**Use of beta-blockers and risk of breast cancer:   
a population-based case-control study**

**Study protocol**

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**Background**

There is some evidence that beta-blocking agents might influence breast cancer risk and prognosis (\*refs). Beta-blockers are used for a number of conditions, notably hypertension, cardiovascular prophylaxis, and arrhythmias (\*refs). Experimental data suggests that beta-blockers may inhibit tumour growth and progression, possibly through \*, but other mechanisms have also been suggested (\*refs). Epidemiological studies evaluating the association between beta-blockers and breast cancer have yielded equivocal results. This prompted us to investigate the association between use of beta-blockers and breast cancer risk in a large population-based case-control study.

**Methods**

The study will be designed as a population-based case-control analysis. By comparing use of beta-blockers among persons diagnosed with cancer (cases) and cancer-free persons (controls), we will estimate odds ratios (ORs) for cancer associated with use of beta-blockers.

We will use data from four Danish nationwide registries: the Danish Cancer Registry, National Patient Register, National Prescription Registry, and Civil Registration System. Virtually all medical care in Denmark is furnished by the national health authorities, whereby these data resources allow true population-based studies covering all inhabitants of Denmark.

**Data sources**

The Danish Cancer Registry (1;2) has recorded incident cases of cancer on a nationwide basis since 1943 and has been shown to achieve almost complete ascertainment of cancer cases (1;3). Cancer diagnoses in the Cancer Registry are recorded according to the International Classification of Diseases, version 10 (ICD-10), and the ICD for Oncology (ICD-O-1-3) for topography and morphology codes.

The Danish National Patient Register contains nationwide data on all non-psychiatric hospital admissions since 1977 and out-patient contacts since 1995. Discharge/contact diagnoses have been coded according to ICD-8 from 1977 to 1993 and ICD-10 since 1994 (4).

The Danish National Prescription Registry (5) contains data on all prescription drugs filled by Danish citizens since 1995. Prescription data includes the type of drug, date of dispensing, and quantity. The dosing information and the indication for prescribing are not available. Drugs are categorized according to the Anatomic Therapeutic Chemical (ATC) index, a hierarchical classification system developed by the WHO, and the quantity dispensed for each prescription is expressed by the number of defined daily doses (DDD) (6).

The Danish Civil Registration System (7) contains data on date of death and migration to and from Denmark, which will allow us to extract controls and to keep track of all subjects.

The above data sources will be linked by the personal identification number, a unique identifier assigned to all Danish residents since 1968 that encodes gender and date of birth (7). All linkages will be performed within Statistics Denmark (5;8).

**Cases and controls**

Cases will be all female residents of Denmark with a histological verified first time diagnosis of breast cancer (ICD10, C50) between January 1, 2000 and December 31, 2009, using the date of the cancer diagnosis as the index date. Inclusion criteria will be 1) age 18-85 years at index date, 2) histological verification of breast cancer and 3) residency in Denmark in 10 years prior to index date. Exclusion criterion will be a previous history of cancer (except non-melanoma skin cancer).

Controls will be selected by use of a risk set sampling strategy. For each case, we will select 10 controls among all Danish citizens matched by gender and birth year and month, and complying with the above inclusion and exclusion criteria for cases.

Subjects will be eligible for sampling as controls before they become cases. Thereby, the calculated odds ratios (ORs) are unbiased estimates of the incidence rate ratios (IRRs) that would have emerged from a cohort study in the source population (9).

**Exposure definition**

Beta-blockers will be defined as any drug within the ATC group C07. This group is mainly comprised of pure beta-blockers (C07A), but also contains combinations of beta-blockers other drugs, such as thiazides and other antihypertensives (C07B, C07C and C07F). The main group C07A can be subdivided into non-selective (C07AA) or selective (C07AB) beta-blockers and combined alpha and beta-blocking agents (C07AG).

For all exposure calculations, we will disregard exposure obtained less than one year prior to the index date in order to reduce the possibility for reverse causation. (10;11).

Ever use of beta-blockers will be defined as filling of one or more prescriptions for any beta-blocker more than one year prior to the index-date. Long-term use of beta-blockers will be defined as ≥5 years of treatment more than one year prior to the index-date.

The expected duration of each prescription is not recorded in the Danish National Prescription Registry. Using a method based on the waiting time distribution (12;13), we have estimated that each beta-blocker prescription should be assigned an approximate duration of 81 days. Consequently, we will define consecutive prescriptions as part of the same continuing treatment period if the prescriptions were dispensed within 81 days after date of the preceding prescription. Longer intervals between prescriptions will be regarded as the treatment had been paused. No adjustment will be made for overlaps between prescriptions. Similarly, the exposure period assigned to single prescriptions or the last prescription in a treatment episode will be 81 days.

**Main analysis**

The analysis will be performed as a conventional matched case-control study. ORs for breast cancer associated with long-term use (≥5 years) of beta-blockers will be calculated using conditional logistic regression adjusting for potential confounders. In the main analyses, use of beta-blockers will compared to non-users of beta-blockers.

The following confounders will be included in the analyses:

a) Use of drugs (≥ 2 fillings prior to index date) known or suspected to modify the risk of breast cancer, including NSAIDs (ATC, M01A excl. M01AX); aspirin (B01AC06, B01AC30, N02BA01 and N02BA51); statins (C10AA); hormone replacement therapy (G03C, G03D, G03F and G03HB01); antidepressants (N06A); and anticoagulants (B01AA).

b) Prior diagnoses of diseases known or suspected to modify the risk of breast cancer, any registered condition related to heavy alcohol abuse (ICD-8: 291, 303, 425.5, 537.5, 571.0, 571.1, 571.2, 571.3, 577.10 ; ICD-10: G31.2, G62.1, G72.1, I42.6, F10.2, K70, K86.0) or any prescription for drugs used to treat alcoholmism (ATC, N07BB); diabetes (composite measure of diagnoses (ICD-8: 249.00, 249.09, 250.00, 250.09; ICD-10: E10-E14) or any prescription of anti-diabetics (ATC: A10)); and history of breast adenoma (ICD-8: 21130-49; ICD-10: D12).

c) Charlson Comorbidity Index (CCI) score (14;15), in which each disease category has an associated weight based on the adjusted risk of one-year mortality.  The level of comorbidity will be defined as none (CCI score: 0), low (CCI score: 1), medium (CCI score: 2); or high (CCI score: ≥3);

d) Parity, classified into the following categories according to number of live births: 0, 1, 2+, or “missing information” (8).

e) Highest achieved education, categorized as 1) basic school, 2) high school, 3) short/medium-term education (11-12 years); 4) long education (≥13 years); or 5) missing or unknown (16;17).

For all confounders, we will disregard exposure less than one year prior to the index date.

All analyses were performed using Stata Release 12.0 (StataCorp, College Station, TX, USA).

**Sensitivity analyses**

We will perform a number of subanalyses/sensitivity analyses.

* First, we will repeat the analysis stratified by age-groups.
* Secondly, we will repeat the analysis excluding patients with certain co-morbidities such as diabetes (see above) or history of ischemic heart disease (ICD-8: 410-414; ICD-10: I20-25).
* Thirdly, we will apply different exposure definitions, e.g., only including pure beta-blockers, selective beta-blockers etc. or using cumulative amount of beta-blocker filled (measured in DDD) or treatment intensity (DDDs filled divided by number of days considered exposed) instead of duration of use.
* Fourthly, we will subdivide cases and their corresponding controls according to subtypes of breast cancer, categorized as ductal carcinomas, lobular carcinomas or other types.
* Fifthly, we will perform analyses stratified by stage, defined as localized or non-localized breast cancer (18).
* Sixthly, we will changed the comparator from never-use of beta-blockers to long-term use (≥5 years) of ACE-inhibitors or ATII-antagonists (ATC, C09A, C09B, C09C and C09D) or vascular calcium-channel blockers (C08CA) respectively. Long-term users of both beta-blockers and ACE/ATII or calcium-channel blockers will be excluded from these analyses.
* Lastly, we will change the one-year lag-time to zero or two years.

**Results**

We identified xx incident breast cancers cases between 1 January 2000 and 31 December 2009. After exclusions we arrived at xx eligible cancers cases (see figure 1), that were matched to xx controls.

The final study sample, i.e. all cases and controls, had filled a total of xx prescriptions for beta-blockers prior to their index dates. As expected, the majority of use was seen within the pure beta-blockers (C07A) which constituted xx (xx%) of all filled prescriptions. Within this group, xx% of use was selective beta-blockers, xx% were non-selective beta-blockers and xx% were alpha and beta-blocking agents. The most used single substances were metoprolol (ATC, C07AB02), propranolol (ATC, C07AA05) and atenolol (ATC, C07AB03) constituting xx%, xx% and xx% of all filled prescriptions respectively.

**Figures and tables**

**Incident breast cancers**

**during 2000-2009:**

xx cases

**Eligible cases:**

xx cases

Age < 18 or > 85 years

- xx cases

No histological confirmation or dead prior to diagnosis

- xx cases

Prior cancer

- xx cases

Recent migrations

- xx cases

**Figure 1: Flow-chart of the selection of cases**

**Table 1**Characteristics of breast cancer cases and their matched controls

|  |  |  |
| --- | --- | --- |
|  | **Cases** | **Controls** |
|  | (n=xx) | (n=xx) |
| Age, median (IQR, years) |  |  |
| Use of beta-blockers ≥ 1  year prior to index-date |  |  |
| Never use |  |  |
| Ever use |  |  |
| Long-term use (≥5 years) |  |  |
| Use of ACE/ARB ≥ 1  year prior to index-date |  |  |
| Never use |  |  |
| Ever use |  |  |
| Long-term use (≥5 years) |  |  |
| Charlson Comorbidity Index (CCI) |  |  |
| None (CCI Score = 0) |  |  |
| Low (CCI Score = 1) |  |  |
| Medium (CCI Score = 2) |  |  |
| High (CCI Score ≥ 3) |  |  |
| Highest achieved education |  |  |
| Short (7-10 years) |  |  |
| Medium (11-12 years) |  |  |
| Long (≥13 years) |  |  |
| Missing or unknown |  |  |
| Reproductive history |  |  |
| 0 births |  |  |
| 1 birth |  |  |
| 2+ births |  |  |
| Missing info |  |  |
| Drugs \* |  |  |
| NSAID |  |  |
| Aspirin |  |  |
| Statins |  |  |
| Hormone replacement therapy |  |  |
| Antidepressants |  |  |
| Anticoagulants |  |  |
| Diagnoses |  |  |
| Alcohol abuse |  |  |
| Diabetes |  |  |
| Breast adenoma |  |  |
| NOTE: IQR = InterQuartile Range; DDD = Defined daily doses; \*) Having filled ≥2 prescriptions more than one year prior to the index date. | | |

**Table 2**Association between exposure to beta-blockers and breast cancer risk,   
specified by exposure pattern within the entire follow-up-period,   
excluding the last year prior to the index date

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Subgroup** | **Cases** | **Controls** | **Adjusted OR 1** | **Adjusted OR 2** |
| Non-use |  |  | 1.00 (ref.) | 1.00 (ref.) |
| Ever use |  |  |  |  |
| Duration of use: |  |  |  |  |
| < 1 year |  |  |  |  |
| 1-4.99 years |  |  |  |  |
| 5-9.99 years |  |  |  |  |
| ≥ 10 years |  |  |  |  |
| Cumulative amount |  |  |  |  |
| 1-199 DDD |  |  |  |  |
| 200-499 DDD |  |  |  |  |
| 500-999 DDD |  |  |  |  |
| 1,000-1,999 DDD |  |  |  |  |
| ≥ 2,000 DDD |  |  |  |  |
| Intensity |  |  |  |  |
| 0.01-0.49 DDD/day |  |  |  |  |
| 0.50-0.99 DDD/day |  |  |  |  |
| 1.00-1.99 DDD/day |  |  |  |  |
| ≥2.00 DDD/day |  |  |  |  |
| Sub-groups (≥5 years) |  |  |  |  |
| Pure beta-blockers |  |  |  |  |
| Combinations |  |  |  |  |
| Selective |  |  |  |  |
| Non-selective |  |  |  |  |
| Alpha and beta |  |  |  |  |
| 1) Adjusted for age and gender (risk-set matching).  2) Fully adjusted model, see section 'Main analysis'. | | | | |

**Table 3**Associations between long-term exposure to beta-blockers (≥5 years)   
and breast cancer risk, specified by patient subgroups

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Subgroup** | **Cases**  **Exposed /unexposed** | **Controls**  **Exposed /unexposed** | **Adjusted OR 1** | **Adjusted OR 2** |
| All |  |  |  |  |
| Age < 50 years |  |  |  |  |
| Age 50 - 69 years |  |  |  |  |
| Age 70+ years |  |  |  |  |
| CCI score = 0 |  |  |  |  |
| No ICH |  |  |  |  |
| No diabetics |  |  |  |  |
| No HRT |  |  |  |  |
| NOTE: CCI = Charlson Comorbidity Index; ICH = ischemic heart disease;  HRT = hormone replacement therapy  1) Adjusted for age and gender (risk-set matching).  2) Fully adjusted model, see section 'Main analysis'. | | | | |

**Table 4**Associations between long-term exposure to beta-blockers (≥5 years)   
and breast cancer risk, specified by type of breast cancer and stage

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Subgroup** | **Cases**  **Exposed /unexposed** | **Controls**  **Exposed /unexposed** | **Adjusted OR 1** | **Adjusted OR 2** |
| All cancers |  |  |  |  |
| Localized\* |  |  |  |  |
| Non-localized\* |  |  |  |  |
| Ductal carcinoma |  |  |  |  |
| Tubular carcinoma |  |  |  |  |
| Other cancers |  |  |  |  |
| \* Only subjects with index dates after 1 Jan 2004.  1) Adjusted for age and gender (risk-set matching).  2) Fully adjusted model, see section 'Main analysis'. | | | | |

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