Study protocol
(Non-interventional study, NIS)

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II. Title of the clinical study:

Non-interventional study on the prevalence of cardiac amyloidosis in patients with severe aortic valve stenosis – evaluation using echocardiography, computed tomography, Tc99-SPECT/CT and cardiac magnetic resonance imaging (CMR)

Short title: Cardiac SPECT/CT/CMR_NIS
Internal study code: NIS-TAVI-AMY
Study protocol version: 3 / 22.06.2026
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1.2 Study synopsis

Study title	Non-interventional study on the prevalence of cardiac amyloidosis in patients with severe aortic valve stenosis – evaluation using echocardiography, computed tomography, Tc99-SPECT/CT and cardiac magnetic resonance imaging (CMR)
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Study centre	Tübingen University Hospital, Department of Radiology
Study design	Monocentric, non-interventional prospective observational study (imaging), IIT according to BO
Main target criteria	Primary: The primary objective is the prospective collection of data from patients who have undergone Tc99-SPECT/CT, CT and, if necessary, cardiac MRI (CMR) in order to determine the prevalence of cardiac amyloidosis in patients with severe aortic valve stenosis – evaluation using non-invasive imaging: echocardiography, photon-counting computed tomography, SPECT/CT or CMR. Secondary: • Correlation of cardiac MRI (T1/T2 mapping) with quantitative SPECT/CT and/or photon-counting CT evaluation. • Comparison of abnormal Tc99 SPECT/CT findings (and their quantification) with quantitative determinations of ECV using photon-counting CT or mapping methods using CMR. • Optimisation of established Tc99 SPECT/CT through the quantitative approach of tracer uptake. • Follow-up of patients with regard to adverse events (death, cardiac death, stroke, hospitalisation due to heart failure) • Evaluation of predictive and prognostic values of SPECT/CT, CT and MRI procedures for tissue characterisation • Influence of acquisition and quantification parameters in SPECT/CT (CT information, NM image, size and positioning of the volume of interest, thresholds) on measured activity concentration and distribution volume • Improvement of diagnostic patient management in cases of suspected cardiac amyloidosis (early detection of amyloidosis)
Patient collective[1]	Patients prior to transcatheter aortic valve implantation (TAVI) with confirmed severe aortic valve stenosis
Number of patients	600
Main inclusion/exclusion criteria	Inclusion criteria: • Patients with severe aortic valve stenosis (valve opening area <1 cm², pmean >40 mmHg, Vmax >4 m/sec) • Suspected cardiac amyloidosis

	• Performance of one or more of the following imaging examinations due to (justifiable) clinical indication: echocardiography, photon-counting computed tomography (CT), Tc99-SPECT/CT, cardiac MRI • Age ≥18 years • Written consent of the patient for prospective data collection regarding Tc99-SPECT/CT, CT, and, if applicable, CMR and follow-up Exclusion criteria: • Patients unable to give consent
Study-relevant examinations	Partial body examination (heart) with echocardiography, Tc99m phosphonate scintigraphy in SPECT/CT; if findings are abnormal, further evaluation using multimodal cardiac MRI (CMR); if TAVI is planned, a CT scan (routine).
Study-related examination or medication	Not applicable, as this is an observational study.
Study duration	54 months (approx. 48 months recruitment period and approx. 6 months subsequent data analysis, publication). Expected start: November 2019 / Expected end: November 2023 According to Amendment 1: approx. 36 months recruitment period and approx. 6 months subsequent data evaluation, publication). Expected start: Q3/2024 / Expected end: Q4/2027

[1]In the context of this text, the masculine form always includes the feminine form.

1.3 Abbreviations used

[^{18}F]-FDG	[^{18}F]-fluorodeoxyglucose
Abb.	Figure
ADC	apparent diffusion coefficient
AE	adverse event
AKE	aortic valve replacement
AKF	Applied Clinical Research (Angewandte Klinische Forschung, intramural funding programme UKT)
AMG	German Medicines Act (Arzneimittelgesetz)
APD	avalanche photodiodes
AS	aortic valve stenosis
BfArM	Federal Institute for Drugs and Medical Devices (Bundesinstitut für Arzneimittel und Medizinprodukte)
BfS	Federal Office for Radiation Protection (Bundesinstitut für Strahlenschutz)
Bq	Becquerel
CA	contrast agent
CCTA	coronary CTA
CMR	cardiac MRI
CRF	case report form
CRO	clinical research organization
CT	computed tomography
CTA	CT angiography
DPD	diphosphono-1,2-propanedicarboxylic acid
DWI	diffusion weighted imaging
ECV	extracellular volume
EKG	electrocardiogram
EMB	endomyocardial biopsy
FFRCT	fractional flow reserve
GFR	glomerular filtration rate
GK	whole body
h	hour
i.v.	intravenous
ICA	invasive coronary angiography
ITT	investigator initiated trial
Chap.	chapter
kg	kilogram
LGE	late gadolinium enhancement
LSO	lutetium orthosilicate
LV-EF	left ventricular ejection fraction
MD	Medical device
MDR	Medical Devices Act (Medizinprodukteverordnung)
min	minute
ml	millilitre
mm	millimetre
MPG	Medical Devices Act
Mon.	months
MPKPV	Ordinance on Clinical Trials of Medical Devices (Verordnung über klinische Prüfungen von Medizinprodukten)
MRI	magnetic resonance imaging
mSv	millisievert
NIS	non-interventional study
PET	positron emission tomography
RNA	ribonucleic acid
ROI	region of interest
RöV	X-ray Ordinance (Röntgenverordnung)
s	second
SAR	specific absorption rate

SNR	signal-to-noise ratio
SOP	standard operation form
SPECT	single photon emission computed tomography
StrlSchV	Radiation Protection Ordinance (Strahlenschutzverordnung)
SUV	standardized uptake value
T	Tesla
TAVI	Transcatheter Aortic Valve Implantation
Tc99m	technetium 99m
TTR	transthyretin
UKT	University Hospital Tübingen

1.4 Summary of the project

Degenerative aortic stenosis (AS) is currently the most common heart valve disease in Western countries. It is defined as an aortic valve opening area <1.0 cm² and/or a mean gradient of 40 mmHg. Older patients with higher-grade AS are primarily treated interventionally using transcatheter aortic valve implantation (TAVI). The outcome does not appear to be worse than with conventional/cardiac surgical aortic valve replacement. A characteristic feature of higher-grade AS is left ventricular hypertrophy. In addition, there is often a restrictive filling pattern, which can be detected by echocardiography. These features are also found in TTR-type (transthyretin-type) cardiac amyloidosis. Amyloid was detected in over 70% of surgically removed heart valves in patients who had undergone aortic valve replacement. The detection of amyloid is clinically significant for the patient, as there are different therapeutic approaches depending on the type of amyloid. Currently, endomyocardial biopsy (EMB) is the gold standard in many places for detecting cardiac amyloidosis. However, this is an invasive procedure with associated risks and limitations (e.g. sampling error). Imaging is often used to detect cardiac amyloidosis based on the results of echocardiography. In the further workflow, cardiac amyloidosis can be confirmed non-invasively based on the calcium-associated storage of technetium 99m (Tc99m)-labelled phosphonates, as traditionally used in skeletal scintigraphy. According to current data, Tc99m phosphonate scintigraphy using SPECT/CT (hereafter referred to as Tc99 SPECT/CT) has a high specificity for the detection of TTR amyloidosis (1,2). If SPECT/CT is available, this non-invasive diagnostic procedure is preferred over EMB; Tc99 SPECT/CT is standard practice at the UKT. Cardiac amyloidosis can also be detected by CT, which is clinically indicated for these patients as part of TAVI planning. The available photon-counting CT, on the other hand, allows for innovative spectral analysis, which enables direct calculation of the myocardial extracellular volume (3-6).

The data available to date indicate a correlation between prognosis and phosphonate storage in the myocardium (7). When using the Perugini score (2), tracer uptake is classified merely as a discrete variable in a rough grid. Quantification of myocardial radiopharmaceutical uptake could provide quantitative parameters that not only better reflect the severity of cardiac amyloidosis, but could also potentially serve as prognostic parameters. Such quantification could be achieved by hybrid imaging using SPECT/CT, which combines anatomical information from computed tomography (CT) with functional information from single photon emission computed tomography (SPECT). This quantification is performed in a post-processing of the acquired data sets.

In addition to Tc99 SPECT/CT, cardiac magnetic resonance imaging (CMR) with its excellent tissue characterisation can also clearly show inflammatory and fibrotic changes in the heart muscle, such as those that can occur in amyloidosis as part of protein deposition. In addition to the established late gadolinium enhancement (LGE) technique, which can primarily image focal fibrotic processes with high accuracy, current CMR tissue characterisation techniques (T1/T2 mapping) can also be used to quantify diffuse myocardial changes and distinguish between fibrotic and inflammatory changes in the heart muscle using T1 or T2 weighting (8). In the event of abnormal Tc99 SPECT/CT findings, the UKT routinely performs a subsequent CMR for verification. This is the case in approximately 10% of patients.

Early detection of cardiac amyloidosis could have a positive impact on the clinical course of patients through timely and appropriate treatment.

This prospective observational study aims to evaluate various imaging and clinical parameters for the early detection of cardiac amyloidosis in patients with aortic stenosis. The prevalence of cardiac amyloidosis is recorded and quantified using the appropriate technique. This is done using SPECT/CT and/or photon-counting CT imaging prior to planned TAVI. In patients with abnormal SPECT/CT or photon-counting CT results, multimodal CMR is used as an additional non-invasive method to make possible cardiac amyloidosis (even) more probable. Standardised follow-up after 6, 12 and 24 months is planned in order to review the prognostic relevance of the parameters determined by imaging techniques (Tc99-SPECT/CT, photon-counting CT (or CMR)).

The observational study does not alter the routine diagnostic management of patients with aortic valve stenosis and suspected cardiac amyloidosis.

1.5 Study organisation / Administration

1.5.1 Principal investigators

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1.5.2 Regulatory aspects

1.5.2.1 Regulatory classification of the study

This is a non-commercial, scientifically driven prospective observational study (non-interventional) in accordance with professional regulations (IIT, Investigator Initiated Trial).

1.5.2.2 German Medicines Act (AMG) and Medical Devices Act (MPG) (Federal Institute for Drugs and Medical Devices, BfArM)

Not applicable.

The SPECT/CT scanner (GE Healthcare Optima 860®) is an approved, CE-marked device (including the software used).

The photon-counting CT scanner (NAEOTOM Alpha®, Siemens Healthineers, Erlangen) is an approved, CE-marked device (including the software used).

The CMR scanners (Siemens) are approved, CE-marked devices with field strengths of 1.5 Tesla and 3 Tesla (including the software used).

No unauthorized drugs are used in this study.

1.5.2.3 Ethics committee

The responsible ethics committee is the

Ethics Committee at the Faculty of Medicine of the Eberhard Karls University and at the University Hospital Tübingen (UKT)
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1.5.2.4 Radiation protection issues

Radiation protection concerns arise from the clinically indicated Tc99 SPECT/CT examination of the patient to be performed in the context of medical treatment, which does not constitute a study-related examination (see section 2.2).

1.5.3 Cooperation

1.5.3.1 GE Healthcare

A cooperation agreement exists with GE Healthcare, regulating collaboration regarding the further development of the combined Tc99-SPECT/CT system in a whole-body system. However, the definition of clinical study content and the conduct of clinical trials remain the sole responsibility of the UKT (University Hospital Tübingen).

1.5.3.2 Siemens Healthineers

A cooperation agreement exists with Siemens Healthineers, regulating collaboration regarding the further development of quantum counting (photon counting) CT. However, the definition of clinical study content and the conduct of clinical studies remain the sole responsibility of the UKT (University Hospital Tübingen).

1.5.3.3 Financing

The study is being initiated, organized, and financed by the Department of Internal Medicine III at the University Hospital Tübingen (Medical Director: Prof. Dr. med. M. Gawaz), the Department of Radiology (Medical Director: Prof. Dr. med. K. Nikolaou), and the Department of Nuclear Medicine and Clinical Molecular Imaging (Medical Director: Prof. Dr. med. C. la Fougère). Following receipt of the necessary approvals, the plan is to initially seek third-party funding for the study from the University Hospital Tübingen's internal funding programs (applied clinical research (AKF) funding programme) and subsequently from external funding organizations.

1.5.3.4 Bayer AG

Bayer AG was secured as an external funding partner in the fourth quarter of 2025. However, the UKT bears sole responsibility for defining the content of clinical trials and conducting them.

2 Introduction and state of research

2.1 Clinical background

Aortic valve stenosis (AS) occurs in up to 3% of people over the age of 75 (9). If patients are symptomatic, they have a significantly reduced prognosis with conservative drug treatment, with an average survival time of 2 years (10-12). Although patients generally benefit from aortic valve replacement (AVR), some patients still have an increased mortality rate despite valve replacement, and the mechanisms behind this are not fully understood.

It is known that characteristics of higher-grade AS such as left ventricular hypertrophy, diastolic dysfunction and atrial fibrillation can also occur in cardiac transthyretin (TTR) amyloidosis. In recent years, it has become apparent that the deposition of amyloid fibrils is much more common than initially thought. Transthyretin (TTR) is synthesised in the liver and, under oxidative stress, forms ß-sheet structures that are then deposited as insoluble complexes in the myocardium, for example (13). As with AS, many patients complain of dyspnoea and leg oedema and these patients also have a very high mortality rate if left untreated. If a patient had both high-grade AS and cardiac amyloidosis but only the AS was treated, e.g. by TAVI, the cardiac amyloidosis could ultimately be responsible for a poor outcome (despite the TAVI procedure).

In addition to initiating treatment for heart failure, therapy for TTR amyloidosis depends on the aetiology of the disease: a) If there is a mutation of the TTR gene (which occurs primarily in younger people), a liver transplant is sought. b) If a wild-type form (senile form) is present, the treatment options have been very limited up to now (e.g. green tea as a possible therapeutic approach). Newer therapeutic approaches (use of small interfering ribonucleic acid (RNA) molecules to suppress TTR molecules or administration of antibodies against serum amyloid) appear to be very promising, but are still in the early stages of clinical application (13).

Accurate diagnosis of cardiac amyloidosis remains a clinical challenge. This is due, in part, to the sometimes non-specific clinical presentation of patients, consisting of general symptoms such as fatigue and reduced performance, as well as nonspecific symptoms such as impaired physical capacity and dyspnoea. Furthermore, initial diagnostic tests (ECG, echocardiography) and laboratory tests may be unremarkable or show nonspecific signs of impaired cardiac function. When cardiac amyloidosis is suspected, myocardial biopsy (EMB) is the gold standard for confirming the diagnosis (13); however, this is an invasive procedure with associated risks; furthermore, diagnostic accuracy may be limited in cases of inhomogeneous amyloid distribution (‘sampling error’).

Non-invasive imaging diagnostics using Tc99-SPECT/CT and, if necessary, CMR therefore play a decisive role in both the primary diagnosis and the assessment of the progression of cardiac amyloidosis. Tc99-SPECT/CT replaces EMB when the appropriate hybrid imaging equipment is available. With timely diagnosis, the myocardial inflammatory and/or fibrotic processes triggered by TTR molecules could potentially be detected at an early stage and reversed with appropriate therapy. Non-invasive quantitative imaging parameters are ideally suited for this purpose, as they not only indicate the severity or burden of cardiac amyloidosis, but are also suitable for monitoring the clinical course (response to therapy).

2.2 Skeletal scintigraphy (Tc99-SPECT/CT) and quantification

Imaging techniques can detect amyloidosis based on the calcium-associated uptake of radioactively labelled Tc99m-labeled phosphonates, as traditionally used in skeletal scintigraphy. According to current data, Tc99m-phosphonate scintigraphy has high specificity for the detection of ATTR amyloidosis (1).

In the scintigraphic diagnosis of amyloidosis (Tc99-SPECT/CT), the assessment of pathology has so far been based solely on the visual impression in the planar image. A 4-point score is usually used, which ranks the myocardial uptake of the tracer in comparison to its osseous uptake (2). Using the Perugini score (2), the tracer uptake is merely classified as a discrete quantity within a coarse grid. Current data already suggest a correlation between phosphonate uptake in the myocardium and patient prognosis (7). Further quantification of myocardial radiopharmaceutical uptake would therefore potentially also have prognostic relevance for the individual patient.

Hybrid imaging using SPECT/CT and PET/CT combines anatomical information from X-ray computed tomography (CT) with functional information from single-photon emission computed tomography (SPECT) or positron emission tomography (PET). Additionally, the CT data are converted into an attenuation matrix to correct the emission data.

Unlike PET/CT, where quantitative parameters such as the standardised uptake value (SUV) are used for clinical evaluation, precise quantification has long been impossible with SPECT/CT. However, semi-quantitative analyses (e.g. in the form of the relative mean pulse rate compared to the opposite side or another reference tissue) have been established for some scintigraphic procedures, which already allow for better standardisation of the assessment (14, 15).

The latest generation of SPECT/CT cameras now offer the option of quantification for the first time, similar to PET/CT (16). This is made possible by correcting the emission data, taking into account the imaging properties of the detector system, and attenuation correction using CT. The SPECT/CT camera (GE Healthcare Optima 860®) that has been in operation in the Department of Nuclear Medicine and Molecular Imaging since June 2018 offers this quantification option. In addition, the use of optimised reconstruction methods improves SPECT and CT image quality, enabling increased sensitivity with reduced radiation exposure. Our own preliminary work shows not only reliable quantification of organ activity in the phantom model, but also initial clinical usability of quantitative SPECT/CT through a significant improvement in the determination of liver-lung shunts in the evaluation of patients for radioembolisation therapy (17).

As part of the proposed study, the data acquired during clinical Tc99-SPECT/CT examinations will be used for quantification. The quantitative parameters determined are the standardised uptake value (SUV) mean, SUVmax, SUVpeak and %ID/ml in the left ventricular myocardium. Quantification is performed by post-processing previously acquired data and does not alter the imaging modalities.

As part of the prospective data collection, only clinically indicated diagnostic Tc99-SPECT/CT scans are performed; i.e. amyloid imaging is performed if it appears appropriate based on the symptoms and cardiac characteristics. In addition to the established Perugini score (2), a precise quantification of myocardial uptake is also performed.

In the event of conspicuous Tc99 SPECT/CT findings, a cardiac MRI (CMR) is also performed at the UKT for verification purposes (see section 2.3). This is necessary in approximately 10% of patients.

2.3 Cardiac magnetic resonance imaging (CMR)

MRI is a non-invasive medical imaging technique that does not require the use of ionising radiation and allows for highly flexible anatomical and functional imaging of tissues. Over the past decade, CMR has become the gold standard for diagnostic imaging of the heart. This is because contrast-enhanced CMR can answer a wide range of questions within a single examination. Morphological sequences enable accurate anatomical imaging of cardiac structures and the identification of oedematous changes in the myocardium. Rapid measurement sequences can be used to dynamically visualise cardiac activity in order to quantify wall motion and stroke volumes. After the administration of gadolinium-containing contrast agents, late gadolinium enhancement (LGE) imaging can also be used to visualise fibrotic or scarred areas of the myocardium, such as those that occur in the context of cardiac amyloid deposition (18).

Despite these advantages, LGE technology has weaknesses in detecting diffuse inflammatory/fibrotic processes, such as those described in the early stages of cardiac amyloidosis. The CE-certified T1/T2 mapping techniques available at the UKT are a suitable option to reliably detect these diffuse myocardial changes using CMR (19-20).

2.4 Photon counting CT

Extracellular volume (ECV) quantification using CT is another attractive method which, like CMR, utilises the properties of extracellular contrast agents to calculate ECV. Photon-counting CT enables ECV calculation even in patients with contraindications for CMR, such as the presence of pacemakers that are not MRI-compatible. Adding a scan with late enhancement (for dual-energy acquisitions) or a non-enhanced and late-enhancement scan (for single-energy acquisitions) to a conventional coronary CT angiography scan can improve risk stratification and requires only minor adjustments to contrast agents and data acquisition protocols at low radiation doses. It has already been shown that quantification of myocardial ECV using CT correlates strongly with ECV quantification using CMR. Myocardial ECV quantification provides incremental prognostic information for various pathologies affecting the heart, such as cardiac amyloidosis (3-6).

CT is routinely performed at the UKT for TAVI planning and is therefore already integrated into the diagnostic workflow for patients with suspected cardiac amyloidosis without increasing their examination burden (see Fig. 1).

2.5 Quantitative parameters in Tc99-SPECT/CT, photon counting CT and CMR

With regard to cardiac diagnostics, Tc99-SPECT/CT, photon-counting CTs and CMR can be used to comprehensively characterise anatomical, functional and metabolic changes in the myocardium. Due to the high specificity of Tc99-SPECT/CT for the diagnosis of cardiac amyloidosis, it would be of great clinical interest to determine whether the quantitative parameters obtained in this way correlate with the quantitative parameters obtained by CT or CMR.

In addition to comprehensive diagnostics for patients, it is hoped that specific, spatially resolved imaging will enable the classification of different stages of the disease – which can range from initial active inflammation to post-inflammatory scarring as an irreversible late stage. This may lead to a better understanding of pathophysiological relationships, which in the long term could result in the further development of diagnostic and therapeutic options.

2.6 Diagnostic work flow in patients with suspected cardiac amyloidosis at the UKT

Fig. 1 shows the diagnostic patient management for degenerative aortic valve stenosis and suspected cardiac amyloidosis at the UKT. This will not be changed as part of the observational study.

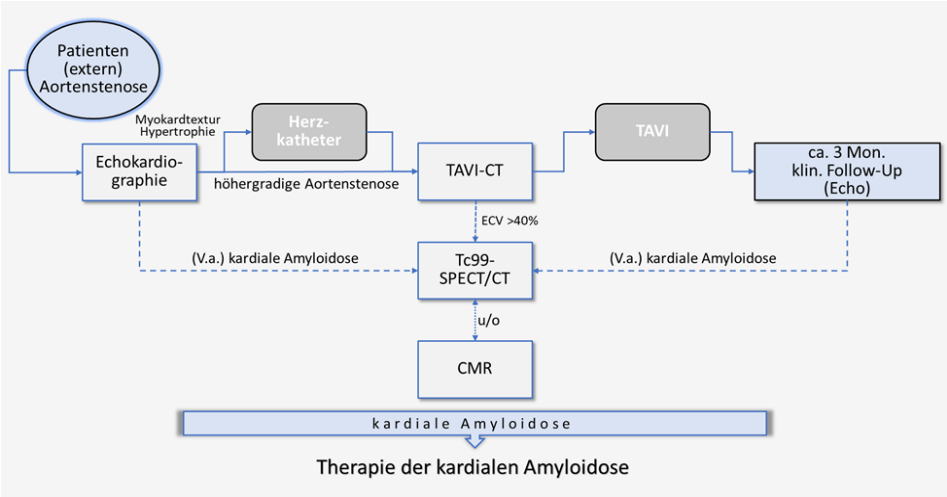


Figure 1: Patient management for degenerative aortic valve stenosis/cardiac amyloidosis at the UKT

Patients with aortic valve stenosis (AS) initially undergo echocardiography in the cardiology department. If severe AS is detected, treatment is carried out using transcatheter aortic valve implantation (TAVI). A CT scan is indicated for TAVI planning in accordance with guidelines. After TAVI, patients are usually followed up, including echocardiography after 3 months. The time frame from initial echocardiography to TAVI at the UKT is approximately 1-2 weeks. If, during the course of the investigations, there is a suspicion of cardiac amyloidosis, e.g., through the diagnosis of extracellular volume (ECV) in the myocardium (>40%), an elective, clinically indicated technetium-99m phosphonate scintigraphy using SPECT/CT (= Tc99-SPECT/CT) and/or cardiac MRI (CMR) is performed. The latter can be performed if the SPECT/CT findings are unclear, provided there are no MRI-relevant contraindications (pacemaker, patient compliance). The decision for Tc99-SPECT/CT or CMR also depends on the fastest possible availability of the equipment. If amyloidosis is confirmed, appropriate therapy is initiated.

3 Prior knowledge relevant to the study

In the nuclear medicine department at the UKT, approximately 1,000 SPECT/CTs are currently performed each year using various tracers, resulting in outstanding expertise in this field. Fig. 2 shows an example of the examination results using Tc99-SPECT/CT in a patient with cardiac amyloidosis.

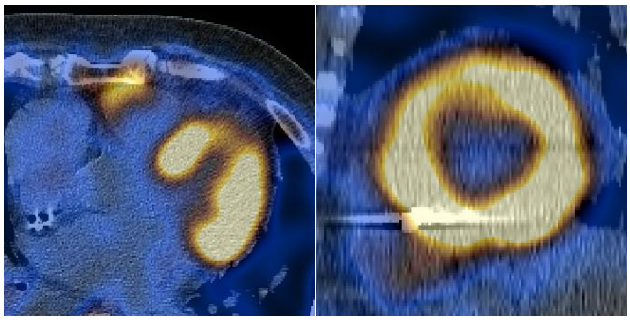


Figure 2: Skeletal scintigraphy with 685 MBq Tc-99m-DPD (TCDPD) in a patient with suspected cardiac amyloidosis:

There is intense tracer accumulation in almost the entire left ventricular myocardium. Myocardial biopsy confirmed the suspected cardiac TTR amyloidosis.

4 Rationale for conducting the study

4.1 Problem statement and rationale

Both severe aortic valve stenosis and cardiac amyloidosis can lead to pronounced septal hypertrophy. Since both diseases are more common in older patients, it is important to know the actual cause of septal hypertrophy, as the two entities differ fundamentally in their therapeutic approach.

Tc99 SPECT/CT has a high diagnostic accuracy for diagnosing cardiac amyloidosis, but involves a certain amount of radiation exposure. In contrast, the established LGE technique (CMR) also has a high accuracy in diagnosing cardiac amyloidosis and does not involve any radiation exposure. Improved CMR techniques with regard to T1/T2 mapping can also quantitatively detect even minor diffuse myocardial changes. In the event of abnormal Tc99-SPECT/CT results, CMR would therefore be used as a confirmatory test to consolidate the diagnosis of amyloidosis and ultimately avoid the need for an invasive myocardial biopsy.

The observational study aims to prospectively collect data on the imaging methods used at the UKT, whose prognostic relevance will be examined in conjunction with the data collected from the follow-up. The focus will be on quantifiable data from the generated Tc99-SPECT/CT and/or photon-counting CT and, if applicable, CMR data sets.

4.2 Study objectives (Summary)

The prospective observational study aims to comparatively evaluate various imaging and clinical parameters in the early detection of cardiac amyloidosis in patients with aortic stenosis, thereby improving the diagnostic workflow for these patients.

This routine diagnostic workflow will be further optimised for patients with significant septal hypertrophy, and the importance of multimodal imaging will be reinforced in this study. The aim is to be able to clearly identify the underlying cause of left ventricular hypertrophy in order to offer each individual patient the most appropriate treatment.

Further objectives of this study are:

- Optimisation of established Tc99 SPECT/CT through the quantitative approach of tracer uptake.
- Comparison of abnormal Tc99 SPECT/CT findings (and their quantification) with the equally quantitative determination of ECV using photon-counting CT or mapping methods using CMR.
- In addition to the purely diagnostic value, the prognostic value is also to be determined by means of a standardised telephone follow-up of patients after 6, 12 and 24 months.

(see Chapter 10)

5 Risk/benefit assessment

Since this is a non-interventional observational study and no additional study-related clinical measures are taken, there is no increased risk for participating patients. The only time commitment required is for the necessary information/consent regarding data protection and data transfer, as well as for the collection of follow-up data via telephone surveys.

The participating patient does not derive any immediate benefit from participating in the study. Since the observational study primarily serves the purposes of quality assurance and improving the diagnostic workflow (confirmed diagnosis of amyloidosis, avoidance of invasive myocardial biopsies), future patients will benefit from the results.

6 Study design

This is a prospective, single-centre observational study (NIS) conducted as an investigator-initiated trial in accordance with professional regulations. The routine diagnostic workflow (see Fig. 1) will not be changed as a result of participation in the study.

7 Study population

These are patients with left ventricular hypertrophy and severe aortic valve stenosis who are scheduled for transcatheter aortic valve implantation (TAVI) and for whom Tc99-SPECT/CT or CMR to determine ECV and CT for TAVI planning (and ECV quantification) are clinically indicated.

7.1 Inclusion criteria

- Patients with left ventricular hypertrophy and severe aortic valve stenosis (valve opening area <1 cm², pmean >40 mm Hg, Vmax >4 m/sec)
- Suspected cardiac amyloidosis
- Performance of one or more of the following imaging examinations based on (justifiable) clinical indications: echocardiography, photon-counting CT, Tc99-SPECT/CT, CMR
- Age ≥18 years
- Written consent of the patient for prospective data collection regarding Tc99-SPECT/CT, CT, CMR if applicable, and follow-up

7.2 Exclusion criteria

- Limited capacity of the patient to give consent

7.3 Justified case number estimate

The study will be evaluated descriptively. The target number of cases is based on the number of examinations that can be realistically achieved.

Based on clinical experience from recent years, the Department of Internal Medicine III (Cardiology and Angiology) anticipates approximately 100-150 potential study participants annually. We expect that around 80% of these will be willing to participate and can be recruited. Furthermore, it is taken into account that in approximately 5% of recruited cases, no data sets suitable for evaluation according to the study protocol can be generated. Therefore, based on the above-mentioned patient numbers, a participation rate of 600 patients within a recruitment period of approximately 4.5 years would be achievable.

7.4 Recruitment

All patients who present to the University Clinic for Internal Medicine III (Cardiology and Angiology) or the Radiology Clinic with high-grade aortic valve stenosis are eligible to participate in the study, and for whom a TAVI is recommended by an interdisciplinary conference decision consisting of specialists in cardiology/cardiac surgery/anaesthesia based on an assessment of the clinical symptoms, the imaging performed so far and the individual course of the disease.

Patients are to be included consecutively for the prospectively planned data collection. It should be possible to approach and recruit patients for the study at any point in the established diagnostic workflow (see Fig. 1). In an initial consultation with the patient, the suitability of participation in the study is assessed. The patient will be informed about the objectives of the non-interventional study and the voluntary nature of participation. If the patient expresses interest in participating in the study, they will be given an information sheet. The responsible study physician will check the inclusion and exclusion criteria after the patient has given their informed consent. The justification for skeletal scintigraphy is provided by a nuclear medicine specialist and, if necessary, a radiologist for the additional CMR.

7.4.1 Procedure for replacing trial participants

Study participants who discontinue the study-relevant examination after giving their consent are counted as recruited patients and documented accordingly. Discontinuation of imaging procedures (Tc99-SPECT/CT, photon-counting CT or CMR) may mean that no evaluable image data sets could be generated. Patients may also be 'lost to follow-up'. In order to ensure that the planned number of cases is reached, an additional number of patients will be recruited to compensate for discontinuations or missing follow-up data.

8 Study-related medication

Not applicable

9 Study procedure and investigation methods

9.1 Study-relevant examinations

The studies relevant to the study are 99Tc-SPECT/CT, photon-counting CT for TAVI planning and, if necessary, CMR. All imaging procedures are performed according to justified clinical indications – i.e. they are not study-related, but are carried out as part of routine care.

9.1.1 Study-relevant blood and tissue sampling

Not applicable

9.2 Type and procedure of study-relevant examinations

9.2.1 Procedure for Tc99-SPECT/CT, photon counting CT and CMR examinations

All imaging procedures are performed in accordance with standard clinical procedures as part of routine clinical practice. There are no study-related changes to the examination procedure.

9.2.1.1 Data processing / post-processing Tc99-SPECT/CT, photon-counting CT or CMR

Tc99-SPECT/CT: Quantification is performed using dedicated software (Q.metrix, GE Healthcare and Hermes Hybrid3D®, Hermes Medical Solutions, Stockholm, Sweden).

Photon-counting CT: Data analysis is performed using clinically approved software (Siemens Syngo.via), which includes spectral analysis, enabling direct calculation of myocardial ECV.

CMR: Data analysis is performed using clinically approved software (Siemens SyngoVia, Erlangen, Germany, or Circle CVI42, Calgary, Canada), which allows functional analysis of CMR data and quantification of mapping data.

The data sets are quantified after data acquisition (post-processing) and have no influence on the examination procedure or diagnosis for the study participants.

9.3 Study-related follow-up

After obtaining the patients' explicit consent, we will conduct a standardised telephone follow-up in the form of a standardised questionnaire; further measures (repeat diagnostics, additional interviews, etc.) are not planned. If necessary, and with the patients' consent, this data set will be supplemented with information from the hospital information system or their general practitioner. The data relevant to the study will initially be recorded in the questionnaire.

The data relevant to the study, demographic data (age, gender) and medical data, including medication, are entered in pseudonymised form (encrypted by patient number without initials and date of birth) in separate documentation forms. Copies of original patient records (medical findings) are filed separately as source documents.

A preliminary data list (identification list) is maintained in which the patient's name is assigned to a patient number. This list and the source documents are only accessible to the study directors and the participating physicians and, if applicable, doctoral students who are subject to confidentiality obligations. Subsequent identification should be possible in the event that patient-relevant findings arise that are not yet foreseeable according to the current state of science and research.

The transfer of the collected data to third parties (i.e. persons entrusted with further processing of the data), the evaluation and publication of the data shall take place exclusively in anonymised form.

The preliminary data list, the standardised questionnaire completed by us by telephone, other study materials and the study results will be stored in the Department of Cardiology and Angiology at the UKT for 10 years after publication. They will then be destroyed in a document shredder or by data deletion.

9.4 Study duration

9.4.1 Duration of the overall study

The study is designed to last approximately 4.5 years*). A recruitment period of approximately 48 months is assumed. The study covers the period for patient recruitment, study information, clinical examination, imaging diagnostics to be performed in Tc99-SPECT/CT or CMR with information and discussion of findings, and multiparametric image data evaluation using special software, as well as standardised follow-up (see appendix).

A further 6 months are estimated for the final interdisciplinary data interpretation and discussion, as well as the publication of the study results.

*) Amendment 1, which was implemented partly due to poor recruitment, doubles the duration of the recruitment phase (start after amendment: Q3/2024 / expected end: Q4/2027).

9.4.2 Individual time commitment for study participants

Approximately 15-30 minutes are estimated for informing and obtaining consent from a participating patient.

Approximately 10-15 minutes are planned for a follow-up telephone call (see section 9.3).

There is no additional time required for the study. If the patient cannot be reached by telephone, the general practitioner will be contacted by telephone or, if necessary, the latest available patient data from the UKT will be reviewed.

9.5 Risks and side-effects

Not applicable

This is a non-interventional observational study.

9.6 Follow-up and further treatment after the end of the clinical study

The study-related follow-up is described in section 9.3.

The follow-up of all included patients after the end of the study will be carried out as part of the clinical therapy and aftercare provided by the Department of Internal Medicine at the UKT. No additional study-related examinations will be performed at these appointments.

10 Definition of the objectives

10.1 Primary objectives

The primary endpoint is the prospective collection of data from patients who have undergone Tc99-SPECT/CT, CT and, if necessary, CMR, in order to determine the prevalence of cardiac amyloidosis in patients with severe aortic valve stenosis. The evaluation is performed using non-invasive imaging:

Tc99-SPECT/CT, photon-counting CT or cardiac MRI. The prospective observational study aims to comparatively evaluate the various imaging and clinical parameters collected in the early detection of cardiac amyloidosis in patients with aortic stenosis, thereby improving the diagnostic workflow for these patients.

10.2 Secondary objectives

- Correlation of cardiac MRI (quantitative T1/T2 mapping) with quantitative Tc99 SPECT/CT and/or photon counting CT evaluation.
- Comparison of abnormal Tc99 SPECT/CT findings (and their quantification) with quantitative determinations of ECV using photon-counting CT or mapping methods using CMR.
- Optimisation of established Tc99 SPECT/CT through the quantitative approach of tracer uptake.
- Follow-up of patients with regard to adverse events (death, cardiac death, stroke, hospitalisation due to heart failure)
- Evaluation of the predictive and prognostic value of SPECT/CT, CT and MRI procedures for tissue characterisation
- Influence of acquisition and quantification parameters in SPECT/CT (CT information, NM image, size and positioning of the volume of interest, thresholds) on measured activity concentration and distribution volume
- Improvement of diagnostic patient management in cases of suspected cardiac amyloidosis (early detection of amyloidosis)

11 Validation methods

11.1 Correlation T1/T2-mapping with Tc99m-SPECT/CT skeletal scintigraphy and photon counting CT

The correlation of the CMR-based parameters of T1 and T2 mapping with Tc99m-DPD skeletal scintigraphy imaging and photon-counting CT is performed both at the level of individual cardiac segments and at the level of individual voxels. Firstly, the consistency of the methods is to be assessed qualitatively within the framework of a visual evaluation. Secondly, the quantitative correlation of the respective measured values will be compared with each other. The spatial distribution of the parameters and their relationship to each other, in conjunction with clinical data, can be used to formulate hypotheses about disease stages and progression.

The following parameters will be evaluated:

- Calcium score of the coronary arteries and aortic valve.
- Stenosis of the coronary arteries in coronary CTA (CCTA) compared to invasive coronary angiography (ICA), if performed pre-interventionally for clinical reasons.
- Calculation of fractional flow reserve (FFRCT) to assess coronary stenosis.
- Spectral analyses of myocardial texture and calculation of myocardial extracellular volume for planning SPECT in the course of TAVI implantation.
- Recording of clinical parameters and cardinal events/outcomes.
- Follow-up after 3 months with clinical visit and additional telephone follow-up examinations after 12 and 24 months.

11.2 Follow-up questionnaire

See attachment.

12 Discontinuation criteria

To assess the feasibility of the study, the number of all discontinuations and the number of all study-relevant examinations completed according to protocol are taken into account. Discontinuations of routine imaging may occur, for example, at the request of the patient or based on a decision by the treating physician, due to the occurrence of intolerable side effects (e.g. acute claustrophobia) or due to technical deficiencies.

12.1 Individual discontinuation criteria

Participation in the study is voluntary for all participants. Consent to participate and to the prospective data collection and scientific evaluation of the results may be withdrawn at any time. Participants are assured that this will not result in any disadvantages regarding their clinical care.

All cases leading to discontinuation of the clinical study must be documented and justified.

13 Data processing and statistical analysis

13.1 Data collection

Patient-related data (demographic data, diagnoses, medication, etc.) is documented as part of the study.

The image data will be archived on electronic media where long-term archiving is technically guaranteed. It should be noted that data protection requirements must be observed for all programmes. Personally identifiable data (in particular names, initials, addresses) must not appear in the files under any circumstances.

13.2 Study monitoring and source data

13.2.1 Study monitoring

This observational study is a scientifically driven study (known as an investigator-initiated trial) conducted at a single centre, the UKT. External monitoring by a CRO is not planned.

13.2.2 Source data

The following verifications will be carried out for all patients recruited in this clinical trial:

- Existence of medical records
- Date of birth
- Gender
- Patient's informed consent for the study and data protection
- Confirmation of the diagnosis being investigated in the clinical trial
- Information on medical history
- Evidence in the patient's file indicating their enrolment in this clinical trial
- Record of adverse and serious adverse events
- Record of study-relevant data

13.3 Statistical design

The statistical analysis is primarily descriptive and exploratory, in line with the study’s prospective observational design. The aim is to determine the prevalence of cardiac amyloidosis in patients with severe

aortic valve stenosis and to comparatively evaluate the diagnostic and prognostic value of the imaging modalities used.

The prevalence of cardiac amyloidosis is reported as the proportion of patients with abnormal imaging or nuclear medicine findings in the total study population, including a 95% confidence interval. Baseline clinical data and imaging parameters from Tc99-SPECT/CT, photon-counting CT, and cardiac MRI are presented descriptively. Continuous variables are reported as mean ± standard deviation or median with interquartile range, depending on the distribution; categorical variables are reported as absolute and relative frequencies.

To address the diagnostic questions, the results of the individual methods are analyzed comparatively. In particular, sensitivity, specificity, positive and negative predictive values, and diagnostic accuracy are determined, provided a suitable reference standard or a consensus clinical diagnosis is available. Agreement between the methods is examined using cross-tabulations and appropriate measures of concordance, particularly Cohen's Kappa. Quantitative measurements such as tracer uptake in SPECT/CT, extracellular volume in photon-counting CT, as well as T1/T2 mapping parameters and ECV in cardiac MRI are compared using Pearson or Spearman correlation analyses. Additionally, Bland-Altman analyses may be used to assess the agreement between quantitative measurement methods.

ROC analyses are performed to evaluate the diagnostic performance of quantitative parameters. These analyses determine the area under the curve, optimal thresholds, and corresponding sensitivity and specificity values. The influence of various SPECT/CT acquisition and quantification parameters, including CT-based information, VOI size and positioning, and thresholds used, is examined using variance analyses, regression models, or appropriate mixed models. For prognostic analyses, clinical endpoints are recorded during follow-up, particularly all-cause mortality, cardiac death, stroke, and hospitalization due to heart failure. Event-free survival times are presented using the Kaplan-Meier method and compared between groups—for example, patients with and without evidence of cardiac amyloidosis—using the log-rank test. The predictive and prognostic value of SPECT/CT, CT, and MRI parameters is examined using Cox regression analyses. In doing so, univariate and, where possible based on the number of events, multivariate models can be created, taking into account clinically relevant covariates such as age, sex, severity of aortic valve stenosis, left ventricular function, and relevant comorbidities.

All analyses are exploratory in nature and use a two-sided significance level of $p < 0.05$. Given the exploratory nature of the study, no formal adjustment for multiple testing is performed; the results are interpreted as hypothesis-generating. Missing values are documented and accounted for using appropriate methods, depending on the extent and structure of the missing data.

The statistical analysis of the study is being conducted in close collaboration with Prof. Dr. Thomas Mayrhofer, Chair of Health Economics at the Mannheim Medical Faculty of Heidelberg University. Prof. Mayrhofer is assisting the study team with statistical analyses to ensure a methodologically sound analysis of the collected data.

13.4 Archiving of data

Patient information and consent forms, as well as information on data protection (including consent), are listed in the appendix. These documents take into account that all patient-related data is subject to medical confidentiality. The records must be protected against unauthorized access and unauthorized modification. In accordance with Section 85 (3) of the Radiation Protection Ordinance (StrlSchV), records of examinations must be kept for ten years, and records of treatment for 30 years after the last examination or treatment. Records of study-related measures should be kept for at least 10 years, according to recommendations from the German Research Foundation (DFG).

Image data is archived on electronic media where long-term archiving is technically guaranteed.

13.5 Final report and publications

The principal investigators are responsible for the final report and any interim reports. The final report must be prepared regardless of the outcome of the clinical trial. It will be written after the follow-up period. A committee will be formed to oversee the publication of the results.

14 Data protection

The Tc99-SPECT/CT or (any) CT and CMR images are pseudonymised (assigned an identification number) immediately after acquisition. The study-related pseudonymised data sets (images) are to be stored for at least ten years and evaluated scientifically. An information sheet is created for each data set, which can be assigned to the corresponding Tc99-SPECT/CT, CT or CMR data sets via the identification number. The participant's body weight, age and gender are entered in this information sheet. It is also planned to transfer the study-relevant clinical diagnoses from the patient's medical records and enter them in the pseudonymised information sheet. The folder containing the pseudonymised information sheets is kept confidentially in a locked cabinet in the principal investigator's office. A confidential list assigning the pseudonymised examinations to the participants remains with the study physician and is kept in another locked cabinet. This is to ensure that any pathologies detected in the participant can be communicated and that the clinical diagnosis can be reviewed at a later date on the basis of the medical records. Access to the medical records shall be permitted for a maximum of six months after the Tc99-SPECT/CT, CT or CMR examination. Written consent will be obtained from each participant for the scientific evaluation of the pseudonymised data sets and the review of the clinical diagnoses from the medical records after the Tc99-SPECT/CT, CT or CMR examination. Consent to the evaluation of the collected data can be revoked by the participant at any time without giving reasons.

To create a database for scientific exchange with other locations, the anonymisation of data records is planned solely for the technical post-processing of the data records. Anonymised data records will then be made available to research institutions, universities and companies registered with the University of Tübingen.

Personal data (names, date of birth, address, previous findings, study-related findings including imaging procedures, results of study-related genetic tests, etc.) will be collected and processed as part of the study.

Where necessary, medical data will also be included in the processing. The documentation and archiving of the data is carried out pseudonymously in a protected electronic database, to which only authorized employees, including doctoral candidates and research assistants bound by professional and data secrecy obligations, have access. All employees involved are subject to confidentiality obligations.

The information obtained in the course of this study may also be transferred for scientific purposes to cooperation partners within the scope of the European General Data Protection Regulation and to cooperation partners outside the European Economic Area, i.e. to countries with a lower level of data protection (this also applies to the USA). If the data is transferred to countries with a lower level of data protection, the controller will take all necessary measures to ensure the level of data protection. If this is not possible, the data will only be transferred if the study patients expressly consent to the proposed data transfer after being informed of the existing potential risks of such a data transfer.

The processing and storage of personal data by the participating researcher, Prof. Mayrhofer (Heidelberg University's Mannheim Medical Faculty), are carried out in accordance with the highest security standards compliant with the GDPR. The data is transmitted in encrypted form via the UKT's own cloud. Access to the stored data is restricted; only a small, selected group of staff members involved in the analysis has access. The data is deleted after transmission to the UKT at Heidelberg University Hospital.

The research results from the study will be published in anonymised form in specialist journals or scientific databases. The identity of the study patients will not be disclosed when the research results are published. However, the local investigators can trace the data back to the study patients with the help of a patient list if they have any queries.

Study patients may request information about their stored data at any time, request a free copy, and have the right to have incorrect data corrected. They may also request at any time that their data be deleted or anonymised so that it can no longer be linked to their person. These rights are restricted in accordance with Section 13 of the State Data Protection Amendment Act and Section 27 of the Federal

Data Protection Amendment Act to the extent that these rights are likely to render the achievement of the respective research purposes impossible or seriously impair them, and the restriction is necessary for the fulfilment of the respective research purposes. Furthermore, the right to information does not apply if the data is required for scientific research purposes and providing the information would require a disproportionate effort.

The principal investigator is responsible for data processing and compliance with statutory data protection regulations.

In the event of complaints, study participants may contact the data protection officer at Tübingen University Hospital or the state data protection officer for Baden-Württemberg.

Your express consent is required for the collection, storage, use and disclosure of data by signing the data protection consent form.

Note for testing centres subject to the LDSG (UKT):

The legal basis for the processing of your data is Articles 6, 7, 9 and 89 of the General Data Protection Regulation in conjunction with Sections 4, 5, 6, 8, 9, 12 and 13 of the State Data Protection Act of Baden-Württemberg in the version applicable from 25 May 2018.

Note for testing centres subject to the BDSG (BG, Robert Bosch Hospital, etc.):

The legal basis for the processing of your data is Art. 6, 7, 9, 89 of the General Data Protection Regulation in conjunction with Sections 22, 27, 29, 32, 33, 34, 35, 36, 38 in the version of the EU Data Protection Adaptation and Implementation Act of 30 June 2017, Federal Law Gazette I, p. 2097 ff.

15 Insurance

Patient insurance is not required due to the lack of risk.

No compensation is provided for study participants.

16 Ethical issues: Informing subjects/consent to participate in the study and data storage

This study will be conducted in accordance with the guidelines of the Declaration of Helsinki in its current version from 2013 (Fortaleza, Brazil). This includes all strict criteria for patient information and the involvement of the relevant ethics committees.

Study participants will receive an information sheet about the study-specific objectives and risks, as well as a general standard information sheet about the performance of Tc99-SPECT/CT or CMR. Participants in the study must sign a consent form for pseudonymised data storage and anonymised data transfer to registered research institutions and companies. This consent can be withdrawn at any time.

The patient information and consent forms, as well as the information on data protection (including consent), are listed in the appendix.

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18 List of appendices

- Patient information
- Patient consent, including information on data protection
- Standardised follow-up form