Title	Prevalence and characteristics of transthyretin amyloidosis in patients with left ventricular hypertrophy of unknown etiology TTRACK
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Research question and objectives	**Primary objective** • To assess the prevalence of patients with cardiac fixation on a radionuclide bone scintigraphy and/or Single Photon Emission Computed Tomography (SPECT) performed with ^{99m}Tc-DPD or ^{99m}Tc-PYP or ^{99m}Tc-HMDP* among patients with (left ventricular hypertrophy) LVH from an undiagnosed etiology. **Secondary objectives** • To assess the prevalence of AL or ATTR amyloidosis in patients with cardiac fixation at the bone scintigraphy (visual grade 1 to 3) and/or SPECT; • In patients diagnosed with ATTR Amyloidosis to assess the prevalence of hereditary (ATTRv) and wild-type (ATTRwt) ATTR amyloidosis; • To describe *TTR* genetic mutations** in patients with ATTRv amyloidosis; • To assess the prevalence of patients with familial history of known cardiomyopathy (CM), polyneuropathy (PN), sudden cardiac death (SCD) among their relatives (ie, parents, siblings and 2nd /3rd degree family members); • To assess the prevalence in patients with positive cardiac fixation at the bone scintigraphy or SPECT of patients

	with concomitant signs or symptoms of ATTR amyloidosis, ie,: • Sensori-motor Polyneuropathy (PN); • Carpal Tunnel syndrome (CTS); • Autonomic dysfunction; • Cardiological manifestations; • Laboratory signs; • Others. • To compare the clinical and biochemical characteristics between patients with positive scintigraphy (cardiac fixation at the bone scintigraphy grade 1, 2 or 3) and/or SPECT. • To asses the level of discrepancy in the evaluation of the scintigraphy and/or SPECT images by different evaluators. * Bisphosphonate (99mTc-DPD/99mTc-PYP/99mTc-HMDP) Scintigraphy. ** Variant and pathogenic mutation per sequencing the coding parts.
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2. LIST OF ABBREVIATIONS

Abbreviation	Definition
^{99}mTc-DPD	99mTechnetium-3,3-diphosphono-1,2-propanodi-carboxylic acid
^{99}mTc-PYP	99mTechnetium-pyrophosphate
^{99}mTc-HMDP	99mTechnetium-hydroxymethylene diphosphonate
AA	Serum Amyloid A Protein
AL	Light chain amyloidosis
ATTR	Transthyretin amyloid
ATTRm	Mutant transthyretin related amyloidosis
ATTRwt	Wild type transthyretin related amyloidosis
AVA	Aortic valve area
BNP	Brain natriuretic peptide
CA	Cardiac amyloidosis
CM	Cardiomyopathy
CMR	Cardiac magnetic resonance
CT	Computed tomography
CTS	Carpal Tunnel syndrome
EC	Ethics Committee
ECG	Electrocardiogram
ECHO	Echocardiography
e-CRF	Electronic Case Report Form
EDP	Exposure during pregnancy
EF	Ejection fraction
EMB	Endomyocardial biopsy
FPFV	First Patient First Visit
HCM	Hypertrophic cardiomyopathy
hs-cTnT	High sensitivity cardiac troponin T
AICD	Automatic Implantable Cardiac Defibrillator
ICD	Inform Consent Document
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IFE	Immunofixation electrophoresis
LGE	Late gadolinium enhancement
LPLV	Last Patient Last Visit
LV	Left ventricular
LVEF	Left ventricular ejection fraction
LVH	Left ventricular hypertrophy
LVOTO	Left ventricular outflow tract obstruction
MRI	Magnetic resonance imaging
MWT	Maximal wall thickness
NT-proBNP	N-terminal pro-brain natriuretic peptide

Abbreviation	Definition
PET	Positron emission tomography
PN	Polyneuropathy
SCD	Sudden cardiac death
sFLC	Serum free light chain
SPECT	Single Photon Emission Computed Tomography
TTE	Transthoracic echocardiography
TNNI3	Troponin I, cardiac muscle
TNNT2	Troponin T, cardiac muscle
TPM1	Tropomyosin alpha-1 chain
TTR	Transthyretin
ATTR-CM	Transthyretin amyloid cardiomyopathy
ATTR-FAP	Transthyretin amyloid polyneuropathy
TTRv	Variant transthyretin
TTRwt	Wild-type transthyretin

3. RESPONSIBLE PARTIES

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4. ABSTRACT

Title

Prevalence and characteristics of transthyretin amyloidosis in patients with left ventricular hypertrophy of unknown etiology (TTRACK**)**

Rationale and background

In an adult, hypertrophic cardiomyopathy (HCM) is defined by the presence of a left ventricular (LV) wall thickness ≥15 mm in one or more LV myocardial segments that is not explained solely by abnormal loading conditions.[11] Five to ten percent of adult cases are caused by genetic disorders including inherited metabolic and neuromuscular diseases, chromosome abnormalities and genetic syndromes. Some patients have non-genetic disorders that present similarly to genetic forms of the disease, for example, wild-type ATTR amyloidosis (ATTRwt, formerly known as senile amyloidosis) and light chain amyloidosis (AL).[45]

The diagnosis of HCM is based on the detection of increased LV wall thickness by any imaging modality.[11]

The three most frequent and clinically challenging types of systemic amyloidosis are acquired monoclonal immunoglobulin light chain amyloidosis (AL), the hereditary mutant ATTR (ATTRv), and the non-mutant ATTR, also known as systemic "senile" amyloidosis or ATTRwt.[42]

AL and ATTR amyloidosis can affect the heart in isolation or with multiorgan involvement.

Clinical features of transthyretin amyloid cardiomyopathy (ATTR-CM) are varied. Although heart failure symptoms predominate, suspicion of ATTR-CM can also be prompted by syncope, arrhythmias, or unexplained LV wall thickening on echocardiography (ECHO).[17]

Transthyretin (TTR) amyloidosis (ATTR amyloidosis) is a rare protein misfolding disease. It presents with a wide spectrum of clinical manifestations.[8,49] TTR, the protein implicated in ATTR amyloidosis, is a tetrameric transport protein. Misfolded TTR protein forms amyloid fibrils causing tissue damage by direct compression and obstruction.[49]

The clinical spectrum of ATTR amyloidosis varies widely from an exclusive neurological involvement to a predominant cardiac presentation.[45] In the first case, the disease is called ATTR polyneuropathy (ATTR-PN). In the second case, the disease is called ATTR cardiomyopathy (ATTR-CM).

ATTR-CM may occur alone or in conjunction with ATTR-PN. ATTR-CM can be associated with genetic variants of *TTR* and can also occur in the absence of a specific mutation, which is known as wild-type TTR (TTRwt).[19,47]

ATTR amyloidosis is likely underdiagnosed, mainly because the disease can mimic other causes of left ventricular hypertrophy (LVH), including hypertensive heart disease and hypertrophic cardiomyopathy (HCM).

The diagnosis of ATTR-CM remains challenging. The electrocardiograms (ECGs), transthoracic ECHO (TTE) and cardiac magnetic resonance (CMR) of ATTR-CM show similar findings to cardiac AL. The diagnosis strategy for ATTR amyloidosis combines pathology and molecular genetic testing. *TTR* is a gene of small size (7 kB), including four exons, and its screening by full sequencing of the coding parts is now easily accessible. In asymptomatic ATTR carriers, CMR may detect early cardiac involvement by focal myocardial late gadolinium enhancement (LGE).[32]

Routine laboratory testings are helpful. High levels of brain natriuretic peptide (BNP) [16] N-terminal pro-brain natriuretic peptide (NT-proBNP)[6] and high sensitivity cardiac troponin T (hs-cTnT) are associated with cardiovascular events, heart failure and death. Despite comparable values of LV wall thickness, plasma BNP values are three to five fold higher in patients with ATTR-CM than in patients with other causes of HCM.

Non-invasive techniques have been developed in the last few years to assist in the diagnosis of ATTR-CM. One of the imaging techniques is bone scintigraphy which has been shown to have excellent results in the diagnosis of ATTR-CM.

Several studies have suggested that ATTR-derived fibrils show avidity for bone tracers, in particular, recent systematic evaluation of bone scintigraphy suggests that ^{99m}Tc labeled 3,3-diphosphono-1,2-propanodicarboxylic acid (^{99m}Tc-DPD), ^{99m}Tc-labeled pyrophosphate (^{99m}Tc-PYP), and ^{99m}Tc-labeled hydroxymethylene diphosphonate (^{99m}Tc-HMDP) may be remarkably sensitive and specific for diagnosing ATTR-CM and may reliably distinguish other causes of CM that mimic amyloid such as HCM.[33,41,43,1,38]

For this reason, ^{99m}Tc-tracer scintigraphy (^{99m}Tc-DPD or ^{99m}Tc-PYP or ^{99m}Tc-HMDP) should be considered in patients in whom ATTR-CM is suspected, supporting the diagnosis when histology is not or hardly achievable.[12]

Bone scintigraphy enables the reliable diagnosis of ATTR-CM in the absence of histological data, in patients who have been evaluated for and do not have a monoclonal gammopathy. Non-invasive diagnostic criteria for ATTR-CM are applicable to the majority of patients with this disease.[17]

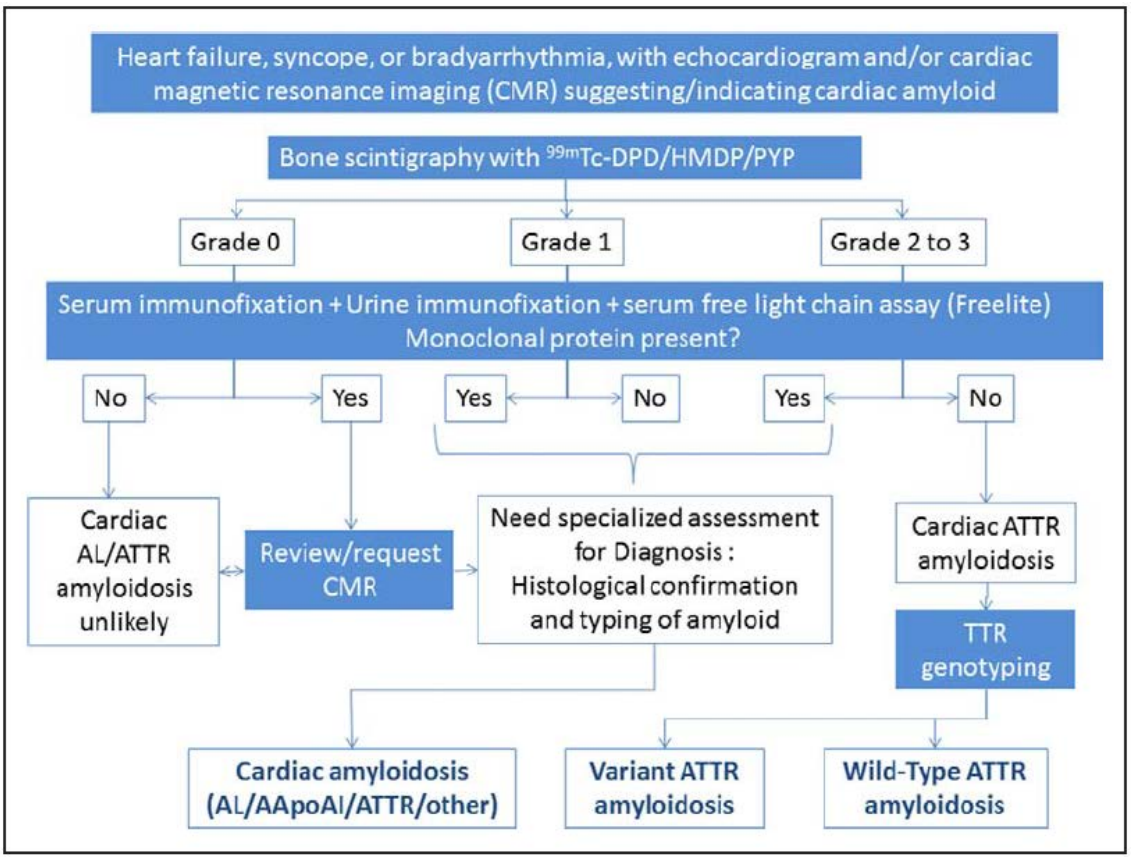
Nuclear medicine bone scans differentiate ATTR cardiomyopathy (ATTR-CM) from light chain cardiac amyloidosis and other myocardial disorders, helping to make the diagnosis without biopsy. Standard bone scans are not absolutely quantitative, so are assessed by comparing the heart to other tissues.[40]

The standard visual scoring system compares heart to bone. This accurately diagnoses ATTR-CM and has been validated in a multicenter study, but has limitations. Semiquantitative techniques including heart/contralateral thorax (H/CL) and heart/whole

body ratio (H/WB) improve on visual scoring but still rely on extracardiac sites as comparators. Absolute quantitation of myocardial uptake using quantitative SPECT should help overcome these shortcomings. In ATTR-CM, this technique is practical, accurately makes the diagnosis and provides information that is not identical to visual scores.[40]

Additional SPECT/CT significantly improved the diagnostic efficacy of ^{99m}Tc-DPD scintigraphy. Uptake grade of involved organs and degree of background activity might help to differentiate between AL and ATTR subtypes.[26]

Figure 1. Diagnostic Algorithm for Patients with Suspected Amyloid Cardiomyopathy (Gillmore 2016)[17]



Echocardiographic features suggesting/indicating cardiac amyloid include (but are not limited to) increased left ventricular wall thickness, restrictive filling pattern, abnormal left and right ventricular longitudinal strain, and atrial septal thickening.

Features suggesting/indicating cardiac amyloid on cardiac magnetic resonance imaging (CMR) include (but are not limited to) restrictive morphology, abnormal gadolinium kinetics, and extracellular volume expansion based on T1 mapping.

AApoA1 indicates apolipoprotein A-I; DPD, 3,3-diphosphono-1,2-propanodicarboxylic acid; HDMP, hydroxymethylene diphosphonate; and PYP, pyrophosphate; TTR, transthyretin; ATTR, TTR amyloidosis.

Research question and objectives

Primary objective

- To assess the prevalence of patients with cardiac fixation on a radionuclide bone scintigraphy and/or Single Photon Emission Computed Tomography (SPECT) performed with ^{99m}Tc-DPD or ^{99m}Tc-PYP or ^{99m}Tc-HMDP* among patients with left ventricular hypertrophy (LVH) from an undiagnosed etiology;

Secondary objectives

- To assess the prevalence of AL or ATTR amyloidosis amyloidosis in patients with cardiac fixation at the bone scintigraphy (visual grade 1 to 3) and/or SPECT;
- In patients diagnosed with ATTR Amyloidosis to assess the prevalence of hereditary (ATTRv) and wild-type (ATTRwt) ATTR amyloidosis;
- To describe TTR genetic mutations** in patients with ATTRv amyloidosis;
- To assess the prevalence of patients with familial history of known cardiomyopathy (CM), polyneuropathy (PN), sudden cardiac death (SCD) among their relatives (ie, parents, siblings and 2nd/3rd degree family members);
- To assess the prevalence in patients with cardiac fixation at the bone scintigraphy/SPECT of concomitant signs or symptoms of ATTR amyloidosis, ie,:
    - Sensori-motor Polyneuropathy (PN);
    - Carpal Tunnel syndrome (CTS);
    - Autonomic dysfunction;
    - Cardiological manifestations;
    - Laboratory signs;
    - Others;
- To compare the clinical and biochemical characteristics between patients with positive scintigraphy (cardiac fixation at the bone scintigraphy grade 1, 2 or 3) and/or SPECT.

- To asses the level of discrepancy in the evaluation of the scintigraphy and/or SPECT images by different evaluators

* Bisphosphonate (99mTc-DPD/99mTc-PYP/99mTc-HMDP) Scintigraphy

** Variant and pathogenic mutation per sequencing the coding parts

Study design

Multi-center and multi-national epidemiological study.

Principal investigators will be cardiologists experienced in the diagnosis of cardiomyopathies or physician specialized in diagnosing patients with LVH.

Patient population. Inclusion and exclusion criteria:

1. **Inclusion Criteria.**

   a. Patient signed inform consent.

   b. Males and Females.

   c. Age ≥50 years.

   d. Left ventricular hypertrophy (LVH) defined as end-diastolic LV maximum wall thickness (MWT) ≥15mm in Echocardiogram.

   e. Plan to undergo or recently underwent radionuclide bone scintigraphy and/or SPECT with any of the following radio labelled tracers: ^{99m}Tc-DPD or ^{99m}Tc-PYP or ^{99m}Tc-HMDP.

2. **Exclusion Criteria:**

   a. Etiological diagnosis explaining the LVH prior to patient inclusion (eg, Sarcomeric HCM, Myeloma, Fabry disease, Sarcoidosis, any type of amyloidosis [AA, AL, TTR])

   b. Severe aortic stenosis defined as aortic valve area (AVA) < 1.0 cm2

Variables– include exposures, outcomes, and key co-variates

- Date Informed Consent Document signed.
- Inclusion and exclusion criteria met.
- Patient demographic data (Age, Gender, Weight, Height, Race).

- Patient family history.
    - Relatives (parents, siblings and $2^{nd}/3^{rd}$ grade family) with known cardiomyopathy (CM), Polyneuropathy (PN), and/or Sudden cardiac death (SCD).
- Patient medical history.
- Results of Cardiological assessments, if performed.
    - Blood pressure
    - Bone scintigraphy with radiolabelled Technetium tracer (^{99m}Tc-DPD or ^{99m}Tc-PYP or ^{99m}Tc-HMDP) visual grading score and/or SPECT
    - ECG (last ECG performed).
    - Echocardiograhy.
    - Magnetic resonance imaging (MRI).
- Neurological assessments:
    - Symptoms and clinical signs of peripheral neuropathy (small fibres).
    - Autonomic symptoms and clinical signs.
    - Carpal tunnel syndrome (CTS) history and symptoms.

Additional set of variables from the patient clinical records as available will be included in the CRF of those patients with cardiac fixation in bone scintigraphy (Grade 1, 2 or 3) and/or SPECT:

- Results from blood sample testing (Creatinine, Haemoglobin, BNP, NT proBNP, Troponin I & T);
- Results from monoclonal protein studies (in urine and blood);
- Results from *TTR* gene sequencing.

Data sources

Data will be collected from medical records completed during routine clinical practice. As an epidemiological study, there are no specific requirements with regard to patient procedures to be performed or the treatment regimen. Data from each patient will be reported on an electronic case report form (e-CRF).

Study size

The study is designed to enroll approximately 1500 patients.

Considering a prevalence of ATTR-CM around 10%, the screening of 1.500 patients will provide a precision of ±1.5% in the estimation of this prevalence with a 95% two-sided confidence interval (primary objective). In addition, considering such a prevalence, the prevalence of the different symptoms or additional signs within the population of ATTR-CM (other objectives) will be estimated with a precision of ±7.3 % for any sign/symptom with a frequency of 30% or lower.

Data analysis

Detailed methodology for statistical analyses of data collected in this study will be documented in a Statistical Analysis Plan (SAP), which will be dated, filed and maintained by the sponsor. The SAP may modify the plans outlined in the protocol; any major modifications of primary endpoint definitions or their analyses would be reflected in a protocol amendment.

- Descriptive analysis will be performed for all the variables;
- Analysis will be stratified by age in years (50 to ≤55; 55 to ≤60; 60 to ≤65; 65 to ≤70; 70 to ≤75; 75 to ≤80; >80), gender and cardiac symptomatology at baseline;
- Patients with a final diagnosis of ATTR-CM will be compared with the other "negative" screened patients in order to understand possible diagnostic red flags;
- Factors associated with ATTR amyloidosis will be identified by univariate and multivariate logistic regression. A significance level of 10% and 5% will be used for the univariate and multivariate analyses, respectively.

Milestones

The study will enroll approximately 1500 patients.

Milestone	Planned date
Start of data collection	September 2017
End of data collection	June 2022 or as soon as 1000 patient will be screened in the study

5. AMENDMENTS AND UPDATES

Amendment number	Date	Protocol section(s) changed	Summary of amendment(s)	Reason
N°1	14 JUN 2018			New European legislation
N°2	11 FEBRUARY 2020	Inclusion/ Exclusion criteria Variables CRF ICD Milestone	Clarification of the protocol process to be in line with the requirement of a NIS protocol Addition of the SPECT in the inclusion criteria Inclusion period extension	Protocol clarification and deletion of the biological sample collection
N°2.1	22 OCTOBER 2020	Milestone	Extension for the data collection	Due to the international pandemy and to allow to reach the goal of the number of patient screened, it was decided to extend the inclusion period
N°2.2	21 MAY 2021	Milestone	Extension for the inclusion period and data collection	Due to the international pandemy and to allow to reach the goal of a minimum of one thousand patient screened, it was decided to extend the inclusion period. Participating country list updates
N°2.3	04 NOVEMBER 2021	Milestone	Extension for the inclusion period and data collection	Due to the international pandemy and to allow to reach the goal of a minimum of one thousand patient screened, it was decided to extend the inclusion period.

6. MILESTONES

Milestone	Planned date
Feasibility meeting	06 – 07 October 2017
Start of data collection	May 2018
End of data collection	June 2022
Final study report	December 2022

7. RATIONALE AND BACKGROUND

Transthyretin (TTR) amyloidosis (ATTR amyloidosis) is a rare protein misfolding disease, (*Conceicao 2016; Sekijima 2015*).[8,49] The protein implicated in ATTR amyloidosis, is a tetrameric transport protein for thyroxine and the retinol-binding protein–retinol (vitamin A) complex (*Monaco 1995*).31 Destabilisation and dissociation of the native TTR tetramer can result in misfolding and the formation of amyloid fibrils and progressive amyloid deposition in tissues (*Connelly 2010, Klabunde 2000, Johnson 2005, Sekijima 2008*).[9,22,20,48]

Misfolded TTR protein forms amyloid fibrils causing tissue damage by direct compression and obstruction (*Sekijima 2015*).[49] The resulting tissue damage is largely irreversible (*Coelho 2013; Plante-Bordeneuve 2014*).[5,37]

The clinical spectrum of ATTR amyloidosis varies widely from an exclusively neurological involvement to a predominantly cardiac presentation. This heterogeneity is linked to several factors including specific TTR mutations, patient and transmitting parent gender, geographical distribution, and endemic/non-endemic aggregation. (*Rapezzi 2013*).[45]

When the peripheral nerves are affected predominantly, the disease is termed transthyretin amyloid polyneuropathy (ATTR-PN). When the heart is primarily affected, the disease is called transthyretin amyloid cardiomyopathy (ATTR-CM).

ATTR-PN is a fatal illness resulting from autosomal dominantly inherited single-point mutations on the *TTR* gene. This disease may strikes people from their 30s. A person affected by this disease will develop a slow, steady, and devastating neurologic decline leading ultimately to death with a mean survival of 10 to 15 years. Symptoms in the early stage of disease are not always easy to recognize and are difficult to interpret on clinical grounds due to their subjective nature (*Conceicao 2008*).[7] By the time clear symptoms are present, the loss of function has already been achieved (*Leufacheur 2013; Kodaira 2011*).[27,23] Early intervention is therefore critical to stabilize the disease (*Coelho 2013; Plante-Bordeneuve 2014*).[5,37]

ATTR-CM may occur in isolation or may occur in conjunction with ATTR-PN. ATTR-CM may be associated with genetic variants of *TTR* but can also occur in the absence of a specific mutation, which is known as wild-type TTR (TTRwt) (*Jacobson 1997, Saraiva 1995*).[19,47]

More than 100 amyloidogenic TTR point mutations have been identified in patients with ATTR-PN. The most common genetic variant associated with polyneuropathy is Val30Met (valine replaced by methionine at position 30 of the amino acid chain), which accounts for ≈85%of ATTR-PN cases worldwide. Generally, non-Val30Met mutations are associated with greater cardiac involvement than Val30Met mutations, and patients with non-Val30Met mutations and a cardiac phenotype tend to be older men and have lower survival rates (*Merlini 2013*).[28]

Median survival from diagnosis for patients with ATTR-CM was reported as 41 months for patients with a Val122Ile mutation (valine replaced by isoleucine at position 122 of the amino acid chain) and 46 months for patients with wild-type ATTR-CM (*Connors 2011*),[10] with most patients dying from cardiac causes, including sudden death, congestive heart failure, and myocardial infarction (*Kyle 1996, Smith 1984*).[25,51]

ATTR amyloidosis is likely underdiagnosed, mainly because the disease can mimic other causes of left ventricular (LV) hypertrophy (LVH), including hypertensive heart disease and HCM.

In the majority of cases, hypertrophic cardiomyopathy (HCM) is inherited as an autosomal dominant genetic trait with a 50% risk of transmission to offspring *(Richard 2003)*.[46] In patients fulfilling HCM diagnostic criteria, sequencing of sarcomere protein genes identifies a disease-causing mutation in up to 60% of cases *(Richard 2003, Van Driest 2003)*.[46,52] The likelihood of finding a causal mutation is highest in patients with familial disease and lowest in older patients and individuals with non-classical features.

In an adult, hypertrophic cardiomyopathy (HCM) is defined by the presence of a LV wall thickness ≥15 mm in one or more LV myocardial segments—as measured by any imaging technique (echocardiography (ECHO), cardiac magnetic resonance imaging (CMR) or computed tomography (CT))—that is not explained solely by abnormal loading conditions (*Elliott 2014)*.[11] In up to 60% of adolescents and adults with HCM, the disease is an autosomal dominant trait caused by mutations in cardiac sarcomere protein genes (*Brito 2012*),[3] but five to ten percent of adult cases are caused by other genetic disorders including inherited metabolic and neuromuscular diseases, chromosome abnormalities and genetic syndromes*)*. Some patients have non-genetic disorders that present similarly to genetic forms of the disease, for example, wild-type ATTR amyloidosis (ATTRwt, formerly known as senile amyloidosis) and light chain amyloidosis (AL). (*Rapezzi 2013*).[45]

AL and hereditary ATTR amyloidosis can affect the heart in isolation or with multiorgan involvement, whereas wild-type ATTR amyloidosis predominantly affects the heart.

The diagnosis of HCM rests on the detection of increased LV wall thickness by any imaging modality. Due to the diverse etiology of the disease, detection of increased LV wall thickness that is unexplained by loading conditions should prompt a systematic search for its underlying cause. In many patients, this work-up should include specialized laboratory testing and, in some circumstances as ATTR amyloidosis, genetic analysis. (*Elliott 2014).*[11]

The common feature of the amyloidosis is the extracellular accumulation of fibrillary proteins, leading to loss of normal tissue architecture and myocardial involvement with clinical implications (*Rapezzi 2009).*[42]

Clinical features are varied, and although heart failure symptoms predominate, suspicion of cardiac amyloidosis can also be prompted by syncope, arrhythmias, or unexplained LV wall thickening on ECHO (*Gillmore 2016*).[17]

Amyloidosis may be systemic or localized and is currently classified according to the type of precursor protein. The most frequent types of systemic amyloidosis associated with clinically relevant cardiac involvement are: AL, AA and ATTR amyloidosis (*Rapezzi 2009*).[42]

ATTRm is transmitted as an autosomal dominant trait with variable penetrance (*Mohty 2013*).[32] Clinical manifestations and age of onset vary depending on the *TTR* mutation, sex, parental gene transmission and geographical area (*Plante-Bordeneuve 2011*).[36] The Val30Met ATTR amyloidosis in Portuguese patients occurs in their second or third decade, with peripheral neuropathy and cardiac conductive disorders. In other geographical areas, the same Val30met mutation presents as late onset ATTR amyloidosis (at age 50—70years) and the clinical manifestations combine peripheral neuropathy, cardiac conductive disorders and heart failure (*Plante-Bordeneuve 1998*).[35] In other *TTR* mutations (Thr60Ala and Val122Ile), cardiac involvement is predominant (*Mohty 2013*).[32]

The diagnosis of ATTR-CM remains challenging. However, because of the overlapping symptoms, sole reliance on clinical manifestations for diagnosis is imprudent (*Kapoor 2011*).[21] The electrocardiograms (ECGs), transthoracic echocardiography (TTE) and CMR of ATTR-CM show similar findings to cardiac AL. The diagnosis strategy for ATTR amyloidosis combines pathology and molecular genetic testing. *TTR* is a gene of small size (7 kB), including four exons, and its screening by full sequencing of the coding parts is now easily accessible. In asymptomatic ATTR amyloidosis carriers, CMR may detect early cardiac involvement by focal myocardial late gadolinium enhancement (LGE) (*Mohty 2013*).[32]

Routine laboratory testing aids. High levels of brain natriuretic peptide (BNP) *(Geske 2013)*,[16] N-terminal pro-brain natriuretic peptide (NT-proBNP) *(Coats, 2013)*[6] and high sensitivity cardiac troponin T (hs-cTnT) are associated with cardiovascular events, heart failure and death. Despite comparable values of ventricular wall thickness, plasma BNP values are three to five times higher in patients with cardiac amyloidosis than in those with other causes of HCM.

The diagnosis of amyloidosis is also obtained through biopsy of a clinically affected organ, with Congo red histology demonstrating pathognomonic green birefringence. However, when amyloidosis is suspected clinically, biopsy of subcutaneous fat, salivary gland, or rectum yields the diagnosis in 50% to 80% of patients with AL amyloidosis (*Ansari-Lari 2004, Gillmore 2016*).[1,17] A much lower yield in patients with ATTR amyloidosis frequently results in a requirement for endomyocardial biopsy (EMB) to confirm the diagnosis (*Fine*

2014, Gillmore 2016).[13,17] EMB is associated with a risk of complications, including myocardial perforation and tamponade that may be fatal and requires expertise that can introduce diagnostic delay (*Maurer 2015, Gillmore 2016*).[30,17] ECGs are abnormal in 90% of cases with cardiac involvement (*Mohty 2013*). The low precordial and limb lead voltage is primarily due to the destruction and replacement of myocytes by the electrically inert amyloid. Although this pattern is exhibited by approximately 70% of AL-associated cardiac amyloidosis, only one third of patients with hereditary amyloidosis and wild-type amyloidosis show low voltage. The pseudoinfarction pattern as evidenced by QS waves in 2 consecutive leads is another common finding (*Kapoor 2011*).[21] Differential diagnosis between HCM and cardiac amyloidosis is aided by measuring the ratio between QRS voltages and LV wall thickness (*Elliott 2014*).[11]

ECHO, although a valuable and widely accessible tool for investigating heart failure, is neither sensitive nor specific for cardiac amyloidosis. Typical findings on ECHO include thickening of ventricular walls, restrictive filling, abnormal left and right ventricular longitudinal strain, and atrial septal thickening (*Falk 2014*).[12] In hereditary amyloidosis, marked LVH may be seen on echocardiogram even in the absence of significant heart failure (*Kapoor 2011*).[21] Low voltage on ECHO and interventricular septal thickness of >19.8 mm on ECHO together have a sensitivity of 72% and specificity of 91% for cardiac amyloidosis (*Rahman 2004*).[39] Cardiac magnetic resonance imaging (CMR) has much greater diagnostic value in cardiac amyloidosis, but false-positive and false-negative CMRs are not infrequent (*Maceira 2005*). Typical findings include restrictive morphology, abnormal gadolinium kinetics, and extracellular volume expansion on T1 mapping. Furthermore, CMR is costly, is available only in specialist centers, is contraindicated in a substantial proportion of patients, and cannot reliably distinguish different types of amyloid (*Gillmore 2016*).[17]

The ventricular myocytes of the "amyloid heart" have shown augmentation of both atrial and brain natriuretic peptide gene expression. Although nonspecific from a diagnostic utility standpoint in cardiac amyloidosis, NT-proBNP is a sensitive (93%) prognostic biomarker (*Kapoor 2011*).[21] Regarding nuclear imaging, the major clinical contribution is the detection of ATTR-CM. TTR is a tetrameric plasma transport protein synthesized in the liver and is the precursor protein in wild-type and familial TTR-related amyloidosis. Several studies have suggested that TTR-derived fibrils show avidity for bone tracers, in particular ^{99m}Technetium labeled tracers, whereas there is no uptake of tracer in the hearts of patients with HCM caused by sarcomeric protein gene mutations.

Recent systematic evaluation of bone scintigraphy suggests that ^{99m}Tc-labeled 3,3-diphosphono-1,2-propanodicarboxylic acid (DPD), ^{99m}Tc-labeled pyrophosphate (PYP), and ^{99m}Tc-labeled hydroxymethylene diphosphonate (HMDP) may be remarkably sensitive and specific for imaging cardiac TTR amyloid and may reliably distinguish other causes of cardiomyopathy that mimic amyloid such as HCM (*Perugini 2005, Rapezzi 2008, Rapezzi 2011, Bokhari 2013, Quarta 2012*).[33,41,43,1,38] Indeed, radionuclide bone scintigraphy may identify cardiac ATTR deposits early in the course of the disease, sometimes before the development of abnormalities on ECHO or CMR (*Glaudemans 2014, Fontana 2014*),[15,14] and has been used to diagnose ATTR amyloidosis among patients with heart failure and

preserved ejection fraction (*Castaño 2015*).4 Cardiac localization of radiotracer occurs in a small proportion of patients with AL amyloidosis, and although usually low grade, it can confound distinguishing between cardiac ATTR and AL types of amyloid (*Bokhari 2013)*.[1]

For this reason, bone scintigraphy (^{99m}Tc-DPD or ^{99m}Tc-PYP or ^{99m}Tc-HMDP) should be considered in patients in whom ATTR amyloidosis is a possibility (age >65 years, history of bilateral carpal tunnel syndrome (CTS), absent family history of HCM, and features consistent with cardiac amyloidosis on ECG and cardiac imaging) (*Rapezzi 2011, Elliott 2014*),[43,11] supporting the diagnosis (*Falk 2014*).[12]

Gillmore analyses of 374 patients with EMBs, corroborated in the whole cohort of 1.217 patients, indicate that cardiac uptake (grade 1, 2, or 3) on a radionuclide bone scan is >99% sensitive but not completely specific for cardiac ATTR (68% specificity compared with EMB histology); the low specificity results largely from low-grade uptake in patients with cardiac AL or cardiac apolipoprotein A-I amyloidosis (*Hutt 2014*).[18] The specificity for cardiac ATTR of grade 2 or 3 cardiac uptake on radionuclide imaging increases to ≈87%, but the sensitivity falls to 91%. Because it is absolutely essential to avoid misdiagnosis of cardiac ATTR in a patient who actually has cardiac AL requiring chemotherapy, the primary aim of the proposed diagnostic criteria was to achieve very high diagnostic specificity. The specificity and positive predictive value for ATTR-CM of the combination of grade 2 or 3 cardiac uptake on a radionuclide scan and the absence of a detectable monoclonal protein despite serum immunofixation electrophoresis (IFE), urine IFE, and serum free light chain (sFLC) assay were 100% (positive predictive value confidence interval, 99.0–100%) in this cohort of 1.217 patients and were also 100% among each of the 3 different radiotracer cohorts. Although we acknowledge that further validation of ^{99m}Tc-HMDP scintigraphy against EMB histology is required, the presented preliminary data for ^{99m}Tc-HMDP indicate that it behaves identically to ^{99m}Tc-DPD and ^{99m}Tc-PYP.

The diagnosis of ATTR-CM is often delayed or missed as a result of the poor sensitivity and specificity of ECHO (*Kristen 2010*),[24] coupled with the current requirement for histological confirmation of amyloid in a tissue biopsy. The diagnosis of ATTRwt is particularly challenging because it presents in older age (when confounding comorbidities such as coronary heart disease, hypertension, and aortic stenosis frequently coexist) (*Pinney 2013, Gillmore 2016*).[34,17]

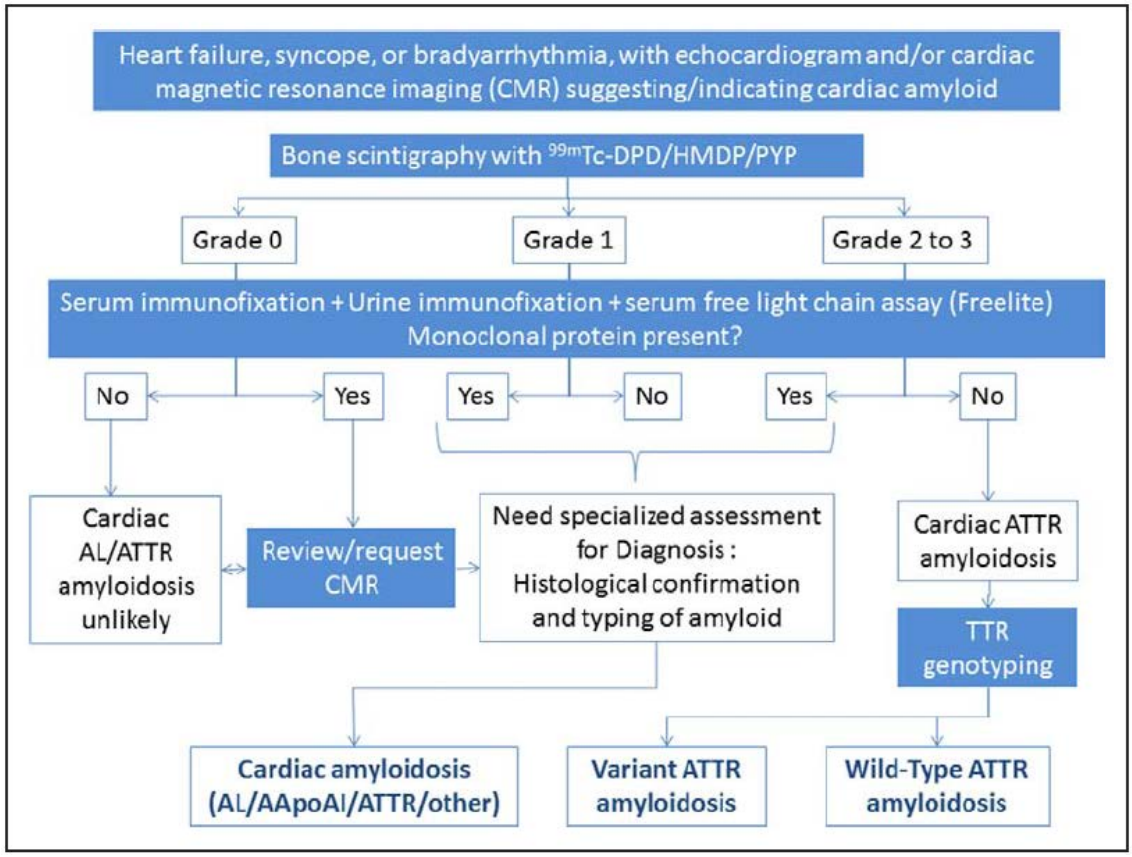
Bone scintigraphy enables the diagnosis of ATTR-CM to be made reliably without the need for histology in patients who do not have a monoclonal gammopathy. Non-invasive diagnostic criteria for ATTR-CM are applicable to the majority of patients with this disease (*Gillmore 2016*).[17]

Nuclear medicine bone scans differentiate ATTR cardiomyopathy (ATTR-CM) from light chain cardiac amyloidosis and other myocardial disorders, helping to make the diagnosis without biopsy. Standard bone scans are not absolutely quantitative, so are assessed by comparing the heart to other tissues. (Ramsay et al. 2019).[40]

The standard visual scoring system compares heart to bone. This accurately diagnoses ATTR-CM and has been validated in a multicenter study, but has limitations. Semiquantitative techniques including heart/contralateral thorax (H/CL) and heart/whole body ratio (H/WB) improve on visual scoring but still rely on extracardiac sites as comparators. Absolute quantitation of myocardial uptake using quantitative SPECT should help overcome these shortcomings. In ATTR-CM, this technique is practical, accurately makes the diagnosis and provides information that is not identical to visual scores. (Ramsay et al. 2019).[40]

Additional SPECT/CT significantly improved the diagnostic efficacy of 99mTc-DPD scintigraphy. Uptake grade of involved organs and degree of background activity might help to differentiate between AL and ATTR subtypes. (Lee J, 2020).[26]

Figure 1. Diagnostic Algorithm for Patients with Suspected Amyloid Cardiomyopathy (Gillmore 2016)[17]



Echocardiographic features suggesting/indicating cardiac amyloid include (but are not limited to) increased left ventricular wall thickness, restrictive filling pattern, abnormal left and right ventricular longitudinal strain, and atrial septal thickening.

Features suggesting/indicating cardiac amyloid on cardiac magnetic resonance imaging (CMR) include (but are not limited to) restrictive morphology, abnormal gadolinium kinetics, and extracellular volume expansion based on T1 mapping.

AApoA1 indicates apolipoprotein A-I; DPD, 3,3-diphosphono-1,2-propanodicarboxylic acid; HDMP, hydroxymethylene diphosphonate; and PYP, pyrophosphate.

Moreover, the data clearly indicate that the combined finding of grade 2 or 3 cardiac uptake on radionuclide scintigraphy and the absence of a monoclonal protein by serum immunofixation electrophoresis (IFE), urine IFE, and sFLC (Freelite) assay was 100% specific for presence of ATTR-CM (Gillmore 2016).[17]

A diagnosis of ATTR-CM should be followed by TTR genotyping in all patients to differentiate between wild-type and variant ATTR-CM.

The main purpose of this study is to determine the prevalence of ATTR-CM among patients admitted due to LVH of unknown etiology when radionuclide bone scintigraphy or SPECT is used.

8. RESEARCH QUESTION AND OBJECTIVES

Primary objective

- To assess the prevalence of patients with cardiac fixation on a radionuclide bone scintigraphy and/or Single Photon Emission Computed Tomography (SPECT) performed with ^{99m}Tc-DPD or ^{99m}Tc-PYP or ^{99m}Tc-HMDP* among patients with (left ventricular hypertrophy) LVH from an undiagnosed etiology.

Secondary objectives

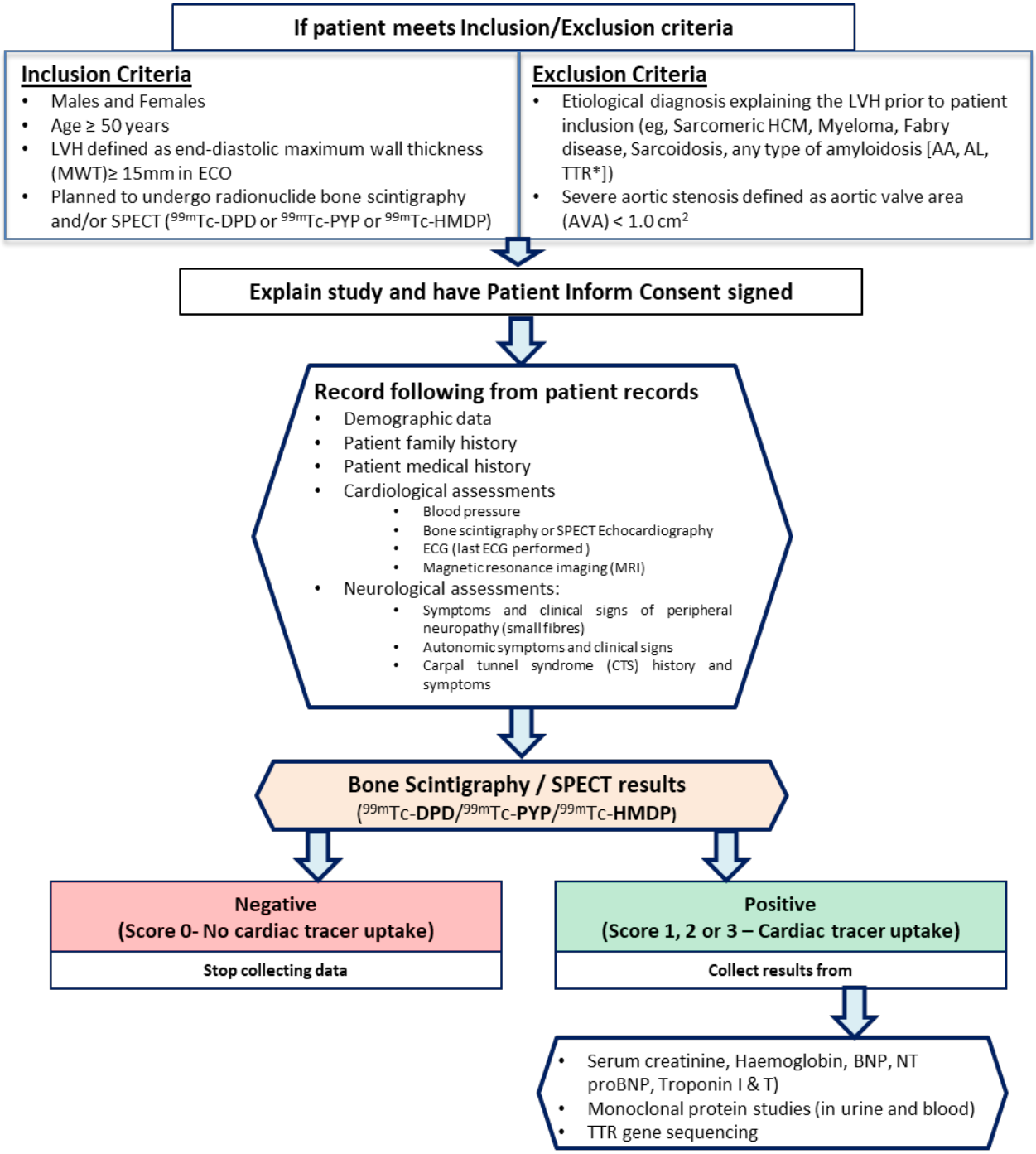
- To assess the prevalence of AL or ATTR amyloidosis amyloidosis in patients with cardiac fixation at the bone scintigraphy (visual grade 1 to 3) and/or SPECT;
- In patients diagnosed with ATTR Amyloidosis to assess the prevalence of hereditary (ATTRv) and wild-type (ATTRwt) ATTR amyloidosis;
- To describe TTR genetic mutations** in patients with ATTRv amyloidosis;
- To assess the prevalence of patients with familial history of known cardiomyopathy (CM), polyneuropathy (PN), sudden cardiac death (SCD) among their relatives (ie, parents, siblings and 2nd /3rd degree family members).

- To assess the prevalence in patients with positive cardiac fixation at the bone scintigraphy and/or SPECT of patients with concomitant signs or symptoms of ATTR amyloidosis, ie,:
    - Sensori-motor Polyneuropathy (PN);
    - Carpal Tunnel syndrome (CTS);
    - Autonomic dysfunction;
    - Cardiological manifestations;
    - Laboratory signs;
    - Others.
- To compare the clinical and biochemical characteristics between patients with positive scintigraphy (cardiac fixation at the bone scintigraphy grade 1, 2 or 3) and/or SPECT.
- To asses the level of discrepancy in the evaluation of the scintigraphy and/or SPECT images by different evaluators

* Bisphosphonate (99mTc-DPD/99mTc-PYP/99mTc-HMDP) Scintigraphy.

** Variant and pathogenic mutation per sequencing the coding parts.

Figure 2. Study Flow Chart



9. RESEARCH METHODS

9.1. Study Design

TTRACK is an epidemiological multicenter and multinational study.

Patients can be enrolled from several countries listed in Appendix 5. This list is non-exhaustive and additional countries may be added to this list.

The management of this protocol will be aligned with country legislation and regulatory procedures.

Investigators will be cardiologists experienced in diagnosis of cardiomyopathies or physicians specialized in diagnosing patients with LVH. The sites involved (at least when performing feasibility) will be representative of their respective country in terms of practice. Participating physicians will not be influenced in their decision making and routine proceedings in any way.

The study will include approximately 1500 patients.

All assessments described in this protocol are performed as part of routine clinical practice or standard practice guidelines for the patient population and healthcare provider specialty in the countries where this non-interventional study is being conducted. Data, including individual study results, will be collected from these assessments, if performed.

9.2. Setting

9.2.1. Site Initiation

The cardiologists who agreed to participate in the study will receive a letter containing:

- A cover letter with a reply slip to be returned to Pfizer's logistics center;
- A study synopsis;
- The participating physicians' contracts, one copy of which should be returned to Pfizer's logistics center;
- A pre-paid envelope.

Once the contract has been signed and returned to Pfizer's logistics center, it will be validated by Pfizer.

A site initiation visit will be organized to present the protocol and its documentation.

This documentation will be sent by mail few days before initiation visit and it will be composed with:

- All study documents in the paper version (protocol, regulatory agreements, etc ...);

- Briefings and patient consent;
- Monitoring documentation (Screening and enrollment log, Subject identification code list, Site visit log, etc).

To initiate the site, Pfizer will have to receive some mandatory documents such as:

- Protocol Acceptance Form signed by the physician;
- The certificate of safety training;
- The signature sheet and delegations of duties log;
- The Curriculum Vitae of the physician and all the people involved in the project.

After reception of these documents, the physician and all the people involved in the study will receive the study website address and his/her own access code. They will find the following documents on the website:

- Regulatory authorities opinion;
- Ethics committee (EC) opinion;
- The study protocol and protocol amendment;
- The serious adverse events (SAE) declaration form.

In addition, data relating to the physician (year of registration, year of doctoral thesis, town where practicing etc.) can be uploaded to the website as well as the anonymized data regarding the patients.

Each physician investigator will be a single number (0001 to 00XX).

Each patient screened in the study will be assigned a number by order of inclusion in the study.

Therefore, each patient will be identified in the study by the center number and patient number (for example 0002-05 is the 5th patient included by the second site)

The physicians will be provided with a hotline number as well as an email address where they can ask questions about the conduct of the study.

9.2.2. Inclusion Criteria

The study will enroll patients according to the eligibility criteria in participating countries. The recruiting centers will be representative of each country involved in terms of care management systems, of size and of practices (at least when performing feasibility).

To ensure equitable recruitment, each site will be limited to a 100 screened patient maximum.

All patients who met the inclusion and exclusion criteria will be invited to participate in the study.

An informed consent document, will be signed by each patient before any data collection (Appendix 1).

If a patient isn't included in the study for any reason, the non-inclusion register has to be completed (Appendix 2).

The enrollment visit will be performed at the time of inclusion, after the patients' acceptance of enrollment in the study and Informed Consent Document signature.

Data will come from medical records and will be collected in routine clinical practice. The enrollment visit will comprise a first section on patients' eligibility for every patient and a second section on the patient information, family history and clinical evaluation at the inclusion.

After the patient undergoes a Bone Scintigraphy and/or SPECT as part of standard of care (Annex 3) the images will be evaluated by the nuclear medicine specialist of the investigational center.

Scans will be defined at each site according to the internationally accepted grading:

Grade 0 = absent cardiac uptake

Grade 1=mild uptake less than bone

Grade 2=moderate uptake equal to bone

Grade 3=high uptake greater than bone

For all patients with cardiac fixation at scintigraphy and/or SPECT the following data will be collected and incorporated in the CRF if available:

- Answers from questions regarding ATTR amyloidosis red flags
- Results from clinical laboratory tests and genotyping.

The patient will finish the study after this visit.

9.2.3. Centralized Review of Bone Scintigraphy (for all patients)

In this study, all the scintigraphy/SPECT tests will be additionally reviewed by two external evaluators.

The objective of this evaluation will be to asses the level of discrepancy in the evaluation of the scintigraphy/SPECT images by different evaluators

All the scintigraphy/SPECT tests will be uploaded in a dedicated database via the study web site.

Two experts from the scientific committee members will connect to the study web site with personalized connection access. They'll review the scintigraphies/SPECT and enter their evaluation.

9.2.4. Blood Samples Analyses

Following the standard practice guidelines for the patient population with positive scintigraphy, blood and urine analyses are usually done to rule out AL amyloidosis. If performed, results of these tests will be collected.

Moreover, to differentiate between wild-type and variant ATTR-CM, genotyping are usually completed. If performed, results will be collected.

9.2.5. Inclusion/exclusion Criteria

Patients with LVH from undiagnosed etiology and the following inclusion/exclusion criteria:

1. **Inclusion Criteria**.

    a. Patient signed inform consent

    b. Males and Females

    c. Age ≥50 years

    d. Left ventricular hypertrophy (LVH) defined as end-diastolic LV maximum wall thickness (MWT) ≥15mm in Echocardiogram.

    e. Plan to undergo or recently underwent radionuclide bone scintigraphy and/or SPECT with any of the following radio labelled tracers: ^{99m}Tc-DPD or ^{99m}Tc-PYP or ^{99m}Tc-HMDP*.

        ** Scintigraphy will be defined at each site according to the standard grading:*

        Grade 0 = absent cardiac uptake

        Grade 1=mild uptake less than bone

        Grade 2=moderate uptake equal to bone

        Grade 3=high uptake greater than bone

2. Exclusion Criteria:

a. Etiological diagnosis explaining the LVH prior to patient inclusion (eg, Sarcomeric HCM, Myeloma, Fabry disease, Sarcoidosis, any type of amyloidosis [AA, AL, TTR**]).

b. Severe aortic stenosis defined as aortic valve area (AVA) <1.0 cm2.

*** TTR diagnosis at exclusion criteria: Direct relative (siblings or parents) of a carrier with a known hereditary mutation in the TTR gene or any patient already diagnosed by any of the following: cardiac fixation at the bone scintigraphy, TR mutation at the genetic testing, biopsy with amyloidosis (positive red congo) and positive TTR staining.*

9.2.5.1. Follow-up Visit

Patients will undergo follow-up to collect results of biological and genetic tests if there is cardiac fixation on a radionuclide bone scintigraphy and/or SPECT.

9.3. Variables

Variable	Data source(s)
Patient informed consent date	**PATIENT INCLUSION**
Patient visit date	**PATIENT INCLUSION**
Age ≥50 years	**PATIENT INCLUSION**
LVH **Echo demonstrated end-diastolic LV MWT ≥15mm**	**PATIENT INCLUSION**
Maximal end diastolic wall thickness	**PATIENT INCLUSION**
Sarcomeric HCM (Genetic testing)	**PATIENT INCLUSION**
Plasma cell dyscrasia	**PATIENT INCLUSION**
Fabry disease	**PATIENT INCLUSION**
AL, AA or any other amyloidosis	**PATIENT INCLUSION**
Severe aortic stenosis (defined as) Maximal aortic velocity >4 m/s or Mean gradient >40 mmHg	**PATIENT INCLUSION**
Birth date (mm-yyyy)	**PATIENT INFORMATION**
Gender	**PATIENT INFORMATION**
Weight	**PATIENT INFORMATION**
Height	**PATIENT INFORMATION**
Ethnic Origin	**PATIENT INFORMATION**
Date	**CLINICAL EVALUATION**
Diastolic blood pressure	**CLINICAL EVALUATION**
Systolic blood pressure	**CLINICAL EVALUATION**
Diagnosed hypertension	**CLINICAL EVALUATION**
Antihypertensive medication	**CLINICAL EVALUATION**
Coronary artery disease	**CLINICAL EVALUATION**
Renal insufficiency	**CLINICAL EVALUATION**
Diabetes Mellitus	**CLINICAL EVALUATION**
Lumbar spinal stenosis 1	**CLINICAL EVALUATION**
CTS – surgery history 2	**CLINICAL EVALUATION**
New York heart Association (NYHA) class	**CLINICAL EVALUATION**
Atrial Fibrillation (AF)	**CLINICAL EVALUATION**

Variable	Data source(s)
Pacemaker	**CLINICAL EVALUATION**
AICD (Automatic Implantable Cardiac Defibrillator)	**CLINICAL EVALUATION**
LGE (Late Gadolinium Enhancement)	**CLINICAL EVALUATION**
Heart rate	**CLINICAL EVALUATION**
Sinus rhythm	**CLINICAL EVALUATION**
PR Interval	**CLINICAL EVALUATION**
QRS interval	**CLINICAL EVALUATION**
Sokolow index	**CLINICAL EVALUATION**
Pseudo-MI pattern	**CLINICAL EVALUATION**
Poor precordial R wave progression	**CLINICAL EVALUATION**
LBBB RBBB Paced Intraventricular conduct delay	**CLINICAL EVALUATION**
Left Ventricular Ejection Fraction (LVEF)	**CLINICAL EVALUATION**
Left Ventricular Obstruction Tract (LVOT)	**CLINICAL EVALUATION**
Longitudinal strain done?	**CLINICAL EVALUATION**
LV end-diastolic diameter	**CLINICAL EVALUATION**
MWT	**CLINICAL EVALUATION**
MWT at septum	**CLINICAL EVALUATION**
MWT posterior wall	**CLINICAL EVALUATION**
Hypertrophic pattern **Apical, Concentric, Asymmetric, Mix**	**CLINICAL EVALUATION**
LV mass index	**CLINICAL EVALUATION**
Aortic valvular stenosis	**CLINICAL EVALUATION**
Pericardial effusion	**CLINICAL EVALUATION**
Patient scintigraphy date	**SCINTIGRAPHY**
^{99m}Tc-DPD, ^{99m}Tc-PYP, ^{99m}Tc-HMDP	**SCINTIGRAPHY**
Grade 0=absent cardiac uptake **Grade 1=mild uptake less than bone** **Grade 2=moderate uptake equal to bone** **Grade 3=high uptake greater than bone**	**SCINTIGRAPHY**
Walking ability: No Impairment, Impaired but ability to walk without a stick or crutches, Walking only with the help of one stick or crutch, Walking with the help of two sticks or crutches, Confined to a wheelchair or bedridden	**ATTR RED FLAGS**
SPECT date	**SPECT**
SPECT Result	**SPECT**
Paresthesia / Numbness / Sensory loss *	**ATTR RED FLAGS**
Pain*	**ATTR RED FLAGS**
Temperature sensory loss*	**ATTR RED FLAGS**
Muscle weakness*	**ATTR RED FLAGS**
Carpal tunnel syndrome symptoms	**ATTR RED FLAGS**
Erectile dysfunction / Vaginal dryness	**ATTR RED FLAGS**
Orthostatic / postural hypotension (dizziness, syncope)	**ATTR RED FLAGS**
Urinary difficulties (i.e. dysuria, incontinence…)	**ATTR RED FLAGS**
Sweating abnormalities	**ATTR RED FLAGS**
Digestive dysautonomia: Constipation, alternating constipation and diarrhoea, nausea, vomiting…	**ATTR RED FLAGS**
Early satiety / Gastro paresis	**ATTR RED FLAGS**
Unintentional weight loss	**ATTR RED FLAGS**
Visual disturbances: Dry eye / Glaucoma	**ATTR RED FLAGS**
Creatinine	**CLINICAL LABORATORIES**
Haemoglobin	**CLINICAL LABORATORIES**

Variable	Data source(s)
BNP	**CLINICAL LABORATORIES**
NTproBNP	**CLINICAL LABORATORIES**
Troponin I	**CLINICAL LABORATORIES**
Troponin T	**CLINICAL LABORATORIES**
IFE of serum	**CLINICAL LABORATORIES**
IFE of urine	**CLINICAL LABORATORIES**
***sFLC assay**	**CLINICAL LABORATORIES**
Electrophoresis performed?	**CLINICAL LABORATORIES**
Date	**TTR gene sequencing**
Result	**TTR gene sequencing**

9.4. Data sources

Data will be collected from medical records and routine clinical practice. As an epidemiological study, there are no specific requirements with regard to patient procedures to be performed or the treatment regimen. Data from each patient will be reported on an electronic case report form (e-CRF).

An electronic Case Report Form (e-CRF – Appendix 3) will be used for data recording. In this protocol, the term e-CRF should be understood to refer to medical records in electronic data form. The data collection method used will be an e-CRF for physicians in English.

The visit after scintigraphy/SPECT should be documented only when the bone scintigraphy is positive with tracer uptake (Grade 1, 2 or 3) or SPECT shows cardiac fixation.

It is the investigator's responsibility to ensure completion and to review and approve all e-CRFs. E-CRFs must be signed by the investigator or by an authorized staff member These signatures serve to attest that the information contained on the e-CRFs is true. Anytime, the investigator has the final personal responsibility for the accuracy and authenticity of all clinical and laboratory data entered into the e-CRF.

9.5. Study Size

Considering a prevalence of ATTR-CM around 10%, the screening of N =1500 patients will provide a precision of ±1.5% in the estimation of this prevalence with a 95% two-sided confidence interval (first objective). In addition, considering such a prevalence, the precision of the estimation of the different symptoms or additional signs within the population of ATTR-CM (other objectives) will be estimated with a precision of ± 7.3% for any sign/symptom with a frequency of 30% or lower.

9.6. Data Management

The database and data management plan will be generated to include the following as a minimum:

- Data Flow Plan;

- Case Report Form Completion Guidelines;
- Data Entry Methods and Guidelines;
- Data Validation Document;
- Data Handling Conventions.

A Data Clarification Form (DCF) process will be used for handling data discrepancies.

Data management and statistical analysis will be performed with SAS software.

9.6.1. Case Report forms (CRFs)/Data Collection Tools (DCTs)/Electronic Data Record

As used in this protocol, the term CRF should be understood to refer to either a paper form or an electronic data record or both, depending on the data collection method used in this study.

A CRF is required and should be completed for each included patient. The completed original CRF are the sole property of Pfizer and should not be made available in any form to third parties, except for authorized representatives of Pfizer or appropriate regulatory authorities, without written permission from Pfizer. The investigator shall ensure that the CRFs are securely stored at the study site in encrypted electronic form and will be password protected to prevent access by unauthorized third parties.

The investigator has ultimate responsibility for the collection and reporting of all clinical, safety, and laboratory data entered on the CRFs and any other data collection forms (source documents) and ensuring that they are accurate, authentic/original, attributable, complete, consistent, legible, timely (contemporaneous), enduring, and available when required. The CRFs must be signed by the investigator or by an authorized staff member to attest that the data contained on the CRFs are true. Any corrections to entries made in the CRFs or source documents must be dated, initialed, and explained (if necessary) and should not obscure the original entry.

In most cases the source documents are the hospital or the physician's chart. In these cases, data collected on the CRFs must match those charts.

In some cases, the CRF may also serve as the source document. In these cases, a document should be available at the investigator site and at Pfizer that clearly identifies those data that will be recorded on the CRF, and for which the CRF will stand as the source document.

9.6.2. Record Retention

To enable evaluations and/or inspections/audits from regulatory authorities or Pfizer, the investigator agrees to keep records, including the identity of all participating patients (sufficient information to link records, eg, CRFs and hospital records), all original signed informed consent documents, copies of all CRFs, safety reporting forms, source documents, detailed records of treatment disposition, and adequate documentation of relevant

correspondence (eg, letters, meeting minutes, and telephone call reports). The records should be retained by the investigator according to local regulations or as specified in the clinical study agreement (CSA), whichever is longer. The investigator must ensure that the records continue to be stored securely for so long as they are retained.

If the investigator becomes unable for any reason to continue to retain study records for the required period (eg, retirement, relocation), Pfizer should be prospectively notified. The study records must be transferred to a designee acceptable to Pfizer, such as another investigator, another institution, or to an independent third party arranged by Pfizer.

Investigator records must be kept for a minimum of 15 years after completion or discontinuation of the study or for longer if required by applicable local regulations.

The investigator must obtain Pfizer's written permission before disposing of any records, even if retention requirements have been met.

9.7. Data analysis

Detailed methodology for summary and statistical analyses of data collected in this study will be documented in a Statistical Analysis Plan (SAP), which will be dated, filed and maintained by the sponsor. The SAP may modify the plans outlined in the protocol; any major modifications of primary endpoint definitions or their analyses would be reflected in a protocol amendment.

- Descriptive analysis will be performed for all the variables;
- Analysis will be stratified by age [50 to ≤55 ; 55 to ≤60; 60 to ≤65; 65 to ≤70; 70 to ≤75; 75 to ≤80; >80 years old], gender and cardiac symptomatology at entry;
- Patients with a final diagnosis of ATTR-CM will be compared with the patients who do not have a final diagnosis of ATTR-CM in order to highlight possible diagnostic red flags;
- Factors associated to ATTR amyloidosis will be identified by univariate and multivariate logistic regression. A significance level of 10% and 5% will be used for the univariate and multivariate analyses, respectively.

An interim analysis of the data collected will be performed for all the variables as soon as 75 patients will be selected with a scintigraphy grade 1, 2 or 3 and with completed e-CRF.

9.8. Quality Control

9.8.1. Investigational Site Set Up

Appropriate training relevant to the study will be given to investigational staff. Any new information relevant to the performance of this Study will be forwarded to the staff during the study.

9.8.2. Investigational Site Monitoring

Regular contacts with the sites will be planned to provide information and support to the investigator(s) and verify that study sites procedures are compliant with the protocol and that data are being accurately recorded in the CRFs.

Additional monitoring tasks will be described in a monitoring plan according to Pfizer SOP; monitoring visit at investigators sites will ensure that:

- information letters have been given to the patients and consents have been signed;
- study is conducted according to the protocol;
- data reported on case report forms is compliant with source documents;
- study documents are correctly archived in accordance with the investigator's study file.

9.8.3. Study Coordination

Regular progress reports presenting key indicators at a National and International level will be regularly prepared and forwarded to the study coordinating team. Based on these status reports, different actions will be decided by the study coordinating team to ensure a satisfactory progress and an appropriate quality of data.

Different signals (Protocol deviations as identified by monitoring) will be used as potential identification of low protocol compliance by investigators.

9.8.4. Quality and Accuracy of Records

The investigator will have the responsibility for collecting and reporting of all clinical, safety and laboratory data entered in the e-CRFs and/or any other data collection forms (source documents) and ensuring that they are accurate, authentic/original, attributable, complete, consistent, legible, timely (contemporaneous), enduring and available when required.

To enable evaluations and/or audits from regulatory authorities or Pfizer, the investigator agrees to keep records, including the identity of all participating subjects (sufficient information to link records, eg, CRFs and hospital records), all original signed informed consent forms, copies of all CRFs, serious adverse event forms, source documents, and detailed records of treatment disposition, and adequate documentation of relevant correspondence (eg, letters, meeting minutes, telephone call reports).

9.8.5. Storage of Record

The records should be retained by the investigator according to local regulations, and/ or as specified in the Clinical Study Agreement.

9.9. Limitations of the Research Methods

9.9.1. Investigational Site Selection

The voluntary participation of physicians constitutes a selection bias observed for this type of study. Investigational sites will be recruited within a list of the country's centers, representative in terms of size, care management system and practices.

9.9.2. Patient Selection

This constitutes another potential selection bias classically associated with studies. Voluntary or involuntary selection of patients in a study by investigators is inevitable, but this bias can be limited by systematic attempts to enroll patients in the study.

9.9.3. Patients Lost to Follow-up

The pragmatic nature of this study (which involves non-intervention on usual patient management practices) may increase the number of patients lost to follow-up after the scintigraphy results reported.

Monitoring on site, remote monitoring and warnings implemented in the e-CRF should minimize this.

9.9.4. Measurement Biases

The assessment of the primary endpoint is based on the bone scintigraphy reading, which implies an evaluator-dependent variability and may create a bias.

In order to measure this bias scintigraphy reviews by external evaluators have been planned. Digitalized images will be reviewed by two external evaluators to analize level of concordance/discrepancies among different specialists as part of a secondary objective for the study.

Secondary endpoints are based on laboratory analyses and may constitute a bias as each site has their own process, units and norms. They are also based on symptom assessment that may differ from an investigator to another and may constitute a bias.

9.10. Other Aspects

If a physician agrees to participate in this study, a written agreement will be concluded with this physician containing the amount of allowance paid for the documentation of one subject. The agreed allowance is paid for the workload involved in the documentation of the treatment on the specific e-CRF.

Since no other examinations will be performed than the usual clinical examinations and laboratory tests, the medical services provided and the drugs will be reimbursed by the health insurer.

10. PROTECTION OF HUMAN SUBJECTS

10.1. Patient Information

All parties will comply with all applicable laws, including laws regarding the implementation of organizational and technical measures to ensure protection of patient personal data. Such measures will include omitting patient names or other directly identifiable data in any reports, publications, or other disclosures, except where required by applicable laws.

The personal data will be stored at the study site in encrypted electronic form and will be password protected to ensure that only authorized study staffs have access. The study site will implement appropriate technical and organizational measures to ensure that the personal data can be recovered in the event of disaster. In the event of a potential personal data breach, the study site shall be responsible for determining whether a personal data breach has in fact occurred and, if so, providing breach notifications as required by law.

To protect the rights and freedoms of natural persons with regard to the processing of personal data, when study data are compiled for transfer to Pfizer and other authorized parties, patient names will be removed and will be replaced by a single, specific, numerical code, based on a numbering system defined by Pfizer. All other identifiable data transferred to Pfizer or other authorized parties will be identified by this single, patient-specific code. The investigator site will maintain a confidential list of patients who participated in the study, linking each patient's numerical code to his or her actual identity. In case of data transfer, Pfizer will maintain high standards of confidentiality and protection of patients' personal data consistent with the Clinical Study Agreement and applicable privacy laws.

10.2. Patient Consent

The informed consent documents and any patient recruitment materials must be in compliance with local regulatory requirements and legal requirements, including applicable privacy laws.

The informed consent documents used during the informed consent process and any patient recruitment materials must be reviewed and approved by Pfizer, approved by the institutional review board (IRB)/independent ethics committee (IEC) before use, and available for inspection.

The investigator must ensure that each study patient or his or her legally acceptable representative fully informed about the nature and objectives of the study, the sharing of data relating to the study and possible risks associated with participation, including the risks associated with the processing of the patient's personal data. The investigator further must ensure that each study patient or his or her legally acceptable representative is fully informed about his or her right to access and correct his or her personal data and to withdraw consent for the processing of his or her personal data.

Whenever consent is obtained from a patient's legally acceptable representative/parent(s) or legal guardian, the patient's assent (affirmative agreement) must subsequently be obtained

when the patient has the capacity to provide assent, as determined by the IRB/IEC. If the investigator determines that a patient's decisional capacity is so limited that he or she cannot reasonably be consulted, then, as permitted by the IRB/IEC and consistent with local regulatory and legal requirements, the patient's assent may be waived with source documentation of the reason assent was not obtained. If the study patient does not provide his or her own consent, the source documents must record why the patient did not provide consent (eg, minor, decisionally impaired adult), how the investigator determined that the person signing the consent was the patient's legally acceptable representative, the consent signer's relationship to the study patient (eg, parent, spouse), and that the patient's assent was obtained or waived. If assent is obtained verbally, it must be documented in the source documents.

The investigator, or a person designated by the investigator, will obtain written informed consent from each patient or the patient's legally acceptable representative, parent(s), or legal guardian and the patient's assent before any study-specific activity is performed. The investigator will retain the original of each patient's signed consent document.

10.3. Patient Withdrawal

Patients may withdraw from the study at any time at their own request, or they may be withdrawn at any time at the discretion of the investigator or sponsor for safety, behavioral, or administrative reasons. In any circumstance, every effort should be made to document patient outcome, if applicable. The investigator would inquire about the reason for withdrawal and follow-up with the patient regarding any unresolved adverse events.

If the patient withdraws from the study, and also withdraws consent for disclosure of future information, no further evaluations should be performed, and no additional data should be collected. The sponsor may retain and continue to use any data collected before such withdrawal of consent.

10.4. Institutional Review Board (IRB)/Independent Ethics Committee (IEC)

According to the involved country legislation, It is the responsibility of PFIZER or the investigator to have prospective approval of the study protocol, protocol amendments, and informed consent forms, and other relevant documents, (eg, recruitment advertisements), if applicable, from the IRB/IEC. All correspondence with the IRB/IEC should be retained by PFIZER or the investigator. Copies of IRB/IEC approvals should be forwarded to Pfizer or the investigator.

10.5. Ethical Conduct of the Study

The study will be conducted in accordance with legal and regulatory requirements, as well as with scientific purpose, value and rigor and follow generally accepted research practices described in Guidelines for Good Pharmacoepidemiology Practices (GPP) issued by the International Society for Pharmacoepidemiology (ISPE), Good Epidemiological Practice (GEP) guidelines issued by the International Epidemiological Association (IEA), Good Practices for Outcomes Research issued by the International Society for Pharmacoeconomics

and Outcomes Research (ISPOR), International Ethical Guidelines for Epidemiological Research issued by the Council for International Organizations of Medical Sciences (CIOMS), European Medicines Agency (EMA) European Network of Centres for Pharmacoepidemiology and Pharmacovigilance (ENCePP) Guide on Methodological Standards in Pharmacoepidemiology, and Food and Drug Administration (FDA) Guidance for Industry: Good Pharmacovigilance and Pharmacoepidemiologic Assessment, FDA Guidance for Industry and FDA Staff: Best Practices for Conducting and Reporting of Pharmacoepidemiologic Safety Studies Using Electronic Healthcare Data Sets, Guidance for Industry: Patient-Reported Outcome Measures: Use in Medical Product Development to Support Labeling Claims and/or equivalent.

11. MANAGEMENT AND REPORTING OF ADVERSE EVENTS/ADVERSE REACTIONS

11.1. REQUIREMENTS

The table below summarizes the requirements for recording safety events on the case report form and for reporting safety events on the non-interventional study (NIS) adverse event monitoring (AEM) Report Form to Pfizer Safety. These requirements are delineated for three types of events: (1) serious adverse events (SAEs); (2) non-serious AEs (as applicable); and (3) scenarios involving exposure to a Pfizer product, including exposure during pregnancy, exposure during breast feeding, medication error, overdose, misuse, extravasation, and occupational exposure. These events are defined in the section DEFINITIONS OF SAFETY EVENTS".

Safety event	**Recorded on the case report form**	**Reported on the NIS AEM Report Form to Pfizer Safety within 24 hours of awareness**
SAE	All (regardless of whether the event is determined by the investigator to be related to any Pfizer product)	Only events determined by the investigator to be related to a Pfizer product
Non-serious AE	All (regardless of whether the event is determined by the investigator to be related to any Pfizer product)	Only events determined by the investigator to be related to a Pfizer product

Safety event	Recorded on the case report form	Reported on the NIS AEM Report Form to Pfizer Safety within 24 hours of awareness
Scenarios **involving exposure to a Pfizer product**, including exposure during pregnancy, exposure during breast feeding, medication error, overdose, misuse, extravasation; lack of efficacy; and occupational exposure	All (regardless of whether associated with an AE), **except occupational exposure**	All (regardless of whether associated with an AE) involving exposure to a Pfizer product

For each AE, the investigator must pursue and obtain information adequate both to determine the outcome of the adverse event and to assess whether it meets the criteria for classification as a SAE (see section "Serious Adverse Events" below).

Safety events must be reported to Pfizer within 24 hours of awareness of the event by the investigator as described in the table above. In particular, if the SAE is fatal or life-threatening, notification to Pfizer must be made immediately, irrespective of the extent of available event information. This timeframe also applies to additional new (follow-up) information on previously forwarded safety event reports. In the rare situation that the investigator does not become immediately aware of the occurrence of a safety event, the investigator must report the event within 24 hours after learning of it and document the time of his/her first awareness of the events.

For those safety events that are considered serious or that are identified in the far right column of the table above that are reportable to Pfizer within 24 hours of awareness, the investigator is obligated to pursue and to provide any additional information to Pfizer in accordance with this 24-hour timeframe. In addition, an investigator may be requested by Pfizer to obtain specific follow-up information in an expedited fashion. This information is more detailed than that recorded on the case report form. In general, this will include a description of the adverse event in sufficient detail to allow for a complete medical assessment of the case and independent determination of possible causality. Information relevant to the event, such as concomitant medications and illnesses must be provided. In the case of a patient death, a summary of available autopsy findings must be submitted as soon as possible to Pfizer or its designated representative.

11.2. Reporting Period

For each patient, the safety event reporting period begins at the time of the patient's informed consent, which is obtained prior to the patient's enrollment in the study, and lasts through the end of the observation period of the study; a report must be submitted to Pfizer Safety (or its

designated representative) for any of the types of safety events listed in the table above occurring during this period. If the investigator becomes aware of a SAE occurring at any time after completion of the study and s/he considers the serious AE to be related to a Pfizer product, the SAE also must be reported to Pfizer Safety.

11.3. Causality Assessment

The investigator is required to assess and record the causal relationship. For all AEs, sufficient information should be obtained by the investigator to determine the causality of each adverse event. For AEs with a causal relationship to a Pfizer product, follow-up by the investigator is required until the event and/or its sequelae resolve or stabilize at a level acceptable to the investigator, and Pfizer concurs with that assessment.

An investigator's causality assessment is the determination of whether there exists a reasonable possibility that a Pfizer product caused or contributed to an adverse event. If the investigator's final determination of causality is "unknown" and s/he cannot determine whether a Pfizer product caused the event, the safety event must be reported within 24 hours.

If the investigator cannot determine the etiology of the event but s/he determines that a Pfizer product did not cause the event, this should be clearly documented on the case report form and the NIS AEM Report Form.

11.4. DEFINITIONS OF SAFETY EVENTS

11.4.1. Adverse events

An AE is any untoward medical occurrence in a patient administered a medicinal product. The event need not necessarily have a causal relationship with the product treatment or usage. Examples of adverse events include but are not limited to:

- Abnormal test findings (see below for circumstances in which an abnormal test finding constitutes an adverse event);
- Clinically significant symptoms and signs;
- Changes in physical examination findings;
- Hypersensitivity;
- Progression/worsening of underlying disease;
- Lack of efficacy;
- Drug abuse;
- Drug dependency.

Additionally, for medicinal products, they may include the signs or symptoms resulting from:

- Drug overdose;
- Drug withdrawal;
- Drug misuse;
- Off-label use;
- Drug interactions;
- Extravasation;
- Exposure during pregnancy;
- Exposure during breast feeding;
- Medication error;
- Occupational exposure.

Abnormal test findings

The criteria for determining whether an abnormal objective test finding should be reported as an adverse event are as follows:

- Test result is associated with accompanying symptoms, and/or
- Test result requires additional diagnostic testing or medical/surgical intervention, and/or
- Test result leads to a change in study dosing or discontinuation from the study, significant additional concomitant drug treatment, or other therapy, and/or
- Test result is considered to be an adverse event by the investigator or sponsor.

Merely repeating an abnormal test, in the absence of any of the above conditions, does not constitute an adverse event. Any abnormal test result that is determined to be an error does not require reporting as an adverse event.

11.4.2. Serious Adverse Events

A serious adverse event is any untoward medical occurrence in a patient administered a medicinal or nutritional product (including pediatric formulas) at any dose that:

- Results in death;
- Is life-threatening;

- Requires inpatient hospitalization or prolongation of hospitalization (see below for circumstances that do not constitute adverse events);

- Results in persistent or significant disability/incapacity (substantial disruption of the ability to conduct normal life functions);

- Results in congenital anomaly/birth defect.

Medical and scientific judgment is exercised in determining whether an event is an important medical event. An important medical event may not be immediately life-threatening and/or result in death or hospitalization. However, if it is determined that the event may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above, the important medical event should be reported as serious.

Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

Additionally, any suspected transmission via a Pfizer product of an infectious agent, pathogenic or non-pathogenic, is considered serious. The event may be suspected from clinical symptoms or laboratory findings indicating an infection in a patient exposed to a Pfizer product. The terms “suspected transmission” and “transmission” are considered synonymous. These cases are considered unexpected and handled as serious expedited cases by PV personnel. Such cases are also considered for reporting as product defects, if appropriate.

Hospitalization

Hospitalization is defined as any initial admission (even if less than 24 hours) to a hospital or equivalent healthcare facility or any prolongation to an existing admission. Admission also includes transfer within the hospital to an acute/intensive care unit (eg, from the psychiatric wing to a medical floor, medical floor to a coronary care unit, neurological floor to a tuberculosis unit). An emergency room visit does not necessarily constitute a hospitalization; however, an event leading to an emergency room visit should be assessed for medical importance.

Hospitalization in the absence of a medical AE is not in itself an AE and is not reportable. For example, the following reports of hospitalization without a medical AE are not to be reported.

- Social admission (eg, patient has no place to sleep);

- Administrative admission (eg, for yearly exam);

- Optional admission not associated with a precipitating medical AE (eg, for elective cosmetic surgery);

- Hospitalization for observation without a medical AE;

- Admission for treatment of a pre-existing condition not associated with the development of a new AE or with a worsening of the pre-existing condition (eg, for work-up of persistent pre-treatment lab abnormality);

- Protocol-specified admission during clinical study (eg, for a procedure required by the study protocol).

11.4.3. Scenarios Necessitating Reporting to Pfizer Safety Within 24 Hours

Scenarios involving exposure during pregnancy, exposure during breastfeeding, medication error, overdose, misuse, extravasation, lack of efficacy, and occupational exposure are described below.

Exposure during pregnancy

An exposure during pregnancy (EDP) occurs if:

1. A female becomes, or is found to be, pregnant either while receiving or having been exposed to (eg, environmental) a Pfizer product, or the female becomes, or is found to be, pregnant after discontinuing and/or being exposed to a Pfizer product (maternal exposure).

   An example of environmental exposure would be a case involving direct contact with a Pfizer product in a pregnant woman (eg, a nurse reports that she is pregnant and has been exposed to chemotherapeutic products).

2. A male has been exposed, either due to treatment or environmental exposure to a Pfizer product prior to or around the time of conception and/or is exposed during the partner pregnancy (paternal exposure).

As a general rule, prospective and retrospective exposure during pregnancy reports from any source are reportable irrespective of the presence of an associated AE and the procedures for SAE reporting should be followed.

If a study participant or study participant's partner becomes, or is found to be, pregnant during the study participant's treatment with a Pfizer product, this information must be submitted to Pfizer, irrespective of whether an adverse event has occurred, must be submitted using the NIS AEM Report Form and the EDP Supplemental Form.

In addition, the information regarding environmental exposure to a Pfizer product, in a pregnant woman (e.g., a subject reports that she is pregnant and has been exposed to a cytotoxic product by inhalation or spillage) must be submitted using the NIS AEM Report Form and the EDP supplemental form. This must be done irrespective of whether an AE has occurred.

Information submitted should include the anticipated date of delivery (see below for information related to termination of pregnancy).

Follow-up is conducted to obtain general information on the pregnancy, in addition, follow-up is conducted to obtain information on EDP outcome for all EDP reports with pregnancy outcome unknown. outcome. A pregnancy is followed until completion or until pregnancy termination (eg, induced abortion) and Pfizer is notified of the outcome. This information is provided as a follow up to the initial EDP report. In the case of a live birth, the structural integrity of the neonate can be assessed at the time of birth. In the event of a termination, the reason(s) for termination should be specified and, if clinically possible, the structural integrity of the terminated fetus should be assessed by gross visual inspection (unless pre-procedure test findings are conclusive for a congenital anomaly and the findings are reported).

If the outcome of the pregnancy meets the criteria for an SAE (eg, ectopic pregnancy, spontaneous abortion, intrauterine fetal demise, neonatal death, or congenital anomaly [in a live born, a terminated fetus, an intrauterine fetal demise, or a neonatal death]), the procedures for reporting SAEs should be followed.

Additional information about pregnancy outcomes that are reported as SAEs follows:

- Spontaneous abortion includes miscarriage and missed abortion;
- Neonatal deaths that occur within 1 month of birth should be reported, without regard to causality, as SAEs. In addition, infant deaths after 1 month should be reported as SAEs when the investigator assesses the infant death as related or possibly related to exposure to investigational product.

Additional information regarding the exposure during pregnancy may be requested. Further follow-up of birth outcomes will be handled on a case-by-case basis (eg, follow-up on preterm infants to identify developmental delays).

In the case of paternal exposure, the study participant will be provided with the Pregnant Partner Release of Information Form to deliver to his partner. It must be documented that the study participant was given this letter to provide to his partner.

Exposure during breastfeeding

Scenarios of exposure during breastfeeding must be reported, irrespective of the presence of an associated AE. An exposure during breastfeeding report is not created when a Pfizer drug specifically approved for use in breastfeeding women (eg, vitamins) is administered in accord with authorized use. However, if the infant experiences an AE associated with such a drug's administration, the AE is reported together with the exposure during breastfeeding.

Medication error

A medication error is any unintentional error in the prescribing, dispensing or administration of a medicinal product that may cause or lead to inappropriate medication use or patient harm while in the control of the health care professional, patient, or consumer. Such events may be related to professional practice, health care products, procedures, and systems including: prescribing; order communication; product labeling, packaging, and nomenclature; compounding; dispensing; distribution; administration; education; monitoring; and use.

Medication errors include:

- Near misses, involving or not involving a patient directly (eg, inadvertent/erroneous administration, which is the accidental use of a product outside of labeling or prescription on the part of the healthcare provider or the patient/consumer);

- Confusion with regard to invented name (eg, trade name, brand name).

The investigator must submit the following medication errors to Pfizer, irrespective of the presence of an associated AE/SAE:

- Medication errors involving patient exposure to the product, whether or not the medication error is accompanied by an AE.

- Medication errors that do not involve a patient directly (eg, potential medication errors or near misses). When a medication error does not involve patient exposure to the product the following minimum criteria constitute a medication error report:

    - An identifiable reporter;

    - A suspect product;

    - The event medication error.

Overdose, Misuse, Extravasation

Reports of overdose, misuse, and extravasation associated with the use of a Pfizer product are reported to Pfizer by the investigator, irrespective of the presence of an associated AE/SAE.

Lack of Efficacy

Reports of lack of efficacy to a Pfizer product are reported to Pfizer by the investigator, irrespective of the presence of an associated AE/SAE or the indication for use of the Pfizer product.

Occupational Exposure

Reports of occupational exposure to a Pfizer product are reported to Pfizer by the investigator, irrespective of the presence of an associated AE/SAE.

12. PLANS FOR DISSEMINATING AND COMMUNICATING STUDY RESULTS

A final report will present the results of analyses concerning follow-up of the patient cohort and will address the study objectives.

Abstracts will be submitted to scientific society congresses where posters or oral communications will be presented. Study results will be presented in publications submitted to peer-reviewed European and international journals.

COMMUNICATION OF ISSUES

In the event of any prohibition or restriction imposed (eg, clinical hold) by an applicable Competent Authority in any area of the world, or if the investigator is aware of any new information which might influence the evaluation of the benefits and risks of a Pfizer product, Pfizer should be informed immediately.

In addition, the investigator will inform Pfizer immediately of any urgent safety measures taken by the investigator to protect the study patients against any immediate hazard, and of any serious breaches of this NI study protocol that the investigator becomes aware of.

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ANNEX 1. LIST OF STAND ALONE DOCUMENTS

Number	Document Reference Number	Date	Title
Appendix 1	CT24-WI-GL07-RF02 1.0	19-November 2019	B3461058_TTRACK Study_ICD_15112019
Appendix 2	Non-Inclusion Register	19-November 2019	B3461058_TTRACK_Non-inclusion Register_15112019
Appendix 3	Case Report Form	28 Jue 2021	B3461058_TTRACK_Case Report Form_28062021
Appendix 4	Safety Report Form	August 2016	Non-interventional Study Adverse Even Report Form For protocols without Stipulated Actvive Collection of Adverse Events
Appendix 5	Coutries list	21 May 2021	B3461058_TTRACK_Countries List

ANNEX 2. BONE SCINTIGRAPHY TECHNOLOGY

^{99m} Tc-DPD Bone Scintigraphy Methodology

The patient receives 740MBq (+/- 10%) of ^{99m} Tc-DPD intravenously.

A gamma camera (single- or double-headed gamma camera) is used equipped with low-energy, high resolution collimator (LEHR collimator).

Whole body scan is obtained 5 min (early) and 3 h (late after injection. The acquisition parameters used for planar imaging are: matrix 1024x512 and velocity 10 cm/min. anterior (ANT) and left anterior oblique (LAO) planar views, with the heart centered in the field of view, are obtained for a totoal of 750-1000 Kc. The acquisition parameters used forWhole body scan are 256x256 matrix with 1.46 zoom factor.

In patients showing cardiac uptake, a myocardial single-photon emission computer tomography (SPECT) is acquired after the late whole-body scan. Acquisition parameters for SPECT imaging are LEHR collimators, matrix 64x64 with 1.46 zoom. In the reconstruction, a Butterworth filter is used with a cut-off of 0.50 and order 5.00.

Visual scoring of cardiac retention is obtained according to the classification of Perugini:

- ✓ Score 0, absent cardiac uptake and normal bone uptake
- ✓ Score 1, mild cardiac uptake, inferior to bone uptake
- ✓ Score 2, moderate cardiac uptake accompanied by attenuated bone uptake
- ✓ Score 3, strong cardiac uptake with attenuated bone uptake.

Scintigraphy grading (0 to 3)

- ✓ Grade 0 = absent cardiac uptake
- ✓ Grade 1 = mild uptake less than bone
- ✓ Grade 2 = moderate uptake equal to bone
- ✓ Grade 3 = high uptake greater than bone

For a semi-quantitative primary analysis, a circular ROI is drawn over the heart, copied and mirrored over the contralateral chest for normalize for the spillover from the ribs. Mean total absolute counts are measure correcting for background counts, and the fraction of mean counts in the heart ROI-to-contralateral chest ROI are calculated as the heart-to contralateral (H/CL) ratio.

Semi-quantitative analysis of heart retention (HR), whole body retention (WBR) and heart to whole-body retention ration (H/WB) is evaluated from region of interest (ROI) drawing in the standard manner.

On anterior images rectangular ROIs are drawn over the heart, and irregular ROIs over the kidneys and bladder. These ROIs are copied and mirrored on posterior images, and for each ROI geometric means of the two projections are calculated. All ROIs are corrected for background counts. Total counts in the images are considered as whole-body counts. Early whole-body is used to represented the injected activity. Whole-body retention (WBR) is evaluated by comparing counts in the late images (corrected for decay and subtracting the activity in the urinary tract and bladder) with the counts in the early whole-body images.

Heart retention (HR is evaluated by comparing decay-corrected counts of the heart in late images with counts in early whole-body images.

Heart to whole-body retention ration (H/WB) values are calculated by dividing counts in the heart by counts in late whole-body images.

ANNEX 3 – SCINTIGRAPHY EXTERNAL REVIEW

In this study, all the scintigraphy tests will be additionally reviewed by two external evaluators.

The objective of this evaluation will be to asses the level of discrepancy in the evaluation of the scintigraphy or SPECT images by different evaluators

All the scintigraphy tests will be uploaded in a dedicated database via the study web site.

Two experts from the scientific committee members will connect to the study web site with personalized connection access. They'll review the scintigraphies and enter their grading evaluation.