STATIN THERAPY AND COVID-19 INFECTION (STACOV PROJECT)

Background:

Statins reduce intracellular cholesterol synthesis by interfering with the limiting enzyme HMGCoA reductase. A lower intracellular cholesterol concentration leads to activation of the transcription factor SREBP 2 upregulating LDL receptor synthesis. In general, intracellular cholesterol homeostasis achieves a new physiological equilibrium at lower cholesterol concentrations. Moreover, the effect of cholesterol pathway inhibition has also an effect on farnesyl and geranyl molecules formation influencing protein prenylation leading to changes on inflammation and immunomodulation in vitro. Changes in intracellular cholesterol alter cell membrane composition, particularly the structures referred to cholesterol rafts that accommodate a huge number of cell surface proteins as receptors. Theoretically, alterations in cholesterol rafts could derange the function of some receptors Some preliminary studies on cell models have suggested that statins could interfere the activity of some membrane viral receptors blunting its entry to cell (Berraondo P et al CIMA nonpublished data). SARS-cov-2 goes into cells through the Angiotensin Converser Enzyme 2 (ACE2) which is located in the surface of several cells including lung cells. It has been suggested that simvastatin could have a role in SARS-cov-2 infection by blocking the virus entry to cell. However, atorvastatin has been shown to increase ACE2 expression in animal models [1]. Moreover, intracellular cholesterol content seems to influence the virus uptake [2]

Severe SARS-cov-2 infection is mediated by an inflammatory storm resulting in a deep tissue injury, endothelial damage, prothrombotic state and multiorgan failure. As mentioned above, statins also have some potent anti-inflammatory effects as modulating TNF, the NFkB transcription factor or blocking some members of the Tool Like Receptor family as TLR4-9 and its downstream cofactor MYD88 [3][4]. This anti-inflammatory effect has been implicated in a better prognosis of some diseases as HBV or HCV chronic infection, limiting the progression of hepatic damage to chronic liver disease or hepatocarcinoma [5]. The impact of statin use on influenza epidemics has been repetitively assessed but contradictory or non-conclusive results have been obtained [6][7]. The combination of statins and angiotensin receptor blockers have shown an important protective effect on other epidemics as Ebola, probably to their action on endothelium protection [8]. A protective effect of statins on pulmonary

hypertension development in a primate HIV model has also been reported [9]. Although, in general all these pleiotropic effects of statins have been shown in vitro and its clinical impact is not clear, a clinical assay to test the efficacy of simvastatin on SARS-cov-2 is going on. Recently an observational study including more than 8000 patients infected by Sars-Cov-2 showed the protective effect of being on statins or ACE inhibitors [10]. Taken into account its widespread use and putative effects on viral entry, inflammation, immune mechanisms and endothelial function, the use of standard therapies as statins have been postulated to target the host response to new virus pandemics [8][11].

Hypothesis:

Considering that simvastatin, and probably statins in general, interfere with SARS-cov-2 cellular uptake and some inflammatory pathways activated by the virus, those patients on statin therapy should be less vulnerable to infection and their clinical course and prognosis should be better than that in individuals not on statin therapy.

Primary objective:

Assess the difference in the WHO SARS-cov-2 scale of severity (9 steps) achieved by Covid-19 infected patients, admitted in the hospital, with and without background statin therapy comparable in age and gender distribution.

Secondary objectives:

- Compare in Covid-19 infected patients with/without statins the number of patients developing pneumonia.

- Compare in Covid-19 infected patients with/without statins the number of patients requiring non-invasive respiratory support.

- Compare in Covid-19 infected patients with/without statins the number of patients requiring invasive respiratory support.

- Compare in Covid-19 infected patients with/without statins the number of patients developing hyperinflammatory syndrome (Ferritin > 800; DD> 1500; CRP> 10; or IL-6 > 40)

- Compare in Covid-19 infected patients with/without statins the number of patients developing multiorgan failure.

- Compare in Covid-19 infected patients with/without statins the number of deaths due to the disease.

- Compare in Covid-19 infected patients with/without statins the mean hospital stays of those discharged.

- Impact of lipid profile (last available) on the previous variables.

Methods

This is a retrospective observational multicentre study based on clinical records review. The following centres will take part in the study:

LIPIDCAS Group:

- Hospital Universitari Sant Joan
- Hospital Universitari Joan XXIII
- Hospital Sant Pau i Santa Tecla
- Hospital Verge de la Cinta
- Pius Hospital de Valls
- Hospital del Vendrell

Other centres from the LIPID AND ARTERIOSCLEROSIS UNITS NET (XULA) from Catalonia.

Inclusion criteria:

Patients ≥ 18 years old with a PCR or immunological confirmation of Covid-19 infection, admitted in the hospital for at least 24 hours, will be included.

Exclusion criteria:

Patients < 18 years old.

Procedures in data collection:

A database (excel) has been developed for the collection of data common to all participating centres. Patient data will be anonymized. Each centre will identify its patient with its own code of the centre, for example, the code of Reus will be number 3 and first patient included will be the 001 and so on successively (3-001,3-002, etc.). Once all centres have completed the review, the databases will be merged. The data will be stored on the server of the website of the Lipid Units of Southern Catalonia (LípidCas) shared by all participating centres and hosted on the server of the Hospital Universitari Sant Joan de Reus.

Confidentiality

The principal investigator undertakes that Hospitals and researchers are responsible for ensuring that confidentiality regarding the identification and data of the participant is maintained at all times. The name and details that will identify the patient only appear in the medical history. Researchers use identification codes without knowing the name of the person to whom the sample belongs. Researchers use identification codes without knowing the name of the person to whom the sample belongs. The data will be obtained from the histories of patients in the different centers and entered into a database anonymously. Once the data is entered, they are completely dissociated from the source. The fully anonymized database will remain on a server (HUSJ) from which will access the people who will work with the data to harmonize the database and perform statistical studies that will be performed by staff outside the health institutions (IISPV-URV) that will not be in contact with the original source nor will there be traceability to identify the source of the data.These procedures are subject to the provisions of Organic Law 3/2018 of 5 December on the protection of personal data and Royal Decree 1720/2007, of 21 December (data protection regulations). However, the provisions of Regulation (EU) 2016/6799 on physical protection with regard to the processing of personal data will be observed. The personal information collected for the study will be identified with a code, in accordance with the procedure established in the Personal Data Protection Act. These types of data are called dissociated data.

Variables to be recorded:

- Basic anthropometry and clinical antecedents including comorbidities will be recorded.
- Biochemical, including the last available lipid profile, and clinical variables including WHO SARS-cov-2 disease stages and other data associated to Covid-19 infection will be recorded according a preestablished data base
- Detailed information about baseline therapies will be also collected.
- A common data base to introduce information will be prepared, consensual and distributed to all centres. (Annex - *pending*).

Statistical analyses

The main objective is a reduction on WHO Sars-cov-2 index (0-8), calculating a mean score in hospital admitted patients of 4,6 (SD:1,12) a 30% reduction on categories 5 to 8 would result in a mean value of 4.2 (SD: 1.02). Accepting a relation between statin and non-statin group of 0.3 an alpha risk of 0.1 and a beta risk of less than 0.2 in a bilateral contrast, **216** subjects are needed in the non-statin group and **109** in the statin group to detect a difference equal to or greater than 0.4 units. The common standard deviation is assumed to be 1.07. Taking into account an estimated dropout rate of 10% **we aim to include 250 patients in the non-statin group and 120 in the statin group**
Continuous variables will be tested for normality using the Kolmogorov-Smirnov test. Data are presented as means and percentiles 25 and 75 for continuous variables not normally distributed or the mean and standard deviation (SD) when distributed normally. Unless otherwise noted, categorical variables are expressed as percentages. Differences between groups will be analyzed using the non-parametric Mann-Whitney test or the Student's parametric t test for continuous variables and the chi-square test or Fisher's exact test for categorical variables. Adjusted differences will be investigated by covariance analysis (ANCOVA). The Pearson correlation test for parametric variables or Spearman for nonparametric variables will be performed to analyze the association between continuous variables.

Multivariate analyzes will be performed to evaluate the impact of statins on the severity of Sars-cov-2 infection adjusted for the major confounding covariates. These analyzes will be of two types, on the one hand, regularized linear regressions with cross-

validation to model the severity of the infection continuously, using the score from 0 to 8. In addition, survival analyzes will be performed using models of Cox and competitive risks in order to measure the relative risk of different infection outcomes (discharge, death). Statistical analyzes will be carried out with the SPSS 25 package (IBM, Madrid Spain) and the R 3.6 package. A p value less than 0.05 will be considered statistically significant.

Expected results

We aim to obtain a statistically significant lower WHO severity index in those Sars-cov-2 patients admitted to hospital. This difference should be maintained after adjusting for confounding covariates. Statin use should be identified by multivariate analyses as a protective factor independently of cofactors.

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