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Cardiac β_1-adrenoceptor autoantibodies in human heart disease: rationale and design of the Etiology, Titre-Course, and Survival (ETiCS) Study

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Aims Evidence for a pathophysiologic relevance of autoimmunity in human heart disease has substantially increased over the past years. Conformational autoantibodies stimulating the cardiac β1-adrenoceptor (β1-aabs) are considered of importance in heart failure development and clinical pilot studies have shown their prognostic significance in human 'idiopathic' cardiomyopathy.

Methods We recently developed a novel highly sensitive fluorescence-based functional assay to detect stimulating β1-aabs. We will use this method to assess Etiology, Titre-Course, and effect on Survival (ETiCS) of β1-aabs in a prospective multicentre study with serial follow-up of patients after a first acute myocarditis or myocardial infarction. Several European core laboratories will jointly study the hypothesis that both disorders may trigger autoimmune reactions leading to the generation of β1-aabs and/or other heart-directed aabs. Further, sera from healthy controls and well-characterized patient cohorts with dilated, ischaemic, or hypertensive cardiomyopathy will be analysed retrospectively for β1-aab prevalence, incidence, persistence, and/or clearance.

Conclusion ETiCS is so far the largest clinical diagnostic study projected to address cardiac autoimmunity. It attempts to unravel the pathophysiology of cardiac autoantibody formation and persistence/clearance. ETiCS will enhance current knowledge on autoimmunity in human heart disease and promote endeavours to develop novel therapies targeting cardiac aabs.

Keywords Autoimmunity • Autoantibodies • Beta-adrenergic receptor • Dilated cardiomyopathy • Myocarditis • Myocardial infarction

Introduction

High prevalence, morbidity, and mortality render heart failure a major health problem.[1] Still, the pathogenesis of the various cardiac disorders leading to this final common path is not fully understood. About two-thirds of cases are due to ischaemia, and the remainder to non-ischaemic myocardial damage. In about 30% of the latter cases, clear concepts for the genesis and perpetuation of the disease are lacking despite recent efforts to re-classify these 'cardiomyopathies of unknown origin' into genetic,

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non-genetic, acquired, and mixed forms.[2] This applies particularly to non-genetic forms of dilated cardiomyopathy (DCM).

Recent research has substantially advanced the understanding of autoimmune mechanisms in DCM.[3,4] It has been shown that an autoimmune attack directed against the cardiac β_1-adrenergic receptor may in fact cause DCM.[5] As a proof of pathogenetic principle, isogenic transfer of agonist-like acting β_1-adrenoceptor antibodies (β_1-aabs) from cardiomyopathic into healthy rats also transferred the disease.[6] More recent animal experiments[7,8] and first clinical pilot studies[9,10] further support their pathogenetic and predictive potential in DCM.[11,12] However, the true prevalence and prognostic value of functionally active cardiac autoantibodies (aabs) in human heart disease are still unclear, as are the events leading to their formation, their frequency of appearance, and their patterns of clearance and/or persistence.[3,4] We postulate that a first significant inflammatory or ischaemic myocyte damage—through liberation of a critical amount of cardiac self-antigens previously hidden to the immune system—might induce and perpetuate disease-causing and/or -modulating autoimmune reactions that deteriorate cardiac function and ultimately result in progressive heart failure.[13]

The Etiology, Titre-Course, and effect on Survival (ETiCS) of functional β_1-aabs (and other cardiac aabs) study is the largest European diagnostic study initiated so far in the field of cardiac autoimmunity. Its prospective part will provide a thorough analysis of 400 patients after a first inflammatory or first ischaemic cardiac event to test the hypotheses (a) that agonist-like β_1-aabs arise following acute myocarditis (AMitis) or myocardial infarction, and (b) that agonist-like β_1-aabs have an impact on cardiac remodelling and patient outcome. Expanding the scope of ETiCS beyond adrenoceptor-directed autoimmunity, the prospective part will also assess the clinical impact of other potentially relevant humoral effectors, including aabs directed against M2 acetylcholine receptors,[14] troponin I/T,[15] anti-heart muscle aabs,[16] and cardiodepressant aabs.[17] Moreover, the prospective approach will allow for an analysis of components of the patients' immune system involved in the generation of cardiac aabs—including a search for pre-disposing genotypes.[18,19]

The *retrospective part* of ETiCS will determine the prevalence and titre-course of agonist-like β_1-aabs in 900 well-characterized patients with ischaemic (ICM) or DCM, and hypertensive heart disease (HHD) enrolled in different trials of the German Competence Network Heart Failure (CNHF).[20] The serological findings will then be correlated with the respective clinical data sets, change in cardiac function, and patients' outcome.

The combined prospective and retrospective approach in ETiCS, together with the access to large, well-characterized patient cohorts is expected to enhance the current knowledge on autoimmunity in human heart disease and to stimulate the development of novel therapies targeting cardio-noxious aabs.[13,21,22]

Study design

The ETiCS study is an investigator-initiated, prospective multicentre diagnostic study under the auspices of the CNHF. It has an independent Data Monitoring and Endpoint Committee (DMEC) (Appendix A) and complies with standards of Good Clinical Practice (GCP).

Study flow

The ETiCS study comprises a prospective and a retrospective part. The **prospective** study will include 400 patients with a first AMitis or a first acute myocardial infarction (FAMI) (*Figure 1*). For individual patients, study-duration is 12 months; after inclusion and baseline assessment, three follow-up visits (FUP) are scheduled at (2−)3, 6, and 12 months (*Figure 2*). Suspected AMitis has to be substantiated by at least one major and two minor clinical criteria or symptoms as defined in *Table 1*, and must be confirmed by endomyocardial biopsy (EMB), evaluated according to the WHO/ISFC[23] or Dallas criteria.[24] First acute myocardial infarction is defined as acute ST-elevation myocardial infarction (STEMI) in patients with no history or signs of previous myocardial infarction—regardless of localization and/or extent of myocardial damage. Diagnosis of STEMI will follow the 2004 AHA/ACC-guidelines.[25] After review of the inclusion and exclusion criteria (*Table 1*) and written informed consent, baseline assessment includes clinical status, standardized questionnaires (SF-36 and PHQ-9[26]), results from cardiac catheterization (left and right heart pressures/haemodynamics), echocardiography, electrocardiogram (ECG), Holter-ECG, blood sampling, and histologic/molecular evaluation of EMBs in myocarditis patients. Cardiac magnetic resonance imaging (cMRI) will be performed in all patients within 96 h of hospitalization. Follow-up visits at (2−)3, 6, and 12 months comprise patient's history including medication, physical examination, SF-36/PHQ-9 questionnaires, echocardiography, ECG, Holter-ECG, and blood sampling (*Figure 2*). At FUP 12 months, a second cMRI will be performed.

The **retrospective** study serves to analyse the prevalence and titre course of β_1-aabs at inclusion and after 12 months of follow-up in 900 thoroughly characterized patients already enrolled in different trials of the CNHF with (a) ICM ($n = 300$ ICM, CNHF subproject 6a, Würzburg University), (b) DCM ($n = 400$ DCM, CNHF subprojects 9a and 6a, Universities of Marburg and Würzburg), or (c) HHD ($n = 200$ HHD, CNHF subproject 7, Göttingen University) (*Figure 1*). In these cohorts, prevalence and persistence or clearance of β_1-aabs will be related to cardiac functional parameters and outcomes as determined at inclusion and after 12 months of follow-up. Both, the prospective and the retrospective parts of ETiCS will study the time to occurrence of a series of pre-specified endpoints (for definitions see *Table 2*).

Control group. Prospective and retrospective results will be compared with data from 300 healthy subjects (*Figure 1*, male to female ratio 1:1) sampled from a population-based longitudinal CNHF study (assoc. CNHF-project ischaemia and coronary arteriosclerosis). Eligibility criteria for control subjects were: normal blood pressure, ECG, and exercise-stress test, and absence of myocardial infarction, diabetes, or peripheral vascular disease. Baseline and 12-month serum samples will be analysed.

Diagnostic procedures

Since the β_1-receptor molecule contains several potential immunogenic epitopes,[27] both, binding characteristics and functional properties of a β_1-aab need to be characterized. It has been shown that β_1-aabs may bind to cell surface β_1-receptors without affecting receptor function, or they bind and allosterically modulate adrenergic signalling (e.g. by stabilizing an active or inactive receptor

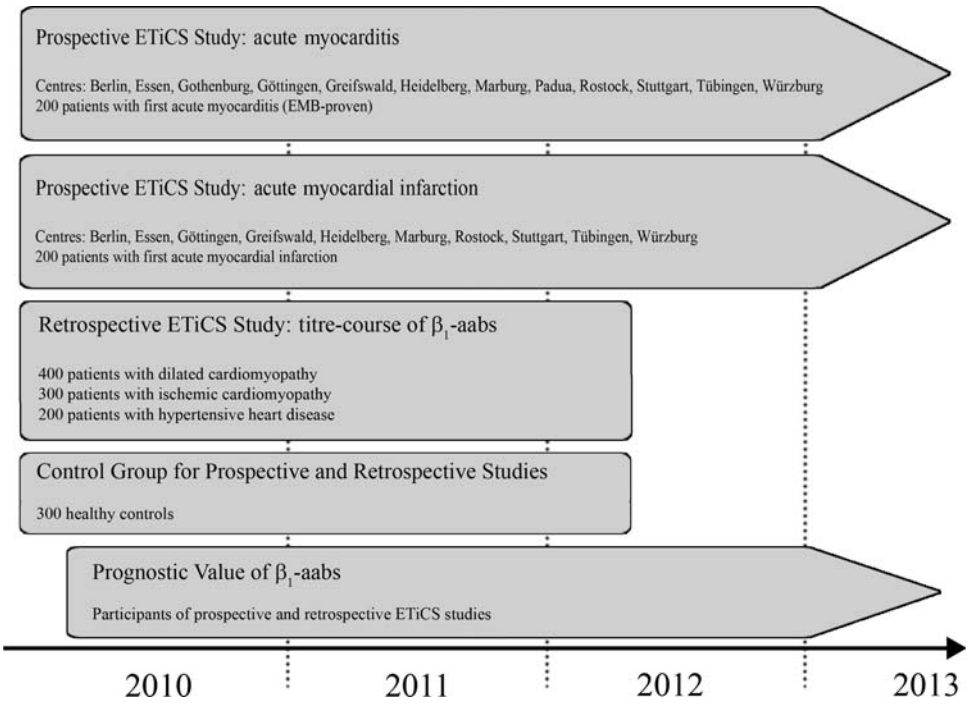

Figure 1 Time frame of the prospective and retrospective parts of the ETiCS study. The scheme depicts the recruitment periods (box and big arrows) for inclusion into the ETiCS study. During this period, serum-screening for functional β_1-aabs will be performed continuously.

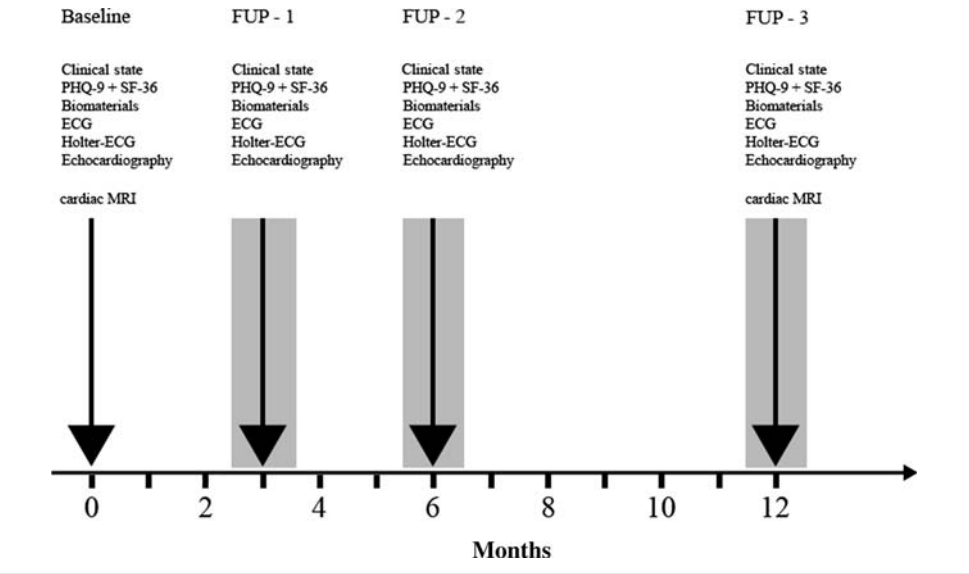

Figure 2 Time schedule of the prospective part of the ETiCS study. The scheme shows the time schedule of follow-up visits (arrows). Grey boxes indicate the approved time intervals for follow-up examinations indicated above each box. At baseline and each follow-up visit, the patient's sera will be screened for functional β_1-aabs and other cardiac aabs. ECG, electrocardiogram; FUP, follow-up visit; SF-36, quality of life questionnaire; PHQ-9, patient health questionnaire; MRI, magnetic resonance imaging.

conformation[12]). Most hitherto identified β_1-aabs had agonist-like effects (hence termed 'stimulating'), although antagonist-like properties are also conceivable. Differences in the screening-methods employed (e.g. a simple peptide-based ELISA-approach or a bioassay with neonatal rat cardiomyocytes or recombinant cells expressing human β_1-receptors[7,9,10]), and the relatively small patient numbers analysed contribute to the high variability in the currently available data on β_1-directed autoimmunity.

Table 1 Inclusion and exclusion criteria for the prospective and retrospective parts of the ETiCS study

Global inclusion criteria
Age >18 years
Written informed consent
Additional inclusion criteria for the prospective study
I. First event of acute myocarditis (one major and two minor criteria fulfilled)
Major criteria
ST/T wave changes
Ventricular arrhythmia
Pericardial effusion on echocardiography
Impairment of LVEF on echocardiography with CAD excluded by coronary angiography (no stenosis >50%)
Minor criteria
Dyspnoea, new onset within the past 30 days
Chest pain, new onset within the past 30 days
Palpitations, new onset within the past 30 days
History of infection within the past 30 days
Fever >38.0°C within the past 30 days
II. First acute myocardial infarction (all criteria fulfilled)
STEMI criteria: ST-elevation in at least two adjacent leads (≥0.1 mV in limb leads, ≥0.2 mV in chest leads) OR
Newly documented left bundle branch block with typical symptoms of myocardial infarction
No myocardial infarction in patient history
Confirmation of acute myocardial infarction by coronary angiography
New onset of chest pain within the past 7 days
Inclusion criteria for the retrospective study
DCM
LVEF ≤40% as determined by echocardiography
Exclusion of CAD by coronary angiography (no stenosis >50%)
HHD
LVEF ≥55% as determined by echocardiography
Arterial hypertension (RR syst >140 mmHg, RR diast >90 mmHg) or therapy with at least two antihypertensive agents
LV hypertrophy with IVS plus LVPW >25 mm wall-thickness
No history of myocardial infarction
Exclusion of CAD by coronary angiography (no stenosis >50%)
ICM
LVEF ≤40% as determined by echocardiography
History of myocardial infarction
LVEF ≤40% or relevant coronary artery disease with stenoses >50% and/or complete occlusion of the coronary artery
Controls
No history of cardiovascular disease, in particular no self-reported myocardial infarction
Normal ECG and normal exercise-stress test
No pharmacologically treated diabetes or peripheral vascular disease
Exclusion criteria
Malignancy or any other disease limiting the expected survival to less than 1 year
End-stage renal failure or chronic haemodialysis
Significant rheumatic or autoimmune disease, requiring immuno-modulatory therapy
Congenital neuromuscular disorders and myasthenia gravis
Graves' disease
Ongoing drug or alcohol abuse
Pregnancy
Inability to give informed consent, any physical or mental inability to supply essential information or expected low compliance

CAD, coronary artery disease; DCM, dilated cardiomyopathy; ECG, electrocardiogram; HHD, hypertensive heart disease; ICM, ischaemic cardiomyopathy; IVS, interventricular septum; LVEF, left ventricular ejection fraction; LVPW, left ventricular posterior wall; MI, myocardial infarction; RR diast, diastolic blood pressure; RR syst, systolic blood pressure.

Table 2 Endpoints of the ETiCS study

Primary endpoints
Prospective studies (AMitis and FAMI): titre course of functionally active β_1-aabs
Retrospective study: titre change of functionally active β_1-aabs (baseline vs. 12 months)
Prognostic analysis combining all subjects from prospective and retrospective studies: time to first life-threatening cardiovascular event[a]
Secondary endpoints for prospective and retrospective studies
Time to all-cause death
Occurrence of ventricular arrhythmias[b]
Change of NYHA classification
Minimum left ventricular ejection fraction during observation period (echocardiography)
Change in left ventricular ejection fraction (echocardiography)
Change in left ventricular end-diastolic diameter (echocardiography)
Left ventricular remodelling (cMRI)
Quantitative tissue loss after myocardial infarction (cMRI)
Severity of inflammation (EMB)

[a]Components are: cardiovascular death from myocardial infarction or progressive heart failure or arrhythmia, sudden cardiac death, resuscitation (successful or not successful), appropriate ICD discharge, cardiac transplantation.
[b]Components are: appropriate ICD discharge, sudden cardiac death, ventricular tachycardia (>15 beats).
cMRI, cardiac magnetic resonance imaging; EMB, endomyocardial biopsy; ICD, implantable cardioverter defibrillator; NYHA, New York Heart Association.

Therefore, the ETiCS study will employ a stringent screening process to assess both the presence and the receptor-modulating potential of conformational β_1-aabs. Our diagnostic

approach comprises screening of patient IgG by fluorescence-activated cell sorting (FACS) with β_1-expressing human cells to assess recognition of native cell surface receptors. Further, we will utilize a novel fluorescent cAMP sensor to assess the effects of the same IgG preparations on receptor-mediated signalling in living cells. Cyclic AMP exerts its cellular effects via protein kinase A, cAMP-gated ion channels, and/or directly activated exchange proteins (Epac1 and 2). The novel sensor utilizes enhanced cyan (CFP) and yellow fluorescent protein (YFP) fused to either end of the cAMP binding domain of Epac1, yielding a high sensitivity and excellent temporal resolution of cAMP measurements.[12] Binding of cAMP generated after stimulation of the β_1-adrenoceptor by agonist-like β_1-aabs leads to a conformational change in the sensor molecule resulting in a significant decrease in fluorescence resonance energy transfer (FRET, see also *Figure 3*). Reproducibility and reliability of the novel FRET-method have been published elsewhere.[12]

Ethical considerations

ETiCS is a non-interventional diagnostic study complying with GCP standards. Added patient risk is almost negligible and limited to complications of blood sampling and possible concerns of data privacy protection. In patients with AMitis, endomyocardial biopsies (EMBs) will be obtained according to the recommendations of the AHA/ACCF/ESC,[28] which recently have been endorsed by studies underscoring the importance of EMBs for patient outcome and therapeutic decisions.[29,30] All parts of the study conform to the Declaration of Helsinki[31] and have been approved by the respective Ethics committees of the participating institutions (Appendix B).

Biometry and statistics

Effect size and sample size

In different patient cohorts, prevalence and cardiovascular event rates vary widely. Effect sizes assumed for power and sample

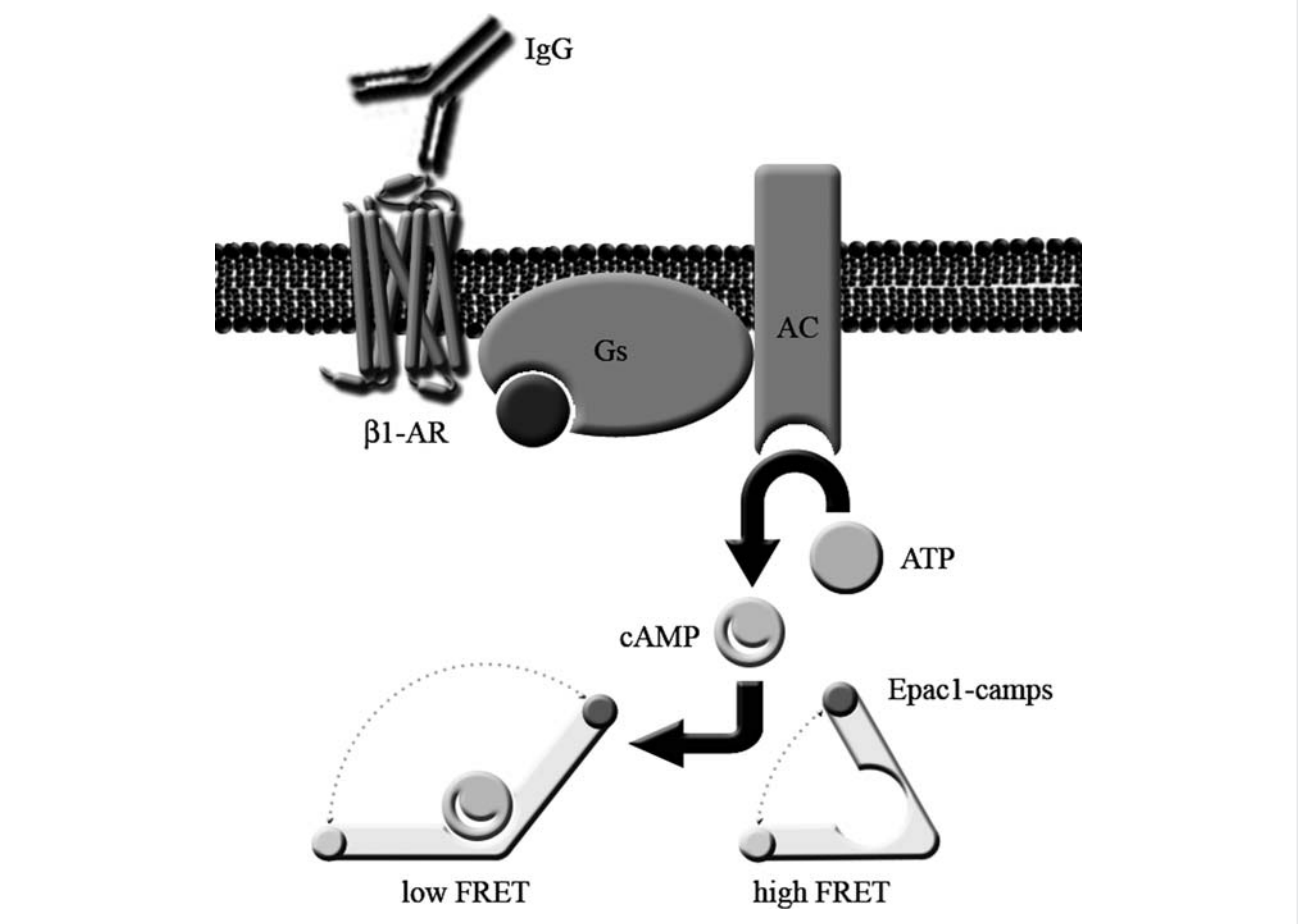

Figure 3 Mode of operation of the diagnostic FRET assay. The scheme depicts the adrenergic signalling cascade supposedly activated by stimulating β_1-aabs. Binding of the antibody induces or stabilizes an active β_1-receptor conformation and increases cytosolic cAMP concentration via activation of Gs-protein and adenylate cyclase. Newly generated cAMP binds to the fluorescent cAMP-sensor (Epac1-camps), thereby reducing FRET between the chromophores cyan and yellow fluorescent protein (CFP/YFP) at either end of the molecule. AC, adenylate cyclase; β_1-AR, β_1-adrenergic receptor; ATP, adenosine 5′-triphosphate; cAMP, cyclic adenosine monophosphate; Gs-Protein, stimulatory G-protein; Epac1, cytosolic cAMP-binding protein 1; FRET, fluorescence resonance energy transfer.

Table 3 Prevalence and cardiovascular event rates assumed for sample size and power analysis

Cohort	Antibody prevalence (%)	Life-threatening cardiovascular event rates in 24 months (%)
Prospective part of the ETiCS study		
170–200 patients with acute myocarditis (AMitis)	>33	30
190–200 patients with first acute myocardial infarction (FAMI)	>12	>40
Retrospective parts of the ETiCS study		
400 patients with dilated cardiomyopathy	33	>40
300 patients with ischaemic cardiomyopathy	12	>40
200 patients with hypertensive heart disease	<5	30
Control group serving as comparator for both parts		
300 healthy control subjects	<2	–

size calculations were derived from the available pilot studies[9,10] and are given in *Table 3*.

Levels of significance and power

The power for the most critical situations (smallest samples and effect sizes) is as follows ($\alpha = 5\%$).

For the prospective part of the ETiCS study, the power will be 0.96 (0.91) to detect a difference in β_1-aab prevalence of 12 vs. 2% in 150 (100) FAMI patients vs. 300 controls; the power will be 0.95 to detect a difference in β_1-aab prevalence of 33 vs. 12% in 100 AMitis vs.100 FAMI patients. In case that β_1-aabs disappear in the course of the study (assuming that up to 50% of initially β_1-aab-positive subjects may become negative at follow-up), the power to detect that the vanishing rate equals or exceeds 10% in 18 (12) of β_1-aab positive FAMI patients is 0.95 (0.80). The percentage of newly generated β_1-aabs in subjects who were negative at baseline is also unknown. The power to demonstrate that the incidence of new β_1-aab generation during follow-up is >5% in 67 AMitis patients is 0.97 (0.81), given that the true population incidence is 20% (15%). For the (potential) prognostic value of β_1-aabs, assuming a population of $n = 800$ patients (400 DCM, 100 AMitis, 200 ICM, and 100 FAMI), an overall β_1-aab prevalence of 20%, and an overall rate of life-threatening cardiovascular events of 30%, the power to detect a hazard ratio of 2 is 0.86–0.97 given that the variance explained by other covariables (e.g. type of disease, LVEF) is 20–50%. In all other relevant situations, the power is higher because of larger effect sizes and numbers of patients.

Drop-outs

Most patients included into the retrospective part of the ETiCS study are recruited into currently ongoing CNHF studies. Biomaterials are collected prospectively and stored in the central CNHF bank of biomaterials. Experience from completed CNHF studies shows that the loss to follow-up or incomplete biomaterial asservation is <2%. Previous recruitment experience at the participating centres indicates that the inclusions planned for the prospective part of ETiCS are likely to be achieved within the anticipated time frame. Based on a recent pilot study, drop-out rate for the prospective study will be <5%. Drop-outs will be replaced by newly recruited patients until the minimal total number of patients is attained in each prospective cohort (AMitis, $n = 170$ patients; FAMI, $n = 190$ patients).

Concomitant scientific projects

Because both cellular and humoral immunity are implicated in immune-mediated heart disease,[4,13] several European research groups will contribute to the prospective part of ETiCS with substudies addressing the immunobiology of β_1-aabs and other humoral effectors of heart-directed autoimmunity in all 400 prospectively included patients.

Immunobiology of cardiac autoreactivity

This substudy attempts to unravel the immunological step-by-step changes after inflammatory or ischaemic myocardial injury, and the time-course and cell types (T and B cell subpopulations) involved in the generation of functional β_1-aabs. The reactivity and prevalence of receptor (auto)antigen-specific T and B cell populations will be serially assessed by antigenic recall assays, and by FACS and Elispot analyses performed with lymphocytes isolated from whole blood (obtained at FUP 3 and 12 months). From all prospectively included patients, the activated immunologic pathways will be analysed by sequential determination of the respective Th1/Th2/Th17 (serum) cytokines.

Since cytotoxic T-lymphocyte antigen 4 (CTLA-4) is a potent (indirect) suppressor of the immune system, its mutation or hampered expression might favour hyperreactivity to autoantigens.[32] Thus, in all prospectively included patients, CTLA-4 expression and CTLA-4 alleles will be determined by FACS and by PCR, respectively. Finally, in all patients, a genomic screen for HLA-DR/DQ and MHC class II haplotypes[18,19] will be performed, and then compared with the occurrence and titres of distinct cardiac autoantibodies in order to uncover genetic constellations that increase the individual susceptibility for heart-directed autoreactivity. The primary endpoint of this substudy will be descriptive (hypothesis-generating), focusing on the characteristics of the individual immune system and its association with the development and titre-course of potentially cardio-noxious aabs.

Other cardiac autoantibodies

Patients with cardiac disorders often suffer from alterations in cellular and humoral immunity.[3,4] In this context, a substantial fraction of these patients has been found to develop cross-reacting antibodies and/or autoantibodies to a variety of cardiac antigens, including mitochondrial carrier proteins,[33] proteins of the contractile apparatus,[15,16] and/or membrane proteins.[10,14] However, only a few selected antibodies seem capable of inducing direct or indirect myocyte damage and subsequent heart failure. Embedded in the ETiCS study, several core centres (Universities of Heidelberg, Gothenburg, Berlin MDC, Greifswald, and Padua) will screen for (a) aabs directed against troponin T/I,[15] (b) aabs against the muscarinic acetylcholine receptor 2,[14] (c) aabs against the angiotensin-1 and the endothelin receptor,[17] (d) cardiodepressant abbs, and (e) organ-specific and skeletal muscle cross-reactive anti-heart-aabs (AHA), aabs against intercalated disks (AIDA), and against cardiac endothelial cells (AECA).[16] The endpoint of cross-sectional analyses is the association of the respective antibody status at diagnosis with the change in cardiac function as derived from sequential echocardiograms and cMRI (baseline vs. 12 months), and the severity and clinical course of the index disease. Longitudinal endpoints are titre-changes of the above cardio-noxious aabs over time, conversion rates (persistence/clearance), and the 'time to first life-threatening cardiovascular event' (see *Table 2* for definition of study endpoints).

Discussion

Rationale of the study design

In the last two decades, much knowledge has been accumulated regarding the potential pathophysiologic and clinical implications of cardiac aabs.[3–5,13] However, no prospective study has to date addressed the central question whether structural damage to the heart muscle is a mandatory pre-requisite for the formation of these aabs. Usually, the immune system does not attack cardiac self-proteins. On a susceptible genetic background, however, this self-inhibition of immune effector cells after cardiac injury may be hampered. It is presently unclear, whether this autoreactivity depends on the amount or 'dose' of self-antigens presented (derived from the extent and severity of myocyte damage as assessed by cMRI and/or EMB) or the kind and quality of subsequently activated immunologic pathways.

In AMitis, diffuse or focal inflammatory processes cause structural damage to the heart, and—if persistent—may cause DCM.[13] Acute ischaemia also causes structural damage to the heart, and ICM represents the most common aetiology of heart failure.[1] Thus, investigation of cellular and humoral immunity in patients with a first event of either disease appears most promising to address the above questions. The prospective part of ETiCS will analyse the time-course and sequentially engaged immunologic processes in patients with AMitis or FAMI, and will correlate these findings with the extent of myocardial damage and the evolution of cardiac morphology and function over a 12-month period. Patients will be followed (2–)3, 6, and 12 months after their index event, as development of auto-reactive IgG is expected to occur within the first 6–8 weeks after cardiac injury. The retrospective part of ETiCS will make use of serial clinical data and biomaterials from 900 selected patients and 300 healthy control subjects participating in the CNHF. This enables the most comprehensive longitudinal study so far on receptor-directed autoimmunity attempting to revisit prevalence, titre-course, and clinical impact of cardiac aabs in well-characterized patient cohorts.

Rationale for endpoint selection

The central hypothesis is that both inflammatory or ischaemic myocardial injury trigger a sequence of immunologic reactions resulting in the generation of functionally active β_1-aabs.[3,4,13] Thus, in the prospective part of ETiCS, the pre-specified primary endpoint is the titre of functional β_1-aabs at diagnosis and (2–)3, 6, and 12 months after the index event. Evidence indicates that a substantial number of DCM and ICM patients, but only very few healthy subjects exhibit elevated titres of activating β_1-aabs,[10,27] and that such aabs are associated with impaired cardiac function,[10] a higher incidence of life-threatening ventricular arrhythmias,[9] and an increased risk for (cardio-vascular) death.[11] Therefore, pre-specified secondary endpoints in the prospective part of ETiCS comprise changes in clinical status (NYHA class), changes in cardiac diameters and function, occurrence of ventricular arrhythmias, and time to all-cause death. Left heart catheterization and cMRI will permit to relate cardiac aab titres to the extent of myocardial infarction, and EMBs will allow for a correlation with the severity of myocardial inflammation.[23,24,29] The potential prognostic value of agonist-like β_1-aabs will be assessed by combining all patients included into the prospective and retrospective part of the ETiCS study. The hypothesis is that functionally activating β_1-aabs independently predict an adverse clinical outcome. Thus, the 'time to first life-threatening cardio-vascular event' has been chosen as the pre-defined primary endpoint of the prognostic analysis.

Perspectives

The results of ETiCS will contribute to a large range of diagnostic, pathophysiologic, and prognostic issues in autoantibody-mediated heart disease.[4] We expect insights on the influence of cardiac events in triggering autoimmune processes and on the contribution of autoimmune processes to the initiation and/or progression of heart failure.[3,13] In particular, we may better understand whether the specific target of an autoantibody, or its titre and biological activity, or its conversion rate (persistence/clearance) relates to the complex processes of cardiac wounding and healing. More precise prognostic markers for patients with an unfavourable course of autoimmune heart failure might be recognized from this study in order to optimize conventional treatment modalities earlier in these patients.

Despite recent progress, the pharmacotherapy of heart failure is often unsatisfactory. This has stimulated the search for causal treatment strategies aiming to block or neutralize factors thought to be involved in heart failure progression.[13,21] In many neurologic, rheumatologic, and endocrine disorders, autoimmunity has been recognized as the main pathogenic factor. Its relevance in human heart disease and failure, however, still needs to be substantiated.[3,4] Therapeutic strategies known from other autoimmune disorders (e.g. application of peptide-ligands[34] or immunoadsorption[35])

might also be helpful in certain human cardiac diseases. In animal models[36,37] and preliminary clinical studies with DCM patients,[13,22] such novel approaches provided encouraging results. Hence, the results from ETiCS may also furnish a basis for the development of novel therapeutic strategies targeting cardio-noxious aabs. Moreover, by venturing different groups in the field of cardiac autoimmunity, ETiCS could equally serve as a starting point for future European research activities on the interrelationship between the immune system and human heart disease.

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Conflict of interest: all authors had full access to the data and have read and approved submission of the final manuscript and have declared no conflicts of interest. The University of Würzburg has filed for patent protection of the diagnostic FRET-method described herein.

Appendix A: committees and study offices

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University of Rostock

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University of Tübingen

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University of Würzburg

Georg Ertl MD (Chair), Roland Jahns MD (local PI, Co-Chair), Christiane E. Angermann MD (Co-Chair), Stefan Störk MD PhD (Clinical Coordinator/Biometry), Stefan Frantz MD, Oliver Ritter MD, Dominik Berliner MD, Nikolas Deubner MD, Department of Cardiology; Christoph Wanner MD, Frank Breunig MD, Vera Krane MD, Department of Nephrology. Valérie Jahns PhD (Clinical Coordinator), Martin J. Lohse MD (Chair, FRET Core-Lab), Viacheslav O. Nikolaev PhD, Angela Schlipp MSc, Alexander Zürn PhD, Institute of Pharmacology and Toxicology; Thomas Hünig PhD (Chair), Thomas Kerkau PhD, Beyersdorf Niklas MD, Vladimir Kocoski DVM, Institute of Virology and Immunobiology.

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