

**Takotsubo cardiomyopathy—Differentiation from acute
coronary syndrome by electrocardiography and
biochemistry**

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ACADEMIC DISSERTATION

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CONTENTS

LIST OF ORIGINAL PUBLICATIONS	5
LIST OF ABBREVIATIONS	6
ABSTRACT	7
LYHENNELMÄ.....	9
1. INTRODUCTION.....	11
2. REVIEW OF THE LITERATURE	13
2.1. Acute coronary syndrome	13
2.1.1. Epidemiology and classification.....	13
2.1.2. Pathophysiology and risk factors	14
2.1.3. Symptoms, diagnosis and treatment	14
2.2. Takotsubo cardiomyopathy.....	15
2.2.1. Definition	15
2.2.2. Pathophysiology.....	15
2.2.2.1. Catecholamine theory	15
2.2.2.2. Aborted MI or microvascular thrombosis.....	17
2.2.2.3. Coronary spasm.....	18
2.2.3. Epidemiology.....	19
2.2.4. Triggers	19
2.2.5. Presentation	20
2.2.6. Variants	20
2.2.7. Diagnosis.....	20
2.2.7.1. Electrocardiography	20
2.2.8. Treatment, recurrence and prognosis	22
2.3. Cardiac catheterization	22
2.4. Biochemistry.....	23
2.5. Blood coagulation	24
2.5.1. Acute-phase reaction	27
2.5.2. Left ventricular thrombus in TTC	27
2.6. Matrix metalloproteinases	27
2.6.1. MMPs and TIMPs in ACS	28
2.6.2. MMPs and TIMPs in LV remodeling	28
3. AIMS OF THE STUDY	30
4. METHODS	31
4.1. Population	31
4.2. Left ventriculography.....	32
4.3. Electrocardiography	33

4.4.	Biochemistry.....	34
4.4.1.	Coagulation studies (II).....	34
4.4.2.	Matrix metalloproteinases (III)	35
4.5.	Statistical analyses	35
5.	RESULTS.....	36
5.1.	Clinical features.....	36
5.2.	Trigger factors	38
5.3.	Left ventriculography.....	40
5.4.	Electrocardiography	40
5.5.	Biochemistry.....	43
5.5.1.	Coagulation study (II).....	43
5.5.2.	Matrix metalloproteinases (III)	45
6.	DISCUSSION	49
6.1.	Electrocardiography (I).....	49
6.2.	Coagulation (II).....	51
6.3.	Matrix metalloproteinases (III)	53
6.4.	Limitations and future studies	54
7.	CONCLUSIONS.....	56
8.	ACKNOWLEDGEMENTS	57
9.	REFERENCES.....	59

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original articles, which are referred to in the text by their Roman numerals.

- I. Parkkonen O, Allonen J, Vaara S, Viitasalo M, Nieminen MS, Sinisalo J. Differences in ST-elevation and T-wave amplitudes do not reliably differentiate takotsubo cardiomyopathy from acute anterior myocardial infarction. *J Electrocardiol* 2014; 47:692-699.
- II. Parkkonen O, Mustonen P, Puurunen M, Valkonen K, Nieminen M, Sinisalo J. Coagulation changes in takotsubo cardiomyopathy support acute phase reaction and catecholamine excess, but not thrombus production. *Int J Cardiol.* 2014 Dec 20;177(3):1063-5.
- III. Parkkonen O, Nieminen MT, Vesterinen P, Tervahartiala T, Perola M, Salomaa V, Jousilahti P, Sorsa T, Pussinen PJ, Sinisalo J. Low MMP-8/TIMP-1 reflects left ventricle impairment in takotsubo cardiomyopathy and high TIMP-1 may help to differentiate it from acute coronary syndrome. *PLoS One.* 2017 Mar 9;12(3):e0173371.

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LIST OF ABBREVIATIONS

ACS	Acute coronary syndrome
BAR	Beta adrenergic receptor
CAG	Coronary angiogram
cAMP	Cyclic adenosine monophosphate
CK-MBm	Creatine-kinase MB-mass
CRP	C-reactive protein
ECG	Electrocardiography
ECM	Extracellular matrix
EF	Ejection fraction
HF	Heart failure
IVUS	Intravascular ultrasound
LAD	Left anterior descending coronary artery
LV	Left ventricle
LVG	Left ventriculogram
MI	Myocardial infarction
MMP-8	Matrix metalloproteinase-8
MRI	Magnetic resonance imaging
No-CAD	No coronary artery disease
OCT	Optical coherence tomography
SD	Standard deviation
sGPV	Soluble glycoprotein-V
STEMI	ST-elevation myocardial infarction
TAT	Thrombin-antithrombin-complex
TIMP-1	Tissue inhibitor of matrix metalloproteinase-1
TnI	Troponin I
TnT	Troponin T
TTC	Takotsubo cardiomyopathy
vWF	vonWillebrand factor

ABSTRACT

Takotsubo cardiomyopathy (TTC) is an acute cardiac condition resembling in symptoms acute coronary syndrome (ACS), but without obstructive coronary artery disease. TTC develops almost solely in post-menopausal women and usually after preceding stress. Of all patients with ACS symptoms, TTC incidence is 2%. Due to similar symptoms and findings, differential diagnosis requires coronary angiography (CAG).

The pathophysiology of TTC is unknown. Even though the accumulated evidence suggests a causative role for a catecholamine surge, other theories exist. Aborted myocardial infarction (MI) produces similar electrocardiography (ECG) and biochemical findings as in TTC. In such cases, because of non-stenotic coronary artery plaques, a dissolved coronary thrombus might show no any signs in the CAG, which could lead to an assumption of non-atherothrombotic etiology for the heart attack. In ACS, altered levels of proteolytic enzyme called matrix metalloproteinase 8 (MMP-8), and its inhibitor, the tissue inhibitor of matrix metalloproteinase 1 (TIMP-1), associate to plaque rupture. Their direct comparison between ACS and TTC remains unknown.

The purpose of this thesis is to test whether either of two non-invasive methods: the ECG (I) or MMP-8 and TIMP-1 (III), could differentiate TTC from ACS. We also set out to find whether, after all, what is responsible for the TTC is transient thrombosis in the coronary circulation (II).

Both our prospective and retrospective collection of patients resulted in 92 TTC cases. The demographics of our material were similar to those of other TTC reports worldwide. In the ECG study (I), a review and comparison of 57 TTC and ACS acute ECGs resulted in criteria differentiating the two with 63% sensitivity and 93% specificity. In cases of suspected ST-elevation myocardial infarction, such accuracy is insufficient for a decision against coronary intervention.

Blood samples from 45 TTC patients and matching numbers of ACS patients and controls were analyzed for coagulation markers. Despite the similar acute-phase reaction in TTC and ACS patients, the TTC d-dimer levels matched those of controls, and were lower and less frequently above reference level than with those in ACS. The blood samples were further analyzed for MMP-8 and TIMP-1 levels showing, on admission, better differentiation between TTC and ACS by TIMP-1 than by troponin T (TnT). In TTC, low ejection fraction (EF) correlated with low MMP-8/TIMP-1 ratio.

In conclusion, ECG lacked the ability to differentiate TTC from ACS that would have allowed avoidance of invasive diagnostics. Secondly, the coagulation results supported catecholamine and argued against the thrombosis theory. Finally, TIMP-1 emerged as a potential future biomarker in differentiation between ACS and TTC. Furthermore, in some TTC patients MMP-8 and TIMP-1 levels may explain more severe left-ventricle (LV) impairment.

LYHENNELMÄ

Takotsubo kardiomyopatia (TTK) on akuutti sydänkohtaus, joka muistuttaa oireiltaan sydäninfarktia. Myös monet sydänfilmiä löydökset, kuten ST-tason muutokset, ovat samankaltaisia. Toisin kuin tavallisessa sydäninfarktissa, TTK-potilailla ei todeta merkittävää sepelvaltimotautia. TTK-potilaiden tyyppilöydös on sydänlihaksen laaja-alainen lamautuminen, joka normaalistuu seurannassa.

TTK:n ilmaantuvuus on noin 2% kaikista sydäninfarktin oirein sairaalaan päätyvistä potilaista. Sydäninfarktin luotettava erottaminen TTK:sta vaatii samankaltaisten ensivaiheen oireiden ja löydösten vuoksi sepelvaltimoiden varjoainekuvauksen. Potilaiden jatkohoito eroaa merkittävästi, minkä vuoksi diagnoosin pääseminen varhain on tärkeää.

Oireyhtymän syntymekanismi on tuntematon. Sitä esiintyy lähes yksinomaan vaihdevuosi-ikäen ylittäneillä naisilla, ja usein kohtausta edeltää akuutti stressitilanne. Monista teorioista todennäköisimpänä pidetään suurien katekoliamiinipitoisuuksien aiheuttamaa sydänlihaksen lamautumista. Myös keskeytynyttä sydäninfarktia on pidetty mahdollisena selityksenä. Tällöin nopeasti liukeneva sepelvaltimotukos aiheuttaisi ohimenevän sydämen lamautumisen, muttei olisi enää nähtävissä sepelvaltimoiden varjoainekuvauksessa.

Väitöskirjatyön tavoitteena oli selvittää, voidaanko TTK erottaa sydäninfarktista kajoamattomilla tutkimusmenetelmillä. Pyrimme myös selvittämään veren hyytymisen ja mahdollisen keskeytyneen sydäninfarktin roolia TTK:n synnyssä.

Tutkimuksen aineisto sisälsi 92 TTK-potilasta. Ensimmäisessä osatyössä vertailimme TTK:ta ja sydäninfarktia sairastavien potilaiden akuuttivaiheen sydänfilmiä muutoksia. Muodostamamme kriteeristö pystyi erottelemaan TTK:n sydäninfarktista 63%:n herkkyydellä ja 93%:n tarkkuudella. Tämä tarkkuus ei kuitenkaan riitä siihen, että potilaan päivystyksellisestä sepelvaltimoiden varjoainekuvauksesta voitaisiin pidättäytyä ST-nousun yhteydessä.

Toisessa osatyössä analysoimme TTK- ja sydäninfarktipotilaiden sekä terveiden verrokkien veren hyytymistekijäpitoisuuksia. Akuutin vaiheen reaktion merkkiainetasot vastasivat potilasryhmillä toisiaan. Verisuonitukoksissa kohoava d-dimeeripitoisuus oli TTK-potilailla samaa tasoa kuin verrokeilla, mutta merkittävästi matalampi kuin infarktipotilailla. Tulokset eivät tue teoriaa keskeytyneestä sydäninfarktista TTK:n laukaisijana.

Viimeisessä osatyössä vertasimme sydänlihaksen ja sepelvaltimoiden rakenteeseen vaikuttavien entsyymien, matriksin metalloproteiinaasi 8:n (MMP-8) ja tämän estäjän (TIMP-1)

pitoisuuksia TTK- ja sydäninfarktipotilailla. TIMP-1 pitoisuus pystyi erottamaan TTK:n ja sydäninfarktin toisistaan kliinisessä käytössä olevaa Troponiini T:tä paremmin. Lisäksi havaitsimme, että TTK potilaiden vasemman kammion heikentynyt supistuvuus oli yhteydessä matalaan MMP-8/TIMP-1 suhteeseen.

Tulokset vahvistivat aiempia käsityksiä katekoliiniamiinien tärkeästä roolista TTK:n synnyssä. MMP-8 ja TIMP-1 pitoisuuksien käyttöä TTK:n erotusdiagnostiikassa ja vaikeusasteen arvioimisessa on syytä tutkia tarkemmin.

1. INTRODUCTION

History and folklore contain numerous stories of heart attack and even sudden death at moments of extreme emotion. The concept of dying from grief or horror is strongly embedded in our folk wisdom. Even the saying “scared to death” is common in our every-day lives. A weak heart presents a traditional explanation for such an event. In recent years, however, research has implicated a possible modern foe behind the old concept.

Takotsubo cardiomyopathy (TTC) was first described in Japan 25 years ago in female patients after emotionally stressful situations [1]. Throughout the 1990’s, the literature mainly consisted of small reports [2-4]. It was not until 2001 that Tsuchihashi et al released the first larger study of TTC and made the condition more widely known [5]. The name takotsubo was chosen to describe the typical motion abnormality in the left ventricle resembling a traditional Japanese octopus fishing pot with its narrow neck and round bottom. Other more descriptive names were stress cardiomyopathy, apical ballooning syndrome, and broken-heart syndrome were used, but over the years, takotsubo has become established as its more unique name [6]. The European Society of Cardiology classifies TTC under unclassified cardiomyopathies, and the American Heart Association under both primary and acquired cardiomyopathies [7,8].

Through the 2000s an increasing amount of knowledge about this peculiar syndrome emerged from all over the globe [9]. To date, TTC has been described in populations both in North America and Europe, in various Asian countries, in Australia, as well as in South America [5,9-14].

TTC incidence grew steadily from 2000 due to better recognition of the syndrome and increasing availability of coronary angiography (CAG). Because ACS is the major differential diagnosis for TTC, TTC diagnosis is almost solely based on the exclusion of occlusive coronary disease by CAG. TTC mimics ACS with similar symptoms and findings. Even with the current non-invasive methods, reliable distinction of TTC from ACS remains impossible. Such methods, however, could potentially spare patients unnecessary medications and examinations, as well as cut medical costs.

Despite vigorous efforts and increasing research data, the underlying mechanisms of TTC remain unknown [15-20]. The plot, however, thickens, and some pieces of the puzzle have started to fall in place. High levels of catecholamines, especially epinephrine, have been implicated in various study settings, but direct evidence of the mechanism in humans is missing. Furthermore,

what makes some patients susceptible to TTC under high catecholamine situation also remains unclear.

TTC was considered a benign disease despite its dramatic presentation, and the patients usually fully recover within weeks. Even though the majority of the TTC studies report infrequent complications, more recently, studies show a larger proportion of patients with severe complications as well as substantial in-hospital mortality [21]. The in-hospital mortality of 2 to 5% appears to be on par with that of ACS [21-26]. Such a finding, among others, emphasizes how much still needs to be learned from this intriguing syndrome.

2. REVIEW OF THE LITERATURE

2.1. Acute coronary syndrome

2.1.1. Epidemiology and classification

Coronary artery disease (CAD) remains the leading cause of mortality in developed countries. Despite improved treatment and better management of risk factors, CAD causes over 8 million deaths annually worldwide [27]. CAD mortality has decreased over recent decades; but variation between demographic groups exists, with slow progress occurring especially in younger women [28].

CAD causes reduced oxygen supply to the myocardium. ACS manifests when the supply suddenly decreases beyond the point when myocardial necrosis will begin. Such a lack of supply usually results from rapid narrowing or blockage in a coronary artery, leading to decreased or complete loss of blood flow to the myocardium [29]. ACS is divided into ST-elevation and non-ST-elevation types ACS depending on the ECG findings (Figure 3). Usually, STEMI results from complete blockage of a coronary artery and NSTEMI and UAP from reduced blood flow [30,31].

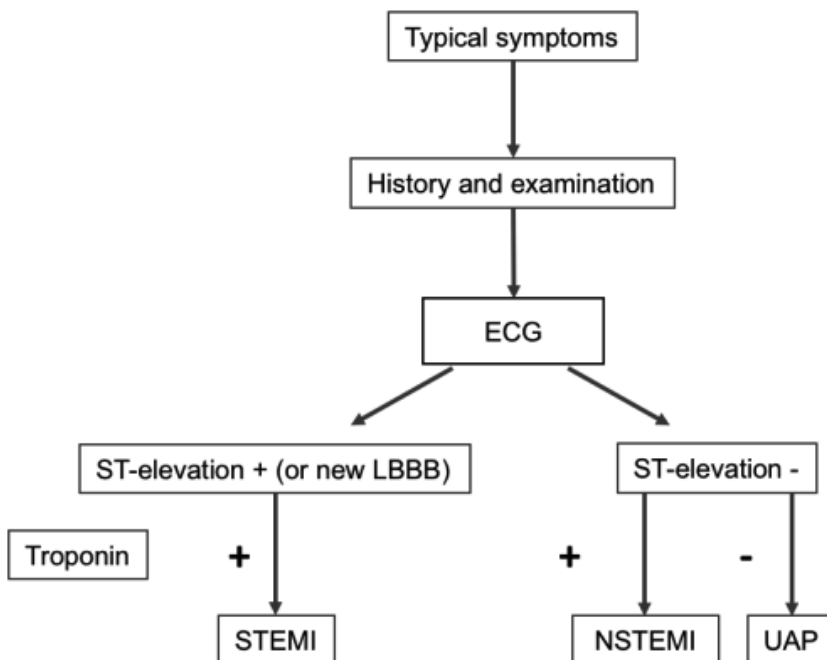


Figure 3 Classification of acute coronary syndrome based on ECG and troponin. ECG = electrocardiography, LBBB = left-bundle branch block, NSTEMI = non-ST-elevation infarction, STEMI = ST-elevation infarction, UAP = unstable angina pectoris.

2.1.2. Pathophysiology and risk factors

In atherosclerosis, which causes coronary artery disease, cholesterol, accompanied by inflammation, accumulates in the endothelium of the arteries. This accumulation leads to formation of atherosclerotic plaques, consisting of a lipid core and a fibrotic cap on the luminal side. This process leads to calcification of the vessels and disruption of their normal structure and function [32].

Coronary artery thrombosis is the most common ACS cause, and thrombosis results from disruption of atherosclerotic plaque within coronary artery wall. The atherosclerotic plaques may grow inwards, toward the coronary artery vessel lumen. Such concentric plaques narrow the lumen and tend to cause angina symptoms during stress. Concentric plaques tend to be more stable due to a thick fibrotic cap and a smaller lipid core. Fibrous cap erosion, however, may provoke thrombosis and lead to ACS--usually, to NSTEMI or UAP [33].

Atherosclerotic plaques, that grow outwards do not narrow the vessel lumen and thus do not cause stress-related symptoms. Such eccentric plaques tend to have thin, weak fibrous gaps and large lipid cores. Such plaques are more vulnerable to rupture. Rupture reveals a large amount of thrombotic material into the vessel lumen and provokes thrombosis. Usually such thrombosis causes complete blockage of the coronary and results in STEMI [32].

The risk for CAD and ACS consists of genetic and lifestyle-related factors [34]. High cholesterol, especially low-density lipoprotein, is the key risk factor for atherosclerosis development [35]. Other risk factors include high blood pressure, diabetes, and smoking, often accompanied by minimal physical activity and a vegetable-poor diet [36,37]. These risk factors both promote the development of atherosclerosis and increase the workload of and oxygen demand on the myocardium.

2.1.3. Symptoms, diagnosis and treatment

Symptoms include chest pain, often radiating to the left arm or neck, shortness of breath, and nausea. The presentation may also be vaguer with symptoms of discomfort, palpitations or gastric pain. Myocardial ischemia may also present with ventricular tachycardia and lead to sudden death [30,31].

The initial diagnosis is based on typical symptoms, ECG, and serum troponin levels. When ACS is suspected, antithrombotic-, nitro-glycerine-, beta-blocking- and pain medication are useful. CAG confirms the diagnosis. NSTEMI and UAP patients undergo CAG within a few days [30]. STEMI patients require immediate CAG and subsequent percutaneous coronary intervention to

open the clotted artery. If CAG is unavailable, thrombolytic medication can dissolve the thrombotic clot in the coronary artery [31].

Optimal secondary prevention of ACS patients reduces risk for further events and improves prognosis. Prevention requires management of risk factors as well as favorable lifestyle changes. Medication includes statins, angiotensin convertase enzyme inhibitors, beta-blockers, and antiplatelet therapy [38-40].

2.2. Takotsubo cardiomyopathy

2.2.1. Definition

Despite many proposed criteria for TTC, no international consensus exists [20]. We applied the following most commonly used modified Mayo Clinic criteria [41,42]. All four must be present for a TTC diagnosis:

1. New electrocardiographic (ECG) change (ST-elevation or T-wave inversion or both) or cardiac-enzyme release (troponin or creatine kinase) or all of these.
2. Transient hypo-, dys-, or akinesis of the left ventricular midsection with or without involvement of the apex extending over a single epicardial coronary vascular bed
3. Absence of obstructive coronary artery disease (coronary luminal narrowing less than 50%) or plaque rupture confirmed by coronary angiogram (CAG)
4. Absence of myocarditis or pheochromocytoma

The Taskforce on Takotsubo Syndrome of the Heart Failure Association of the European Society of Cardiology further redefined the criteria by adding elevated serum natriuretic peptide [43]. Recent suggestion is that the contraction abnormality in pheochromocytoma represents TTC, and thus can classify as a physical stressor [20].

2.2.2. Pathophysiology

2.2.2.1. Catecholamine theory

Since the findings of Wittstein et al in 2005, TTC has been associated with high levels of circulating catecholamines [44]. That small study presented 2 to 3 fold higher epinephrine and norepinephrine levels in TTC than in matched myocardial infarction (MI) patients. TTC patients' catecholamine

levels remained elevated for a follow-up period of 7 to 9 days. The metabolite levels of the catecholamines, metanephrine and normetanephrine, showed elevation, but did not differ from the MI group levels.

Madhavan et al. in 2009 reported opposite results. They found subacute circulating fractionated catecholamine levels in 19 TTC patients similar to 10 age- and sex-matched STEMI patients. For the TTC patients, all of their metanephrine and 74% of the normetanephrine levels were normal. In the same study, 24-hour urine samples of fractionated catecholamines and metanephrines were normal in all but the two TTC patients, who had mildly elevated metanephrine levels [45].

The most recent catecholamine study reported normal or only moderately elevated values in the majority of the 33 TTC patients; epinephrine was elevated in 3, norepinephrine in 13, metanephrine in 5, and normetanephrine in 9 patients. Sampling occurred within 1 to 7 days after admission. As the delay from admission to blood sampling affected the selection of blood work, not all samples were not available for all patients. The trigger factor or TTC localization did not influence the results, however; patients complicated by heart failure had higher normetanephrine levels [46].

The histology of TTC patients supports the catecholamine excess theory. Serial endomyocardial tissue samples of 8 TTC patients showed acute morphological alterations indicative of catecholamine excess-derived microcirculatory dysfunction and direct cardiotoxicity. Such transient changes disappeared by the time of the second sampling after functional recovery. No signs of necrosis were evident [47]. Whether the alterations affected the whole depth of the myocardium, and what was the severity of changes in different parts of the myocardium remains unknown.

A high level of circulating epinephrine is thought to activate a cardio-protective mechanism in the myocytes [48]. Under normal conditions, coupling of epinephrine to beta-adrenergic receptor (BAR) activates the stimulating $G_{\alpha S}$. $G_{\alpha S}$ promotes the action of adenylate cyclase, which produces cyclic AMP (cAMP). The elevated concentration of cAMP leads to activation of protein kinase A (PKA). The activation opens the L-type Ca^{2+} -channels leading to muscle contraction. G-protein receptor kinase 5 controls the activation of BAR by phosphorylation followed by coupling to arrestin. Arrestin inactivates the BAR and promotes its internalization from the cell membrane. Under high circulating epinephrine concentrations, however, the binding of epinephrine leads to activation of $G_{\alpha_{\text{beta1}}}$, which prevents the cAMP production and shuts down the signaling [49].

The possible mechanism behind TTC was described in a rat model, where a TTC-like contraction abnormality could be triggered with a high-dose intravenous epinephrine bolus, but not

with norepinephrine. The effect of such an epinephrine bolus could be inhibited by pre-treatment with pertussis toxin, an inactivator of $G_{\alpha\text{phat}}$ [50]. Whether such a finding applies to humans is unknown.

2.2.2.2. Aborted MI or microvascular thrombosis

Aborted MI results from the spontaneous dissolving of a coronary thrombosis. The lack of oxygen in the ischemic region may cause a temporary abnormality in myocardial contraction described as stunning. Some studies have suggested such a thrombotic mechanism to be responsible for TTC [51,52]. Hypothetically, aborted MI in a long wrapping left anterior descending coronary vessel could cause an apical stunning similar to that seen in the TTC. An acute fast-dissolving thrombus, especially from coronary artery wall erosion, could be missed by CAG. Such thrombi are the cause of occlusion in 30 to 35% of ACS patients [53].

Coronary artery wall structures can be visualized by two catheter-based imaging modalities. Intravascular ultrasound (IVUS) uses soundwaves to assess possible disruptions in artery walls. Due to insufficient resolution, however, the intravascular ultrasound may also miss a thrombus on top of an area of vessel erosion [54]. One IVUS study showed a ruptured atherosclerotic plaque in the left anterior descending coronary artery of 5 TTC patients [52], but another showed no signs of plaque rupture or thrombi in 10 TTC patients [54]. Optical coherence tomography (OCT) is an optical based method otherwise like intravascular ultrasound, but with up to 10-fold resolution. At the moment, it is the most reliable method for finding changes in the coronary artery walls such as coronary erosion [33]. Only one case report of TTC and OCT exists, and the results showed no signs of erosion, plaque rupture, positive remodeling, or thrombi in the LAD vessel [55].

Supportive evidence for the aborted MI theory behind TTC came from a study comparing ECGs of 20 TTC, 20 spontaneous LAD-reperfusion, and 20 mechanically reperfused LAD MI patients. ECGs recorded from hospital admission up to 6 days of hospitalization showed similar results between the TTC- and aborted MI groups. TTC patients differed from the mechanically reperfused group in T-wave voltage and QT-interval length [56].

Widespread microcirculatory thrombosis beyond single-vessel territory could in theory cause broad myocardial stunning, despite normal coronary angiography and absence of plaque rupture. The role of microvascular dysfunction in TTC pathogenesis has been debated and evaluated by Doppler guide wire, TIMI frame count and myocardial perfusion grade, nuclear imaging, and contrast echocardiography [41,57-60]. The results show impaired perfusion, which normalizes in

follow-up after 3 to 4 weeks [58,59]. In the affected myocardium, Yoshida et al. (2007) reported extensive metabolic defects exceeding the volume of the perfusion defect. This result favors a metabolic origin for the microvascular dysfunction as opposed to a thrombotic origin [60]. Whether microvascular dysfunction is the cause or is secondary to TTC remains unknown.

2.2.2.3. Coronary spasm

Early reports proposed the coronary spasm as the underlying mechanism for TTC since in the CAG no obstruction of the coronaries was apparent. The fact that the contraction abnormality involves an area larger than provided by a single coronary artery and that the unlikeliness of multivessel coronary spasm presented later questions as to this hypothesis.

Reports of coronary spasm provocation tests with ergovine and acetylcholine show an incidence of provoked spasms ranging from 0 to 71%. Altogether 7 studies comprised 112 patients. Coronary vessel spasm was provoked in 30% of the patients and multivessel spasm in 10% (Table 1) [15]. Only 3 patients exhibited spontaneous spasm during CAG. These researchers performed provocation tests in either the acute or chronic phase, which may have affected results.

Table 1 *Results of coronary-spasm provocation test reports [5,61-66]*

Reference	Provocative test	Method
Abe 2003	14% (1)	ach
Akashi 2005	0% (0)	ach
Athanasiadis 2006	59% (10)	ach
Kawai 2000	29% (2)	N/A
Kurisu 2002	71% (single 4 / multi 6)	ach 12 / erg 2
Sato 2006	0% (0)	erg
Tsuchihashi 2001	21% (single 5 / multi 5)	ach

ach = acetylcholine, erg = ergovine, multi = multivessel, single = single vessel
% (n)

A small endomyocardial biopsy series revealed endothelial cell apoptosis in conjunction with reduced perfusion [67]. Another small study showed increase in peripherally measured vasoconstriction and endothelial dysfunction during a mental stress test in follow-up TTC patients compared to controls

[68]. These data suggest that TTC may be accompanied by an impaired endothelial function related to endothelial cell damage causing microvascular spasm.

If a coronary or microvascular spasm plays a role in the pathogenesis of the TTC, it may require other co-existing factors to emerge in the acute phase. Alternatively, the spasm could be a contributing factor in some TTC patients.

2.2.3. Epidemiology

TTC comprises 2 to 2.5% of patients with symptoms and findings of ACS. Because 90% of TTC patients are women, most of the peri- or post-menopausal, the incidence in that particular population is up to 6% [69]. Even though initially reported in Japan, TTC cases occur worldwide with the highest number per capita in Europe [9].

2.2.4. Triggers

Acute emotional stress precedes TTC in roughly one third of all patients. According to same review, commonly reported stressors include news of death or serious illness of a family member or a friend or a frightful situation e.g. being a victim of a mugging. Third of the patients have a physical stressor, which can be either physically strenuous situation such as unusual exercise or non-cardiac comorbidity like subarachnoid hemorrhage or cancer. A distinct acute stress-factor is missing from the remaining third of the patients, and some patients report only prolonged mental stress or fatigue. Men have high rate of preceding physical stress or another medical condition, whereas women tend to have emotional stress or no stressor at all [70].

The incidence of stress factors varies among reports [5,11,12,20,71]. More appropriately, TTC can be divided into primary and secondary depending on whether the TTC is the primary reason for hospitalization [43]. TTC case reports feature various distinct stressors such as therapeutic and accidental intravenous epinephrine injections [72-74]. And several reports also contemplate the role of insect, spider, snake, and jellyfish bites or stings [75-82], but whether any venom or the stressful situation itself triggers TTC, remains unknown. Interestingly, TTC incidence rises after natural disasters such as earthquakes [83-85].

2.2.5. Presentation

TTC is an acute cardiac condition with symptoms resembling those of the ACS. Besides 90% of the patients' being women, [70] of an average age of 67 years, reported cases range from 16 months to 98 years [71,86,87]. Chest pain is the most common symptom in 75.9% followed by dyspnea in 46.9% and fainting in 7.7% [71]. Other symptoms have been reported in smaller numbers such as nausea or vomiting, and fatigue [11,12,71]. The presentation depends on the severity of the symptoms, which may range from mild discomfort to cardiogenic shock. TTC incidence is highest in the afternoon and during summertime [22,88].

2.2.6. Variants

The early reports defined TTC as an apical ballooning syndrome. The classical form comprised hyper-contractile basal part and a- or dyskinetic apical and mid-ventricular part of the left ventricle. After Hurst et. al. published the first case with mid-ventricular TTC in 2006, recognition of atypical forms increased [89]. Currently overall of 5 different types other than apical have been reported in 18-40% of the TTC patients [12,90-92]. The etiology of different TTC patterns remains unknown and recurring cases may have a different pattern than initial event [93,94]. Right-ventricle involvement appears in roughly one third of the patients and usually accompanies worse LV function and a higher complication rate [90,95].

2.2.7. Diagnosis

2.2.7.1. Electrocardiography

ECG findings in TTC and in ACS have a high resemblance. In the TTC patients, ST-elevation and T-wave inversion are the most common findings in approximately 70% of patients. Third of the patients exhibit abnormal Q-waves [96], and the evolution of the ECG changes are similar in TTC and ACS: the resolution of the ST-elevation followed by development or deepening of T-waves or both [51]. ECG in TTC normalizes during the follow-up. Electrophysiological changes on a cellular level in TTC are unknown.

ST-elevation myocardial infarction (STEMI) requires fast decision-making and immediate revascularization. By many ECG-based criteria, studies have tried to differentiate TTC from STEMI in order to help in the assessment of necessary procedures and care. The studies used

ST-segment and T-wave changes, Q-waves, QT-interval measurements, and QRS morphology [56,97-106]. The results were controversial and due to insufficient sensitivity and specificity, most researchers would not recommend to their clinical use.

Kosuge et al. suggested (2010), for the differentiation of TTC from STEMI, the lack of ST-elevation in V₁ and ST-depression in aVR. This criterion has the highest specificity and sensitivity to date (91% and 96%, respectively) [98]. Another study used ST-elevation amplitude <1.75mm in V₂ and < 2.5mm in V₃ for the differentiation, with sensitivity of 67% and specificity of 94% [107]. This procedure was replicated in another study providing a sensitivity of 79% and specificity of 73%, while other criteria offered less impressive results than in the many original publications [108]. The studies are difficult to compare, because some studies use measurement criteria differing from the guidelines [31], like J-point + 0.08s for the ST-elevation [56,98,99,104]. The differing measurement method may result higher amplitudes and differing sensitivities and specificities.

Negative T-wave results show higher variation than does the ST-segment; the incidence of negative T-waves ranges on admission from 17% to 71% [99,103,109]. Such controversy suggests the current ECG studies may offer an unreliable picture of the T-wave changes. Moreover, one of the studies did not even report negative T-waves [98].

The time from symptom onset to ECG ranges between the studies from 3.4 ± 2.0 to 11.0 ± 8.6 hours [97,98,100,110], while some studies report only the usage of on-admission ECGs without any specific time-interval [56,99,101,103,105]. Serial ECGs from TTC patients revealed an evolution from acute phase ST-elevation to T-wave inversion in the subacute phase; therefore, the variability of the ECG changes on admission may have resulted from different time-intervals from symptom onset to ECG [56]. Dib et. al. (2009) compared the time-interval from symptom onset to ECG based on the primary ECG finding: ST-elevation, T-wave inversion or non-specific ST-T-changes [104]). The groups had a similar time delay from the beginning of the symptoms to ECG, which suggests the primary ECG finding is not solely time-dependent [104].

Despite the variable amount of impaired left ventricle (LV) in the mid-ventricular TTC, the ECG changes remain few. Even the more severe cases show ST-elevation in leads I, aVL and V₂₋₄ only [105]. ECG, however, records the mid-ventricular area of the myocardium inaccurately. The area is only recorded horizontally by lead V₂ and in frontal plane by leads I and aVL while the apical part is much better represented by the chest leads.

The absence of abnormal Q-waves has been proposed to favor a TTC diagnosis over ACS due to the lack of necrosis in TTC [99]. A later study, however, reported one third of TTC

patients to have Q-waves despite no signs of necrosis by magnetic resonance imaging (MRI). Most commonly, Q-waves were in leads V2-3, but no specific information existed as to morphology or of the leads affected. [101].

A common finding in TTC is QT-interval prolongation. Its incidence ranges from 50 to 100%, but in TTC torsades de pointes is rare[111]. Arrhythmias, in general, are not uncommon: atrial fibrillation appears in 5 to 15%, ventricular arrhythmias in 4 to 9% and cardiac arrest in 4 to 6% of the patients [112-114]. Risk for life-threatening arrhythmias associates with male sex [113].

2.2.8. Treatment, recurrence and prognosis

TTC has no specific treatment, but standard heart-failure treatment applies [115]. No medications have proven universally useful in prevention of recurrence [116], though ACE-inhibitors/angiotensin-receptor blockers may reduce recurrence [117]. The annual recurrence rate is 1 to 2 % [117].

Previously, TTC was considered as a benign disease. Recently Singh et. al. (2014), however, reported initial hospitalization mortality as high as 4.5% similar to that of a STEMI. Factors associated with mortality included secondary TTC and male sex [21]. Sharkey et. al. (2015) discovered that 20% of TTC patients, who were hemodynamically unstable on admission, had a higher risk for complications. Out of these patients 20% died during the initial hospital stay [118].

2.3. Cardiac catheterization

First coronary angiography was performed in the late 1950s [119]. Nowadays, CAG is routine in the diagnosis of acute coronary syndrome, and coronary intervention via angioplasty is the primary treatment when applicable. The function of the LV can be assessed in the same session as left ventriculography (LVG).

The introduction of emergency CAG in clinical practice improved the diagnostics of ACS and led to discovery of new entities like TTC. Because TTC patients present symptoms and non-invasive findings similar to ACS patients, the differential diagnosis is based on the CAG. The CAG of TTC reveals no obstructive coronary artery disease, but the LVG shows a wide contraction abnormality composed of the mid-ventricular part with or without the involvement of the apical part. A hypercontractile basal part usually accompanies the contraction abnormality [20]. Patients with >50% stenosis in their coronary arteries, but with their contraction abnormality out of proportion to

the coronary status, may be considered having TTC especially if cardiac MRI shows no ischemic damage [120].

Unless CAG is performed in the acute phase, a TTC patient with ST-elevation, would be treated within the guidelines for ST-elevation myocardial infarction (STEMI). If a patient receives thrombolytic therapy, no TTC diagnosis can occur afterwards due to possibility of a dissolved atherothrombotic infarction (aborted MI). Therefore, in the diagnosis of TTC, the role of emergency CAG is pivotal.

2.4. Biochemistry

ACS diagnosis relies on history, ECG, and cardiac enzymes. For many decades, the improvement in accuracy of MI diagnostics is attributed to the development of myocardium-specific enzyme assays. First the creatine kinase MB-isoform, and then the troponins have allowed swift and reliable recognition of myocardial damage [121,122]. Elevated cardiac enzyme levels are apparent in various cardiac situations such as myocarditis and myocardial infarction. On admission cardiac enzyme levels in TTC are similar to ACS levels, and like the ECG changes, are unable to distinguish between acute TTC and ACS [45].

During congestive heart failure, cardiac myocytes produce brain natriuretic peptide (BNP) and its precursor pro-peptide (pro-BNP). In TTC, their levels are also elevated and correlate with severity of the LV dysfunction and are higher than in ACS patients. The ratio of BNP to either troponin or creatine kinase showed the potential to distinguish between TTC and ACS [45,123].

Many acute conditions exhibit elevated C-reactive protein (CRP) levels, and TTC is no exception. CRP is an acute-phase protein synthesized by hepatocytes during inflammation and infection. On admission, TTC patients' CRP levels are elevated and correlate with BNP and normetanephrine levels. Such findings suggest that inflammation plays a role in the pathophysiology of TTC [124,125].

2.5. Blood coagulation

The circulation is a closed system providing oxygen and nutrients to tissues. Damage to this system leads to disruption of the function and may have catastrophic consequences. An elaborate coagulation system reacts swiftly by forming a blood clot at the site of damage, a process called thrombosis. The resultant hemostasis allows repair of the underlying vascular damage and return of normal function.

Dysfunction in the coagulation system may lead to bleeding disorders or excessive thrombosis. Thrombosis is an intricate interplay between circulating coagulation factors, thrombocytes, and the structure of the blood vessel. Damage to the vessel wall releases tissue factor and reveals thrombogenic material such as collagen. Endothelial cells at the site release von Willebrand factor (vWF), which attracts and activates circulating thrombocytes (Figure 1 and 2). Release of tissue factor from the endothelium activates the coagulation cascade, leading to formation of crosslinked fibrin. Activated thrombocytes aggregate to the site of injury and the fibrin formation stabilizes the plaque [126].

Many pathologies such as cancers and inflammatory diseases predispose to thrombosis. Atherosclerosis is characterized by inflamed calcified lipid plaques in artery walls and accompanies a thrombogenic state. Coronary artery disease is marked by atherosclerotic plaques in the coronary circulation. Rupture of such a plaque leads to revelation of thrombogenic material and subsequent formation of an intracoronary thrombus. This thrombus disrupts the coronary blood flow and results in ACS.

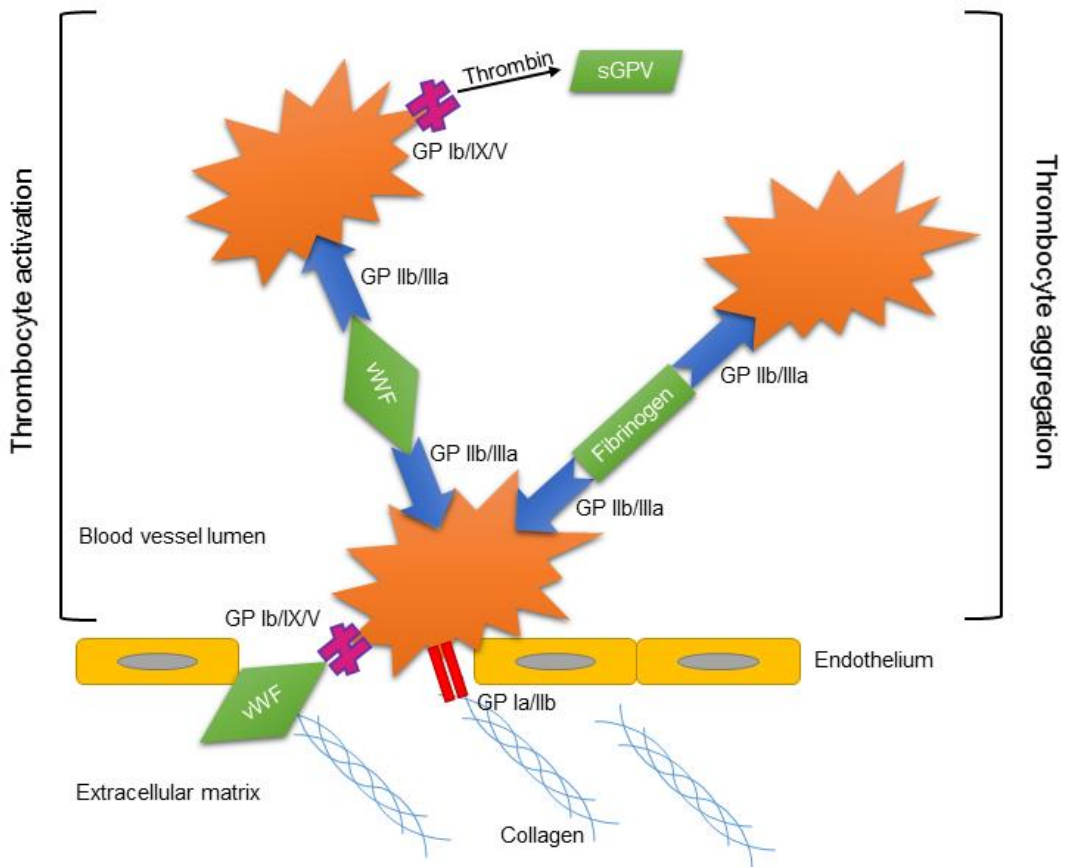


Figure 1. *Thrombocyte activation and aggregation in the arterial damage site. The green boxes indicate the relevant molecules. GP = glycoprotein, sGPV = soluble glycoprotein V, vWF = von Willebrand factor. (Illustration by the author)*

Fibrinolysis is a reaction that is reverse to thrombosis. A specific degradation product of cross-linked fibrin is d-dimer. Elevated level of d-dimer indicates a hypercoagulable state and thrombus formation and degradation. D-dimer values are elevated in ACS patients [127,128]. In a small series of 15 TTC patients, D-dimer was elevated in 60% [60].

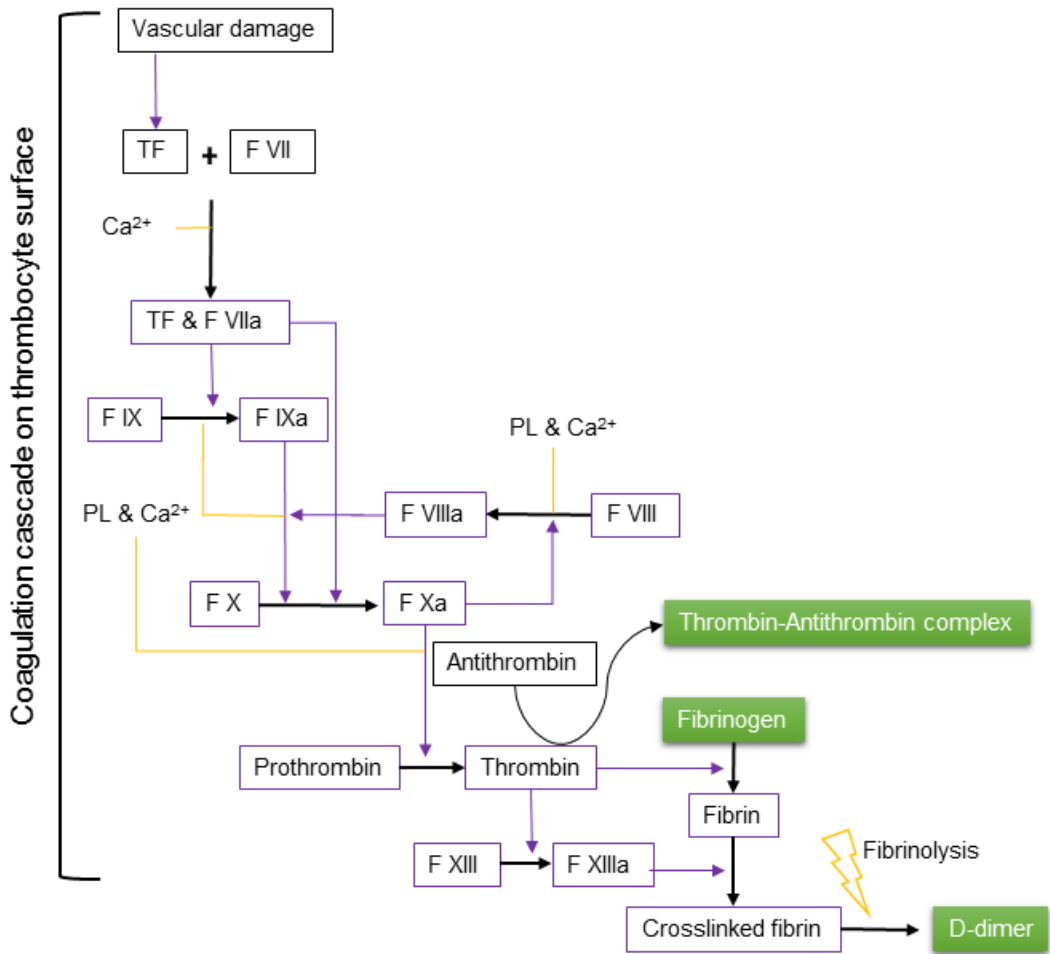


Figure 2. Coagulation cascade on a thrombocyte surface. The green boxes indicate the relevant molecules. F = coagulation factor, PL = phospholipide, TF = tissue factor. (Illustration by the author)

Activated platelets form the main component of an arterial thrombus. Elevated levels of soluble glycoprotein V (sGPV), a plasma marker of platelet activation, associate with MI [129,130]. The platelet surface glycoprotein (GP) IbIXV complex mediates initial platelet adhesion to subendothelial von Willebrand factor. Upon platelet activation, sGPV is proteolysed from the GP IbIXV complex and accumulates in plasma [131].

2.5.1. Acute-phase reaction

Acute-phase reaction, a defense reaction against toxic or infectious agents, is characterized by a cytokine-induced increase of acute phase proteins, such as C-reactive protein and coagulation factors like F VIII, von Willebrand factor, prothrombin, and fibrinogen. An acute-phase reaction is also a natural consequence of stress-induced myocardial damage.

Evidence exist as to the stress hormones, epinephrine and norepinephrine, directly influencing the coagulation system and platelet function [132-136]. Catecholamines are especially known to release F VIII and von Willebrand factor from endothelial cells [137].

2.5.2. Left ventricular thrombus in TTC

The reduced blood flow in the LV apex that is due to contraction abnormality may cause thrombus formation. Such thrombi are reported in 2-12% of cases and may cause stroke or other systemic embolism [24,138-141]. Thrombi usually develop during the subacute phase, but also report of a new thrombus after 14 days following nearly recovered LV function exists [142]. Risk for thromboembolic complications associates in women with a physical trigger, and in both sexes with ST-elevation and elevated C-reactive protein levels [140]. Therefore, anticoagulation should be considered during hospitalization and after discharge until a follow-up visit [115].

2.6. Matrix metalloproteinases

Matrix metalloproteinases (MMP) are a family of zinc-dependent endopeptidases with the ability to cleave all kinds of extracellular matrix proteins and bioactive molecules. MMPs are responsible for extracellular matrix (ECM) turnover by degradation of structural proteins such as collagens. In addition to their role in degrading and repairing of the ECM, they take part in pathological processes. Inflammatory cells are the main source of MMPs, but smooth muscle and endothelial cells also secrete them [143].

MMPs are powerful enzymes with potent a destructive capability. Due to their power, their activity is tightly controlled at the transcription level and by endogenous inhibitors. Tissue inhibitors of MMPs (TIMP) perform a vital part in their regulation. Any imbalance between MMPs and TIMPs may lead to an alteration in the net proteolytic effect and disrupt normal ECM properties. In the myocardium, the ECM provides the scaffolding for myocyte attachment and proper alignment.

Furthermore, the ECM participates in signaling and contains many bioactive molecules [143]. The ECM affects both the form and function of the heart, which in conjunction with improper MMP and TIMP levels, are altered in many cardiovascular diseases (CVD) [144-146].

2.6.1. MMPs and TIMPs in ACS

Atherosclerosis, unless interrupted, is a progressive disease marked by inflammation and dynamic changes in artery walls [147]. Rupture of an atherosclerotic plaque reveals thrombotic material from the plaque and vessel wall, which leads to thrombotic occlusion in the vessel and MI. The fibrous cap of the plaque separates the lipid core from the vessel lumen and contains mostly type-I collagen, which is mainly degraded by MMP-8, also known as neutrophil collagenase [148]. MMP-8 was implicated in the shoulder region of advanced inflamed atherosclerotic plaques, the areas most prone to rupture [149-151]. MMP-8 is specifically controlled by TIMP-1, which also has growth-factor-like- and pro-inflammatory effects [152,153]. ACS patients show elevated serum MMP-8 and TIMP-1 levels [144,154]. These pieces of evidence suggest that MMP-8 and TIMP-1 play roles in the weakening of an atherosclerotic plaque and development of ACS.

2.6.2. MMPs and TIMPs in LV remodeling

Changes in the composition, form, and function of the myocardium in a variety of heart diseases are collectively termed left ventricle (LV) remodeling. Changes in the ECM play a pivotal role in their process. Instead of a passive structure, the ECM forms an active scaffolding for myocardial cells that directly affects myocardial contraction. Furthermore, the ECM takes part in cellular communication and contains various active biomolecules [143].

Different etiologies like arterial hypertension and coronary artery disease cause LV remodeling, that causes impaired myocardial function and may ultimately lead to heart failure. Such changes in ECM structure in conjunction with altered MMP and TIMP levels, suggests their involvement in the pathogenesis [145,146]. An imbalance between MMPs and TIMPs leads to an increase or decrease in fibrotic ECM content depending on etiology.

Serial endomyocardial biopsies of TTC patients show, during the acute phase, inflammatory cells and transient interstitial fibrosis. Biopsies after functional recovery show normalization of ECM fibrotic content [47,155]. Another study also measured MMP-2, MMP-9, and TIMP-3 levels from tissue samples. In the acute phase, MMP-9 levels were high, while others remained unchanged [155].

Earlier serum MMP and TIMP profiles in 10 TTC patients showed patterns similar to those in hypertension and diastolic HF patients. TIMP-4 was elevated and MMP-1 and MMP-8 are low. TIMP-1 levels remained unchanged. That study included no direct comparison with HF or ACS patients [156]. The associations of the transient fibrosis, LV function, and MMP and TIMP levels remains unknown.

3. AIMS OF THE STUDY

The studies were performed to elucidate the pathogenesis behind TTC and improve the selection of differential diagnostic tools between TTC and ACS. The main goals of the conducted four studies were to discover

- 1) whether if TTC can be differentiated from ACS by ECG (**I**)
- 2) the role of thrombosis in TTC pathogenesis (**II**)
- 3) whether TTC can be differentiated from ACS by MMP-8 and TIMP-1, what is their role in TTC severity (**III**)

4. METHODS

4.1. Population

The complete TTC population comprise 92 patients. The studies included a varying number of study subjects depending on available data. Study sample came from from two populations.

Population I comprised patients from the prospective Corogene study [157]. A total of 5276 consecutive patients assigned for CAG were included in this study in Helsinki University Central Hospital between June 2006 and March 2008. The patients comprised 3000 elective patients (no coronary artery disease 1201 and stable coronary artery disease 1799) and 2276 acute patients (acute coronary syndrome 2090 and ACS-like presentation without coronary artery disease 204). The CAGs of the ACS-like patients were carefully reviewed, and 47 patients met the TTC criteria (see 2.3). Of the ACS- and ACS-like patients, TTC incidence in the Corogene population was 2.0%. Patients filled in family history- and risk-factor questionnaire. Examinations were performed during their hospitalization.

For Population II, a retrospective search for TTC patients was performed in the Helsinki University Central Hospital cardiology registry between May 2001 and June 2006, and April 2008 and December 2009. A total of 196 possible candidates appeared in this registry, which was in line with the incidence from the Corogene study. After the review of the CAG and LVG, however, only 96 patients met TTC criteria, of whom 7 had died. The remaining patients were reached by phone, and 41 were willing to participate in the study. Reasons for refusal were: 22 unreachable, 14 deciding not to attend, 12 in too frail condition for a clinic visit. Patients attended a clinic visit during which they were interviewed and examined. An additional 4 prospective TTC patients were enrolled between January and June 2010.

Co-morbidities, coronary risk factors, clinical characteristics, medications, trigger factors, and echocardiography information for left ventricle recovery assessment were acquired from patient records and by interview.

All patients signed an informed consent. Study protocols were approved by the ethics committee of Helsinki University Central Hospital.

4.2. Left ventriculography

CAG and LVG were performed in the acute or sub-acute phase for all 92 TTC patients. In TTC, the delay from symptom onset was 55.9 ± 49.4 h. Information on delay in ACS patients was unavailable. For the STEMI patients in our hospital, the average time from first medical contact to reperfusion was 119 minutes.

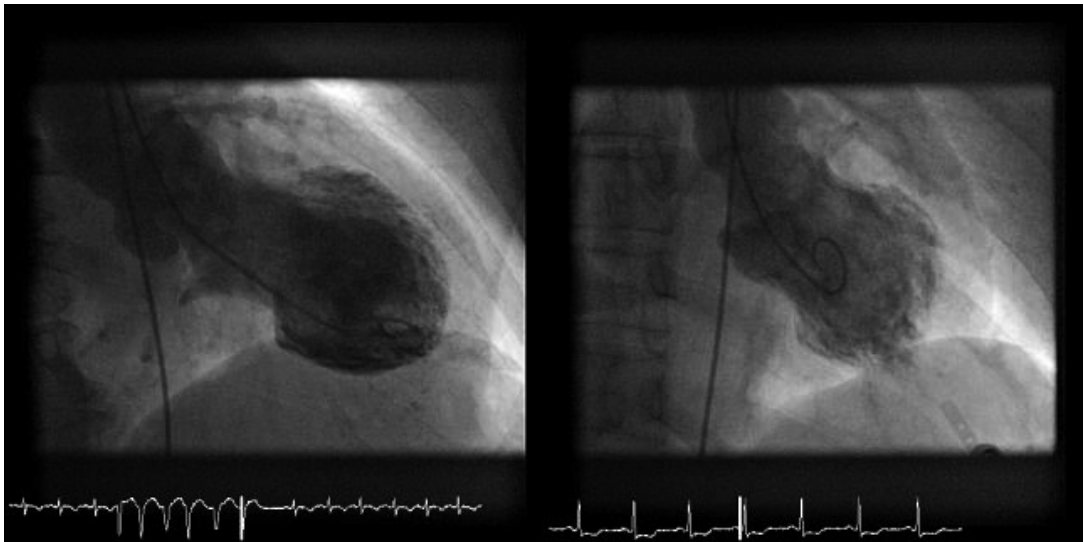


Figure 4 *On the left apical and on the right a mid-ventricular TTC during systole.*

The type and extent of the contraction abnormality was assessed from LVG by adapting a method previously described for tomographic heart imaging. The left ventricle was divided into three parts: the apical 30%, mid 35%, and basal 35% [158]. Four differing TTC patterns were found: 1) broad apical (i.e. mid and apical akinesia/dyskinesia), 2) small apical, 3) symmetric mid-ventricular, and 4) asymmetric mid-ventricular. Due to the small number of mid-ventricular TTC patients, apical (1,2) and mid-ventricular (3,4) types were combined before the statistical analysis (Figure 4). LVG was performed with the Siemens AXIOM Artis dFC system. Ejection fraction (EF), end diastolic volume, end systolic volume, and stroke volume were measured with Siemens AXIOM Sensus software (Siemens AG, Forchheim, Germany). The software was unable to perform the measurements for 10 patients out of the 92.

4.3. Electrocardiography

For Study I, TTC and anterior-STEMI patients' acute-phase ECG recordings (<24h from symptom onset) were reviewed. Such an ECG was available for 57 of the 92 TTC patients—29 from population I and 28 from population II.

A random selection was performed of 96 anterior-STEMI patients with available acute ECG recording from the Corogene study. STEMI was defined by European Society of Cardiology guidelines: typical symptoms and ST-elevation ≥ 2 mm in men, or ≥ 1.5 mm in women in leads V2–V3 or ≥ 1 mm in other leads or both [31]. Patients underwent acute coronary angiography with the following findings: a left anterior descending coronary artery occlusion (LADa, $n = 43$ or LADb, $n = 53$); (TIMI 0 flow, $n = 47$) or stenosis (TIMI 1–3 flow: $n = 49$). All participants received percutaneous coronary intervention.

Pain-onset to ECG interval was longer in apical-TTC, 7.6 ± 6.7 h, than in anterior-STEMI patients, 4.7 ± 5.5 h. In this regard, the mid-ventricle-TTC and anterior-STEMI 10.3 ± 8.6 h vs 4.7 ± 5.5 h patients did not differ. The two TTC groups also had a similar delay.

ST-segment measurements were made at the J-point, following the European Society of Cardiology guidelines [31]. The minimum requirements for each measured parameter were: ST-segment elevation ≥ 1.0 mm, negative T-wave ≥ 0.5 mm, Q-wave amplitude ≥ 2 mm, and duration ≥ 0.02 s (≥ 0.04 s for III-lead).

We sought answers to the following questions concerning ST-elevation and negative T-waves:

- i. How many patients had at least one lead affected by a specific ECG change?
- ii. How frequently was each lead affected?
- iii. What was the amplitude of one specific ECG change?

Abnormal Q-waves were defined as length ≥ 0.02 s (≥ 0.04 s for III-lead) and amplitude > 2 mm. Both QT-interval length as well as incidence of prolongation were compared. A linear regression model described by Sagie et al. serve to adjust the QT-interval to a heart rate of 60 beats per minute [159]. QT-interval length of over 440 ms was defined as prolonged. QT-interval was measured from lead V4.

One physician (O.P.) analyzed all the ECGs in random order. For the most important ECG variables, the ST-elevation and negative T-wave amplitude, 20 randomly selected ECG's were re-analyzed, which yielded an intra-observer variability of 1.9%.

4.4. Biochemistry

Cardiac enzyme levels on admission and the highest values during hospitalization were collected: Troponin T (TnT) from 86, Troponin I (TnI) from 5, and CK-MBm from 7 TTC patients. The available N-terminal prohormone of brain natriuretic peptide level was collected. Blood sampling was performed following the laboratory standards of the Helsinki University Central Hospital.

Population I patients were enrolled in Studies **II** and **III**. The blood samples were drawn from the arterial cannula in the beginning of the CAG in the catheterization laboratory. Before analysis, the citrated plasma was stored at -80°C .

Both studies included ACS patients from the same Corogene study. The ACS definition was: 1) episode of chest pain typical for ischemia, and $> 50\%$ stenosis in at least one coronary artery, 2) typical ischemic ECG changes for unstable angina (UAP), non-ST-elevation myocardial infarction (NSTEMI), or ST-elevation myocardial infarction (STEMI), and 3) elevated level of cardiac biomarkers in NSTEMI or STEMI.

4.4.1. Coagulation studies (II)

Equal numbers of age- and sex matched ACS patients, and patients without coronary artery disease (No-CAD) were selected from the same Corogene study. Overall this was 6 UAP, 29 NSTEMI, and 12 STEMI patients. Blood sampling was similar to that of TTC patients.

All ACS and TTC patients were on standard antiplatelet and anticoagulation medication. CAG was conducted in all TTC and ACS patients during the acute phase (median 48 hours, (IQR 24 – 96) vs. median 48 hours, (IQR 24 – 90); $p = 0.94$), respectively. Coronary intervention was performed when applicable [30,31]. The no-CAD patients were scheduled for elective CAG due to chest pain, valve problems, or cardiomyopathy

We measured fibrinogen (Fg) (STA®-Fibrinogen 5, Diagnostica Stago, Asnieres sur Seine France), Thrombin-antithrombin complex (TAT) (Enzygnost® TAT micro, Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany), D-dimer (Asserachrom® D-Di, Diagnostica Stago), soluble glycoprotein V (sGPV) (Asserachrom® Soluble GPV, Diagnostica Stago), and vonWillebrand factor –antigen (vWF:ag) (STA-Liatest® vWF:Ag –reagent, Diagnostica Stago), high-sensitivity C-reactive protein (hsCRP) (Orion Diagnostica, Espoo, Finland) from the same arterial sample.

4.4.2. Matrix metalloproteinases (III)

In addition to TTC population I, 2072 ACS patients were enrolled from the Corogene study: 722 STEMI, 1123 NSTEMI, and 226 UAP. The same blood samples were used as in Study II.

Controls were, 1000 subjects from the 1997 population-based health-survey FINRISK [160]. These subjects were free of cardiovascular diseases during their 13-year follow-up. Blood samples of the controls were drawn during a clinic visit at the Disease Risk Unit in the National Institute for Health and Welfare, Helsinki [161]. The FINRISK study was approved by the ethics committee of the National Public Health Institute. All patients gave their written informed consent.

Time-resolved immunofluorometric assay (IFMA) and enzyme-linked immunosorbent assay (ELISA; R&D Systems, Minneapolis MN, USA) served to determine the serum MMP-8 and TIMP-1 concentrations from all study participants [162].

4.5. Statistical analyses

Statistical analyses were performed with SPSS 16.0, 21.0 and 22.0 (SPSS Inc., Chicago IL, USA). Significance of the differences in the quantitative variables between cases and controls, and within TTC patients were analyzed depending on the normality and size of the study groups by Student's t-test or the Mann-Whitney U-test. When applicable, non-parametric variables were log-transformed before analysis. Categorical variables were analyzed either with the chi-square or Fisher exact test. Relations between variables were separately tested with Pearson correlation or Spearman's rho for cases and controls. A p-value less than 0.05 was considered statistically significant.

Receiver-operating characteristics (ROC) served in assessment of the diagnostic ability of the MMP-8, TIMP-1 and MMP-8/TIMP-1 ratio to distinguish cases from controls. C-statistics assessed whether such determinations had any value over the traditional risk factors. Associations between the tertiles of the determined concentrations and diagnoses were analyzed with an adjusted multivariate logistic model. All parametric values are given as mean \pm standard deviation (SD) and non-parametric as median (interquartile range (IQR)). A p-value < 0.05 was considered statistically significant.

5. RESULTS

5.1. Clinical features

A female dominance of 91.3% was evident, as expected, and patients were mostly post-menopausal (66 years \pm 10). Chest pain (81.5%) was the main symptom on admission, whereas 19.6% reported dyspnea and 2.2% nausea. Thorax X-rays of 34 patients of the available 78 showed excessive plethoricity (15.4%), congestion (26.9%), or pleural effusion (5.1%). Eight patients received non-invasive ventilation (continuous positive airway pressure or bi-level positive airway pressure ventilation) and 7 inotropic medications. Treatment of 2 patients required intubation and mechanical ventilation. No intra-aortic balloon pumps were necessary (Table 2).

Classic cardiovascular risk factors were as follows: hypercholesterolemia (total cholesterol >5 or low-density lipoprotein >3) (45; 48.9%), diabetes (14; 15.2%), hypertension (47; 51.1%), and smoking: current (18; 19.6%), ex-smoker (25; 27.2%). History of coronary disease in the family was present in 39.1% of the patients, but none reported confirmed cases of TTC within the family. All patients survived their initial hospitalization (Table 2).

Of the 21 patients who used beta-blocking drugs daily, 12 took beta-1-selective and 9 non-selective beta-blockers. In addition, 4 had a prescription for propranolol occasional use due to rhythm disturbances or anxieties. A total of 22.8% of patients were taking beta-blockers at the time of TTC onset, which suggested the limited power of beta-blockers in TTC prevention. Other medications are described in Table 1. Statin use was higher in population I than II probably due to increasing overall usage during the 2000s (Table 2).

Table 2. Demographics of both TTC populations, adapted from Parkkonen et. al. 2014 with publishers permission (I).

	Valid cases	All TTC (n = 92)	Population I (Corogene) (n = 47)	Population II (n = 45)	p-value
Age, years, mean (\pm SD)	92	65 (\pm 10)	66 (\pm 9)	64 (\pm 11)	0.237
Female	92	84 (91.3)	42 (89.4)	42 (93.3)	0.499
Family history (MCC)	92	36 (39.1)	19 (40.4)	17 (37.8)	0.795
Prior medications					
Regular β -blocker	92	22 (23.9)	11 (23.4)	11 (24.4)	0.134
Propranolol when necessary	92	4 (4.3)	4 (8.5)	0 (0.0)	0.134
β -sympathomimetic (long-term)	92	7 (7.6)	1 (2.1)	6 (13.3)	0.043
β -sympathomimetic (short-term)	92	6 (6.5)	3 (6.4)	3 (6.7)	0.956
Atroidual near TTC	92	6 (6.5)	5 (10.6)	1 (2.2)	0.102
ACE-inhibitor	92	22 (23.9)	14 (29.8)	8 (17.8)	0.177
AT-blocker	92	12 (13.0)	7 (14.9)	5 (11.1)	0.590
Calcium channel blocker	92	4 (4.3)	3 (6.4)	1 (2.2)	0.328
Diuretic	92	25 (27.2)	15 (31.9)	10 (22.2)	0.296
Metformin	92	9 (9.8)	4 (8.5)	5 (11.1)	0.675
Insulin	92	3 (3.3)	2 (4.3)	1 (2.2)	0.583
Statin	92	36 (39.1)	26 (55.3)	10 (22.2)	0.001
Co-foundings					
FA	92	9 (9.8)	8 (17.0)	1 (2.2)	0.017
TIA	92	1 (1.1)	0 (0.0)	1 (2.2)	0.304
DM; n (%)	92	14 (15.2)	8 (17.0)	6 (13.3)	0.514
Hypertension	92	47 (51.1)	25 (53.2)	21 (46.7)	0.532
Hypercholesterolemia	92	45 (48.9)	23 (48.9)	22 (48.9)	0.996
Asthma	92	9 (9.8)	4 (8.5)	5 (11.1)	0.675
COPD	92	3 (3.3)	2 (4.3)	1 (2.2)	0.583
Thorax X-ray					
Normal	80	45 (56.3)	21 (50.0)	24 (63.2)	0.491
Excessive plethoricity	80	12 (15.0)	7 (16.7)	5 (13.2)	0.491
Congestion	80	23 (28.7)	14 (33.3)	9 (23.7)	0.491
Treatment					
Time to therapy (h) (from onset of the pain)	91	56 (\pm 49)	58 (\pm 44)	53 (\pm 55)	0.633
Sympathomimetics / Inotropics	92	8 (8.7)	3 (6.4)	5 (11.1)	0.421
Non-invasive ventilation	92	8 (8.7)	5 (10.6)	3 (6.7)	0.286
Intubation	92	2 (2.2)	2 (4.3)	0 (0.0)	0.286
LV - Cineangiography					
EDV (ml)	82	102.9 (\pm 23.9)	104.1 (\pm 26.0)	101.4 (\pm 20.6)	0.609
ESV (ml)	82	49.9 (\pm 20.2)	52.6 (\pm 22.1)	46.6 (\pm 17.4)	0.179
SV (ml)	82	53.0 (\pm 18.1)	51.5 (\pm 18.0)	54.9 (\pm 18.3)	0.410
EF (%)	82	52 (\pm 15)	50 (\pm 16)	54 (\pm 13)	0.240
ECHO - Cardiography					
EF (%) (admission)	42	44 (\pm 12)	42 (\pm 13)	46 (\pm 11)	0.224
Normalized (days)	58	47.7 (\pm 40.4)	51.5 (\pm 42.1)	43.8 (\pm 39.0)	0.472

Data are expressed as n (%) or mean \pm SD.

ACE = angiotensin-converting enzyme; AT = angiotensin; COPD = chronic obstructive pulmonary disease; DM = diabetes mellitus; EF = ejection fraction; EDV = end diastolic volume; ESV = end-systolic volume; FA = atrial fibrillation; MCC = morbus coronarius cordis; SV = stroke volume; TIA = transient ischaemic attack; TTC, Takotsubo cardiomyopathy

5.2. Trigger factors

Triggering factors we divided into three categories; emotional, physical, and no acute trigger. A preceding emotional stressor accounted for 55.5% of the events; the most common reasons were news of a death or an acute medical condition of a family member or friend. Heavy physical exertion preceded it in 7.6% and another non-cardiac medical condition in 12.0% of the patients. No recent cerebrovascular event or head trauma occurred, but one patient had a migraine attack and another an epileptic seizure. Patients' meticulous interview did not identify an acute trigger in 25% of the patients. Table 3 describes the stressors in detail.

Inhaled beta-agonist medication was routinely used by 14.1%: short-acting (such as salbutamol) 6.5% and long-acting (such as salmeterol) 7.6%. Salbutamol was administered to 7.6% on admission for dyspnoea. Physical trigger factors and use of beta-agonist drugs showed no relationship. One suspected iatrogenic TTC event followed possible intravenous administration of adrenaline including lidocaine for local anesthetic.

Table 3. *Trigger factors among TTC patients*

Emotional (51 patients)		Physical (18 patients)
Anxiety/fear/panic (30)	Argument (9)	Excercise/physical activity (7)
Anxiety attack in sauna	Argument	Dancing at son's birthday party
Being mugged	Argument with nurse after cancer surgery	Climbing stairs
Experience of sexual harassment	Fight with a tenant	Dusting a carpet
Car accident (no physical trauma)	Fight with husband	Fall from pier into ice cold water
Change of job	Fight with a customer	Heavy woodwork
Fear of upcoming surgery	Fight with a dog seller	Nordic walking excercise
Fear of possible layoff	Fighth with son	Physi"cally heavy" day, "a long day"
Frustration after doctors appointment	Fight with siblings	Non-cardiac medical condition (11)
Insomnia	Heated conversation	Infection/allergic reaction (4)
Threat with an axe and a crossbow	Death of a close person (12)	Local anesthetic with adrenaline
News of husband's illness	Death of a friend (3 patients)	Heavy flu
Nervousness due to occupied restroom	Death of a mother	Stung by wasp; allergic to wasps'
News of sister's cerebral hemorrhage	Death of a husband (4 patients)	Traveler's diarrhea
News of daughter's divorce	Death of a cousin	Central nervous system-related (2)
News of father's illness and daughter's pregnancy	Death of a daughter	Epileptic seizure
Overwhelming emotion	Mother's funeral	Migraine attack
Overwhelming d'isappointment	Son's funeral	Cardiorespiratory related (2)
Relapse of an alcoholic husband		Acute exacerbation of COPD
Recollection of recently drowned father while diving		Electrical cardioversion a week earlier
Shock over husband's chest pain		Post-surgical (1)
Stressful period in life		After hip surgery
Stress over hosting an event		Other (2)
Stress over moving		Alcohol abuse, 3-month binge
Stress over daughter's moving		Painful leg cramps
Uncertainty over employment		
Unpleasant news at work place		
Witnessing a fire at home		
Witnessing a bicycle accident		
Worry in family (2 patients)		

* 23 patients had no acute trigger

5.3. Left ventriculography

Four TTC patterns emerged from the LVG analysis. The classic broad apical (77.2%) was the most common, and only a few small apical types (3.3%) appeared. Symmetric mid-ventricular (12.0%) and asymmetric mid-ventricular (7.6%) types were frequent. For further analyses, due to the small number of cases in most of the subtypes, the apical and mid-ventricular subtypes were combined. Contraction abnormality type was unrelated to medication, presenting symptoms, coronary risk factors, sex, trigger factors, cardiac enzymes, or thorax roentgenogram. Intraventricular thrombus was absent in all LVG's.

The mean EF was $51 \pm 15\%$. EF and stroke volume were higher in mid-ventricular than apical-TTC patients ($62 \pm 10\%$ vs $49 \pm 15\%$; $p=0.03$, and 67 ± 23 ml vs 50 ± 18 ml; $p=0.04$; respectively). Angiotensin-II receptor-blocker users' stroke volume (SV) was higher than non-users' (66.1 ± 23.5 ml vs 50.4 ± 17.5 ml; $p=0.04$), however, angiotensin-convertase blockage affected no other results. Other medications showed no relation to LVG measurements. The measurement of 2-dimensional LVG resulted in an EF of 0% for one patient, which suggested a contraction movement directed only towards the angle of view.

5.4. Electrocardiography

Acute 12-lead electrocardiography (ECG) was available for 57 TTC patients. Analysis showed ST-elevation in 72%, negative T-wave 68% or q-wave in 37%, or both in at least one ECG-lead. Incidence of QT-interval prolongation was 49%. Naturally all anterior-STEMI patients had ST-elevation present; negative T-waves apparent in 44% and q-waves in 38%. QT-interval was prolonged in 13%. Atrial fibrillation occurred in 2 patients. Both frequencies and amplitudes of ST-elevations and negative T-waves are shown in Figure 5.

Apical and mid-ventricular TTC did not differ in ST-elevation incidence (75% vs 56%, $p=0.23$). ST-elevations occurred more frequently, and the amplitudes were higher in anterior-STEMI than in TTC patients in leads V_1 to V_5 (Figure 5). ST-elevation distribution between ECG leads showed similarities between anterior-STEMI and TTC except for lead V_1 . In mid-ventricular-TTC, the few ST-elevation changes were small and presented only in leads I and/or V_2 . In both TTC types, ST-elevation frequency was low in lead V_1 . Different TIMI flow grades did not significantly change the amplitude or frequency of ST-elevation or negative T-waves in STEMI ECGs. Interestingly, the

ECG criteria for STEMI were not obvious in any of the mid-ventricular or in 25 of the apical-TTC patients. ST-depression occurred only in lead aVR of 3 apical-TTC patients.

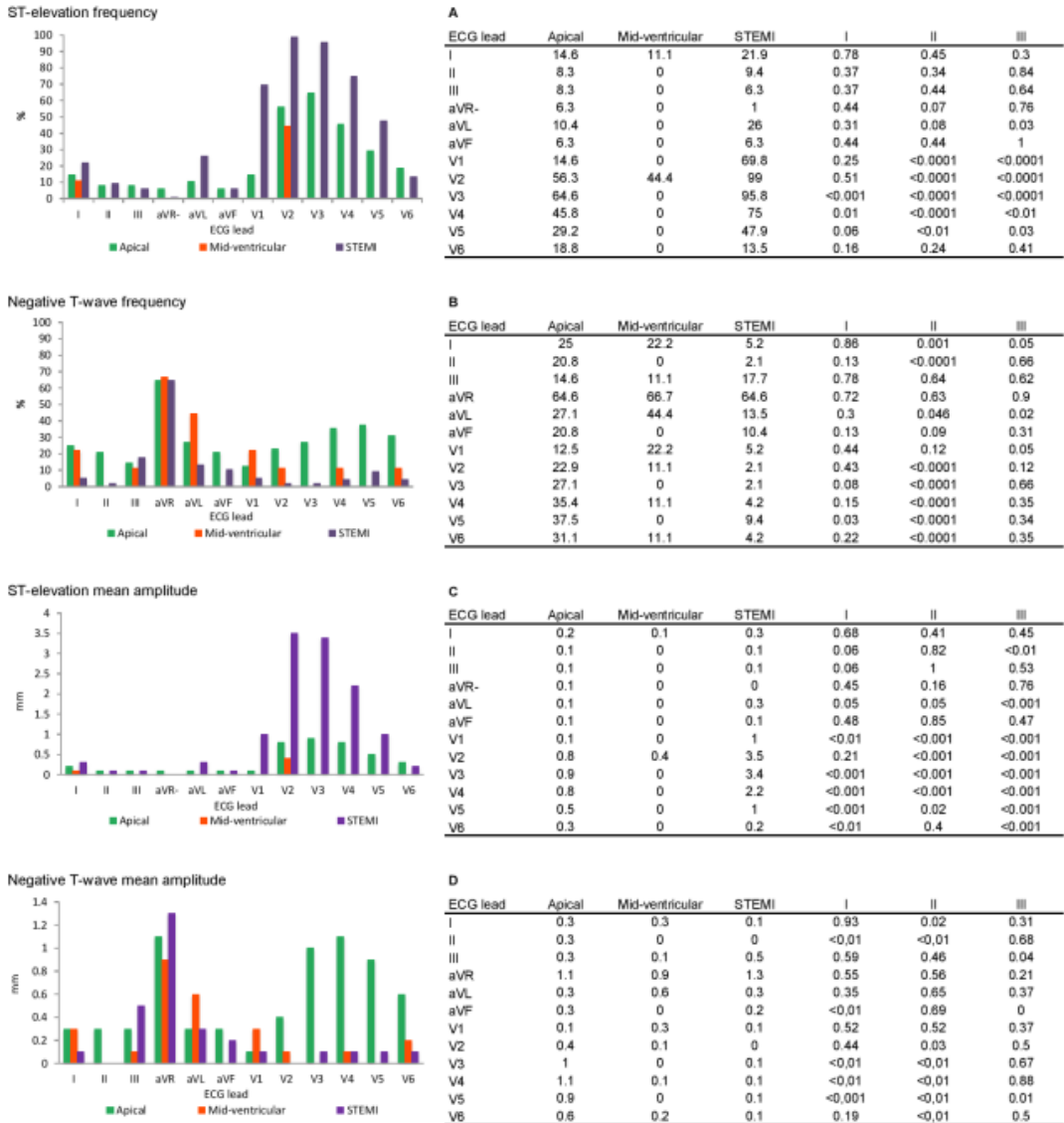


Figure 5. (A) ST-elevation and (B) negative T-wave frequencies in percentages. (C) ST-elevation and (D) negative T-wave mean amplitudes in millimeters (mm). I = Apical vs. Mid-ventricular, p value; II = Apical vs. STEMI, p value; III = Mid-ventricular vs. STEMI, p value. STEMI = anterior STEMI. Adapted from Parkkonen et. al. 2014 with publisher's permission (1).

Of the 3 groups on admission, the apical-TTC patients had the most frequent and highest negative T-wave amplitudes occurring in leads I, II, and V₂ to V₆. Negative T-wave featured in all mid-ventricular-TTC patients in at least one lead, but the amplitudes were mostly small—the more prominent showing in lead aVL. The negative T-wave frequency in lead aVL was higher in both apical- and mid-ventricular-TTC than in anterior-STEMI patients (p=0.046 vs p=0.016; respectively). Such finding, however, identified TTC with only 30% sensitivity and 86% specificity with 57% predictive value.

Neither Q-wave incidence, QRS duration, nor axis differed between TTC groups and anterior-STEMI (Table 4)

Table 4. *QRS duration and axis degree, Q-wave incidence. STEMI, ST-elevation myocardial infarction*

	Apical	Mid-ventricular	Anterior-STEMI	I	III	III
QRS duration, ms	88.2 ± 11.3	93.2 ± 11.1	90.6 ± 15.4	0.35	0.23	0.62
QRS axis, °	90.6 ± 15.4	12.2 ± 61.6	18.1 ± 57.9	0.82	0.84	0.77
Q-wave, n (%)	17 (35)	4 (44)	36 (38)	0.71	0.86	0.73

STEMI, ST-elevation myocardial infarction

I = Apical vs. Mid-ventricular, p-value; II = Apical vs. Anterior-STEMI, p-value; III = Mid-ventricular vs. Anterior-STEMI, p-value

Corrected QT-intervals were longer in apical-TTC (0.439 ± 0.046 ms) and mid-ventricle-TTC (0.430 ± 0.032 ms) than in anterior-STEMI patients (0.405 ± 0.042 ms) (p<0.0001 and p=0.04; respectively). The TTC groups did not differ in this regard. Prolonged QT-interval occurred in 13% anterior-STEMI, 49% apical-TTC, and 56% mid-ventricular-TTC patients. The difference between TTC groups and STEMI was significant (p<0.001), but not between TTC groups.

Of the patients with ST-elevation, the lack of ST-elevation in lead V₁ identified TTC with 83% sensitivity, 70% specificity, and a predictive value of 54%. ST-elevation amplitude <2 mm in lead V₂ identified TTC from anterior-STEMI with 73% sensitivity, 89% specificity, and 73% predictive value. The two combined: lack of ST-elevation in V₁ and ST-elevation amplitude < 2mm in V₂ identified TTC with 63% sensitivity and 93% specificity with a predictive value of 79%.

Time interval from onset of pain to ECG was longer in apical-TTC ($7.6 \text{ h} \pm 6.7$) than in anterior-STEMI patients ($4.7 \text{ h} \pm 5.5$). Mid-ventricle-TTC ($10.3 \text{ h} \pm 8.6$) and anterior-STEMI patients as well as the two TTC groups did not differ.

5.5. Biochemistry

On admission, TnT was elevated ($>0.03 \text{ } \mu\text{g/L}$) in 81 patients ($0.623 \text{ } \mu\text{g/L} \pm 0.927$, range <0.03 to 7.76); peak TnT release was ($0.730 \text{ } \mu\text{g/L} \pm 1.317$, range <0.03 to 11.91). Of the 5 patients with available TnI levels, 4 had elevated values ($>0.3 \text{ } \mu\text{g/L}$), $0.768 \text{ } \mu\text{g/L} \pm 0.506$ (range 0.26 to 1.41), and peak TnI release was $1.356 \text{ } \mu\text{g/L} \pm 1.313$ (range 0.26 to 3.53). CK-MBm values were measured in 7 patients, and 5 had elevated values ($>9 \text{ } \mu\text{g/L}$), $14.57 \text{ } \mu\text{g/L} \pm 10.25$ (range 4.0 to 29.0); peak CK-MBm release was $20.86 \text{ } \mu\text{g/L} \pm 24.05$ (range 4.0 to 72.0).

Pro-BNP was available for 18 patients (median 3547 ng/L , IQR $1050 - 6029$). All values were elevated ($>300 \text{ ng/L}$).

5.5.1. Coagulation study (II)

The only marker of hemostasis and thrombosis that differed between TTC and ACS was D-dimer. The number of patients with elevated ($> 0.5 \text{ } \mu\text{g/mL}$) D-dimer was similar between TTC (14) and No-CAD (15), but lower than in ACS (20). Both TTC and ACS had similar elevated hsCRP, fibrinogen, and vWF:Ag values, which were higher than was No-CAD (Table 5).

Coronary risk factors in TTC differed from STEMI in lower BMI (26.1 kg/m^2 ($22.8 - 29.0$) vs. 26.9 kg/m^2 ($24.2 - 31.6$), $p=0.025$) and less dyslipidemia (57% vs. 83% , $p=0.007$); and from No-CAD in lower BMI (26.1 kg/m^2 ($22.8 - 29.0$) vs. 26.8 kg/m^2 ($24.5 - 30.5$), $p=0.038$). The time from symptom onset to CAG was similar between TTC and ACS patients (48 hours ($24 - 96$) vs. 48 hours ($24 - 90$); $p=0.94$). Non-significant obstructions ($\leq 50\%$) in CAG were evident in 47% of the TTC and 15% of the No-CAD. CAG of ACS patients showed single-vessel disease in 28% , two-vessel disease in 21% and three-vessel disease in 51% . From highest to lowest, pro-BNP values were in TTC 2528 ng/L ($1397 - 5767$), in ACS 810 ng/L ($268 - 2372$), and in No-CAD 197 ng/L ($111 - 533$) and differed among the groups ($p<0.0001$).

Table 5. *Coagulation and acute-phase markers from plasma*

	TTC	ACS	No-CAD	I	II	III
44sCRP (mg/L)	7.13 (1.99-23.00)	9.44 (1.48-30.52)	1.44 (1.02-3.00)	0.200	<0.0001	<0.0001
D-Dimer (µg/L)	0.31 (0.22-0.59)	0.39 (0.24-1.20)	0.38 (0.22-0.56)	0.019	0.370	0.064
vWF:AG (%)	174.0 (139.0-215.0)	151.0 (132.0-210.0)	130.0 (98.0-169.0)	0.210	<0.0001	0.005
Fibr (g/L)	3.90 (3.19-4.70)	4.40 (3.47-5.29)	3.52 (3.12-3.79)	0.092	0.005	<0.0001
TAT (µg/mL)	3.80 (3.00-5.50)	4.10 (3.30-6.80)	3.60 (2.90-7.80)	0.500	0.290	0.560
sGPV (ng/mL)	38.8 (33.50-55.20)	40.10 (33.00-56.20)	36.70 (27.30-53.30)	0.540	0.190	0.100

F = fibrinogen, *hsCRP* = high-sensitivity C-reactive protein, *sGPV* = soluble glycoprotein V, *TAT* = thrombin antithrombin complex, *vWF* = von Willebrand factor

I, *TTC* vs. *ACS*, *p*-value; *II*, *TTC* vs. *No-CAD*, *p*-value; *III*, *ACS* vs. *No-CAD*, *p*-value
Median (interquartile range)

For anticoagulant and antithrombotic medication see Table 6. All statistically differing parameters (low-molecular weight heparin, clopidogrel, aspirin, glycoprotein inhibitor, BMI, and dyslipidemia) were examined in multivariate analysis, but none predicted the laboratory parameters.

Table 6. *Number of patients on anticoagulation and antithrombotic medications*

	TTC	ACS	No-CAD	I	II	III
Clot lysis (<24h)	1 (2)	3 (6)	0 (0)	0.620	1.000	0.240
Warfarin	5 (11)	0 (0)	4 (9)	0.060	1.000	0.120
Aspirin	42 (89)	46 (98)	34 (72)	0.090	0.060	0.001
Clopidogrel	32 (68)	38 (81)	6 (13)	0.150	<0.0001	<0.0001
LMWH	45 (96)	42 (89)	1 (2)	0.240	<0.0001	<0.0001
Glycoprotein IIb/IIIa inhibitor	18 (38)	19 (40)	0 (0)	0.830	<0.0001	<0.0001

LMWH = low-molecular weight heparin

I = *TTC* vs. *ACS*, *p*-value; *II* = *TTC* vs. *No-CAD*, *p*-value; *III* = *ACS* vs. *No-CAD*, *p*-value

Median (interquartile range).

5.5.2. Matrix metalloproteinases (III)

MMP-8 levels in ACS and TTC were similar, but were lower in controls. TIMP-1 levels showed a stepped rise from low in controls to TTC and highest in ACS (Figure 6). MMP-8 and TIMP-1 levels, and the MMP-8/TIMP-1 molar ratio were used to divide patients into tertiles. TIMP-1, but not MMP-8 and MMP-8/TIMP-1 molar ratio, associated with ACS over TTC (3rd tertile vs 1st OR 4.52 (1.86 - 11.00), $p < 0.002$). TIMP-1 remained independently associated with ACS in multivariate analysis.

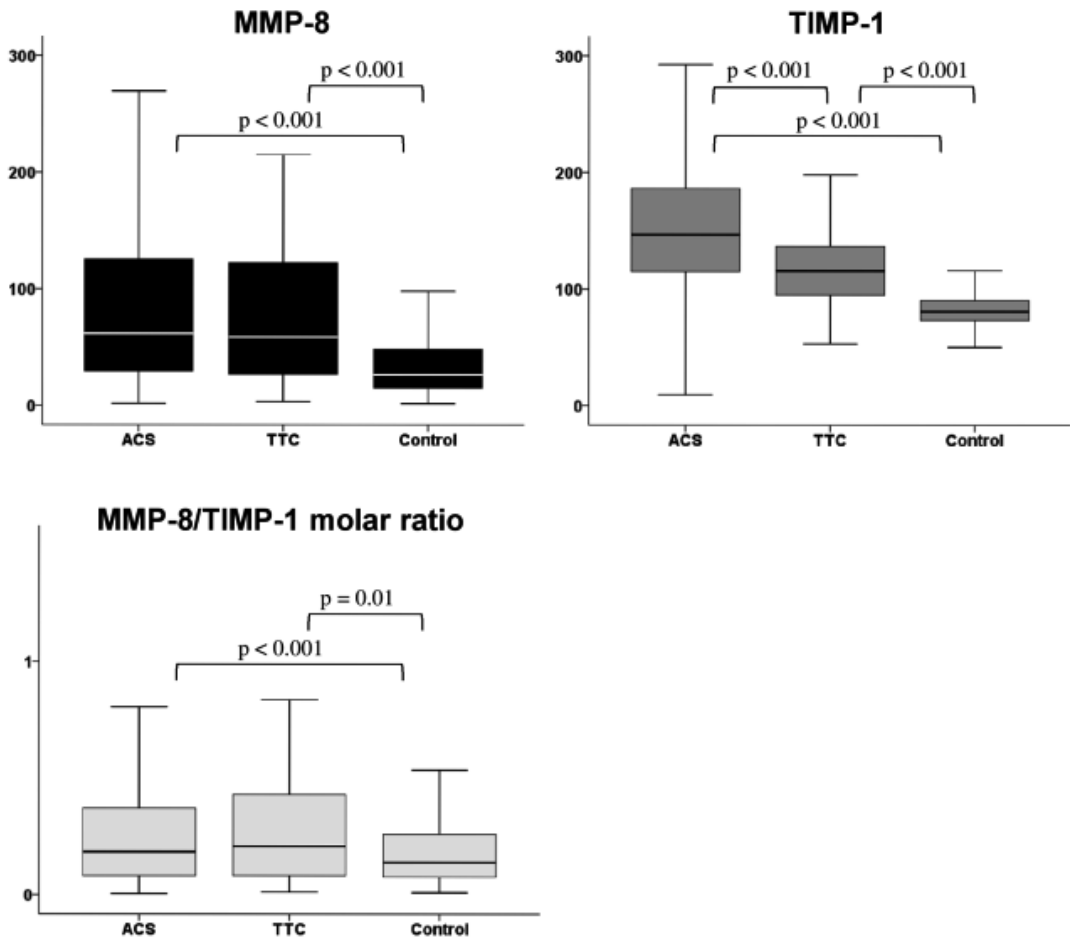


Figure 6. MMP-8 and TIMP-1 levels (ng/mL) and their ratio between ACS, TTC and controls. MMP-8 = matrix metalloproteinase 8, TIMP-1 = tissue inhibitor of matrix metalloproteinase 1. Adapted from Parkkonen et. al. 2016 (III).

On-admission TnT levels were similar (0.3 µg/L (0.1-0.9) vs. 0.2 µg/L (0.1-0.6); $p = 0.7$) and unable to differentiate ACS and TTC (area under the curve (AUC) 0.522 (0.442-0.602; $p > 0.05$). TIMP-1 differentiated ACS from TTC with an area under the curve (AUC) of 0.679 ($p < 0.0001$) (Figure 7). TIMP-1 also improved the sensitivity and specificity of other significant differences (age, sex, smoking) in the c-statistics (AUC 0.821, 0.764 - 0.879 ($p < 0.0001$) vs. 0.844, 0.783 - 0.906 ($p < 0.0001$), ($p < 0.01$) (Figure 7).

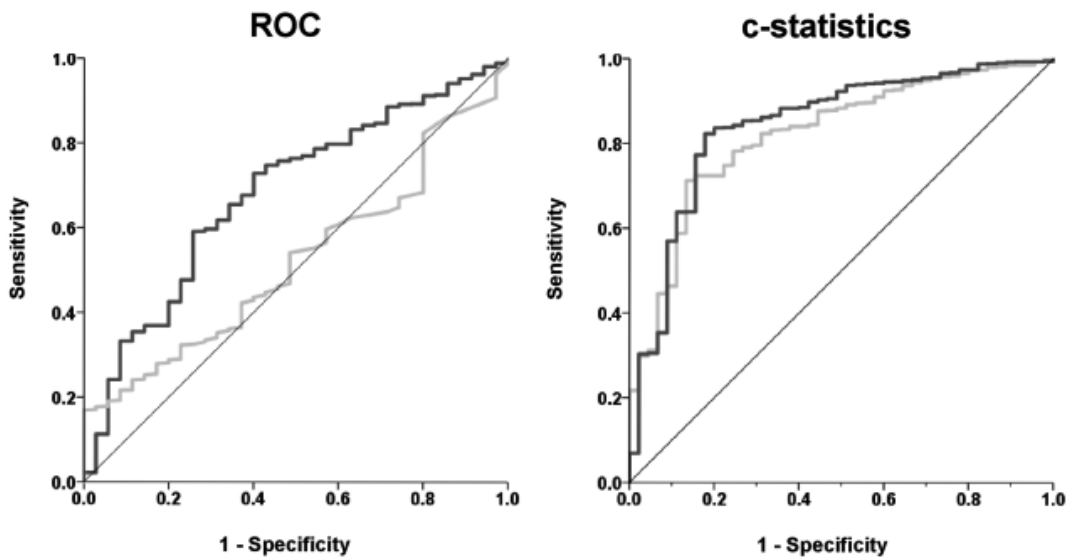


Figure 7. *Left: In recognition of ACS, ROC curves show better sensitivity and specificity of on-admission TIMP-1 (dark grey) over TnT (light grey). Right: In c-statistics, combination of TIMP-1 and other risk factors (age, sex and smoking) improved the differentiation of ACS and TTC compared to other risk factors alone (light grey). Adapted from Parkkonen et. al. 2016 (III).*

EF from the LVG in TTC patients was abnormal (<50%) in 17 patients. The median EF was 53% (35 – 62). MMP-8 and TIMP-1 individually showed no association with LV function; however, MMP-8/TIMP-1 molar ratio correlated with EF ($r=0.358$; $p=0.02$). Of end-diastolic, end-systolic, or stroke volume, the EF was the only LVG measurement that correlated with the MMP-8/TIMP-1 molar ratio. MMP-8, TIMP-1 levels and ratio between apical and mid-ventricular TTC were similar. The echocardiography EF in ACS had no association with MMP-8, TIMP-1 or MMP-8/TIMP-1 molar ratio.

TTC with low < 50% EF had high TIMP-1 levels similar to those of ACS, but significantly lower MMP-8 levels. In fact, the MMP-8/TIMP-1 ratio was significantly lower in low-EF TTC and higher in normal-EF TTC than was in ACS. In normal-EF TTC, the TIMP-1 was lower than and MMP-8 similar to ACS.

Table 7 TTC patients by ejection fraction (EF) compared to ACS and Controls

	TTC EF < 50%	TTC EF > 50%	ACS	Control	I	II	III	IV	V
MMP-8 (ng/ml)	28.5 (17.4-69.7)	72.9 (34.9-164.0)	61.5 (29.2-125.4)	26.3 (14.9-48.2)	0.01	0.01	<0.001	n.s.	<0.001
TIMP-1 (ng/ml)	136.8 (102.6-169.1)	113.3 (94.3-128.3)	146.7 (115.0-186.3)	80.9 (73.2-90.4)	0.01	n.s.	<0.001	<0.001	<0.001
MMP-8/TIMP-1 molar ratio	0.08 (0.05-0.20)	0.27 (0.13-0.51)	0.18 (0.08-0.37)	0.14 (0.08-0.26)	0.04	0.04	0.07	0.01	<0.001
TnT admission (µg/L)	0.3 (0.1-0.7)	0.2 (0.1-0.6)	0.3 (0.1-0.9)	N/A	n.s.	n.s.	N/A	n.s.	N/A
TnT highest (µg/L)	0.2 (0.2-0.6)	0.3 (0.2-0.9)	0.84 (0.26-2.47)	N/A	0.01	<0.001	N/A	0.01	N/A
CK-MBm (µg/L)	11.0 (6.5-17.5)	11.0 (5.5-16.0)	8.0 (4.0-26.0)	N/A	n.s.	n.s.	N/A	n.s.	N/A
hsCRP (mg/L)	5.8 (0.9-24.8)	7.2 (2.4-18.8)	5.8 (1.9-20.5)	0.8 (0.4-1.9)	n.s.	n.s.	<0.001	n.s.	<0.001

CK-mbm = creatine kinase mb-mass, hsCRP = high sensitivity c-reactive protein, MMP-8 = matrix metalloproteinase 8, TIMP = tissue inhibitor of I = TTC EF < 50% vs. TTC EF > 50%, II = TTC EF < 50% vs. ACS, III = TTC EF < 50% vs. Control, IV = TTC EF > 50% vs. ACS, V = TTC EF > 50% vs. Control.

Data as median (IQR)

n.s., not significant; N/A, not available

6. DISCUSSION

During the time we conducted our studies, the number of TTC studies has increased substantially. The elegant animal model by Paur et al. (2009) has been one of the most interesting reports, shedding new light onto the pathogenesis of TTC [50]. Further studies hopefully can confirm whether the same mechanism applies to humans as well. The availability of larger cohorts and multicenter studies has revealed new insights. Recent large cohort reports have recognized a subset of patients at high risk during hospitalization, in opposition to the common perception of a benign disease [118,163].

Our work supports many of the previous concepts and theories in TTC. The complete material showed qualities in demographics, co-morbidities and medications similar to those in earlier reports, thus allowing direct comparison with others' findings [5,11,12,90].

6.1. Electrocardiography (I)

In the search for TTC ECG criteria, we found ST-elevation less than 2 mm in lead V2 in the absence of ST-elevation in lead V1 was a specific finding in TTC patients, however, its sensitivity was less impressive. Overall, ST-elevation changes in anterior-STEMI patients were more distinctive and frequent than in TTC. Even though less frequently, ST-elevations were apparent in similar pattern in apical TTC as anterior STEMI with the exclusion of lead V1. Mid-ventricular TTC clearly differed—the patients had very modest ST-elevations and they appeared in only few leads.

Temporal evolution of ECG changes in TTC follow similar pattern to that of STEMI. T-wave inversion normally follows ST-elevation, and changes normalize within 1 to 2 weeks [56]. Primary negative T-waves could, therefore, be considered a sign of a longer delay to seek medical attention. Dib. et al. (2009) found no relation between delay from symptom onset and primary ECG finding, which suggests variability in primary ECG findings in TTC regardless of delay [104]. In many cases, however, the moment of pain onset is a very subjective perception. Therefore, the role of the delay remains a debatable in TTC ECG interpretations [164,165].

The point of measurement for ST-elevation affects the amplitude as well as the frequency. Current guideline of the European Society of Cardiology recommends using the J-point [31]. Many reports used the J-point + 80 milliseconds for measurement point, which may result in higher amplitude and frequency in cases with an upward ST-segment [98,99,104,105], thus making direct comparison of our results difficult. The best comparison is offered by the results of Tamura et

al. (2011) and Johnson et al. (2013), which utilize the same measurement point as does the current study [108,166]. Usage of similar measurement points in the future, and analysis of ST-segment morphology, could lead to more accurate results.

ST-depression is a rare finding in TTC, and some of the milder changes could possibly be explained by normal variation of the baseline. ST-depression appeared in only 6 TTC patients—3 of those in lead aVR suggesting that in TTC reciprocal changes are rare, if not non-existent. The most promising earlier ECG criteria identified TTC with good sensitivity and specificity by combining ST-depression in aVR and lack of ST-elevation in V1 [98]. Our results strongly disagree, due to the low incidence of ST-depression. Strangely, no T-wave changes were reported in the same study, raising the question whether their ST-depression changes were merely a combination of ‘late’ measurement point and negative T-wave.

Compared to anterior STEMI, apical TTC patients had frequent and high amplitude negative T-waves, especially in the chest leads. Mid-ventricular TTC had relatively scarce negative T-wave changes, which were in line with ST-segment findings. The only lead that differentiated both TTCs from the STEMI was aVL. This finding was rather specific, but its sensitivity was inadequate for clinical decision-making.

Although mid-ventricular TTC involves a large area of affected myocardium in LVG, the ECG changes remain few. The standard 12 leads cover mid-ventricular myocardium poorly. The affected myocardium projects horizontally to lead V₂ and in the frontal plane to leads I and aVL, which explains the scarce ECG changes. Fittingly, of the 12 ECG leads ST-elevation showed in only 2 (I and V₂) and negative T-waves in 3 (I, aVL and V₂) in line with others’ findings [105]. ST-elevation was present in half and negative T-waves in all our mid-ventricular TTC patients. None of the mid-ventricular patients fulfilled the criteria for ST-elevation myocardial infarction [31]. Mid-ventricular TTC patients could potentially pose a diagnostic problem. Mild symptoms leading to longer delay from symptom onset to medical contact and few or non-existent ECG findings could result in misdiagnosis of non-cardiac chest pain. Luckily, in the absence of severe heart failure, TTC has a good prognosis.

A surprisingly low number of our TTC patients fulfilled the ECG criteria for STEMI. Another study focused on those TTC patients fulfilling the STEMI criteria, comparing female TTC patients were compared to female STEMI patients with poor results. In that study, trial of previous criteria by Kurisu et al and Johnson et al. (2009) produced less impressive results than in their original publications [105,108]. Vervaat et. al. (2015) had the best result with a frontal plane ST-vector 60

degrees distinguishing TTC from STEMI with 49% sensitivity and 93% specificity [167]. Although a smaller number of TTC patients than initially thought caused a differential diagnostic problem with STEMI, no ECG interpretation should delay the angiography and treatment in cases with ST-elevation.

In MI, Q-waves are related to a large area of stunned myocardium and small infarct size, which is usually seen after a rapid revascularization [51,101]. Such a finding could support the aborted-MI theory behind TTC. Sharkey et al (2008), however, found that despite, a similar incidence of Q-waves in TTC and ACS, TTC patients showed no signs of necrosis in magnetic resonance imaging [101]. In our study, the incidence of Q-waves did not differentiate ACS from TTC; and therefore could not rule out the hypothetical aborted MI.

6.2. Coagulation (II)

To rule out any atherothrombotic event behind the TTC, we compared TTC and ACS patients in the acute phase for coagulation factors. The more systemic approach allowed us to tackle the shortcomings of previous imaging studies assessing the role of aborted MI and microvascular thrombosis. TTC associated with a significant increase in coagulation factors known to be acute-phase proteins, but showed no plausible signs of abnormal fibrin formation. These results argue against an acute thrombotic situation and are fitting for the catecholamine-excess theory.

Elevated D-dimer level marks a hypercoagulable state, thrombus formation, and degradation. In our study, the TTC- and No-CAD patients both had less frequent elevated D-dimer levels than ACS patients had. D-dimer values were also higher in ACS than in TTC, despite similar medication and acute phase reaction. These results suggest less thrombosis, if any, in TTC than in ACS.

A preceding excessive-stress event or other increased sympathetic activity in the majority of TTC cases manifests as elevated catecholamine levels. The coagulation system and platelet function are directly influenced by the levels of epinephrine and norepinephrine. The catecholamines, especially epinephrine, lead to the release of F VIII and von Willebrand factor from the endothelial cells [137]. In a stressful situation, such physiological pro-thrombotic changes cause no thrombosis in healthy vessels, but may exaggerate thrombosis in the presence of endothelial erosion or atherosclerotic plaque rupture. In our study, TTC and ACS patients both had high levels

of von Willebrand factor. TTC patients, however, had no convincing signs of thrombus formation in other laboratory assays or in angiography—unlike ACS patients.

An acute phase reaction is a defensive reaction against toxic or infectious agents. Stress-induced myocardial damage is also characterised by a similar cytokine-induced increase in acute phase proteins like C-reactive protein and in coagulation factors such as fibrinogen, von Willebrand factor, F VIII and prothrombin. Elevated levels of such proteins were akin in TTC and ACS and were higher than in No-CAD. In conjunction with D-dimer findings, these results suggest that TTC exhibits a similar acute phase reaction than ACS but not thrombosis.

Platelet activation plays an important role in arterial thrombosis both the platelets forming the main part of the arterial thrombus. Two studies have compared platelet activation between ACS and TTC in the acute phase with controversial results. The first study showed elevated platelet activation marker CD26P expression and higher plasma interleukin-6 levels reflecting enhanced platelet activation in ACS compared to activation in TTC in the acute phase [168]. The other study revealed platelet activation evaluated by integrin glycoprotein IIb/IIIa and P-selectin expression in the acute phase as similar for TTC and for ACS patients [169]. Interestingly, platelet activation at follow-up was related to elevated acute-phase epinephrine levels [169].

In our TTC population, sGPV, a marker for platelet activation, was not increased; nor did we observe any significant difference in sGPV values between the ACS and TTC groups. ACS patients had borderline higher levels of sGPV than did non-CAD patients, as expected. Our results did not suggest platelet activation in TTC, but the difference between ACS and controls was only moderate, thus challenging the sGPV's ability to identify platelet activation in ACS.

No LV thrombus was detectable in any of the TTC patients. However, none of our patients underwent cardiac magnetic resonance imaging, which would have allowed more comprehensive evaluation by LVG [138].

We found in TTC patients no biochemical or angiographic evidence of thrombus formation or degradation, which in conjunction with others reports of negative imaging findings of TTC patients' coronaries, suggests a non-thrombotic cause for TTC. An extensive metabolic defect exceeding the perfusion defect in the TTC-affected myocardium, favors a metabolic origin for the microvascular dysfunction and opposes any thrombotic origin [60]. Furthermore, a larger study later reported similar coagulation results [170]. Although the role of microvascular dysfunction and perfusion defect remains unclear in the pathogenesis of TTC, our results here support a non-

thrombotic cause and argue against the existence of microvascular thrombosis or atherosclerotic plaque erosion.

6.3. Matrix metalloproteinases (III)

MMP-8 and TIMP-1 have been implicated in ACS. Due to the probable difference between TTC and ACS pathophysiology, we hypothesized that MMP-8 and TIMP-1 can differentiate TTC from ACS. Only one small series of TTC patients' MMP and TIMP profiles exists and it contains no direct comparison to ACS [156]. Furthermore, the effects of MMPs and TIMPs in HF and other cardiomyopathies led us to explore whether MMP-8 and TIMP-1 levels affect the severity of the LV impairment in TTC.

TIMP-1 was significantly higher in ACS than in TTC, but curiously TTC also had higher levels than did controls. On admission, TIMP-1 could differentiate TTC from ACS with better sensitivity and specificity than did TnT. TnT is cardiac specific, but not specific to ACS. Within a few hours after ischemic myocardial damage, TnT levels rise. Therefore, on admission, TnT levels might not be able to differentiate ACS from other heart diseases. In the current study, the highest TnT value differed between ACS and TTC, but the on-admission levels were similar and in both cases low. The differing TIMP-1 levels provided improvement in early non-invasive diagnostics of ACS vs TTC. TIMP-1 also improved the accuracy of differentiation more than other significant differences (age, sex, and smoking) between the groups did.

Heart failure (HF) is marked by changes in the ECM, which serves as barrier against pathologic stress, but ultimately contributes to pathogenesis. The changes depend upon the etiology of the condition [145,171]. In pressure-overload HF (for instance from hypertension), the decreased proteolysis leads to increased ECM volume, especially accumulation of type-I collagen. In such cases, the myocardium exhibits a pro-fibrotic low-MMP and high-TIMP balance [145,146]. The increased fibrosis causes myocardial stiffness. Similar fibrosis in hypertrophic cardiomyopathy linearly associates with decreased regional contractility [172].

In TTC patients, endomyocardial biopsies show inflammation and transient interstitial fibrosis in the acute phase, which resolves after functional recovery [47,155]. The increased fibrosis mainly comprises type-I collagen and subsequently shows an increased type-I/III collagen ratio. In the ECM, the type-III collagen provides the elastic properties and type-I the load bearing properties, and such an altered ratio associates with increased stiffness [155]. None of the endomyocardial biopsy

studies found signs of post-ischemic necrosis; however, contraction band necrosis, also associated with pheochromocytoma, did occur [47,155,173]. MRI of TTC patients supports such findings and reveals signs edema and inflammation, but no convincing signs of necrosis fitting to ischemic damage [90].

In our results, in TTC patients, the MMP-8/TIMP-1 molar ratio was associated to EF. Low-EF TTC had lower MMP-8 levels and higher TIMP-1 levels than did normal-EF TTC. Such a pro-fibrotic ratio may reflect the increased interstitial fibrosis seen in previous endomyocardial biopsies. Moreover, such an increased fibrosis may explain the worse LV function in some proportion of TTC patients. The correlation of MMP-8/TIMP-1 ratio with EF supports this theory.

MMP-8 differentiated ACS and TTC patients from controls, but not from each other. Clearly, MMP-8 plays a role in both ACS and TTC, but their origin appear to differ. Evidence suggests that acute-phase MMP-8 and TIMP-1 ACS originate from atherosclerotic plaques [144,154]. In TTC, however, the relationship of acute MMP-8/TIMP-1 molar ratio to LV EF, in conjunction with the previous endomyocardial-biopsy findings, suggest myocardium as their origin. Direct proof of what ultimately leads to the altered MMP-8 and TIMP-1 levels is lacking. HsCRP correlated with MMP-8 and TIMP-1. Accordingly, the alteration in TTC patients' MMP-8 and TIMP-1 levels may in fact be linked to inflammatory response. On the other hand, elevated catecholamines, especially norepinephrine, are known to affect MMP and TIMP levels. The elevated catecholamine levels in TTC may be cause of the altered MMP-8 and TIMP-1 levels [174-176].

6.4. Limitations and future studies

The greatest limiting factor in our study was sample size. Even with the rising TTC incidence in the 7 years of our study, a collection of a large population in a single center would require years of patient enrollment. Such restrictions call for large multicenter studies in the future to achieve more accurate view of this syndrome.

Even though the catecholamine hypothesis seems the most plausible explanation for TTC pathogenesis of TTC, some of the results display variability similar to that in coronary spasm and microvascular dysfunction. The catecholamine theory is based heavily on one study with a relatively small number of high-catecholamine-level patients [177], later studies, however, show such results in only a small proportion of TTC patients [45,46]. Our results (**I, II**) were in line with the catecholamine theory, but the uncertain high acute levels in TTC need confirmation in a larger population.

Two of our studies (**I**, **II**), had common problems associated with their retrospective study setting. The dynamic changes in ECG and biochemical measures were difficult to evaluate, due to partial information concerning time delays in seeking medical care and blood sampling. Moreover, no serial sampling to assess the temporal effect was possible. Some of the data were not available for all the patients, which limited sample size in some cases. A well-designed prospective study with standardized serial samples would solve some of these limitations.

The methods of Studies **II** and **III** allowed us to observe the possible pathophysiologic mechanisms by means of circulating biomarkers. The approach and the study setting, however, prevented us from verifying our findings at the cellular level, or by intracoronary imaging or by endomyocardial biopsies. Such methods would have provided more solid evidence for these results and further studies with such methods are, therefore, warranted.

The recently proposed multifactorial theory of TTC pathophysiology seems appealing in the light of these results [20,178]. Many of the suggested mechanisms for TTC, such as aborted MI, microvascular dysfunction, and coronary spasm are recognized as individual entities and known as MI without obstructive coronary atherosclerosis [179]. Although all our patients fulfilled the TTC criteria, cardiac magnetic resonance imaging, IVUS/OCT, and endomyocardial biopsies would have benefitted their assessment. Moreover, such methods would have allowed better evaluation of each patient's individual characteristics and could have provided new associations supporting the multifactorial theory. In a clinical setting, these entities tend to overlap, due to their very similar symptoms and findings and may also be underestimated after MI rule-out.

As the hunt for the mechanisms regarding TTC pathophysiology continues, the results of this work support the most probable theory, which is the catecholamine theory. Moreover, these findings suggesting fibrosis in the MMP study reveal a possible multifactorial pathogenesis. The recent increase in TTC populations should offer exciting new insights and may help to unravel the mystery.

7. CONCLUSIONS

- ECG analysis offered no reliable criteria to differentiate acute TTC patients from ACS patients. A surprisingly small percentage of TTC patients posed a true differential diagnostic problem versus STEMI patients. The mid-ventricular TTC patients showed very subtle if not obsolete ECG changes perhaps leading to misdiagnosis.
- TTC and ACS patients displayed similar acute phase reactions according to their laboratory values. Despite their pro-thrombotic state, the d-dimer results differed from those of the ACS patients and were similar to those of healthy controls arguing against thrombosis as the origin of TTC. Our findings supported catecholamine excess theory but oppose a concept of aborted MI as the basis of the TTC pathogenesis.
- A low MMP-8/TIMP-1 ratio may reflect increased ECM fibrotic content and more severely impaired LV function. A high TIMP-1 may be beneficial in differentiation of acute TTC from ACS.

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