knowledge of neither the exposure nor the outcome status at the time of subject selection, minimizes the possibility of subject selection bias.

However, we cannot completely rule out the possibility of selection bias due to the low response rate from the HeNCe Life study sub-cohort. Nonetheless, the distribution of the main risk factors among our cases are similar to other HNC studies suggesting the representativeness of our sample.

7.3.2.2 Selection Bias Due to Loss to Follow Up

Another common scenario in which selection bias can affect the validity of a cohort study is when subjects who exit the study due to loss to follow up have a different probability of experiencing the outcome compared with the remaining subjects in the study. Unlike subject selection bias, this type of bias can pose a threat to the analysis of a prospective cohort study (247). Regarding our study, if subjects who are exposed to beta-blockers and have experienced mortality have a greater or lower chance of being lost to follow up, our estimated hazard ratio will be overestimated or underestimated, respectively. This will occur when we restrict our analysis to subjects with complete follow-ups.

To assess the extent to which selection bias due to loss to follow up may have impacted our study, we resorted to a rule of thumb. The rule states that when the loss-to-follow-up rate is lower than 5%, the study will suffer from little bias; if the rate is higher than 20%, the study will suffer from a severe bias (248). Out of the 303 subjects who fit our inclusion criteria, there were only 11 cases of loss to follow up that accounted for less than 5% of the entire sample. Therefore, we believe that selection bias due to loss to follow up if any would only have a negligible effect on the validity of our analysis.

7.3.3 Missing Data Bias

Missing data bias is very similar to selection bias due to loss to follow up in nature. It arises when analysis is restricted to subjects without missing information (complete case analysis), and subjects with missing data have a different chance of experiencing the outcome compared with subjects with complete information. Hypothetically, if subjects who are exposed to beta-blockers and have experienced mortality have a greater or lower chance of having missing values, our estimated hazard ratio will be overestimated or underestimated, respectively. Although we could not theoretically link the missing information to neither the exposure nor the outcome, to assess the potential impact of this bias on the validity of our study, we will apply the same rule of thumb mentioned above (see section 6.3.2.2). In our total of 303 subjects, 29 subjects were excluded from the analysis due to missing information, which represented 9.6% of the sample. Therefore, according to the rule of thumb, missing data bias is unlikely to have had a serious impact on the validity of our analysis.

7.3.4 Information Bias

Information bias is caused by measurement errors. For categorical variables, such as the exposure and outcome in our study, the bias is also often referred to as “misclassification”.

Based on the relationship between the measurement error and the exposure and outcome status, misclassification is classified as either non-differential or differential. Misclassification is considered non-differential when the error is only related to one variable (the exposure or the outcome) and not both the exposure and the outcome simultaneously. This type of misclassification affects both cohort and case-control studies.

When the measurement error is simultaneously associated with the exposure and the outcome (the error is a common effect of the two variables), the misclassification is considered differential. This type of bias usually affects only case-control studies.

In our cohort study, the exposure and the outcome status were extracted from two separate sources. The outcome information was extracted by government employees

without knowledge of the exposure status of our subjects. Therefore, it is unlikely that any measurement error of the exposure or the outcome would be linked to both variables simultaneously. Thus, we assume that all misclassification potentially present in the study is non-differential.

The outcome information regarding HNC overall survival in our study was retrieved from a reliable governmental registry. The identities of the individuals in the registry were confirmed by matching full names and dates of birth to those in our database. These facts lead us to be confident in the accuracy of our outcome variable and the assumption that there is no measurement error in our outcome variable.

Our exposure, beta-blocker use, is a binary variable created based on the recall of medication use by our subjects in their consultation appointments. As a result, the accuracy of the exposure variable is susceptible to errors caused by inaccurate recall, especially considering that the majority of our subjects are elderly patients. This scenario describes the type of information bias called “recall bias”, and in the case of our study, a cohort study, the bias will be non-differential. For a binary exposure, a non-differential misclassification will usually bias the estimate towards the null, and the magnitude of the effect is usually determined by specificity, sensitivity, and the prevalence of the exposure (249). The lower the specificity, sensitivity, and prevalence, the greater the extent to which the estimate will be biased towards the null. As a result, we postulate that our HR underestimated the effect of beta-blocker use on HNC survival.

7.3.4 Bias due to confounding

Observational studies allow for possible confounding by indication, which is a special type of confounding in which individuals who are prescribed or taking a medication are fundamentally different from those who do not take the drug. However, this would be unlikely because neither doctors nor patients would be aware of the effect of beta blockers on HNC survival.

7.3.5 Other Limitations and Considerations

Unfortunately, we could not acquire accurate and precise information regarding the prescription time interval and the recommended daily dose for all the drugs analyzed in our study, including the main exposure, beta-blocker use. Instead, we had to rely on patients' recall at the time of HNC diagnosis to determine the type of drugs chronically used by our subjects. In addition to increasing the potential recall bias, it prevented us from studying the potential dose-response relationship between beta-blocker use and HNC survival. Analyzing the dose-response relationship would allow us to account for the effects of beta-blockers in short term and temporary users that were ignored by the binary exposure variable. Moreover, this information would allow us to more precisely assign exposure status to each subject based on time length of exposure, and thus decrease the potential for misclassification.

For our outcome, we could not acquire information regarding the cause of death for each deceased subject, which limited our analyses to overall survival, instead of cancer-specific survival. Although overall survival is an important measure for cancer prognosis, its advantage over cancer-specific survival gradually diminishes with increasing age of a sample due to increasing competing risks. Thus, it could potentially endanger the validity of our analysis. However, given our low statistical power due to the small sample size, it was perhaps more suitable for us to use all-cause mortality as an outcome measure due to its larger number of events compared with cancer-specific mortality.

During the study, we discovered that the great majority of the beta-blockers identified were selective (97%). Although this reflected the fact that selective beta-blockers are a better choice for managing CVD and hypertension, it prohibited us from studying the potential effect of non-selective beta-blockers. All the *in vitro* and animal studies that show positive effects of beta-blockers on neoplasms featured the use of non-selective beta-blockers, propranolol in particular (115, 116, 118). In addition, recent studies investigating the potential antineoplastic properties of beta-blockers demonstrated that there is an emerging trend of exploring the effect of selective and non-selective beta-blockers separately (132, 144, 246). The near-absent proportion of non-selective beta-blockers in our study essentially means that our results can be only used to interpret the effect of selective beta-blockers on HNC survival, not that of the non-selective ones.

7.3.6 Internal and External Validity

The biases mentioned above, including selection bias, information bias and bias due to confounding, are all threats to the internal validity of our study. Internal validity is a measure of the extent to which a measured effect is attributed to a causal relationship between the exposure and the outcome in the source population, not by systematic errors (bias). In contrast, external validity measures how generalizable the study result is to the target population. As we discussed above, we implemented several measures to improve internal validity, which is a prerequisite for external validity. Moreover, representativeness or external validity is not always necessary to generalize the findings. For example, smoking is a major risk factor for many chronic diseases, and its effects are mostly shown by observational studies which, compared to descriptive studies, have low external but high internal validity.

Our target population is HNC patients in general. Our sample features patients with all the different severities of HNC. In addition, the unique healthcare system in Canada provides equal health care for all its citizens, regardless of their SES. This resulted in the large diversity of social classes in our sample. These factors enhanced the generalizability of our results.

7.4 Future study directions

Future studies related to this subject should primarily address the limitations of this study. First, they should incorporate a large sample size that offers sufficient statistical power. For studies using the HeNCe Life database, the investigators should take advantage of the multi-center design and combine data from all the sites (Brazil, Canada and India). Aside from providing a larger sample size, this combination will also offer a more diverse sample. Studying the potential differential effect of beta-blockers on HNC prognosis among patients from different ethnicity, race and cultural backgrounds will render the results more generalizable to all HNC cancer patients.

Second, new studies should acquire accurate and detailed information on beta-blocker use for each subject. The information should include prescribed daily dose and duration

of use, and should be acquired from credible sources, such as pharmacy prescription databases, rather than patient recall. This will enable the studies to allocate the exposure status of each patient more precisely and avoid immortal time bias, a form of misclassification caused by failure to account for exposure status change over time. In addition, it would allow dose-response analysis, which is a crucial criterion for establishing causality.

Third, new studies should secure sufficient numbers of both selective and non-selective beta-blocker users to study the role of beta-blocker selectivity in HNC prognosis. Recent studies exploring the anti-carcinogenic properties of beta-blockers have started to analyze the effects of the selective and non-selective beta-blockers separately. In a study on epithelial ovarian cancer, the survival time associated with non-selective beta-blockers was shown to be three times as long as the one associated with selective beta-blockers. Meanwhile, the survival time of selective beta-blocker users was not significantly longer compared with that of the unexposed patients (144). Such a research strategy is supported by the difference between the expression patterns of beta-1 and beta-2 adrenergic receptors in the human body. Since the great majority of beta-1 adrenergic receptors are located in the heart, its antagonist, the selective beta-blocker, is expected to have very localized effects in this region. In contrast, the beta-2 adrenergic receptors are expressed throughout the sympathetic nervous system, therefore the effect of its antagonist, the non-selective beta-blocker, is expected to be more systemic and widespread. In addition, the majority of *in vitro* and animal experiments that have demonstrated anticarcinogenic properties of beta-blockers have investigated only non-selective beta-blockers; to our knowledge, no studies have tested the effect of selective beta-blockers in these settings.

Finally, after acquiring a large sample size and sufficient statistical power, future studies should examine the effect of beta-blockers on HNC cancer-specific mortality. Although offering fewer outcome events compared with other measures of survival, cancer-specific survival analysis allows researchers to directly and more accurately measure the biological impact of beta-blocker use on HNC prognosis and survival. In comparison, although overall survival offers a larger number of outcome events, the accuracy of the

effect can be compromised by the inclusion of non-cancer mortalities. The compromise will be greater as the age of the subject increases. In addition, given large sample sizes and sufficient power, new studies should also perform analysis that is restricted to HNC patients with hypertension or CVD. Patients with these indications comprise the great majority of long-term beta-blocker users. Therefore, it is in a researcher's best interest to use restriction to eliminate potential confounding by indication when analyzing the effect of beta-blockers.

In addition, future *in vitro* and animal studies should aim to explore the precise expression patterns and locations of beta-1 and beta-2 adrenergic receptors in HNC of different origins (oral cavity, pharynx and larynx). To our knowledge, very few studies have tried to explore this topic. Gaining a solid understanding of the beta-adrenergic receptors is crucial to the establishment of a solid biological rationale, on which logical hypotheses can be based. Moreover, *in vitro* and animal studies should examine the effects of selective beta-blockers on tumours, which, to our knowledge, has never been done before. This will provide researchers with insights into the potential role that beta-blocker selectivity plays in HNC prognosis and survival.

7.5 Public Health Implications

Associated with a high mortality rate, high suicide rate and loss of upper aerodigestive functions, HNC represents a tremendous burden on the healthcare system and a drastic deterioration of a patient's quality of life. The beta-blocker, due to its recently discovered therapeutic effects for treating infantile hemangioma, has rapidly attracted the attentions of cancer researchers. There have been studies on the effect of beta-blockers on the risk, prognosis and survival of a large variety of cancers. In the case of breast cancer, overwhelming positive results indicating the effectiveness of beta-blocker against mortality have led to a clinical trial (125). If proven effective for HNC, beta-blocker use can potentially become an affordable and easy clinical intervention on top of the traditional treatments (chemotherapy, radiotherapy and surgery).

8. CONCLUSION

After analysis with the Kaplan-Meier method and Cox proportional hazards models, we did not find a significant association between committed beta-blocker use and overall survival in HNC among our sample of Canadian subjects.

However, this is the first study attempting to explore this topic. Acting as an exploratory study, it can provide larger observational studies with guidelines for experimental design in the future. In addition, the results from this study can act as a reference for future comparisons of results. Moreover, the combination of databases we created for this study will be valuable not only for future studies related to this topic, but also for a wide range of other research interests.

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10. APPENDIX I

Search Strategy

Database(s): **Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily, Ovid MEDLINE(R) and Ovid OLDMEDLINE(R)** 1946 to Present

Search Strategy:

#	Searches	Results
1	exp "Head and Neck Neoplasms"/	261933
2	((cancer* or tumo?r* or neoplas* or metaplas* or carcinoma* or adenoma* or adenocarcinoma* or metastasi* or sarcoma* or ameloblastoma* or (odontogenic* adj3 keratocyst*) or malignan*) adj5 (head or neck or uadt or "upper aero-digestive" or "upper aerodigestive" or esophag* or oesophag* or face or facial or oral* or intra-oral* or intraoral* or "intra oral*" or mouth or buccal or gingiv* or gum* or lip or lips or labial* or palat* or palatal* or salivary or parotid or lingual* or sublingual* or sub-lingual* or mandib* or submandib* or sub-mandib* or maxill* jaw or jaws or tongue* or glossal* or otor?inolaryngolog* or throat or ear or ears or auricle* or auricular or larynx* or laryngeal* or nose* or nasal* or paranasal* or sinus or hypopharynx or hypopharyngeal* or nasopharynx or nasopharyngeal* or oropharynx or oropharyngeal or tonsil* or parathyroid or thyroid or trachea* or cheek*)).tw.	232632
3	(exp Head/ or exp Neck/) and exp Neoplasms/	29142
4	or/1-3	345429
5	exp Adrenergic beta-Antagonists/	79860
6	(beta adj3 block*).tw.	46755
7	B-blocker.tw.	89
8	or/5-7	99425
9	4 and 8	352
10	limit 9 to english	306

Result 1.

Authors Chow W; Amaya CN; Rains S; Chow M; Dickerson EB; Bryan BA.

Title Growth Attenuation of Cutaneous Angiosarcoma With Propranolol-Mediated beta-Blockade.

Source JAMA Dermatology. 151(11):1226-9, 2015 Nov 1.

Abstract

IMPORTANCE: Patients with stage T2 multilesion angiosarcomas of the scalp and **face** that are larger than 10 cm demonstrate a 2-year survival rate of 0%. To our knowledge, major therapeutic advances against this disease have not been reported for decades. Preclinical data indicate that **blocking** beta-adrenergic signaling with propranolol hydrochloride disrupts angiosarcoma cell survival and xenograft angiosarcoma progression.

OBSERVATIONS: A patient presented with a beta-adrenergic-positive multifocal stage T2 cutaneous angiosarcoma (>20 cm) involving 80% of the scalp, left forehead, and left **cheek**, with no evidence of **metastasis**. The patient was immediately administered propranolol hydrochloride, 40 mg twice a day, as his workup progressed and treatment options were elucidated. Evaluation of the proliferative index of the **tumor** before and after only 1 week of propranolol monotherapy revealed a reduction in the proliferative index of the **tumor** by approximately 34%. A combination of propranolol hydrochloride, 40 mg 3 times a day, paclitaxel poliglumex, 2 mg/m2 infused weekly, and radiotherapy during the subsequent 8 months resulted in extensive **tumor** regression with no detectable metastases.

CONCLUSIONS AND RELEVANCE: Our data suggest that beta-blockade alone substantially reduced angiosarcoma proliferation and, in combination with standard therapy, is effective for reducing the size of the **tumor** and preventing metastases. If successful, beta-blockade could be the first major advancement in the treatment of angiosarcoma in decades.

11. APPENDIX II

FileMaker Database

Country **Id No:**

A. General Information

New Record
Find
Next Section
Comments

A1. Subject Initials

A2. Medicare Number

A3. Hospital/ Recruitment site

- 01 Jewish General Hospital
- 02 Hospital Notre-Dame
- 03 Montreal General Hospital
- 04 Royal Victoria Hospital
- 99 Don't know

A4. Sex

- 00 Female
- 01 Male

A5. Date of Birth Day Month Year

A6. Date of first visit

A7. Date of last visit

A8. Language

- 00 French
- 01 English
- 02 Other (Specify)
- 99 Don't know

A9. Hospital File/Folder Number

Creator Name
Creator Date
Modifier Date
Modifier Name
Modified Time
Created Time

Country **Id No:**

B. Details of Data Collection

B1. Data Collected by (Initials):

B2. Date of Data Collection

Day **Month** **Year**

New Record
Find
Next Section
Previous Section
Comments

C. Disease details

C1. Cancer site:

○ Tongue ○ Floor of mouth ○ Gingiva ○ Buccal mucosa

○ Oropharynx ○ Hypopharynx ○ Supra glotic ○ Glotic ○ Sub glotic ○ Other

○ CONTROL

Specify (Side, extension, etc.)

C2. Date of Diagnosis **Day** **Month** **Year**

C2.2. Date of Diagnosis (Cleaned) **Day** **Month** **Year**

C3. Global TNM Stage **T:** **N:** **M:**

(Tumour, Lymph Nodes, Metastasis)

Type of TNM Staging

○ Clinical Staging ○ Radiological Staging ○ Not Applicable

C4. Date of Diagnosis of metastasis: **Day** **Month** **Year**

C5. Metastasis site: (Neck, Lung, Brain, Breast, etc)

C6. Presence of synchronous second primary cancer?

○ No ○ Yes (Specify site) ○ Not Applicable ○ Don't Know

If Yes, specify site

Country **Id No:**

D. Diagnostic procedures

New Record
Find
Next Section
Previous Section
Comments

D1. Biopsy of primary lesion

Date of Biopsy

Biopsy Report No:

○ No ○ Yes ○ Not Applicable ○ Info Missing

Hospital/clinic where biopsy was done: ○ Notre-Dame ○ Other (Specify):

○ Not Applicable ○ Info Missing

D2. Results of biopsy

Tumor Differentiation ○ Well Differentiated ○ Moderately Differentiated ○ Poorly Differentiated ○ Info Missing

○ Not Applicable

Keratinization ○ Keratinized ○ Non-Keratinized ○ Not Applicable ○ Info Missing

Any other details

D3. Sentinel Lymph node biopsy

Date of SLNB

○ No ○ Yes (Specify results) ○ Not Applicable ○ Don't Know

Details of results

D4. PET Scan

Date of PET Scan

○ No ○ Yes (Specify results) ○ Not Applicable ○ Don't Know

Details of results

Country **Id No:**

D. Diagnostic procedures

New Record
Find
Next Section
Previous Section
Comments

D5. Chest X-ray **Date of Chest X-ray**

(00) No (01) Yes (Specify results) (88) Not Applicable (99) Don't Know

Details of result

D6. Was HPV typing performed? **Date of HPV typing**

(00) No (01) Yes (Specify results) (88) Not Applicable (99) Don't Know

If Yes, Give details of the result

(00) No HPV detected (01) HPV detected (88) Not Applicable (99) Info Missing

HPV Types detected

Any other details of result

D7. Endoscopy **Date of Endoscopy**

(00)No (01) Yes (Specify results) (88) Not Applicable (99) Don't Know

Type of Endoscopy

Details of result

Country **Id No:**

D. Diagnostic procedures

New Record
Find
Next Section
Previous Section
Comments

D8. CT Scan **Date of CT Scan**

(00) No (01) Yes (Specify results) (88) Not Applicable (99) Don't Know

Details of result

D9. MRI **Date of MRI**

(00)No (01) Yes (Specify results) (88) Not Applicable (99) Don't Know

Details of result

D10. Any Other diagnostic test **Date of test**

(00)No (01) Yes (Specify results) (88) Not Applicable (99) Don't Know

Details of test and result

Country **Id No:**

E. Comorbidities

New Record
Find
Next Section
Previous Section
Comments

(Cardiovascular system)

				Date of diagnosis	Details if any
E1. Myocardial Infarction	(00) No	(01) Yes	(99) Missing		
E2. Angina/Coronary Artery Disease	(00) No	(01) Yes	(99) Missing		
E3. Arrhythmias	(00) No	(01) Yes	(99) Missing		
E4. Hypertension	(00) No	(01) Yes	(99) Missing		
E5. Venous Disease	(00) No	(01) Yes	(99) Missing		
E6. Peripheral Arterial Disease	(00) No	(01) Yes	(99) Missing		

(Respiratory system)

E7. Restrictive lung Disease (COPD, Chronic bronchitis, Emphysema or Asthma)	(00) No	(01) Yes	(99) Missing		

(Gastrointestinal system)

E8. Hepatic (Chronic/ Acute hepatitis, cirrhosis, portal hypertension, Encephalopathy, Ascites, Jaundice Bilirubin >2)	(00) No	(01) Yes	(99) Missing		
E9. Stomach/Intestine (Peptic Ulcer, Inflammatory bowel syndrome, Chronic malabsorption syndrome)	(00) No	(01) Yes	(99) Missing		
E10. Pancreas (Acute/Chronic pancreatitis, Bleeding GI)	(00) No	(01) Yes	(99) Missing		

Country **Id No:**

New Record
Find
Next Section
Previous Section
Comments

E. Comorbidities (Continued...)

(Neurological system)

				Date of diagnosis	Details if any
E11. Stroke	(00) No	(01) Yes	(99) Missing		

(Acute/Chronic Stroke with or without neurologic deficit,Past or recent TIA)

E12. Dementia	(00) No	(01) Yes	(99) Missing		

(Mild/Moderate/Severe Dementia)

E13. Paralysis	(00) No	(01) Yes	(99) Missing		

(Paraplegia, Hemiplegia)

E14. Neuromuscular	(00) No	(01) Yes	(99) Missing		

(MS, Parkinson's Myathenia Gravis, or Chronic neuromuscular disorder)

(Renal system)

E15. End-stage renal Disease	(00) No	(01) Yes	(99) Missing		

(Chronic renal insufficiency, Acute/Chronic dialysis)

(Endocrine system)

E16. Diabetes Mellitus	(00) No	(01) Yes	(99) Missing		

If Yes, specify type (01) Type I (02) Type II (99) Info Missing (88) Not Applicable

Diabetes related end-organ failure

(00) None (01) Retinopathy (02) Neuropathy (03) Nephropathy (04) Coronary artery Disease (05) Pheripheral arterial Disease

(99) Info Missing (88) Not Applicable

(Rheumatologic)

E17. (Rheumatoid arthritis, Systemic lupus, Mixed connective tissue disorder, Polymyositis, Rheumatic polymyositis)	(00) No	(01) Yes	(99) Missing		

Country **Id No:**

E. Comorbidities (Continued...)

New Record
Find
Next Section
Previous Section
Comments

(Other relevant hospitalization history / surgical history)

E18. Did the participant ever had any relevant surgical procedure? (00) No (01) Yes (99) Info Missing

Whether the surgery/hospitalization for or related to Cancer? (00) No (01) Yes (99) Info Missing

Specify (site, reason, any complications)

Date of procedure

Any other comorbidities

E19. Does the particiapant have any other comorbidities? (00) No (01) Yes (99) Info Missing

Is it related to Cancer? (00) No (01) Yes (99) Info Missing

Specify (site, reason, any complications)

Date of procedure/ diagnosis

Country **Id No:**

F. Family History of Cancer

New Record
Find
Next Section
Previous Section
Comments

F1. Did any member of participant's family ever had cancer?

(00) No (01) Yes (99) Info Missing

F2. Details

Relationship	Status	Current/ Last Age	Type of Cancer	Age at Diagnosis
(01) Mother	Deceased			
(02) Father	Alive	Info Missing		Info Missing
(03) Sister	(88) NA	(888) NA		(888) NA
(04) Brother				
(05) Daughter				
(06) Son				
(07) Grand Mother				
(08) Grand Father				
(09) Aunt/Uncle				
(88) Not Applicable				

F3. Did any other member of participant's family ever had cancer?

(00) No (01) Yes (99) Info Missing (88) Not Applicable

Country **Id No:**

G. Medication - Beta-blockers

G0. Did the participant ever had taken Beta-blockers? (00) No (01) Yes (99) Info Missing

New Record
Find
Next Section
Previous Section
Comments

	Generic name	Brand name	Dose	No:of tabs/day	Age at starting	Age at stopping
G1.						
G2.						
G3.						
G4.						

Medication - Other

(888 if not applicable; 999 if missing)

G5.						
G6.						
G7.						
G8.						
G9.						
G10.						

H. Allergy to Medication

H0. Did the participant has reported to have any allergy to medication? (00) No (01) Yes (99) Info Missing

H1. Drug 1 **H2. Drug 2** **H3. Drug 3** **H4. Drug 4**

Details if any

Country **Id No:**

I. Habits

New Record
Find
Next Section
Previous Section
Comments

Tobacco Smoking

I1. Did the participant has ever smoked any tobacco product?

(If the participant ever smoked in last one year then considered as current smoker)

(00) None Smoker (01) Current Smoker (02) Past Smoker (99) Info Missing

I2. Average Pack Years **I3. Age at starting** **I4. Age at stopping**

(888 if not applicable; 999 if missing)

Alcohol consumption

I5. Did the participant has ever consumed any alcohol product?

(00) None user (01) Current user (02) Past user (03) Social/Occasional User (99) Info Missing

Type of beverage	Amount/day	Unit/day	Age at starting	Age at stopping

Any other drug consumption

I6. Did the participant has ever consumed any other drugs?

(00) None user (01) Current user (02) Past user (99) Info Missing

Type	Unit/day	Age at starting	Age at stopping

Country **Id No:**

J. Systemic Therapy

New Record
Find
Next Section
Previous Section
Comments

J0. Did participant received systemic therapy?

(00) No (01) Yes (99) Info Missing

J1. Primary Systemic Therapy + Concurrent Radiotherapy

(01) Cisplatin (02) Cetuximab (03) Carboplatin + Infusional 5-FU

(04) 5-FU + Hydroxyurea (05) Cisplatin + Paclitaxel (06) Cisplatin + Infusional 5-FU

(07) Carboplatin/Paclitaxel (08) Weekly Cisplatin

Primary Chemotherapy with Postoperative Chemoradiation

(09) Cisplatin 100mg/m2 IV + radiotherapy

Induction Chemotherapy / Sequential Chemotherapy

(10) Docetaxel + Cisplatin + 5-FU (11) Paclitaxel + Cisplatin + Infusional 5-FU

Single agents

(12) Carboplatin (13) Paclitaxel (14) Docetaxel (15) 5-FU

(16) Methotrexate (17) Cetuximab (18) Cisplatin (19) Ifosfamide

(20) Bleomycin (21) Capecitabine (22) Vinorelbine (88) Not Applicable (99)Info Missing

J2. Date of start of therapy

J3. Date of stop of therapy

Country **Id No:**

K. Radiotherapy

New Record
Find
Next Section
Previous Section
Comments

K0. Did participant received radiotherapy?

(00) No (01) Yes (99) Info Missing

K1. Does of radiotherapy in Gray

K2. Number of fractions

K3. Radio therapy Technique **mv**

K4. Date of start of therapy

K5. Date of stop of therapy

K6. Was the radiotherapy aimed as palliative?

(00) No (01) Yes (88) Not Applicable (99) Info Missing

Any other relevant details

L. Surgery

L0. Did participant received surgery for cancer?

(00) No (01) Yes (99) Info Missing

L1. Date of surgery

Any other relevant details (Type, margins, Neck dissection, etc)

Country **Id No:**

M1. Subsequent Visit

Visit no:

Date of visit:

Type of visit: (00) Pre-treatment (01) During treatment (02) Post treatment

New Record
Find
Next Section
Previous Section
Comments

Functional measures

Dysphagia (00) No (01) Yes (88) Not Applicable (99) Info Missing

If Yes, specify the grade **Medication prescribed**

Radio-Mucositis (00) No (01) Yes (88) Not Applicable (99) Info Missing

If Yes, specify the grade **Medication prescribed**

Radio-Dermatitis (00) No (01) Yes (88) Not Applicable (99) Info Missing

If Yes, specify the grade **Medication prescribed**

Xerostomia (00) No (01) Yes (88) Not Applicable (99) Info Missing

If Yes, specify the grade **Medication prescribed**

KPS **ECOG** **Weight** **Unit**

Neck Examination (Adenopathy) (00) No (01) Yes (88) Not Applicable (99) Info Missing

If Yes, give details **Medication prescribed**

M1

Country **Id No:**

M1.2 Subsequent Visit

ORL (00) No (01) Yes (88) Not Applicable (99) Info Missing

Sites Buccal cavity Oro- pharynx Hypo- pharynx Larynx Other

Lesion (00) No (01) Yes (88) Not Applicable **Details**

RPL (00) No (01) Yes (88) Not Applicable **Details**

New Record
Find
Next Section
Previous Section
Comments

Odynophagia (Pain while swallowing) (00) No (01) Yes (88) Not Applicable (99) Info Missing

If Yes, give details **Medication prescribed**

Dysgegusia (distortion of taste) (00) No (01) Yes (88) Not Applicable (99) Info Missing

If Yes, give details **Medication prescribed**

Dysphonia (distortion of voice) (00) No (01) Yes (88) Not Applicable (99) Info Missing

If Yes, give details **Medication prescribed**

Recurrence of lesion (00) No (01) Yes (88) Not Applicable (99) Info Missing

New Secondary lesion (00) No (01) Yes (88) Not Applicable (99) Info Missing

Any other Test or finding

Any other Details/ Medication

M1

Does subject has any subsequent visit?

(00) No (01) Yes

Country **Id No:**

N. Recurrence - Disease details

New Record
Find
Next Section
Previous Section
Comments

N0. Did subject ever had a recurrence of lesion

(00) No (01) Yes (88) Not Applicable (99) Info Missing

N1. Cancer site:

Tongue Floor of mouth Gingiva Buccal mucosa

Oropharynx Hypopharynx Supra glotic Glotic Sub glotic Other

Not Applicable

Specify (Side, extension, etc.)

N2. Date of Diagnosis **Day** **Month** **Year**

N3. Global TNM Stage **T:** **N:** **M:**
(Tumour, Lymph Nodes, Metastasis)

Type of TNM Staging

Clinical Staging Radiological Staging Not Applicable

N4. Date of Diagnosis of metastasis: **Day** **Month** **Year**

N5. Metastasis site: (Neck, Lung, Brain, Breast, etc)

Country **Id No:**

O. Second Primary - Disease details

New Record
Find
Next Section
Previous Section
Comments

O0. Did participant every had a second primary lesion?

(00) No (01) Yes (88) Not Applicable (99) Info Missing

O1. Cancer site:

Tongue Floor of mouth Gingiva Buccal mucosa

Oropharynx Hypopharynx Supra glotic Glotic Sub glotic Other

Not Applicable

Specify (Side, extension, etc.)

O2. Date of Diagnosis **Day** **Month** **Year**

O3. Global TNM Stage **T:** **N:** **M:**

(Tumour, Lymph Nodes, Metastasis)

Type of TNM Staging

Clinical Staging Radiological Staging Not Applicable

O4. Date of Diagnosis of metastasis: **Day** **Month** **Year**

O5. Metastasis site: (Neck, Lung, Brain, Breast, etc)

Country **Id No:**

Comments

New Record

Find

Previous Section

Question No:	Comment

o10Duplicate 00 No
01 Yes

Additional comments