HIDDEN AURICULAR AMYLOID DEPOSITS AND AORTIC STENOSIS:

CLINICAL AND PROGNOSTIC IMPLICATIONS IN AORTIC VALVE REPLACEMENT SURGERY

February 20, 2023

Study Protocol

The Aortic-Ruti cohort is a prospective single center study of cardiovascular patients diagnosed with severe AS and with indication for isolated SAVR. Patient management was decided by the physicians, following recommended guidelines. The study also included a comparative analysis of two subgroups stratified by the presence or absence of amyloid deposits in intraoperative biopsy samples.

From November 2020 and November 2021, a total of 110 patients over 18 years of age, diagnosed with severe aortic stenosis by echocardiographic criteria, with indication for isolated aortic valve replacement and elective surgery, were included. The echocardiographic criteria for the diagnosis of severe AS were the following: average gradient greater than 40mmHg and/or maximum speed greater than 4m/s. Aortic valve area calculated by the continuity equation: the severity cut-off point established by the guidelines is less than 1cm2 or set by the body surface index <0.6cm2/m2. Those patients undergoing emergency surgery or combined surgery, with extra-cardiac disease with a prognosis of less than 365 days, with moderate-important alcoholism and abnormal laboratory values, were excluded.

During the surgery, atrium and ventricular septum biopsies were collected for histological and Visium Spatial Gene Expression analysis. Blood samples for biomarker analysis were obtained before the surgery, at discharge and at one month and one year of follow-up, centrifuged, and then serum and EDTA-plasma were stored at -80ºC until assay.

Clinical and demographic characteristics were recorded at baseline, discharge, 1 month and 1 year.

Variables were collected prospectively. Intraoperative histological results were recorded, and a dedicated biomarker database was built, incorporating baseline and follow-up serum values collected at each visit.

Data were organized into five datasets per sub-cohort: Baseline, Follow-up, Biomarkers, EuroQuol Quality of Life test (EQ-5D-5L), and Histology.

Follow-up data analysis focused on one-year post-surgery results, except for EQ-5D-5L scores, where a mean of the three global scores (discharge, 1 month, and 1 year) was calculated per patient and compared between sub-cohorts.

Blood samples were collected at admission (n=105), discharge (n=84), one month (n=88), and one year (n=94) post-surgery. Serum and EDTA plasma were stored at −80 °C. Auricular and septal tissue samples were obtained during surgery in 70 patients and fixed in 4% paraformaldehyde before paraffin embedding.

Paraffin-embedded tissue sections (4 µm) were stained using Congo Red, Sirius Red, and Trichrome techniques. For Congo Red staining, sections were treated with haematoxylin and a Congo Red solution, and positivity was confirmed by apple-green birefringence under polarized light. Sirius Red staining involved incubation with picrosirius to visualize collagen types I and III, appearing red/orange and apple green, respectively. Trichrome staining included fixation in Bowin's solution and sequential staining steps to differentiate tissue components, evaluated with a LEICA DMI6000B microscope.

Paraffin-embedded sections (4 µm) were deparaffinized, rehydrated, and blocked with 5% horse serum in PBS-Tween and Triton X-100. Sections were incubated overnight at 4°C with primary antibodies against TTR (1:200), ANP (1:200), and troponin I (1:50). After washing, secondary antibodies (1:500) were applied for 45 minutes at room temperature. Following PBS

rinses, Congo Red staining and mounting were performed. Imaging was carried out using a Leica TCS SP5 confocal microscope.

Midregional Proatrial Natriuretic Peptide (MRproANP). MRproANP serum levels were measured with Midregional ProAtrial Natriuretic Peptide ELISA Kit (ABIN6975642; antibodies online; lot W17065241). The detection range and the minimum detectable dese are 39pmol/L – 2500pmol/L and 9.8pmol/L, respectively.

Meteorin-like (Mtrnl). Serum Mtrnl levels were measured with the Human Meteorin-like/METRNL DuoSet ELISA (DY7867-5; R&D; lot P300680). The detection range and the minimum detectable dese are 15.6 pmol/L – 1000 pg/mL.

Four tissue samples (Positive Congo Red, n=2; Negative Congo Red, n=2) were analyzed using the Visium Spatial Gene Expression system at the Josep Carreras Leukaemia Research Institute (IJC). RNA sequencing data was pre-processed and normalized using the SC Transform (SCT) method to correct for technical variability. Samples were integrated to identify spatially distinct clusters across conditions. Differential gene expression analysis was performed to classify cell populations, mapping them to known cell types such as fibroblasts, cardiomyocytes, endothelial cells, and immune cells. Data visualization was conducted using UMAP dimensionality reduction, and spatial cluster annotation was based on marker gene expression.

Statistical Analysis Plan

Categorical variables were summarized as counts and percentages, and continuous variables as mean ± standard deviation or median (interquartile range), as appropriate. Changes from baseline to 1-year follow-up in clinical, imaging, and biomarker variables were assessed using linear regression models, with and without interaction terms for Congo Red status (positive vs. negative). Estimated marginal means were used to compare adjusted follow-up values. Associations between Congo Red status and clinical outcomes, including hospital readmission and atrial fibrillation, were evaluated using conditional Poisson regression models adjusted for sex, age, LVEF, and baseline NT-proBNP. Left atrial size was additionally included in models assessing atrial fibrillation.

Further linear regression models were used to analyze the relationship between Congo Red status and log-transformed NT-proBNP, Metrnl, and EQ-5D-5L quality of life scores at 1 year, adjusting for key covariates. Model assumptions were verified, and 95% confidence intervals were reported. Analyses were performed using R software (v4.4.0).