CLINICAL STUDY PROTOCOL

Cardiac Amyloidosis Discovery Trial

(CAD Trial)

ClinicalTrials.gov ID:	NCT06469372
Protocol Version:	7.0
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STUDY SYNOPSIS

Protocol Title	Cardiac Amyloidosis Discovery Trial (CAD-Trial)
Protocol Number	7.0
Design	This is a single center, diagnostic clinical trial in which we aim to prospectively validate a deep learning model that identifies patients with features suggestive of cardiac amyloidosis, including transthyretin cardiac amyloidosis (ATTR-CA). Cardiac Amyloidosis is an age-related infiltrative cardiomyopathy that causes heart failure and death that is frequently unrecognized and underdiagnosed. We have developed a deep learning model that identifies patients with features of ATTR-CA and other types of cardiac amyloidosis, including echocardiographic, ECG, and clinical factors. By applying this model to the entire Columbia University Irving Medical Center's patient population, we will identify a list of patients at highest predicted risk for having undiagnosed cardiac amyloidosis. We will then invite these patients for further testing to diagnose cardiac amyloidosis with the goal of recruiting 100 total patients. Consented eligible patients will be evaluated for the presence and clinical manifestations of cardiac amyloidosis. Patients will undergo a single study visit, using amyloid nuclear scintigraphy, monoclonal protein testing and a physical examination. Those who meet criteria for the disease will be given the diagnosis of cardiac amyloidosis. Patients will be counseled on the results of their test findings and appropriate referrals made for further care.
Study Sites	This study is to be conducted at NYP Milstein Hospital and 21 Audubon Clinical Cardiovascular Research Laboratory for the Elderly location.
Time on Study	The duration of patient participation in this study is 1 day.
Primary Objective	To prospectively validate a deep learning model to diagnose cardiac amyloidosis, an underdiagnosed, lethal, and treatable cause of heart failure.
Target Sample Size	100 patients
Inclusion and Exclusion Criteria	Every participant must meet all of the following inclusion criteria to be eligible for enrollment in this study: 1. High predicted probability of having cardiac amyloidosis as determined by deep learning model 2. Age ≥ 50 years 3. Electronically stored ECG and echocardiogram within 5 years of study start date

	4. Ability for the patient or health care proxy to understand and sign the informed consent after the study has been explained. Exclusion criteria 1. Primary amyloidosis (AL) or secondary amyloidosis (AA). 2. Prior liver or heart transplantation. 3. Active malignancy or non-amyloid disease with expected survival of less than 1 year. 4. Previous testing for cardiac amyloidosis such as amyloid nuclear scintigraphy, cardiac, or fat pad biopsy 5. Impairment from stroke, injury or other medical disorder that precludes participation in the study. 6. Disabling dementia or other mental or behavioral disease. 7. Enrollment in a clinical trial not approved for co-enrollment. 8. Inability or unwillingness to comply with the study requirements. 9. Nursing home resident. 10. Other reason that would make the subject inappropriate for entry into this study.
Aims	The specific aims of this investigation include: 1. To prospectively validate that deep learning models can detect undiagnosed cardiac amyloidosis in large populations of patients who have undergone cardiac testing. 2. To incorporate additional data to improve the deep learning model's detection of cardiac amyloidosis.
Safety Assessments	The safety of study participants will be evaluated by: 1. Assessment of adverse events (AEs), including serious adverse events (SAEs). 2. Vital sign measurements (blood pressure, pulse rate, and respiratory rate). 3. 12-Lead electrocardiogram (ECG). 4. Physical examinations 5. Echocardiography

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation or Specialist Term	Explanation
99mTc-PYP	99mTechnetium pyrophosphate
99mTc-HMDP	99mTechnetium hydroxymethylene diphosphonate
AE	Adverse event
AI	Artificial Intelligence
TTR	Transthyretin
AL-CA	Light chain cardiac amyloidosis
ATTRm	Mutant Transthyretin amyloidosis
ATTRwt	Wild-type Transthyretin amyloidosis
ATTR-CA	Transthyretin cardiac amyloidosis

1. INTRODUCTION

4.12. Heart failure is a leading cause of death, morbidity, and health care spending in the United States.

Heart failure is common disease with 6.5 million adults in the United States having a diagnosis of heart failure and accounting for 1 in 8 deaths.[1, 2] Heart failure is a highly morbid disorder, with patients having low quality of life[3] and frequent hospitalizations.[4] It is a leading cause of health care spending with an estimated $30.7 billion spent in 2012 for medications, the cost of health care services, and lost income from missing days of work.[1] Heart failure is subdivided by left ventricular ejection fraction into heart failure with reduced ejection fraction (HFrEF) and heart failure with preserved ejection fraction (HFpEF). HFrEF has a broad array of therapies that have been shown to improve outcomes, whereas there are no treatments which have been proven to reduce mortality in unselected populations with HFpEF. The emerging paradigm of HFpEF is that it is a clinical syndrome with a diverse set of underlying pathophysiologies and multitude of etiologies which require specific and varying treatments.

1.2. Amyloidosis is a leading cause for heart failure and heart rhythm disturbance

Systemic amyloidosis results from deposition of amyloid fibrils in tissues throughout the body, including the heart, nerves, lungs, gastrointestinal tract, and skin. Cardiac amyloidosis is the clinical syndrome that results from amyloid fibril deposition in the heart. Patients most commonly present with a heart failure syndrome with marked LV wall thickening and preserved ejection fraction on imaging. The two most common types of cardiac amyloidosis are transthyretin cardiac amyloidosis (ATTR-CA) and light chain cardiac amyloidosis (AL-CA). Both of these entities were previously thought to be rare, but it has been increasingly recognized that ATTR-CA is far more common than previously thought and may be present in up to 13% of hospitalized patients with HFpEF and an increased wall thickness over the age of 60 years.[5] Misdiagnosis is common, with studies consistently finding significant delays in diagnosis of cardiac amyloidosis.[6-8]

1.3. Biology and pathophysiology

The pathophysiology of cardiac amyloidosis is highly dependent on the type of fibril involved. ATTR-CA results from deposition of misfolded transthyretin (TTR) monomers. In vivo, TTR is primarily synthesized in the liver and forms a tetrameric protein with each subunit containing 127 amino acids. This tetrameric protein is often referred to as “prealbumin” and serves as a carrier for thyroid hormone and part of the complex that carries vitamin A. The TTR tetramer can dissociate into monomers, and in some cases these monomers can become misfolded into an insoluble fibril and irreversibly deposit into tissues. ATTR is divided into two types: wild-type TTR (ATTRwt) and mutated (ATTRm). ATTRwt is predominantly a disease of the elderly and is characterized by high rates of cardiac involvement (ATTRwt-CA).[9] In ATTRm, patients have specific mutations in the TTR gene which

leads to increased rates of tetramer dissociation which subsequently deposit as amyloid fibrils into tissues as in the heart (ATTRm-CA) or nerves. More than 130 genetic mutations have been recognized, with Val122Ile being particularly common in the United States with an estimated 1.3 million African Americans affected.[10]

1.4. Therapy for ATTR-CA is available

Historically, treatment of ATTR-CA was limited to supportive care and organ transplantation. However, recent work has led to the development of amyloid-targeted therapy. Possible treatment strategies for ATTR involve preventing production of the transthyretin protein, stabilizing the TTR tetramer, and removing fibrils from tissues. In 2019, tafamidis (VYNDAMAX™), a small molecule TTR tetramer stabilizer, was found to improve the prognosis of patients with ATTR-CA and became FDA approved as the first therapy targeted for ATTR-CA.[11] Strategies to prevent TTR production using small interfering RNA (patisiran) and antisense oligonucleotides (inotersen) have been shown to improve outcomes in ATTRm hereditary polyneuropathy,[12, 13] and trials are ongoing to study their use or similar compounds in ATTR-CA. Because these treatments prevent progression of the disease rather than reversing fibril deposition that has already occurred, there has been a drive to diagnose patients in the early stage of disease prior to the development of severe end-organ dysfunction.

1.5. Non-invasive nuclear imaging can accurately diagnose ATTR-CA

The diagnosis of ATTR-CA could historically only be made by endomyocardial biopsy, a test that is invasive, expensive, and often only available in tertiary care transplant centers. Cardiac imaging with echocardiography and cardiac magnetic resonance imaging can be suggestive of the diagnosis, but the hallmark features of LV thickening are nonspecific. Work over the last decade has shown that bone seeking radiopharmaceuticals such as technetium-99m pyrophosphate (99mTc-PYP), 99mTc-labeled 3,3-diphos-phono-1,2-propanodicarboxylic acid (99mTc-DPD), or 99mTc-hydroxymethylene diphosphonate (99mTc-HMDP) are specific for ATTR-CA.[14] Non-invasive testing pathways which combine these nuclear scans with monoclonal protein testing to rule out AL-CA have been shown to accurately diagnose ATTR-CA when compared to endomyocardial biopsy. These scans are graded using a semiquantitative scale from 0-3 using visual appearance compared to ribs with planar scans with grade 2 and 3 scans thought to be consistent with ATTR-CA, with grade 0 having no myocardial uptake, grade 1 having some cardiac uptake but less than ribs, grade 2 having cardiac uptake equal to ribs, and grade 3 having cardiac

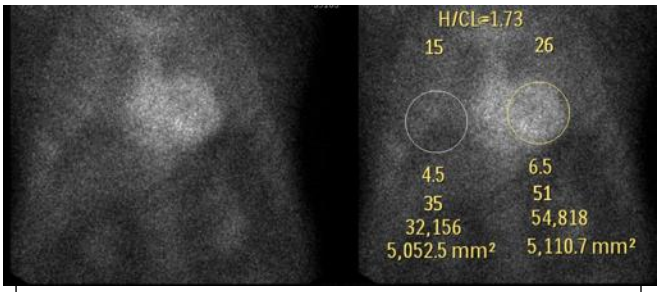

Figure 1: PYP scan. This figure demonstrates a grade 3 99mTc-PYP scan with H:CL ratio of 1.73 which is consistent with a diagnosis of ATTR-CA.

uptake greater than ribs with decreased bone uptake (Figure 1). In addition to visual scoring, quantitative methods can be used by measuring the heart to contralateral chest ratio (H:CL) which measures counts over the heart and comparing it to an equal sized area of interest measured over the contralateral chest. Further refinements of 99mTc-PYP scanning have included the incorporation of single photon emission computed tomography (SPECT) imaging to improve test accuracy. Likely a result of the increasing awareness of ATTR-CA and the availability of non-invasive diagnostic testing, testing volume has increased yearly and as of 2019 more than 130 99mTc-PYP scans are conducted at our center each year with a positive test rate greater than 30%.

1.6. Artificial intelligence

Artificial intelligence (AI) is a general term for computing techniques which seek to simulate human thinking. Historically, these techniques used rule-based algorithms to solve simple problems. Advances in computer power and development of more complex algorithms have allowed AI techniques to be applied to a broad range of problems. Machine learning approaches, which allow for algorithms to be trained using data sets without explicit instruction from humans, have had the most widespread success. Deep learning, a subset of machine learning, has been particularly useful in tasks related to interpreting images. Deep learning takes many layers of mathematical equations to try and interpret some input, such as the color of a pixel. The sum of these layers is often called a neural network due to the model being inspired by the way that neurons are interconnected in the human brain. When trained on large, feature rich datasets in focused problems, the accuracy of deep learning neural networks can outstrip even expert human observers. The success of such neural networks has been demonstrated in the reading of mammograms,[15] ECG algorithms which predict a patient's risk of hypertrophic cardiomyopathy based on a 12-lead ECG,[16] and diagnosis of diabetic retinopathy based on retinal images.[17] The laboratory of Dr. Adler Perotte, a close collaborator in this study, has expertise in computational statistics and artificial intelligence as it applies to electronic health data.

1.7. Informatics pipeline

Over the past 18 months, our group has been working to build an informatics pipeline of patients undergoing cardiovascular testing at Columbia University Irving Medical Center. We have abstracted more than 2 million ECGs, over 200,000 echocardiograms, and more than 750 99mTc-PYP scans and are working to add other cardiovascular and radiologic tests. This database includes ECG physician reports, raw ECG waveforms, ECG reading-software abstracted metadata like QRS voltage and QRS length, echocardiogram reports, chest x-ray images, and 99mTc-PYP scan clinical reports, planar and SPECT images, and concurrently obtained chest CT scans. This interlinked database is a large, feature-rich dataset which is well-suited for using deep learning to answer complex clinical questions such as using the results of a more common testing modality (i.e. echocardiography and electrocardiography) to predict the outcome of a less common one (99mTc-PYP scanning).

2. STUDY OBJECTIVES & STUDY DESIGN

2.1 Aim 1:

To prospectively validate that deep learning models can detect undiagnosed cardiac amyloidosis in large populations of patients undergoing cardiac testing

2.2 Rationale

This study aims to create an automated, end-to-end pathway which can accurately identify patients at high probability of having cardiac amyloidosis who are currently undetected. The specific analytical objectives of this aim include the following: (1) use a deep learning model to identify 100 patients at high probability of having undiagnosed cardiac amyloidosis and invite them for diagnostic testing with the hypothesis that ≥ 20% will be found to have cardiac amyloidosis. A positive detection rate of this amount could likely allow for effective active case surveillance in at-risk populations and result in adoption of the technology at other institutions, potentially leading to thousands of additional diagnoses being made each year.

2.3. Database Structure:

Our informatics pipeline uses three major sources of data for this study: (1) a database of cardiac amyloidosis cases and controls, (2) an ECG database, and (3) an echocardiogram database (Figure). The cardiac amyloidosis cases and controls database includes 753 consecutive patients who underwent 99mTc-PYP scanning. Using this database, we identified 212 patients with definite ATTR-CA by consensus criteria and 455 patients with no cardiac amyloidosis.[18, 19] A manuscript analyzing these patients in detail is in revisions. Additional lists of patients with AL-CA and ATTR-CA were obtained from department records. The ECG database includes over 2 million ECGs. At Columbia University Irving Medical Center, ECGs are stored and analyzed digitally using the MUSE Cardiology Information System (GE Healthcare, Chicago, IL, United States). For each individual ECG, the MUSE database stores high level, quantitative data in a matrix format for all 12 ECG leads in addition to the physician text-based report. Quantitative data includes interval length (such as PR,

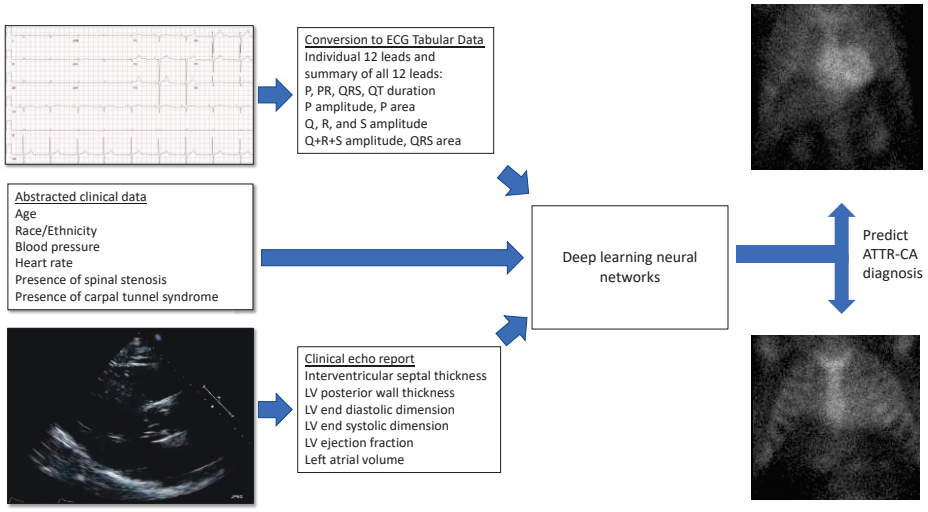

Figure 2. Visual representation of database information. Our database has three significant inputs (ECG, abstracted clinical information, and echocardiography) which are used in our deep learning model to predict one output (presence or absence of ATTR-CA, typically as diagnosed by 99mTc-PYP scanning).

QRS, and QT intervals) and peak wave amplitude (such as P, Q, R, S, and T wave amplitude), and each lead can be analyzed individually or as a sum of all 12 leads. The echocardiogram database includes over 200,000 echocardiograms in more than 100,000 patients. At CUIMC, echocardiograms are stored digitally and analyzed using the Syngo system (Siemens Healthineers, Erlangen, Germany). Our echocardiogram database contains abstracted information from the clinician report, including interventricular septal thickness, LV posterior wall thickness, LV ejection fraction, LV end-diastolic dimension, left atrial volume indexed for body surface area, and aortic root dimensions. With the combination of these three databases, we are able to rapidly conduct experiments to identify ECG, echocardiographic, demographic, and clinical features which distinguish ATTR-CA cases from normal controls.

4.12. Traditional statistical modeling:

The dataset used to create the traditional statistical model has nearly 1,000 variables per individual case. To ensure all cases are included, they must have <10% of data missing including no missing data in variables considered most critical (LV ejection fraction, interventricular septal thickness, posterior wall thickness, age, gender, ECG paced versus unpaced). From there, multiple imputation by chained equations (MICE) is conducted to ensure all cases are included. Data is then separated into training (70%), development (15%), and test (15%) data sets on a per-patient level. Due to limited size of the dataset, 5-fold cross-validation will be utilized. To deal with the high dimensionality of the data, both logit and probit regression models are generated. To minimize overfitting we prefer the use of L1 regularization over L2.

4.12. Deep learning model development

The above methods are utilized to create the same train, development, test dataset with patients identically coded into those groups to allow for direct comparison. The first set of models generated utilize only tabular data and are based off of neural accumulators (NAC) and neural arithmetic logic units (NALU).[20] NACs/NALUs are neural layers designed to learn functions comprised of arithmetic expressions such as addition, subtraction, multiplication, and division. The most promising aspect of NAC/NALU is that by learning generalizable numerical representations, there is hope to build neural networks that are explainable with simple and sparse arithmetic of raw input features. This architecture's inductive bias is dependent upon learning a set of weights which, after some internal operations, yield values near -1, 0, or 1. We have developed a modification of NAC/NALU to decrease overfitting and improve sparsity, believing this would lead to the model to output a simpler equation utilizing fewer features and less variance. To encourage this behavior, we appropriately parameterized Laplace priors on our weights. We refer to these layers as Laplacian-regularized NAC and NALU (LR-NAC, LR-NALU). From there, we plan to include the raw ECG waveform data in the NAC/NALU models as well as develop a convolutional neural network (CNN) model based primarily of the waveform data as discussed in Aim 2. The CNN model will be developed similarly using the

same train/dev/test splits. We will utilize the Adam optimizer and binary cross entropy as the loss function. We will assess convolutions across a single lead as well as a pooled convolution across all leads. We will then evaluate performance characteristics of the model with probability thresholds of 1%, 5%, 10%, 20%, 50%, and 75%.

2.6. Anticipated problems and strategies: Limited subject recruitment: We will continue to recruit patients until we have been able to successfully enroll 100 patients. We believe the recruitment pool of over 100,000 patients receiving an echocardiogram in the past eight years will allow for successful recruitment . False positive or negative amyloid scintigraphy scanning with planar imaging: 99mTc-PYP scanning has been found to have high levels of accuracy compared to biopsy in a large multicenter study which included patients seen at CUIMC.[14] Additionally, we will use SPECT imaging which further reduces the risk of false positive testing.[21] Identification of other types of amyloidosis: Every patient enrolled will undergo monoclonal protein testing to rule out AL amyloidosis and will have a clinical evaluation of assess for the risk of rare types of amyloidosis.

2.7. Aim 2: To incorporate additional data to improve the ability of deep learning models to predict cardiac amyloidosis

2.8. Rationale: Because current therapy for ATTR-CA only prevent disease progression without significantly reversing end-organ damage that has already occurred, it is imperative to attempt to diagnose patients as early as possible in their disease course. However, because the symptoms and structural changes of cardiac amyloidosis are more apparent in late stage disease, it is far more common for the diagnosis to be made in its later stages. In its current form, our deep learning model works incorporates many markers of advanced disease like decreased ECG voltage and increased LV wall thickness. As a result, in the same manner that diagnosis of ATTR-CA is easier with a more extreme phenotype in traditional clinical care, it is likely that our current model will have this same weakness. We believe the incorporation of additional clinical data and raw ECG waveform may improve the ability to detect patients at earlier stages of the disease and with less pronounced cardiac manifestations.

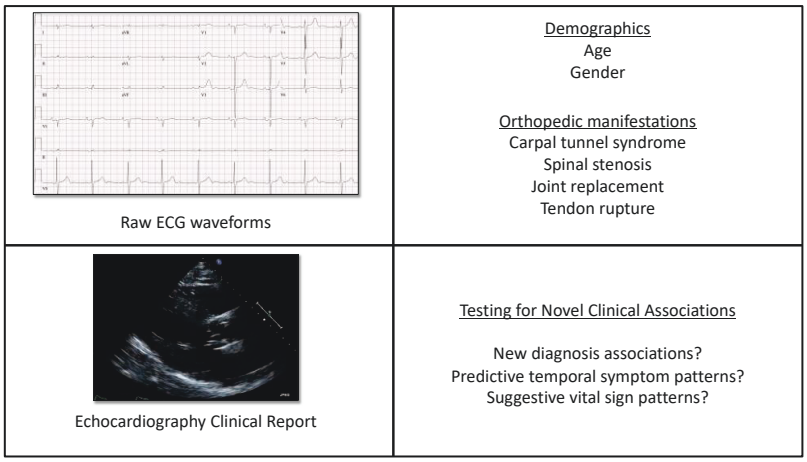

Figure 4: Aim 2. With the addition of raw ECG waveforms electronic health record information to pre-existing data in our pipeline, we aim to improve the predictive capacity of our model and potentially identify novel clinical associations for ATTR-CA.

2.9. Orthopedic manifestations: Previous work has shown that the orthopedic manifestations of carpal tunnel syndrome,[22] spinal stenosis,[23, 24] and hip and knee osteoarthritis requiring arthroplasty[25] can precede the diagnosis of cardiac amyloidosis by up to 5-7 years. Pathophysiologically, the carpal tunnel and multiple areas in the spine are small, compressed spaces. As a result, relatively little amyloid deposition can lead to significant symptoms, and nerve involvement may accentuate pain syndromes. The period of time when patients have symptomatic carpal tunnel syndrome, spinal stenosis, or osteoarthritis but before they have developed symptomatic heart disease could be an ideal time to diagnose systemic amyloidosis and initiate treatment. Preliminary work at our institution using manual chart abstraction has demonstrated that these orthopedic manifestations are more common in ATTR-CA patients than in controls (to be presented at International Society of Amyloidosis 2020 meeting). In order to incorporate these features in our model, we will collect electronic health record data on orthopedic manifestations as documented by Diagnosis Related Group codes, past medical and surgical history, and problem lists all patients seen in the CUIMC system. We will then incorporate this data into our deep learning models with two significant goals: (1) assess improvement in model's capacity to detect ATTR-CA, and (2) determine if it is possible to develop a model which prioritizes detection of patients with earlier cardiac involvement.

2.10. Electrocardiogram waveform analysis: Our current deep learning model uses tabular ECG data from the MUSE system documenting stored voltages and deflections. We believe that improvements to the ECG portion of this model are possible by adding raw waveform data. The waveforms on a 12 lead ECG are feature-rich with ~50,000 discrete data points as opposed to ~700 data points that are stored in the tabular data. Prior work has shown that deep learning analysis of 12 lead ECGs can identify significant pathology that would be essentially invisible to a human interpreter.[16] We plan to include the raw ECG waveform data in the NAC/NALU models as well as develop a convolutional neural network (CNN) model based primarily of the waveform data. The CNN model will be developed similarly using the same train/development/test splits previously discussed. We will utilize the Adam optimizer and binary cross entropy as the loss function. We will assess convolutions across a single lead as well as a pooled convolution across all leads. We will then evaluate performance characteristics of the model with probability thresholds of 1%, 5%, 10%, 20%, 50%, and 75%. By adding raw waveforms data, we aim to increase the AUC of our model to >0.90.

2.11. Testing for novel clinical associations by using deep learning: The most promising aspect of NAC/NALU-based deep learning models is that by learning generalizable numerical representations, there is hope to build neural networks that are explainable with simple and sparse arithmetic of raw input features. Compared to other deep learning models, the output is more interpretable and is essentially an equation. The addition of a Laplacian-regularized filter pushes the model to make valuables more binary, either including them entirely or not at all. Instead of finely tuning hundreds of knobs, the model is asked to flip on switches of fewer input variables that matter.

While this may sacrifice some overall performance, it pushes the model towards simplicity which we believe limits overfitting, improves generalizability, and most importantly points out fewer variables that actually matter. To be used in real clinical settings and also derive insight on novel clinical associations, a model incorporation thousands of variables is unlikely to be successful. With such restrictions in place, we seek to determine if there is at least one novel clinical association that is found to be important in detecting ATTR-CA that is not currently known in the scientific community.

2.12. Future Directions: (1) Clinical trial to detect early stage amyloidosis: Our initial clinical trial (Aim 1) will likely result in identification of patients with later stage cardiac amyloidosis. By adding the orthopedic manifestations and ECG waveform data and tuning the model for earlier stage disease (Aim 2), we hope to be able to repeat this clinical trial but target patients with earlier stage disease (2) Deployment of the model at additional centers: The use of ECG, echocardiographic, demographic data, and clinical diagnoses to identify cardiac amyloidosis is generalizable to other medical centers. We anticipate that this model could be used at other centers to identify further cohorts of patients with cardiac amyloidosis. (3) Application of this research model to other diseases: The use of artificial intelligence analysis of pre-existing clinical data to identify undiagnosed disease has significant potential outside of the study of amyloidosis. It is possible that training similar models targeted at other diseases could have success in increasing identification of rare disease such as primary pulmonary hypertension or diseases that have a latency period that allows for improved treatment with earlier initiation such as cancer.

2.3 Overview of Study Design

We will undertake a prospective cohort study among subjects with heart failure seen at one of two sites: (1) Columbia University Irving Medical Center and affiliated ColumbiaDoctors sites and (2) Allen Hospital of NY Presbyterian. Patients identified as having a high probability of having undiagnosed cardiac amyloidosis will undergo comprehensive cardiovascular examinations including: (1) complete medical history documenting the presence of HF and any symptoms that could be associated with cardiac amyloidosis, as well as any additional co-morbid conditions or symptoms, (2) targeted physical exams including orthostatic blood pressure measurements, assessment for gallops and volume status (e.g. JVP, rales, edema) and determination of functional class, (3) standard 12 lead electrocardiogram, (4) full two-dimensional and three-dimensional echocardiograms with Doppler flow assessment and tissue Doppler and speckle tracking (See procedures for details), (5) cardiac amyloid scintigraphy, and (6) blood testing for monoclonal protein testing.

2.3.1 Patient Recruitment

The CAD-Trial study is designed to identify a sufficient number of cases to prospectively validate that deep learning models can detect undiagnosed cardiac amyloidosis in large populations of patients undergoing cardiac testing. To accomplish this objective, we will undertake an approach to cohort

identification and selection is used that deliberately selects for subjects who are at increased risk for cardiac amyloidosis.

Patient Recruitment: To accomplish the above objectives, the cohort will be identified by examination of all patients who had an ECG and echocardiogram at CUIMC within the last 5 years (Figure 3). The deep learning model will be applied to this dataset of patients to create a list of patients at highest probability of having cardiac amyloidosis. The medical records of these patients will be evaluated for inclusion and exclusion criteria, and the 100 highest probability patients who agree to take part in the study will be enrolled.

Selection criteria: 100 patients will be enrolled in this protocol. *Inclusion criteria* will include age ≥ 50 years, electronically stored ECG and echocardiogram within 5 years of study start date, ability for the patient or health care proxy to understand and sign the informed consent after the study has been explained. *Exclusion criteria* include a previous diagnosis of ATTR, AL, or AA amyloidosis, previous testing for cardiac amyloidosis such as a prior cardiac amyloid scintigraphy, cardiac, or fat pad biopsy, prior organ transplantation, active malignancy, or non-amyloid heart disease with an expected survival of less than one year.

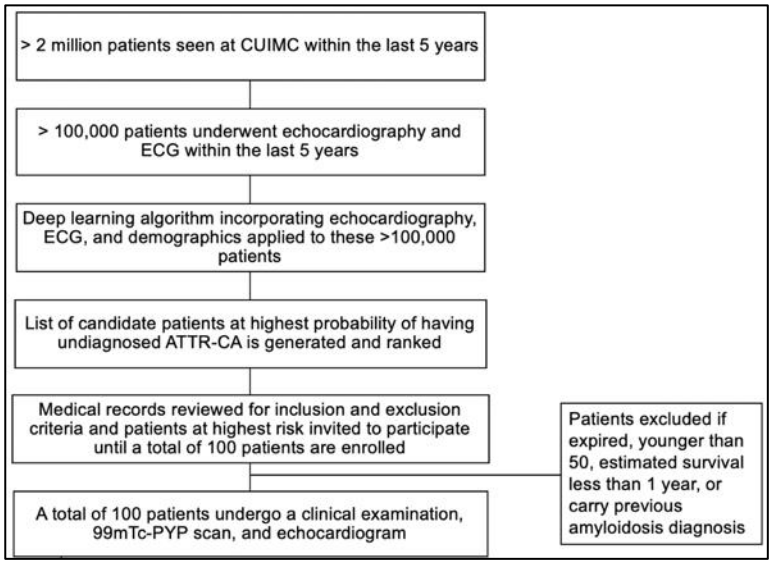

Figure 3: Study Flow. This diagram demonstrates the analysis of patients and data with the goal of performing 99mTc-PYP scanning on 100 patients identified by our deep learning model to be at high probability of having undiagnosed ATTR-CA.

Study procedures: Eligible patients will be identified in the manner above and invited to participate in the study by phone call. During this phone call, they will be assessed for eligibility and undergo an informed consent process. A study visit will then be scheduled. At the study visit, they will undergo a history and physical examination by a physician, 12 lead electrocardiogram, transthoracic echocardiogram, blood testing for monoclonal proteins, blood biobanking, and cardiac amyloid scintigraphy. They may be offered TTR genetic testing by blood or saliva sample if preliminary testing is concerning for amyloidosis, counseling on that decision, and given the choice of whether to have genetic testing without it affecting their ability to participate in the other study procedures. Patients will be given a $100 debit card for their participation in the study and will be provided a car service if needed and feasible for transport to and from the study site. After completion of all testing, patients will be counseled on the findings of their testing and appropriate referrals for follow-up or further testing will be made. For the purposes of the study, a diagnosis of cardiac amyloidosis will be made in the following settings:

- ATTR-CA diagnosis: A diagnosis of ATTR-CA will be made according to consensus guidelines by an amyloidosis expert.[18, 19] These includes imaging criteria with requires that a patient's cardiac amyloid scintigraphy SPECT scan shows myocardial uptake and follow-up monoclonal protein testing shows no evidence of AL amyloidosis or pathologic criteria with a biopsy showing ATTR.
- AL-CA diagnosis: A clinical diagnosis of AL-CA will be by an amyloidosis expert according to society guidelines. These includes a diagnosis made in one of the following settings: (1) cardiac biopsy showing AL deposition and (2) extra-cardiac biopsy showing AL deposition with typical cardiac features on imaging including echocardiography or cardiac magnetic resonance imaging.

2.3.2 Study Procedures

The timing of all procedures in this study is outlined in Table 4. Eligible patients will be identified. Patients who are interested in the study will have a screening visit to evaluate for eligibility and obtain informed consent. If patients qualify for study and do consent they will have their study visit within 4 weeks of screening. At that visit they will have all of the following testing done.

Table 4. Study Procedures

Procedure	Screening (phone visit)	Baseline	Phone wrap-up visit
Informed Consent	X		
Inclusion and Exclusion Criteria	X		
Baseline History		X	
Height and Weight‡		X	
Vitals Signs		X	
Medications		X	
Clinical Examination		X	
Adverse Events Review		X	
Blood draw for monoclonal protein testing, genetic testing, and biobanking		X	
Electrocardiogram		X	
Echocardiogram		X	
^{99m}Tc-PYP administration		X	
^{99m}Tc-PYP Scintigraphy		X	
Patient compensation - $100 debit card		X	
Phone Call Follow-Up			X

Appropriate referral for follow-up in the event of ATTR-CA diagnosis or discovery of other serious pathology			X

2.3.2.1 Clinical Examination

A detailed health investigation will be performed in all patients by a provider experienced in the care of patients with HF. This will include a medical history focused on the presence of HF as defined by standard clinical criteria (NHANES[26] and European Society of Cardiology[27] for HFpEF), etiology of HF, and co-morbid conditions; physical examination including vital signs, volume status, cardiopulmonary examinations and neurologic examination with focus on neuropathic findings. Exams will take ~45 minutes.

2.3.2.2 Cardiac amyloid scintigraphy

A nuclear medicine technologist will perform planar cardiac imaging of the chest using dual-headed gamma cameras equipped with low energy, high resolution collimators. 10-25 mCi of ^{99m}Tc-PYP or 10-20 mCi of 99mTc-HMDP will be administered intravenously and imaging will be performed after approximately 3 hours. The anterior and lateral planar views centered on the heart will be obtained simultaneously for a total of at least 750K counts/view (approx. 3-8 min of imaging). Cardiac retention will be assessed by both a semi-quantitative visual score (range: 0 [no uptake] to 3 [uptake greater than rib]) and a quantitative heart-to-contralateral (H/CL) ratio of total counts in a region of interest (ROI) over the heart divided by background counts in an identical size ROI over the contralateral chest including soft tissue, ribs, and blood pool. A visual score 2 or greater and/or calculated H/CL ratio 1.3 or greater, with myocardial retention of tracer confirmed in all cases by SPECT imaging with or without a CT scan for image registration, will be required to indicate TTR-CA according to prior published data.[28] The details of the imaging procedures for cardiac amyloid scintigraphy imaging are shown in Table 5. Each cardiac amyloid scintigraphy scan will be read by a board-certified nuclear cardiologist blinded to clinical information.

Table 5

Imaging procedures	Parameter
Preparation	No specific preparation. No fasting required.
Scan	Rest scan
Dose of ^{99m}Tc-PYP	10-25 mCi intravenously
Dose of ^{99m}Tc-HMDP	10-20 mCi intravenously
Time between injection and acquisition	3-hour planar and optional SPECT
Imaging parameters	
Field of view	Recommended: cardiac or chest; Optional: whole body planar
Image type	Recommended: cardiac or chest SPECT and planar imaging
Position	Supine
Energy window	140 keV, 15-20%
Collimators	Low energy, high resolution
Matrix	64 x 64 minimum
Pixel size	3.5-6.5 mm
Planar imaging specific parameter	
Number of views*	Anterior, lateral, and left anterior oblique
Detector configuration	90 degrees

Image duration (count based)	750,000 counts
Magnification	1.46
SPECT imaging specific parameters	
Angular range	360 degrees
Detector configuration	180 degrees

ECG gating	Off; nongated imaging
Number of views/detector	40
Time per stop	30 seconds
Magnification	1.0

2.3.2.3 Two- Dimensional Echocardiography with Complete Doppler Analysis

A complete two-dimensional (2D) echo will be performed utilizing a commercially available GE Vivid-9 or Philips iU33 Echocardiography System. A standard imaging protocol will be performed. The following linear echocardiographic measurements will be obtained (by 2D or M-mode) in both end-diastole and end-systole: left ventricular (LV) internal dimension, septal wall thickness (SWT), and posterior wall thickness (PWT). Quantification of LV mass will be derived by the formula of Devereux RB et al[29] and by three dimensional-guided modified biplane Simpson's rule[30, 31]. LV and left atrial (LA) volumes (systolic and diastolic) will be measured by the biplane method of discs (modified Simpson's rule). LV ejection fraction and LA function will be calculated from these volume measurements.

2.3.2.4 Electrocardiogram

A standard 12 lead electrocardiogram will be performed with standard instrument sensitivity of 10 mm = 1 mV. A sum of precordial voltage (sum of S wave in lead V1 plus R wave in lead V_5 or V_6 [SVl + RV_5 or V_6]) will be calculated for all electrocardiograms. This sum will be used to compare data from patients with myocardial retention of ^{99m}Tc-PYP from controls. LVH will be defined when this sum is greater than 35 mm. When the sum was less than 15 mm, low voltage in the precordial leads is present. Additional criteria to define low voltage only in the limb leads include no QRS deflection greater than 5 mm in any limb lead and low voltage in all leads is present when the average voltage in the three limb leads is <5 mm, and the average voltage in the chest leads is <10 mm.[37] Finally, the mass: voltage ratio will be calculated using the LV mass divided by the sum of S wave in lead VI plus R wave in lead V5 or V6 [SV1 + RV5 or V6]. The ECG tracings will be scanned and uploaded in electronically.

2.3.2.5 Blood and saliva testing

During the study visit, subjects will undergo a blood draw with no more than 25 cc of blood drawn and a cheek swab or saliva swab. This blood will be sent for testing for serum kappa and lambda free light chains and serum protein electrophoresis with immunofixation. These tests will assess for the substrate for light chain amyloidosis which can mimic ATTR-CA. If patients are found to have a monoclonal gammopathy, they will be referred for further management by a physician outside the study. In addition, a sample may be drawn for biobanking and TTR genetic testing at the investigator's discretion if preliminary testing is concerning for cardiac amyloidosis.

2.3.2.6 Follow-Up Protocol

Participants will be called after the completion of testing by an investigator to counsel them on the results of their testing. In the event that they are diagnosed with cardiac amyloidosis or are found to have other serious pathology, the following steps will be taken:

- The patient will be told the results of their testing and an appropriate referral for follow-up made. If the patient has a treating physician, they will be instructed to go to their physician for further care as needed. In the event of an amyloidosis diagnosis, the patient will be referred to the Columbia University Amyloidosis Center for further care. If they do not have a treating physician, they will be given an appropriate referral for further care.
- A letter will be generated and mailed to the patient and uploaded to the patient's medical record. This letter will state the results of their testing and recommend that the patient see a physician for further care.

2.3.3 Hypotheses

Specific Aim 1: To prospectively validate that deep learning models can detect undiagnosed cardiac amyloidosis in large populations of patients undergoing cardiac testing

The specific hypothesis is that by applying an cardiac amyloidosis deep learning detection model to the entire Columbia database of patients, we will identify a group of patients at highest likelihood of having undiagnosed cardiac amyloidosis. We will validate the model by prospectively scanning 100 subjects with cardiac amyloid scintigraphy with ≥ 20% being diagnosed with cardiac amyloidosis, a percentage that would potentially allow a cost-effective, wide-scale screening approach. The prevalence of cardiac amyloidosis in an unselected population of older adults who have undergone cardiac testing with an echocardiogram and ECG is unknown but is likely significantly less than 15% based on studies of more selected populations such as older adults with heart failure[5] and aortic stenosis.[38] Based on our model's ability to accurately distinguish between cardiac amyloidosis cases and normal patients in a cohort of patients undergoing cardiac amyloid scintigraphy scanning, we predict that ≥ 20% of patients identified by the model at being of high probability of having cardiac amyloidosis will be diagnosed with cardiac amyloidosis.

Specific Aim 2: To incorporate additional data to improve the deep learning model's detection of cardiac amyloidosis.

Hypothesis 1: Incorporating raw ECG waveform data and clinical variables such as orthopedic manifestations, e.g. carpal tunnel syndrome and spinal stenosis, associated with ATTR-CA, will improve the model's predictive capacity by 5% compared to currently used data in Aim 1.

Hypothesis 2: These deep learning models will allow identification of at least one key variable not currently associated with ATTR-CA that can inform future amyloidosis research.

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