

Research Project Protocol

TITLE	PROSPECTIVE AI-BASED ECHOCARDIOGRAPHY FOR DETECTION OF CARDIAC AMYLOIDOSIS IN PATIENTS UNDERGOING TRANSTHORACIC ECHOCARDIOGRAPHY WITH LEFT VENTRICULAR HYPERTROPHY
STUDY CODE	ORCHESTRA-PROS
PROMOTOR	Hospital University Germans Trias i Pujol
Date	17/12/2025

1. RESUMEN DEL PROTOCOLO

Title and subtitles, version and date of the protocol	PROSPECTIVE AI-BASED ECHOCARDIOGRAPHY FOR DETECTION OF CARDIAC AMYLOIDOSIS IN PATIENTS UNDERGOING TRANSTHORACIC ECHOCARDIOGRAPHY WITH LEFT VENTRICULAR HYPERTROPHY ORCHESTRA Protocol version 2.0 Date: 17/12/2025
Justification and context	Cardiac amyloidosis is characterized by deposition of misfolded protein in the myocardium causing mainly heart failure symptoms with preserved left ventricular ejection fraction. There are also specific clinical (bilateral carpal tunnel syndrome, polyneuropathy, skin bruising, ruptured biceps tendon...), biomarkers (disproportionally elevated NT-proBNP to the degree of heart failure, persistent elevated troponin, proteinuria..), electrocardiographic (reduced voltage of QRS, atrial fibrillation..) and echocardiographic features (concentric left ventricular hypertrophy, dilated atria, reduced global longitudinal strain with typical pattern of apical sparing, diastolic dysfunction...). Early diagnosis of the disease is crucial to identify patients that may benefit from appropriate treatment. Suspected cardiac amyloidosis on echocardiography or on cardiac magnetic resonance needs to prompt the request of serum free-light chain quantification and serum and urine immunofixation as well as single photon emission computed tomography using bone radiotracers.
Hypothesis	The use of artificial intelligence (AI) assisted algorithm applied to echocardiographic data may allow identification of suspected cardiac amyloidosis more precisely as compared to cardiologists with expertise in cardiac imaging.
Objetives	The main objective of this prospective analysis is to estimate the true prevalence of ATTR-cardiac

	<p>amyloidosis among patients referred for echocardiography and who present red flags of cardiac infiltration by amyloid by referring the patients to 99mTc-pyrophosphate (PYP) SPECT and hematological tests.</p> <p>We will also evaluate:</p> <ul style="list-style-type: none"> • The diagnostic accuracy of the AI-based algorithm to identify ATTR-cardiac amyloidosis. • The characteristics of patients in whom the clinician would have not diagnosed the disease with the echocardiography but the AI-algorithm identifies them as suggestive of having the disease. • The impact on the workflow of the patients with suspected cardiac amyloidosis by the implementation of AI-tools at the echocardiography laboratory.
Design	Prospective
Study population	<p>Patients 18 years old or older referred to transthoracic echocardiography and in whom the clinician expert in echocardiography or the AI-tool suggest that there are echocardiographic features that suggest ATTR-cardiac amyloidosis will be referred to the clinically indicated pathway (99mTc-pyrophosphate (PYP) SPECT and hematological tests) as follows (Figure 2):</p> <ul style="list-style-type: none"> • Patients in whom the cardiologist expert in echocardiography and the AI-based tool agree on the suspicion of cardiac amyloidosis will be referred to further analysis with 99mTc-pyrophosphate (PYP) SPECT and hematological tests as clinically indicated. • Patients in whom the cardiologist expert in echocardiography considers there is suspected cardiac amyloidosis but the AI-based tool disagrees will be referred to the referring physician for further control and eventually analysis with 99mTc-pyrophosphate (PYP) SPECT and hematological tests as clinically indicated. • Patients in whom the cardiologist expert in echocardiography considers there is not cardiac amyloidosis but the AI-based tool



	disagrees will be asked for consent to participate in the study with further tests including 99mTc-pyrophosphate (PYP) SPECT and hematological tests.
Sample size	200
Variables	The variables that will be analysed include demographics, clinical (symptoms and signs), laboratory (hemogram and regular biochemistry data such as renal function, glycemia, lipid profile..) and specific tests to confirm or rule out the diagnosis of cardiac amyloidosis.
Source of data	PACS and SAP
Medication or medical device subject to evaluation (when applicable)	NA
Data analysis	Categorical variables are presented as frequencies and percentages, and continuous variables are reported as mean \pm standard deviation or median with interquartile range (IQR) according to the distribution of the variable. The Student T-test will be used for comparison of continuous variables, and the Chi-square test will be used to compare categorical variables. Sensitivity, specificity, negative and positive predictive value will be analysed using a 2x2 contingency table. A p-value of <0.05 will be considered statistically significant for all analyses. All statistical tests will be two-sided.
Stages and schedule	Please see below the scheme

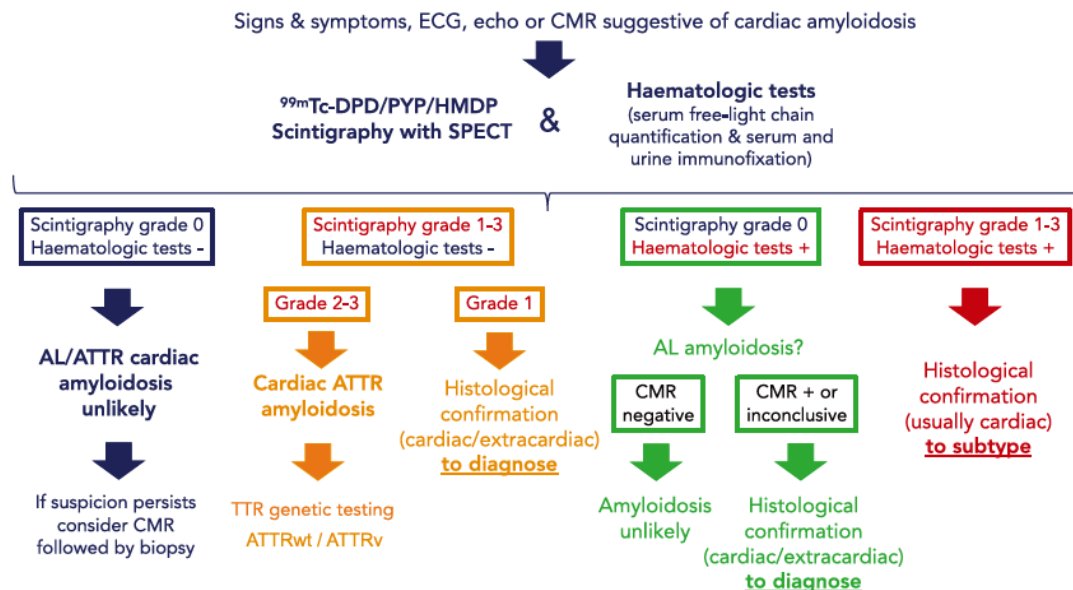
Task	Description	Status	Amount "AF021"	Paid	Billing	October '26	November '26	December '26	January '27	February '27	March '27	April '27	May '27	June '27	July '27	August '27	September '27	October '27	November '27	December '27	October '28
PHASE I (RETROSPECTIVE ANALYSIS)																					
PHASE I (RETROSPECTIVE ANALYSIS) YEAR 1																					
M4	T4.1 Submission of the prospective protocol to the ethical committee	Not Started	(35,792.04)	25/02/2027																	
M5	T5.1 Software usage	Values 92,000 Euros Research	(27,000.72)	09/02/2026																	
M6	T6.1 Prospective Analysis (Clinical use)	Educ data are collected	(76,560.00)	30/02/2026																	
PHASE I (RETROSPECTIVE ANALYSIS) YEAR 2																					
M7	T7.1 Software usage	Values 92,000 Euros Research	(27,000.72)	09/02/2027																	
M8	T8.1 Prospective Analysis (Clinical use)	Educ data are collected	(76,560.00)	30/02/2027																	
M9	T9.1 Publication	Publication of abstract paper demonstrating the results	(35,792.04)	30/02/2028																	

1. BACKGROUND

Cardiac amyloidosis is characterized by deposition of misfolded protein in the myocardium causing mainly heart failure symptoms with preserved left ventricular ejection fraction.¹ There are also specific clinical (bilateral carpal tunnel syndrome, polyneuropathy, skin bruising, ruptured biceps tendon...), biomarkers (disproportionally elevated NT-proBNP to the degree of heart failure, persistent elevated troponin, proteinuria...), electrocardiographic (reduced voltage of QRS, atrial fibrillation...) and echocardiographic features (concentric left ventricular hypertrophy, dilated atria, reduced global longitudinal strain with typical pattern of apical sparing, diastolic dysfunction...).² Early diagnosis of the disease is crucial to identify patients that may benefit from appropriate treatment. Suspected cardiac amyloidosis on echocardiography or on cardiac magnetic resonance needs to prompt the request of serum free-light chain quantification and serum and urine immunofixation as well as single photon emission computed tomography (SPECT) using bone radiotracers.² Echocardiography is the imaging technique of first choice to evaluate patients with dyspnea complaints and suspected heart failure as well as other pathologies. Echocardiography is a technique of first choice to evaluate patients with cardiovascular risk factors such as arterial hypertension and diabetes and many of those patients may have echocardiographic features that can be observed in early phases of cardiac amyloidosis.² Currently, identification of patients with cardiac amyloidosis with available echocardiographic tools remains challenging. However, novel artificial intelligence (AI)-based algorithms applied to echocardiographic images for analysis may help the cardiologists in the identification of early phase of cardiac amyloidosis. Early diagnosis of cardiac amyloidosis is key to implement effective therapies that have demonstrated to improve survival.³ Several studies have demonstrated the accuracy of AI-based algorithms applied to echocardiography for the diagnosis of cardiac amyloidosis.⁴⁻⁶ The hypothesis of the present prospective study is to evaluate the accuracy of the AI-based algorithm to identify patients with echocardiographic findings suggestive of cardiac ATTR amyloidosis using as ground truth the subsequent analysis with imaging techniques that permit its diagnosis such as 99mTc-pyrophosphate (PYP) SPECT and cardiac magnetic resonance as well as hematologic tests. If needed, histological confirmation on cardiac or extracardiac tissue could be performed (Figure 1), as recommended by recent consensus document from the Heart Failure Association of the European Society of Cardiology.

In addition, this study will help to answer the true prevalence of ATTR cardiac amyloidosis among patients referred to transthoracic echocardiography that present red flags for ATTR cardiac amyloidosis. The AI-based algorithm is the software Us2.ai which has been used in other populations for this purpose, as previously published.⁸

Figure 1: Diagnostic algorithm of cardiac amyloidosis.⁷



2. REFERENCES

- 1.- Writing Committee; Kittleson MM, Ruberg FL, Ambardekar AV, Brannagan TH, Cheng RK, et al. 2023 ACC expert consensus decision pathway on comprehensive multidisciplinary care for the patient with cardiac amyloidosis: a report of the American College of Cardiology solution set oversight committee. J Am Coll Cardiol 2023;81:1076–126. 10.1016/j.jacc.2022.11.022
- 2.- Lane T, Fontana M, Martinez-Naharro A, et al. Natural History, Quality of Life, and Outcome in Cardiac Transthyretin Amyloidosis. Circulation 2019; 140:16–26. doi:10.1161/CIRCULATIONAHA.118.038169
- 3.- Ioannou A, Patel RK, Razvi Y, Porcari A, Sinagra G, Venneri L, et al. Impact of earlier diagnosis in cardiac ATTR amyloidosis over the course of 20 years. Circulation 2022;146:1657–70. 10.1161/CIRCULATIONAHA.122.060852
- 4.-Chang RS, Chiu I, Tacon P, Abiragi M, Cao L, Hong G, et al. Detection of cardiac amyloidosis using machine learning on routine echocardiographic measurements. Open Heart. 2024;11:e002884. <https://doi.org/10.1136/openhrt-2024-002884>
- 5.-Goto S, Mahara K, Beussink-Nelson L, et al. Artificial intelligence-enabled fully automated detection of cardiac amyloidosis using electrocardiograms and echocardiograms. Nat Commun 2021; 12:2726. doi:10.1038/s41467-021-22877-8
- 6.-Oikonomou EK, Vaid A, Holste G, Coppi A, McNamara RL, Baloesu C, et al. Artificial intelligence-guided detection of under-recognised cardiomyopathies on point-of-care cardiac ultrasonography: a multicentre study. Lancet Digit Health 2025;7:e113–23. 10.1016/S2589-7500(24)00249-8
- 7.- Garcia-Pavia P, Rapezzi C, Adler Y, Arad M, Basso C, Brucato A, Burazor I, Caforio ALP, Damy T, Eriksson U, Fontana M, Gillmore JD, Gonzalez-Lopez E, Grogan M, Heymans S, Imazio M, Kindermann I, Kristen AV, Maurer MS, Merlini G, Pantazis A, Pankuweit S, Rigopoulos AG, Linhart A. Diagnosis and treatment of cardiac amyloidosis. A position statement of the European Society of Cardiology Working Group on Myocardial and

Pericardial Diseases. Eur J Heart Fail. 2021 Apr;23(4):512-526. doi: 10.1002/ejhf.2140. Epub 2021 Apr 7.

8.-Venneri, L., Aimo, A., Porcari, A., Sezer, I., Ioannou, A., Sheikh, A., Mansell, J., Razvi, Y., Iyer, S.B., Martinez-Naharro, A., Bandera, F., Lim, S.C., Frost, M., Ezekowitz, J., Lam, C.S.P., Moody, W., Whelan, C., Lachmann, H., Wechelakar, A., Emdin, M., Hawkins, P.N., Solomon, S.D., Gillmore, J.D. and Fontana, M. (2025), Artificial intelligence-based echocardiographic assessment for monitoring disease progression in transthyretin cardiac amyloidosis. Eur J Heart Fail. <https://doi.org/10.1002/ejhf.70073>

3. HYPOTHESIS

The systematic use of AI assisted algorithm applied to echocardiographic data will provide an accurate estimate of the true prevalence of ATTR cardiac amyloidosis among patients referred to echocardiography who present red flags of this disease and inform about policies that can help to improve the workflow of these patients and the access to effective therapies.

4. OBJETIVES

4.1. Primary objective

The main objective of this prospective analysis is to estimate the true prevalence of ATTR-cardiac amyloidosis among patients referred for echocardiography and who present red flags of cardiac infiltration by amyloid by referring the patients to 99mTc-pyrophosphate (PYP) SPECT and hematological tests.

4.2. Secondary objective

We will also evaluate:

- The diagnostic accuracy of the AI-based algorithm to identify ATTR-cardiac amyloidosis.
- The characteristics of patients in whom the clinician would have not diagnosed the disease with the echocardiography but the AI-algorithm identifies them as suggestive of having the disease.
- The impact on the workflow of the patients with suspected cardiac amyloidosis by the implementation of AI-tools at the echocardiography laboratory.

5. METHODS

5.1 Type of study

This is an observational, prospective study that uses an AI-based algorithm (Us2.ai) that analyzes echocardiographic data currently available at the hospital. Us2.ai is the most comprehensive AI-

driven echocardiogram clinical interpretation support tool on the market, with the ability to produce a complete and fully automated patient report with clinical findings.

Us2.ai is intended to assist clinicians with the interpretation of the Echo studies and is currently in routine use globally. Us2.ai application was validated at the Brigham and Women's Hospital (results published by Tromp J et al. Nature Communications volume 13, Article number: 6776 (2022)), with high levels of accuracy shown across clinical and real-world cohorts worldwide (results published by Tromp J et al. The Lancet Digital Health, Volume 4, Issue 1, e46 - e54 2021). Us2.V1 including 23 key measurements and HF and PH disease indications based on International Guidelines has received FDA clearance and CE Mark for distribution in addition to country specific regulatory clearances. In April 2024, Us2.ai received FDA Clearance for Us2.v2 an extended version of its software, 22 additional measurements and new features such as Strain, Stress Echo and integrating Cardiac Amyloid detection (measurements based indications on International Guidelines). CE clearance granted in April 2025 - also includes disease detection for HCM and Mitral Regurgitation. In December 2024, the validation study of Us2.ca was presented as Late Breaking Trial at EuroEcho Imaging Congress in Berlin for indications for Cardiac Amyloid using Pattern recognition and Echo measurements Guidelines Directed indications. This model has received CE Mark clearance in April 2025 and is pending FDA clearance. As included in the supplemental material, the device is a software platform that automatically processes, analyses and makes measurements on acquired transthoracic cardiac ultrasound images, producing a full report with measurements of several key cardiac structural and functional parameters. The data produced by this software is intended to be used to support qualified cardiologists, sonographers, or other licensed professional healthcare practitioners for clinical decision-making. The device is indicated for use in adult patients. The device has not been validated for the assessment of congenital heart disease, valve disease, pericardial disease, and/or intra-cardiac lesions (e.g. tumours, thrombi). The fact that the device is CE Mark approved for making measurements of structural and functional parameters and to support clinical-decision making in adult patients, this qualifies the device to provide data that points out towards a diagnosis of cardiac amyloidosis.

In this prospective study, patients referred to transthoracic echocardiography and in whom the clinician expert in echocardiography or the AI-tool suggest that there are echocardiographic features that suggest ATTR-cardiac amyloidosis will be referred to the clinically indicated pathway (99mTc-pyrophosphate (PYP) SPECT and hematological tests) as follows (Figure 2):

- Patients in whom the cardiologist expert in echocardiography and the AI-based tool agree on the suspicion of cardiac amyloidosis will be referred to further analysis with 99mTc-pyrophosphate (PYP) SPECT and hematological tests as clinically indicated.
- Patients in whom the cardiologist expert in echocardiography considers there is suspected cardiac amyloidosis but the AI-based tool disagrees will be referred to the referring physician for further control and eventually analysis with 99mTc-pyrophosphate (PYP) SPECT and hematological tests as clinically indicated.
- Patients in whom the cardiologist expert in echocardiography considers there is not cardiac amyloidosis but the AI-based tool disagrees will be asked for consent to participate in the study with further tests including 99mTc-pyrophosphate (PYP) SPECT and hematological tests.

All patients will be asked to provide informed consent to participate in this prospective observational study for analysis of the clinical data and in those in whom as per current guidelines the additional tests are not mandatory to undergo those tests.

Phase 3: Pragmatic prospective AI-analysis assessment

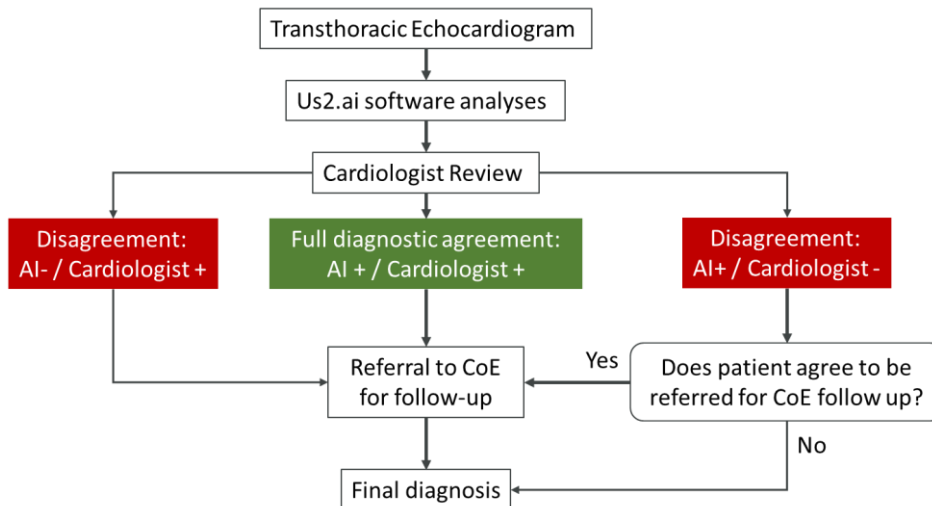


Figure 2: Workflow of patients referred to transthoracic echocardiography.

Clinical data will be pseudoanonymized. Important aspects to bear in mind include:

- The pseudonymization of the collected data in the database is performed by a third person with technical and functional independence from the research team and access to SAP. The data do not leave the hospital at all and it is secured in an institutional (departmental) unit (J:). The echocardiographic data do not leave the hospital and they will be analyzed in hospital. The analysis the software performs does not go back to PACs and it is stored in a an institutional (departmental) unit (J:).
- The software Us2.ai can not perform training during the study. If agreed in future projects, the patient data could be used for training and retraining the software, for which we would reapply the ethical approval and this would require a different version of the software (development mode).
- The hospital has experience with the use of this software. The software is already implemented in the center for another project that received the approval (PI-22-272) and the DOSI was always involved in its implementation. For this new project we will also request the help of the DOSI because in this specific project the software will be physically installed.

5.2 Design

This is an observational, prospective analysis.

5.3 Study population

- **Inclusion criteria**, Patients 18 years old or older with left ventricular hypertrophy defined by a wall thickness of at least 12 mm and other echocardiographic red flags of suspected cardiac amyloidosis
- **Exclusion criteria**, Patients with poor echocardiographic acoustic window to allow proper analysis of the data will be excluded as well as patients with already known cardiac amyloidosis.

5.4 Study variables

5.4.1 Outcome, exposure, or effect variables

The variable of interest will be the prevalence of ATTR-cardiac amyloidosis among patients referred to transthoracic echocardiography and with echocardiographic red flags of this disease.

5.4.1 Other variables

Other variables of interest will include:

- Demographics: age and sex.
- Clinical variables: symptoms of heart failure, blood pressure, heart rate, body mass index, heart rhythm, medications, cardiovascular risk factors and comorbidities (i.e. chronic kidney disease, chronic obstructive pulmonary disease, cancer, stroke...).
- Laboratory variables: hemogram, biochemistry, NT.proBNP, troponin.
- Echocardiographic variables: chamber quantification, presence of significant valvular heart disease, longitudinal strain.
- Hematological tests to rule out/confirm AL amyloidosis
- 99mTc-pyrophosphate (PYP) SPECT Perugini score and quantitative assessment
- Clinical outcomes: heart failure hospitalization, cardiovascular death, all-cause death
- Time to occurrence of the clinical outcome: from the time of the echocardiogram to the occurrence of the clinical outcome.

5.5 Study assessments:

This is a prospective study of echocardiographic data clinically acquired. The results by the expert in echocardiography clinician and the AI-based algorithm will be compared and confirmed using the ground truth that include 99mTc-pyrophosphate (PYP) SPECT and hematological tests. This will allow us to estimate the prevalence of the disease and dimension the need for effective therapies.

5.6 Sample size

The prevalence of cardiac amyloidosis remains unclear and the available data are biased. Therefore, this prospective analysis will help us to dimension the needs for imaging and additional tests as well as the workflows of patients with cardiac amyloidosis that may benefit from effective therapies.

5.7 Statistical analysis

Categorical variables are presented as frequencies and percentages, and continuous variables are reported as mean \pm standard deviation or median with interquartile range (IQR) according to the distribution of the variable. The Student T-test will be used for comparison of continuous variables, and the Chi-square test will be used to compare categorical variables. Sensitivity, specificity, negative and positive predictive value will be analysed using a 2x2 contingency table. A p-value of <0.05 will be considered statistically significant for all analyses. All statistical tests will be two-sided.

5.8 Limitations

There will be a selection bias in those patients in whom the further workflow to diagnose the disease will not be completed.

6. SOURCES OF DATA ACQUISITION AND MANAGEMENT

6.1 Data sources

The source of data is secondary (from the clinically acquired echocardiographic data and stored in PACS).

6.2 Data management and quality control

I do not expect to have missing data.

7. ETHICAL AND LEGAL ASPECTS

We commit to submit the study for evaluation by the accredited ethical committee of the University Hospital Germans Trias i Pujol.

The present study is compliant with the fundamental ethical principles contained in the Declaration of Helsinki (Helsinki, October 2024) and the specific regulations according to the type of study:

- Research involving the analysis and storage of biological material: Law 14/2007 on Biomedical Research and Royal Decree 1716/2011 regulating Biobanks.
- Observational studies involving medicinal products: Royal Decree 957/2020, of November 3, regulating observational studies involving medicinal products for human use.
- Research involving healthcare devices: Law EU 2017/745 i Royal Decree 192/2023

7.1 Benefit-risk assessment for research subjects

The present analysis will inform us about the accuracy of the physicians and the AI-based algorithm for the diagnosis of cardiac amyloidosis and the prevalence of the disease (cardiac amyloidosis) helping us to elaborate workflows that will be more efficient and optimize the resources.

7.2 Information to subjects and informed consent

Patients will be asked to sign the informed consent to participate in the study (please see appendix).

7.3 Confidentiality and data protection

All patient data will be pseudoanonymised and handled in compliance with national and EU data protection laws, including the General Data Protection Regulation (GDPR) and Spanish Organic Law 3/2018. Data will be securely stored, with access restricted to authorized researchers only.

7.4 Interference with prescribing and dispensing habits

Not applicable.

8. MANAGEMENT AND COMMUNICATION OF ADVERSE REACTIONS

This prospective study evaluates a diagnostic workflow with minimal adverse reactions (claustrophobia in case of cardiac magnetic resonance or limited nephrotoxicity in case of patients with renal disease that will undergo cardiac magnetic resonance – improbable since in those patients we will not use gadolinium contrast agent – or derived from the extraction of blood samples for the haematological tests). All these tests will be clinically indicated.

9. OBTAINING AND MANAGEMENT OF BIOLOGICAL SAMPLES

Not applicable.

10. PLANS FOR DISSEMINATION AND COMMUNICATION OF RESULTS

Not applicable.

11. SOURCE OF FUNDING

The study will receive an unrestricted grant from Astra Zeneca, which has no access to the data and does not have influence on the results of the study.

12. WORK PLAN FOR THE EXECUTION OF THE STUDY

Please see below:

Task	Description	Status	Amount €	Fund	Billing	October '26	November '26	December '26	January '27	February '27	March '27	April '27	May '27	June '27	July '27	August '27	September '27	October '27	November '27	December '27	October '28
PHASE 1: RETROSPECTIVE ANALYSIS																					
PHASE 2a: PROSPECTIVE ANALYSIS YEAR 1																					
M4	T4: Submission of the prospective paper to the ethical committee	Not Started	137,273.36																		
M5	T5: Software usage	Not Started	137,273.36																		
M6	T6: Prospective Analysis (Clinical use)	Not Started	137,273.36																		
PHASE 2b: PROSPECTIVE ANALYSIS YEAR 2																					
M7	T7: Software usage	Not Started	137,273.36																		
M8	T8: Prospective Analysis (Clinical use)	Not Started	137,273.36																		
M9	T9: Publication	Not Started	137,273.36																		