PROTOCOL AND STATISTICAL ANALYSIS PLAN

SUBCLINICAL HYPERTHYROIDISM AND FRACTURES IN PRIMARY CARE

Study design and setting

We will conduct a retrospective cohort study using data from the Dutch General Practitioner (GP) Database from the PHARMO Data Network. The GP Database is a longitudinal database comprising data from electronic patient records registered by GPs. Records include information on diagnoses and symptoms, laboratory test results, and prescriptions. Prescription records include information on the type of product, prescription date, strength, dosage regimen, quantity, and route of administration. Drug prescriptions are coded according to the WHO Anatomical Therapeutic Chemical (ATC) Classification System. Diagnoses and symptoms will be coded according to the International Classification of Primary Care (ICPC). The GP Database covers a catchment area representing 3.2 million residents (~20% of the Dutch population). More information has been published elsewhere (Overbeek JA, Swart KMA, Houben E, Penning-van Beest FJA, Herings RMC. Completeness and Representativeness of the PHARMO General Practitioner (GP) Data: A Comparison with National Statistics. Clin Epidemiol. 2023;15:1-11. https://doi.org/10.2147/CLEP.S389598).

Time frame

Data from January 1, 2012, to December 31, 2021, will be analyzed, encompassing a ten-year period.

Study population

The study population consists of patients identified through TSH measurements requested in primary care, excluding those with known thyroid diseases.

Subclinical hyperthyroidism (SHT) is defined by a TSH concentration below the lower limit of the reference interval and an FT4 concentration within the reference interval in the same sample or patients with a recorded ICPC code A91.07 (SHT). Method specific reference intervals will be used for each TSH and FT4 measurement.

Exclusion criteria for subclinical hyperthyroidism are: in the 2 years prior to inclusion (1), use of thyroid medication (ATC starting with H03), amiodarone (ATC C01BD01) or ever recorded use of lithium (ATC N05AN01) or (2) mention of ICPC codes: T85 (hyperthyroidism), T86 (hypothyroidism), A91.06 (subclinical hypothyroidism), A91.07 (subclinical hyperthyroidism) or T71 (thyroid malignancy).

The patients included based on ICPC code could include several misclassifications, where the biochemical diagnosis was subclinical hypothyroidism, in that case, only patients with a laboratory confirmed subclinical hyperthyroidism will be included. Furthermore, patients without available data for the study period or who could not be matched to controls will be excluded from the analysis. In addition, subjects younger than 18 years of age at time of inclusion will also be excluded. In order to minimize the chance of thyroid disorders being related to pregnancy we will exclude patients with subclinical hyperthyroidism which are included three months before to one year after a registered pregnancy (ICPC codes W78 "Desired pregnancy" or W79 "Unwanted pregnancy"). If a pregnancy is

registered in the follow-up TSH and FT4 values will also be excluded in the lab dataset three months before to 1 year after a registered pregnancy.

As a reference group, euthyroid patients will be included, defined as having TSH and FT4 measurements within the reference interval. Exclusion criteria are TSH or FT4 measurements outside of the reference interval or mention of ICPC codes: T85 (hyperthyroidism), T86 (hypothyroidism), A91.06 (subclinical hypothyroidism), A91.07 (subclinical hyperthyroidism) or T71 (thyroid malignancy) during 5-year follow-up. The euthyroid reference group were matched to cases by age, sex, and GP practice with four intended matched subjects for each SHT subject.

A detailed overview of the patient selection process will be presented in order to illustrated the flow diagram of the inclusion and exclusion criteria applied in this study.

Exploratory and confirmatory factor analysis

Exploratory factor analysis (EFA) was conducted on a randomly selected subset comprising 25% of the database. The EFA helped to define the exact variables and develop robust outcome measures. After finalizing the analysis protocol based on the EFA results, the protocol was registered on the ISRCTN registry to maintain transparency and reproducibility.

Once the analysis protocol is published, the remaining 75% of the database, which had been withheld to prevent bias, will be decoded and made available for research. A confirmatory factor analysis (CFA) will then be performed on this larger dataset to validate the factors identified in the EFA and to confirm the consistency and reliability of the defined outcome measures.

Outcomes

The primary outcomes of interest are fractures and osteoporosis, which are categorized as follows:

- Major osteoporotic fracture: fractures of the femur, humerus, vertebrae, or radius/ulna (respective ICPC codes: L75, L75.01, L76.04, L76.06, L72).
- Osteoporotic fracture: major osteoporotic fractures plus fractures of the tibia/fibula, clavicle, ribs, pelvis, and patella (respective ICPC codes: L73, L76.03, L76.05, L76.07, L76.08).
- Any fracture: all osteoporotic fractures plus fractures of hand/foot bones, skull, and nose (ICPC codes: L74, L74.01, L74.02, L76, L76.01, L76.02).
- Hip fracture: ICPC codes L75 and L75.01
- Spine fracture: ICPC code L76.06
- Osteoporosis: Defined by ICPC codes L95 (osteoporosis) or L95.02 (osteoporosis), but not L95.01 (osteopenia), or by the use of specific medications. These include bisphosphonates (M05BA, M05BB), parathyroid hormones and analogues (H05AA), other drugs affecting bone structure and mineralization (M05BX, such as denosumab and romosozumab), and the selective estrogen receptor modulators raloxifene (G03XC01). However, zoledronic acid (M05BA08) will not be included if there are more than two prescriptions in a year, and denosumab (M05BX04) is excluded if there are more than three prescriptions per year, as such cases are likely indicative of prophylactic cancer-related treatment rather than osteoporosis management. A diagnosis of osteoporosis during follow-up will be excluded if this was already present in the year before cohort entry date.

Statistical analysis

Baseline characteristics will be compared between the SHT and euthyroid groups. Categorical variables were summarized as frequencies and percentages, while continuous variables will be reported as means with standard deviations (SD) or medians with interquartile ranges (IQR).

Incidence rates for fractures and osteoporosis will be calculated per 1000 person-years for both groups. Time-to-event analysis will be conducted from the cohort entry date to the first occurrence of a fracture or osteoporosis, with censoring at the date of fracture or osteoporosis diagnosis or end of follow-up. Hazard ratios (HRs) and 95% confidence intervals (CIs) for fractures and osteoporosis will be estimated using Cox proportional hazards models, adjusting for age, sex, hypertension, diabetes mellitus, hypercholesterolemia, and chronic kidney disease (CKD).

Additionally, subgroup analyses will be performed by stratifying for different age groups (50- and 50+ years of age), sex and baseline TSH concentration. Additional subgroup comparisons will be made for patients with persistent SHT, those who progressed to overt hyperthyroidism, and those who experienced TSH normalization. These groups were defined as follows: (1) Progression to overt hyperthyroidism, defined as FT4 levels above the reference range during the follow-up period; (2) Recovery, characterized by TSH levels returning to within the reference range, but never above, at any time during the follow-up; (3) Persisting subclinical hyperthyroidism, defined as persistently suppressed TSH levels with normal FT4 levels throughout the follow-up period. For these groups the TSH and FT4 values in the first four weeks will be excluded, since they are deemed too close to the inclusion date and the Dutch primary care guideline recommends testing after three months.

A sensitivity analysis explored osteoporosis outcomes without considering the medication criteria, as bone-modifying medications could be used for other indications besides osteoporosis.

All estimates were reported with 95% confidence intervals. All statistical analyses were conducted using R version 4.2.2 (2022-10-31 ucrt). The following R packages were used for the analysis: car, dplyr, ggplot2, ggpubr, lubridate, multcomp, purrr, survminer, survival, readr, and tidyr.