Protocol

Risk and course of COVID-19 infection in patients with hypo- or hyperthyroidism. A Danish population-based cohort study

1. Background

A novel coronavirus disease (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has during the last 7 months affected over 8.7 million people, claiming more than 450.000 lives in over 200 countries (www.worldometers.info/coronavirus - 21th June 2020).

At present there are no data regarding the risk and course of COVID-19 in patients with underlying hypo- or hyperthyroidism. The European Thyroid Association (ETA) has issued a consensus statement regarding issues specific to patients with thyroid diseases during the COVID-19 pandemic (www.eurothyroid.com). Patients with hypo- or hyperthyroidism are advised to continue their prescribed medications [Levothyroxine (L-T4) and anti-thyroid drugs (ATD), respectively]. However, for a number of reasons, patients with hypo- or hyperthyroidism may have an increased risk of developing a severe or fatal course of COVID-19. First, the activity of serum angiotensin-converting enzyme (ACE) as well as the tissue distribution of angiotensin-converting enzyme 2 (ACE2) is influenced by the serum levels of thyroid hormones [Thyroxine (T4) and liothyronine (T3)] [1-3]. Accepting that SARS-CoV-2 uses ACE2 as a receptor for host cell entry [4], thyroid dysfunction may influence the course of COVID-19 in the same way and direction as has been suggested for angiotensin converting enzyme inhibitors (ACE-I) and angiotensin ll receptor blockers (ARB), which will upregulate the expression of the ACE2 enzyme. Preliminary reports indicated that the use of ACE-1 or ARB increased the risk of developing a severe or fatal course of COVID-19, but subsequent observations have questioned this association [5,6]. Second, patients with hypo- or hyperthyroidism have an increased burden of co-morbidity, especially with respect to cardiovascular and pulmonary conditions [7-10] which is also reported in several case series of patients with severe and fatal COVID-19 [11,12]. Third, in most patients the etiology of hypo- and hyperthyroidism is due to autoimmunity (95% and 50% for hypo- and hyperthyroidism, respectively) [13,14]. Although patients with autoimmune disorders do not appear to be more

likely to contract COVID-19 [15], they may have severe complications if their immune systems are suppressed, either by their disease or by medications that treat their autoimmune disorder [16]. Finally, albeit rarely, patients using ATD are at risk of developing agranulocytosis [17].

As hypo- and hyperthyroidism are quite common conditions, together affecting up to 4-5% of the Danish population [13,14], any association with risk and prognosis of COVID-19 may have important public health impact. Thus, there is an urgent need to clarify whether there is an increased risk and/or worsened prognosis of COVID-19 in patients with hypo- or hyperthyroidism using high quality population-based data from a uniform tax financed health care system.

2. Objectives

The primary aim is to examine prognosis of COVID-19 in Danish patients being treated for hypo- or hyperthyroidism by examining the association of use of L-T4 or ATD with the risk of hospital admission, Intensive care unit admission, mechanical ventilation, acute renal replacement therapy, treatment with inotropes/vasopressors, and death. The secondary aim is to examine the risk of being diagnosed with COVID-19 in these patients.

3. Methods

3.1. Study design

This nationwide study will include all persons tested for SARS-CoV-2 in Denmark [18]. The impact of hypo- and hyperthyroidism on risk of acquiring COVID-19 will be examined using a test-negative case-control design, while the prognosis will be evaluated in a cohort of COVID-19 positive patients, Figure 1.

3.2. Study period

27 February 2020 (the date of the first identified COVID-19 case in Denmark) to 31th of August 2020.

3.3. Data sources

The study will utilize information from The Danish COVID-19 cohort, identified from the Danish Microbiology Database [19], The Danish National Registry of Patients (DNRP) [20], The Danish Civil Registration System (CRS) [21], The National Prescription Database (NPD) [22], and the Danish Cause of Death Registry [23].

3.4. Study population

The source population is all Danish citizens, approximately 5.8 million. The study population for the risk of COVID-19 in patients with hypo- or hyperthyroidism will be all persons tested for SARS-CoV-2 in the study period. Only persons tested positive for SARS-CoV-2 will be included in the prognosis analysis (cohort analysis).

3.5. Follow-up

Patients will be followed from the date of their positive test for SARS-CoV-2 and the following 30 days for all outcomes.

3.6. Exposure

The study includes two different exposures. Hypothyroidism is defined as current use of L-T4, while hyperthyroidism is defined as current or former use of ATD. Persons who are treated with L-T4 and ATD simultaneously are classified as having hyperthyroidism.

Current use is defined as a filled prescription for L-T4/ATD within 180 days before the SARS-CoV-2 test.

Former use of ATD is defined as a prescription more than 180 days before the SARS-CoV-2 test.

Data on prescriptions are obtained from NPD [22], which includes complete information (i.e date of dispensing, Anatomical Therapeutic Chemical code, and drug quantity to name but a few variables) on all filled prescriptions in Denmark since 1995.

The main exposures will be:

- Current use of L-T4 compared with no use of L-T4
- Current use of ATD (inclusive L-T4+ATD users) compared with never use

Secondary exposure will be:

- Former use of ATD compared with never use
- Ever use of ATD compared with never use

As an additional analysis, ATD will be subdivided into methimazole and propylthiouracil and analyzed separately if the number of exposed subjects is sufficient.

3.7. Outcomes

When evaluating the prognosis of COVID-19 in patients with hypo- or hyperthyroidism the primary outcome is death within 30 days after a positive test for SARS-CoV-2. Additional outcomes include, hospital admission (>12 hours) at the day of or within 30 days after a positive test in patients not already hospitalized. Additional outcomes include Intensive care unit admission, use of mechanical ventilation, renal replacement therapy and/or death at the day of or within 30 days after a positive test for SARS-CoV-2.

The secondary outcome is risk of COVID-19 in patients treated for hypo- or hyperthyroidism.

3.8. Confounding

Confounding will be handled by using propensity score (PS) methods [24]. The PS is the probability of being exposed given the covariate pattern for the patient. The PS will be estimated for each patient hospitalization in each of the examined comparisons using a logistic regression model including calendar time and the covariates listed in the appendix. We apply weighting to equalize the number and covariate distribution between the comparison groups and users of L-T4/ATD.

3.9 Selection and information bias

The risk of selection bias is reduced by including all tested Danish inhabitants. We do not expect that patients with hypo- or hyperthyroidism are more or less likely to be tested than the general background population. When this is said, the criteria for testing have changed during the study period, which will be addressed in a sensitivity analysis stratified by calendar time.

As exposure and outcomes are accurately recorded, any misclassification would be minor and non-differential.

3.10 Statistical analyses

The risk of COVID-19 will be analyzed using a test-negative case-control design among all persons tested for SARS-CoV-2. The odds ratio for current L-T4 or ATD use versus non-use will be estimated using logistic regression models adjusted for covariates.

Only persons tested positive for SARS-CoV-2 will be included in the prognosis analysis (cohort analysis). Patients will be followed from the day of the positive SARS-CoV-2 test and until date of hospitalization, intensive care unit admission, use of mechanical ventilation, death, or end of follow-up (30 days). For each exposure group the 30-day PS weighted cumulative risk of death, hospital admission, intensive care unit admission, and use of mechanical ventilation will be estimated. Since many patients with COVID-19 seem to have a prolonged disease course a sensitivity analysis using a follow up of 60 days will be conducted for each outcome variable.

Subgroup analysis will be conducted to address potential effect modifiers such as age (≤65 and >65 years), and burden of co-morbidity (patients with hypo- or hyperthyroidism with and without any co-morbidity).

If the prognosis of COVID-19 is influenced by the use of L-T4 and/or ATD the specificity of the(se) association(s) will be evaluated by using persons hospitalized for pneumonia or exacerbation in chronic obstructive pulmonary disease who tested negative for SARS-CoV-2 as COVID-19-negative controls. In this approach the risk estimates for relevant out-comes will be compared between COVID-19 positive and negative users of L-T4/ATD.

3.11 Sample size

As of middle of August 2020, approximately 1.500.000 Danes have been tested for SARS-CoV-2 and 14.757 are tested positive. A total of 621 COVID-19 patients have died (4.2%). The frequency of L-T4/ATD users is expected to be 5%.

We retrieved publicly available data on the age- and sex-distribution of persons reaching four different COVID-related outcomes; community-managed infection, hospitalization

without ICU, ICU and death [25]. In addition, we retrieved data on the age- and sex-specific use of ATD and L-T4 in Denmark (www.medstat.dk). Using age- and sex-stratification on these data sources, it is possible to estimate the count of subjects who will experience a COVID-related outcome while being treated with either ATD or L-T4 and assuming no association between these drugs and the COVID outcome. Such figures can be assumed to follow a Poisson distribution. Since other components of the underlying 2 x 2 tables can be assumed to orders of magnitude more frequent, these "outcomes among exposed" are in practice the sole contributors of statistical imprecision, and the final effect estimate can be assumed to inherit the confidence intervals of a Poisson distribution based on these counts. Accordingly, null estimates from our study can be expected to have the following confidence intervals:

Outcome	Expected number of ATD users	Expected number of L-T4 users	Null estimate for ATD	Null estimate for L-T4
Community-managed	30	250	1.00,(0.67 - 1.43)	1.00,(0.88 - 1.13)
Death	7	31	1.00,(0.37 - 2.18)	1.00,(0.67 - 1.43)
Hospitalized – not ICU	16	91	1.00,(0.57 - 1.62)	1.00,(0.80 - 1.23)
ICU	2	11	1.00,(0.03 - 5.57)	1.00,(0.48 - 1.84)

Of note; any positive association between thyroid drug use and one of these outcomes will results in a higher count of exposed outcomes and a relatively more precise estimate.

4. Ethical/data protection issues

According to Danish law, registry-based studies do not require informed consent or ethical committee approval.

All data are anonymized and stored at secured servers at the Danish Health Data Authority. Data management and analyses are performed through secure remote access to these servers. Data management and analyses will be conducted and checked by experienced researchers.

5. Dissemination

Results will be communicated in international peer-reviewed journals and the website of the Danish Medicines Agency. Any evidence of adverse effects of use of L-T4/ATD will be communicated to the Danish Medicines Agency.

6. Research group

The research group consists of researchers from department of Endocrinology, Odense University Hospital (Professor Laszlo Hegedüs and Associate Professor Thomas H. Brix), and the pharmacoepidemiologic unit at Clinical Pharmacology and Pharmacy, University of Southern Denmark (Professor Jesper Hallas and Ph.d-student Lars Christian Lund). The research project will be led by Thomas H. Brix while the data management will be performed or supervised by Lars Christian Lund and Jesper Hallas.

7. References

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