Stroke and atrial fibrillation in Hypertrophic Cardiomyopathy

MD (Res) Thesis

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DECLARATION

I, Oliver Guttmann confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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ABSTRACT

Introduction

Hypertrophic cardiomyopathy (HCM) is an inherited myocardial disease associated with atrial fibrillation (AF) and thromboembolism (TE), which are related to adverse clinical outcomes and reduced survival. Current ESC and ACCF/AHA guidelines recommend anticoagulation in all patients with HCM and atrial fibrillation but the absolute risk of thromboembolism in patients with and without documented AF is unclear. The primary aim of this study was to derive and validate a model for estimating the risk of TE in HCM. Analyses were performed to determine predictors of AF and TE. Exploratory analyses assessed the performance of the CHA_2DS_2-VASc score and outcome with vitamin K antagonists (VKA). A further aim was to investigate the effect of AF on mortality and the efficacy of antiarrhythmic therapy in the development of AF.

Objectives

The aims of the thesis were to:

a) Systematic review of the literature on atrial fibrillation and thromboembolism in patients with HCM and meta-analyse prevalence and incidence of atrial fibrillation and thromboembolism

b) Assess prevalence, incidence and predictors of AF in a large multicentre cohort

c) Investigate the effect of AF on mortality

d) Assess clinical predictors of thromboembolism and derive and validate a risk model for estimating the risk of thromboembolism in patients with and without documented atrial arrhythmia

e) Assess the performance of the CHA_2DS_2-VASc score in this population

f) Assess the effect of therapy with vitamin K antagonists (VKA) in patients with AF and their bleeding risk (HAS-BLED score)

g) Investigate effect of antiarrhythmic therapy on the development of AF

Methods

Retrospective, observational cohort studies. The primary outcomes were a thromboembolic event defined as a composite of cerebrovascular accident (CVA), transient ischaemic attack (TIA) or systemic peripheral embolus as defined in the CHA_2DS_2-VASc score and atrial fibrillation, which was defined as paroxysmal, permanent or persistent AF detected on ECG, Holter monitoring or device interrogation. The secondary outcome was all-cause and cardiovascular mortality (defined as sudden cardiac death, heart failure related death, stroke related death and other cardiac death).

Results

a) Systematic review of the literature on atrial fibrillation and thromboembolism in patients with hypertrophic cardiomyopathy

The meta-analysis of a population of 7381 patients revealed an overall AF prevalence of 22.45% (95% CI 20.13% to 24.77%), I^2=78.9% ($p<0.001$). Overall prevalence of thromboembolism in HCM patients with AF was 27.09% (95% CI 20.94% to 33.25%), I^2=61.4% ($p<0.01$). Overall AF incidence was 3.08% per 100 patients per year (95% CI 2.63% to 3.54%, I^2=86.5%, $p<0.001$) and incidence of thromboembolism in HCM patients with AF was 3.75% per 100 patients per year (95% CI 2.88% to 4.61%), I^2=37.9% ($p=0.1$).

Left atrial (LA) dimension and age were common predictors for AF and thromboembolism. Meta-analysis revealed an LA diameter of 38.03 mm (95% CI 34.62-41.44) in SR and 45.37 mm (95% CI 41.64-49.04) in AF. There were no RCTs of therapy; anticoagulation was associated with lower stroke incidence but data on other interventions were limited and contradictory.

b) *Assess prevalence, incidence and predictors of AF in a large multicentre cohort*

c) *Investigate the effect of AF on mortality*

Of 4248 HCM patients 740 (17.4%) patients reached the primary endpoint of AF within 10 years from first evaluation. Multivariable Cox regression revealed an association between AF and the following: female sex, LA diameter, NYHA class II, NYHA class III and IV, hypertension and vascular disease. The incidence of cardiovascular death was 4.92% in the sinus rhythm (SR) group and 10.9% in the AF group (difference in proportions = 5.9%; 95% CI= [4.1%-7.8%]). The incidence of non-cardiovascular death was 3.2% in the SR group and 5.9% in the AF group (difference in proportions= 2.8%; 95% CI= [0.1%- 4.2%]).

d) *Assess clinical predictors of thromboembolism and derive and validate a risk model for estimating the risk of thromboembolism in patients with and without documented atrial arrhythmia*

e) *Assess the performance of the CHA_2DS_2-VASc score in this population*

Of 4821 HCM patients in the thromboembolism cohort 172 (3.6%) reached the primary endpoint within 10 years. Cox regression revealed an association between TE and age, AF, the interaction between age and AF, TE prior to first evaluation, NYHA class, LA

diameter, vascular disease and maximal LV wall thickness. There was a curvilinear relation between LA size and TE risk. The model predicted TE with a C-index of 0.75 (95% CI: 0.70, 0.80) and the D-statistic was 1.30 (95% CI: 1.05, 1.56). 27.5% of patients had a CHA_2DS_2-VASc score of 0 of whom 9.8% developed TE during follow up.

f) *Assess the effect of therapy with vitamin K antagonists (VKA) in patients with AF and their bleeding risk (HAS-BLED score)*

g) *Investigate effect of antiarrhythmic therapy on the development of AF*

VKA treatment was associated with a 54.8% (CI 31%-97%, p=0.037) relative risk reduction in HCM patients with AF. Calculation of the HAS-BLED score in a population of 536 patients with HCM and AF revealed a score of 0-2 in 500 patients (93.3%) with a score of 3-5 in 36 patients (6.7%).

An intention to treat analysis of β-blockers, calcium channel antagonists, disopyramide and amiodarone at baseline evaluation did not show a significant preventative effect on AF during follow up.

Conclusions

The literature review suggests that AF is common in HCM and associated with high thromboembolic risk. LA dimension and age are independently associated with AF but the existing literature is insufficient to create robust clinical tools to predict AF or thromboembolism. Most data suggest that AF patients should be anticoagulated.

Our study shows that the risk of TE and AF in HCM patients can be identified using a small number of readily available clinical features. This study confirms that AF is associated with a poor prognosis in HCM. LA size, in particular, should be monitored

closely and the assessment and treatment of conventional vascular risk factors should be routine practice in older patients. Exploratory analyses show for the first time evidence for a reduction of TE with VKA treatment. The CHA_2DS_2-VASc score performs poorly in patients with HCM and should not be used to assess TE risk in this population. There is no evidence for the efficacy of antiarrhythmic therapy in development of atrial arrhythmia.

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ABBREVIATIONS

AF:	Atrial fibrillation
ASA:	Alcohol septal ablation
ECG:	Electrocardiogram
EF:	Ejection Fraction
ESC:	European Society of Cardiology
FHSCD:	Family history of sudden cardiac death
FS:	Fractional shortening
HCM:	Hypertrophic cardiomyopathy
ICD:	Implantable Cardioverter-Defibrillator
LA:	Left atrium
LGE:	Late gadolinium enhancement
LV:	Left ventricle
LVH:	Left ventricular hypertrophy
LVOT:	Left ventricular outflow tract gradient
LVOTmax:	Maximum left ventricular outflow tract gradient recorded
LVOTO:	Left ventricular outflow tract obstruction
MWT:	Maximal wall thickness
NSVT:	Non-sustained ventricular tachycardia
NYHA:	New York Heart Association class
RCT:	Randomised controlled trial
SAM:	Systolic Anterior Movement

SBP: Systolic blood pressure

SCD: Sudden cardiac death

SR: Sinus rhythm

TE: Thromboembolism

VF: Ventricular fibrillation

VT: Ventricular tachycardia

1 INTRODUCTION

Hypertrophic cardiomyopathy (HCM) is a common genetic disorder of the heart muscle. It occurs in 1 in every 500 adults. In most individuals, it is inherited as an autosomal dominant trait caused by mutations in cardiac sarcomere protein genes and is associated with an increased risk of sudden cardiac death, stroke, long-term morbidity from progressive ventricular dysfunction and atrial and ventricular arrhythmias [1-3]. The histological hallmark of HCM consists of myocyte disarray, fibrosis and small vessel disease [4]. The hypertrophy is asymmetric in the majority of cases, but apical and concentric hypertrophy have also been described. A proportion of patients exhibit left ventricular outflow tract obstruction, systolic anterior motion of the mitral valve, which is associated with varying degrees of mitral regurgitation [5].

A physician from Geneva, Theophile Bonet (1620–1689) described cardiomegaly as a heart 'larger than that of any bullock' in a coachman who died suddenly [6]. William Harvey is also known to have described a patient with probable LVOT obstruction and symptoms of palpitations and chest pain [7]. Giovanni Maria Lancisi (1654–1720), a physician to Pope Clement XI, described deaths due to hypertrophy, dilatation and valve defects on autopsies of patients with sudden death [8]. Later Alfred Vulpian a physician and neurologist, coined the term ''subaortic stricture'' [9]. Two of his interns Hallopeau and Liouville then proposed the term "subaortic stenosis" [10-12]. Schmincke a German pathologist described left ventricular hypertrophy in the absence of physical exertion, renal or vascular disease [13]. Brock published on functional obstruction of the left ventricle and Teare first described the pathology of asymmetric hypertrophy of the heart in modern times [14]. Teare, who worked as a pathologist at St George's hospital reported on eight

patients with sudden cardiac death and also described 'bizarre arrangements of bundles of muscle fibres running in diverse directions and separated by connective tissue and clefts' [15]. Three of the patients presented with palpitation as early symptom and were found to be in atrial fibrillation.

Already since these times it has become clear that atrial fibrillation and stroke are common in HCM with significant impact on morbidity and mortality [16]. Further information on prevalence, incidence and predictors of AF and thromboembolism in addition to identification of high risk patients is therefore of clinical importance to guide medical therapy and particularly anticoagulation.

1.1 HCM: Definition and diagnostic criteria

HCM is defined as left ventricular hypertrophy in the absence of abnormal loading conditions, such as hypertension and valve disease [1-3;17-19]. The diagnosis relies on morphological assessment mostly by echocardiography and cardiac MRI with a maximal wall thickness of greater or equal to 15mm as usual diagnostic criteria for HCM in adults [3;17]. Familial hypertrophic cardiomyopathy is characterised by an autosomal dominant pattern of inheritance. First degree relatives of an individual with HCM therefore have a 50% probability of being a carrier of the genetic mutation. Often mutation carriers will not fulfil the diagnostic criteria due to incomplete penetrance and expression. In these individuals minor abnormalities on electrocardiography and echocardiography reflect expression of a pathogenic gene mutation in these individuals [20]. This disparity between affected and unaffected family members has several important implication, such as genetic testing, counselling and psychosocial factors. McKenna et al. therefore propose

major and minor criteria for the diagnosis of HCM in first degree family members. These criteria include echocardiography measurement, such as left ventricular wall thickness and systolic anterior motion of the mitral valve and also electrocardiography characteristics [20].

1.2 Epidemiology

Several methodologically diverse studies across the world suggest a prevalence of unexplained increase in LV thickness in the range of 0.02–0.23% in adults. The prevalence appears similar across different racial groups [1;21-25]. An American study of 4111 young patients selected from the general population revealed evidence of HCM in 7 subjects (0.17%) on the basis of identification of a hypertrophied, non-dilated left ventricle and a MWT of 15 mm. Prevalence was higher in men than women (0.26:0.09%) [1]. This male preponderance is common in most studies and remains unexplained, and might reflect study bias secondary to screening. Genetic and hormonal influences could be important as well [3]. A further study from the US of 714 consecutively studied outpatients referred for echocardiography HCM was present in 4 patients (0.5%). This cohort of patients was older with ages between 50 to 69 years [26]. Investigators in Olmsted County state an overall age- and sex-adjusted incidence rate of HCM of 2.5/100,000 person-years [23]. A Japanese study of 12841 worker revealed HCM in 22 out of the 1584 (1.38%) individuals who underwent echocardiography [22]. The prevalence of suspected HCM in 2066 young teenagers screened for the prevention of sudden death was 0.2% [27]. A retrospective study in western Denmark on a population 2 798 000 over a 2-year period revealed 20 subjects with HCM with an overall incidence $3.6/10^6$ population/year [28].

1.3 Histopathology

On a macroscopic level HCM is characterised by increased heart weight and hypertrophy in any distribution. There is anterior displacement of the papillary muscles and mitral valve leaflets [29]. HCM is characterised by myocyte hypertrophy and disarray (Figure 1). The myocytes display nuclear enlargement, pleomorphism and hyperchromasia. Fibrosis, small vessel disease and increased interstitial connective tissue are evident as well. The distribution can be regional and patchy [4;29;30]. The fibrosis leads to expansion of the interstitium and contributes to diastolic dysfunction and coats the small coronary vessels [30]. In addition reduced coronary flow reserve and fibrosis are caused by narrowing of the intramyocardial vessels due to intimal and medial smooth muscle hyperplasia [31].

Figure 1: Histopathology in HCM

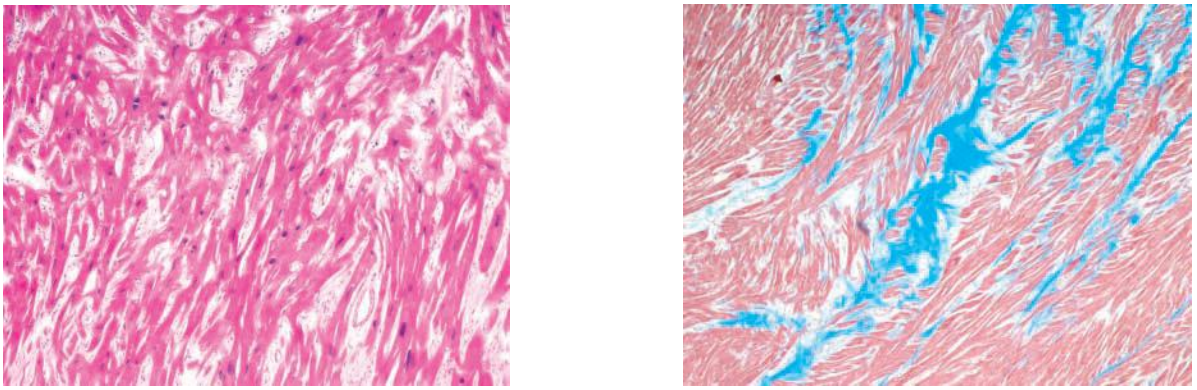


Figure reproduced from Hughes (2004). [29] The image on the left reveals myocyte disarray in a pinwheel pattern. Modified Masson's shows increased interstitial fibrosis (right image, blue).

1.4 Genetics and Pathogenesis

1.4.1. Sarcomere protein gene mutations

Data on genetics of adults suggest that up to 60% of patients have mutations in constituents of the cardiac sarcomere [32]. Hundreds of these mutations have been identified. Most of the sarcomeric protein mutation are missense [2]. Cardiac myosin-binding protein C (*MYBPC3*) and ß myosin heavy chain (*MYH7*) genes account for about 80% of mutations [33]. Others include cardiac troponin-T (*TNNT2*), troponin-I (*TNNI3*), troponin-C, *α*-tropomyosin (*TPM1*), cardiac actin (*ACTC*), titin (*TTN*) and *α* - cardiac myosin heavy chain [2;33-36]. By contrast, mutations in proteins of the thin filament, account for less than 10% of HCM cases. HCM-causing mutations in *TCAP* (telethonin), *MYOZ2* (myozenin-2, also known as calsarcin-1), *ANKRD1* (ankyrin repeat and KH domain-containing protein 1), *PLN* (cardiac phospholamban), *JPH2* (junctophilin-2), and *CAV3* (caveolin-3) have also been described; they encode genes responsible for Z-disk and non-sarcomeric proteins [37].

1.4.2. Genotype-Phenotype associations

The establishment of clinically useful relations between genotype and phenotype remains elusive in HCM. A recent systematic review reported a higher prevalence of family history of HCM and SCD, a younger age at presentation, and greater maximal left ventricular in individuals with a sarcomere gene mutation but no difference was reported in clinical characteristics when comparing *MYBPC3* and *MYH7* mutations. However, these data were limited by the inconsistency of study design and the small size of many study cohorts [38]. Some phenotypic associations have been described in the human or

murine model. *MYH7* mutations are commonly associated with more severe hypertrophy, while *TNNT2* defects only produce mild hypertrophy but are associated with overall higher incidences of sudden cardiac death than other HCM genes [39]. *MYH7* mutations tend to present earlier than patients with *MYBPC3* mutations, who present later in the fifth or sixth decades [40]. The pattern of hypertrophy might be linked to genetic status. Left ventricular apical hypertrophy has been reported in *TNNI3* mutations [41] and midcavity hypertrophy in *ACTC* [42] and *MYL3* mutations [43]. Different mutations in the same gene might predispose to certain phenotypes. Some mutations in *MYH7* are associated with particularly severe hypertrophy and predispose to sudden death (Arg403Gln) and heart failure (Arg719Trp). Mutations in Phe513Cys, Leu908Val, and Gly256Glu are associated with less severe outcomes [44;45]. Homozygous or compound heterozygous patients have more severe phenotypes than heterozygous patients [33;46]. More severe phenotypes can also be associated with an independent second mutation in about 5% of patients; triple mutations have been reported in 0.8% [47;48].

1.4.3. The structure of the sarcomere

Thick myosin filaments, thin actin filaments and titin filaments constitute the cardiac contractile machinery of the sarcomere. Accessory proteins such as *α*-tropomyosin, troponin I, troponin T and troponin C regulate the cyclical interaction between the cross-bridges of the filaments. Individual sarcomeres form myofibrils by connecting in series, which are surrounded by a system of membrane-bound tubules called the sarcoplasmic reticulum (SR) [49;50]. Z-discs are transverse structures, which delineate the lateral borders of sarcomeres and anchor the thin filaments representing the smallest functional units in striated muscle. The core of a Z-disc consists of actin filaments

coming from adjacent sarcomeres which are crosslinked by *α* actinin molecules. They are the centre of the I-band composed of hundreds of different proteins [51;52]. M-bands crosslink the thick filaments in the centre. Elastic titin filaments connect both transverse structures longitudinally [53].

1.4.4. The function of the sarcomere

The contractile machinery is activated by Ca^{2+}-controlled conformational change of the proteins troponin and tropomyosin and subsequent interaction between troponin-tropomyosin units along the actin filament. Calcium enters the cell during the cardiac action potential through depolarization activated Ca^{2+} channels. This results in Ca^{2+} release from the sarcoplasmic reticulum (SR). The raised intracellular Ca^{2+} - concentration causes myofilament protein troponin C binding, which results in a conformational change and contraction. The tropomyosin strand undergoes azimuthal movements on the actin filament and permits cross-bridge cycling.

A decrease in Ca^{2+} - concentration, its removal from troponin C and therefore relaxation is achieved by several mechanisms that results in transport out of the cell. These include reuptake of Ca^{2+} into the SR through Ca^{2+} ATP ase, sarcolemmal Na +/ Ca^{2+} exchange, sarcolemmal Ca^{2+} - ATPase or mitochondrial Ca^{2+} uniport [50;54;55].

1.4.5. Non-Sarcomeric gene mutations

A further 5-10% of adult HCM are caused by genetic disorders including metabolic and neuromuscular diseases, chromosomal abnormalities and genetic syndromes [56].

Metabolic disorders include Anderson-Fabry disease [57]. A further mutation, which can give rise to a phenotype of hypertrophic cardiomyopathy in addition to other characteristics such as glycogen vacuoles on cardiac biopsy, Wolff-Parkinson-White syndrome and conduction disease is found in the gamma-subunit of AMP kinase [58]. Other causes include mitochondrial cardiomyopathies [59] and infiltrative diseases such as amyloid. [60]

In younger patients hypertrophy of the left ventricle is mostly associated with congenital malformations inherited metabolic disorders (glycogen storage diseases), neuromuscular diseases and syndromes [61;62]. Friedreich's ataxia is a neuromuscular disease with neurological and endocrine sequelae associated with left ventricular hypertrophy [63]. Malformation syndrome with autosomal dominant mutations inheritance in tyrosine phosphatase, non-receptor-type II protein (PTPNII) include Noonan's syndrome and LEOPARD syndrome [64;65].

Epigenetic influence and disease modifiers appear to play an important role. They might explain variable disease penetrance in terms of outcome, phenotype and age of onset [66].

1.4.6. Pathogenesis

Impaired contractile function has been implicated as a compensatory cause of hypertrophy leading to diastolic dysfunction [67;68]. Conflicting evidence however exists with mutations, that increase or decrease contractility [69;70]. Impaired biomechanical stress sensing leading to hypertrophy in response to load might also be an important factor. A protein critical in this regard is titin [71].

Abnormal Ca^{2+} flow and sensitivity has also been implicated in the pathogenesis [72]. A HCM mutation in *α*–tropomyosin has been shown to cause hypersensitivity of calcium dependent contraction [73]. Calcium channel antagonists have also been shown to reduce myocardial hypertrophy in transgenic mice with a α-cardiac myosin heavy chain mutation [74]. A further theory of abnormal bioenergetics has been strengthened by the association of cardiac hypertrophy with mitochondrial and AMP kinase mutations [75-77]. Rats with troponin T mutations revealed a 30% reduction of phosphocreatine : ATP ratio by NMR spectroscopy reflecting abnormal cardiac energetics. Similar results are evident in patients with sarcomeric mutations even in absence of a phenotype [78;79]. Fibrosis following apoptosis of myocytes secondary to microvascular ischaemia and hypertrophy leads to focal scarring [80;81]. This can lead to the arrhythmic phenotype in HCM in addition to myocyte disarray and increased muscle mass [82;83]. Interestingly no association between fibrosis arrhythmia and disarray was found in murine models [84;85].

1.5 **Clinical features**

1.5.1. Diastolic dysfunction

Diastolic dysfunction is a major cause of morbidity in HCM and predisposes to the development of atrial fibrillation [86]. It is caused by a decrease and prolongation in isovolumetric left ventricular relaxation and alteration of the filling dynamics [87-89]. Abnormalities of the dissociation of actin and myosin in the early filling phase and abnormal relaxation leading to impaired filling and abnormal compliance and stiffness of the left ventricle are responsible for the increase in left atrial and left ventricular end-diastolic pressures [89-94]. The volume of the left atrium is reflective of the diastolic state of

the left ventricle and an important predictor for the development of AF and thromboembolism [16]. A comprehensive evaluation of diastolic function requires transmitral Doppler, tissue Doppler of the mitral annulus, isovolumetric relaxation time pulmonary vein flow and pulmonary artery pressure [95;96]. These may be abnormal on echocardiography prior to development of the phenotype especially in younger individuals [97-99].

A large proportion of symptoms, such as shortness of breath and chest discomfort, are secondary to diastolic dysfunction, especially if LVOT obstruction is absent. Restrictive cardiomyopathy is a phenotype, in which the diastolic abnormalities dominate the clinical picture [2;100].

Patients with these symptoms commonly respond to therapy with ß-blockers, Ca^{2+} channel antagonist, which prolong diastole and relaxation in addition to diuretics (especially spironolactone) and nitrates [2;101;102]. Metabolic modulators such as perhexiline have been shown to improve diastolic dysfunction and exercise capacity [103].

In a study of 100 patients with HCM 28% showed a progression of left ventricular diastolic dysfunction defined as restrictive filling pattern over a follow up period of 109+-67 months. Diastolic dysfunction was associated with a poor prognosis in terms of death and heart transplantation [104], which represent a therapeutic option in end-stage patients [105].

1.5.2. Systolic dysfunction

Systolic impairment is a common feature of HCM. It is also well recognised that a proportion of patients with HCM progress to a ‘burnout’ or end-stage phase. In a study

of 1080 patients 2.4% of patients presented with systolic impairment. A further 4.8% of the patients undergoing echocardiography developed systolic impairment during a follow up of 66 months. Systolic impairment is a marker of poor prognosis with almost 60% of the patients dying or requiring heart transplantation compared with less than 10% with normal systolic function [106]. A study from the US assessed 293 patients over a median follow up of 6 years. Seventeen percent of patient developed progressive heart failure. Thirty percent of those patients developed end-stage systolic dysfunction and 48% heart failure, which was classified as non-obstructive with preserved systolic function [107].

Age, family history of HCM and wall thickness are risk factors in the evolution of dilated-hypokinetic HCM [108]. Small vessel disease, ischaemia and progressive fibrosis appear to be important in the development of systolic impairment [4;109]. Severe microvascular dysfunction was reported to be a predictor of adverse LV remodelling and systolic dysfunction in a small cohort of patients with HCM assessed by resting and dipyridamole myocardial blood flow [110]. Elevated levels of serum C-terminal propeptide of type I procollagen indicated increased myocardial collagen synthesis and therefore fibrosis in sarcomere-mutation carriers without overt disease. This preceded the development of left ventricular hypertrophy or fibrosis visible on MRI [111].

Mechanism such as necrosis, apoptosis, increased fibroblast proliferation, oxidative stress and cytokines important in the development of heart failure in ischaemic heart disease most likely play a role as well [112;113]. Genetic factors also predispose to systolic impairment. Certain sarcomeric mutations in *α* –tropomyosin, troponin T and ß -myosin

heavy chain have been implicated in hypertrophic cardiomyopathy and dilated cardiomyopathy [114-116].

Ejection fraction (EF) measurement to assess systolic impairment is challenging and is commonly increased when measured in HCM patients in view of increase in wall thickness and decreased chamber size [117]. Simpson's method assumes that LV volume can be calculated by the summation of a stack of elliptical discs, which are affected by hypertrophy. The area is determined by the LV diameter in 2- and 4-chamber views [118]. CMR has advantages in assessment of systolic function in HCM [119]. Doppler and systolic strain can determine systolic abnormalities in patients with HCM despite a normal ejection fraction [120;121].

In patients with normal EF but symptoms of breathlessness rate limiting medication to improve LV filling and loop diuretics are recommended [3]. Patients with systolic impairment and heart failure symptoms should be treated according to the ESC Guidelines for the management of chronic heart failure. Treatment includes diuretics, ß-blockers, angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARB) and mineralocorticoid receptor antagonists (MRA) [122]. If drug therapy fails CRT can be considered in a subset of patients [3]. Cardiac transplantation represents a therapeutic option in end-stage patients [105].

1.5.3. Left ventricular outflow tract obstruction

Approximately a quarter of patients with HCM present with resting left ventricular outflow tract obstruction (LVOTO). The gradient is usually measured by continuous wave Doppler and associated with posteriorly directed mitral regurgitation due to

incomplete leaflet apposition [2]. LVOTO is characteristically labile and dynamic. The severity of the gradient varies spontaneously and may just be evident post-prandially or after haemodynamic challenge [5;123].

The mechanism described involves the anterior mitral valve leaflet, less commonly the posterior leaflet, to undergo Venturi and or drag forces during systole leading to contact of the leaflet with the interventricular septum. A high velocity flow in the outflow tract lifts the mitral valve towards the septum called the Venturi mechanism. The drag mechanism describes the pushing force of flow initiating the anterior motion of the mitral valve [124-130]. Occasionally this can cause thickening and distortion of the mitral valve [131]. Obstruction can also be secondary to abnormal insertion of the papillary muscle to the mitral valve leaflet [132;133]. Right ventricular obstruction is rare but can be observed in patients with Noonan's syndrome [2].

LVOTO has been shown to be a risk factor for HCM related death, specifically stroke and heart failure and a predictor of AF symptomatic progression to NYHA class III and IV [134]. A low sudden death rate has been reported in asymptomatic patients with LVOTO [135]. About two-thirds of patients with symptomatic non-obstructive HCM have been shown to have latent LVOTO. Exercise stress echocardiography is therefore required in this cohort [136].

The consequences of LVOTO are at least partially related to LV wall stress and myocardial ischaemia with subsequent apoptosis of myocytes and fibrotic replacement [137;138]. Intervention is therefore justified in patients with significant LVOTO and significant symptoms on maximal medical therapy [101;139;140].

Medical therapy consists of treatment with ß-blockade, Ca^{2+} channel antagonist and addition of disopyramide if symptoms are not adequately controlled [141-144].

For the minority of patients that require further therapy the gold standard intervention for relief of LVOTO remains a septal myectomy operation. Muscle from the subaortic region is resected to enlarge the LVOT. This abolishes or decreases SAM and subsequently the obstructive gradient [5]. In the Morrow procedure a complete median sternotomy is performed and the hypertrophied interventricular myocardium is accessed via an aoortotomy. Myotomies are performed to excise the myocardial tissue [17;145].

An alternative to the surgical approach is a percutaneous transluminal septal myocardial ablation also termed alcohol septal ablation (ASA) [146]. Alcohol is injected in the first septal branch through the central lumen of an over-the-wire balloon catheter inflated in the target vessel. The optimal septal branch is identified via contrast echocardiography [147]. About 90% of patients have an acute reduction of their LVOT gradient following the procedure [148-151]. As part of the remodelling process a further reduction of the gradient can be expected within 12 months. During this time patients show significant improvement in NYHA class, exercise capacity and peak oxygen consumption [152;153]. Evidence also exists for reduction in systemic pulmonary artery pressure, left atrial dimensions, improvement in left ventricular diastolic dysfunction and improved myocardial blood flow [151;154;155]. Following ASA a right bundle branch patterns is common, whereas left bundle branch block is seen in patients following myectomy [152;156].

A meta-analysis of 12 studies to compare outcomes of both procedures does not report any significant difference between short and long-term mortality, functional status post-

procedure, ventricular arrhythmias, the need for re-intervention and mitral regurgitation. Importantly a significant increase in RBBB and need for permanent pacing was found in the alcohol septal ablation group along with a higher residual gradient in the left ventricular outflow tract [157].

In patients who are not suitable for surgery or ASA, without significant hypertrophy or sigmoid shape of the interventricular septum and in elderly patients it has been recognised that right ventricular pacing with short AV-delay can reduce the LVOT gradient and improved symptoms have been reported [3;158;159]. A comparison of DDD pacing with myectomy showed a greater reduction in the LVOT and symptomatic improvement in patients undergoing surgery [160]. Recent data have re-examined the efficacy of dual chamber pacing for refractory symptomatic LVOTO [161;162]. In a recent Cochrane review it was noted that all data derive from small studies and that the few randomised trials concentrate on physiological outcome measures rather than hard clinical end-points [163;164].

1.5.4. Arrhythmia

1.5.4.1. Ventricular arrhythmias

Ventricular arrhythmias are common in patients with HCM. Non-sustained ventricular tachycardia (NSVT) occurs in about a quarter of patients. It is typically comparatively slow and asymptomatic. This is defined as ≥3 consecutive ventricular beats at ≥120 beats/min lasting <30 seconds. NSVT is an independent predictor of SCD [135;165-167], which is not influenced by frequency, duration or rate [165;168] . The prevalence increases with age and is linked to left ventricular wall thickness and also the presence of late gadolinium enhancement on cardiac magnetic resonance imaging [165;169].

Asymptomatic NSVT does not usually require antiarrhythmic therapy but if it is poorly tolerated ICD therapy and treatment with ß-blockers or amiodarone should be considered [17]. If a focal origin is suspected an EP study and ablation may be of value [170]. Exercise induced VT carries a high risk of SCD [171]. Documented sustained monomorphic VT, defined if lasting longer than 30 seconds is uncommon but may be associated with LV apical aneurysms [172;173]. A study of 178 patients with HCM revealed 31% of patients with evidence of NSVT on Holter monitoring. This was associated with degree of LVH and severity of symptoms and was associated with sudden death [174].

1.5.4.2. Atrial fibrillation

Atrial fibrillation is the most common arrhythmia in HCM. An Italian registry reports AF in 18% of 1677 patients over a follow up period of 9.7 years [175]. Another study on 900 patients reveals a prevalence of 21.3% [176]. A similar proportion is reported in a cohort of 480 patients [177]. Several predictors have been identified for AF. These include age [178], LA dimension [179], NYHA class [177], LVOT gradient and p-wave duration [134], LV wall thickness [180] and LV dimensions and fractional shortening [181]. Genotype has also been implicated in the development of AF [180;182;183].

Onset of AF can cause symptomatic deterioration and heart failure. One mechanism for this is the loss of the atrial component of cardiac filling [184]. AF is also a significant predictor of heart-failure related mortality, stroke and severe functional disability. A mortality of 15.4% of HCM related causes in patients with HCM compared with a 35% mortality of HCM related causes in patients with AF in 480 patients has been reported.

The annual HCM mortality was 3% in patients with AF as compared to 1% in patients in sinus rhythm [177].

Treatment consists of ß-blockade and non-dihydropyridine Ca^{2+} channel antagonism for rate- and amiodarone for rhythm control [177;179;184;185]. A small study has shown efficacy of sotalol in suppression of supraventricular arrhythmias [186]. Data on ablation therapy in these patients reveals beneficial outcomes with persistence of sinus rhythm in about 60% of patients at 1 year follow up [187]. Due to the high risk of thromboembolism current guidelines recommend that all patients with HCM and AF should be anticoagulated [3;17]. One study on 900 patients reports a cumulative incidence of thromboembolism among non-anticoagulated patients twice that of patients on anticoagulation [176]. Interestingly LVOT obstruction has been shown to be associated with enhanced thrombin generation and platelet activity in patients with HCM in sinus rhythm indicating a general procoagulant state of HCM patients [188].

1.5.4.3. Brady-arrhythmia

Sinus node and atrio-ventricular node dysfunction are uncommon in HCM. Treatment is according to recent guidelines for cardiac pacing [189]. A study investigating 451 patients with HCM for severe conduction disease reports sinus node disease or atrioventricular conduction abnormalities in 10.6% of patients. Eight percent of patients underwent implantation of a pacemaker for primary brady-arrhythmia [190]. An EP study on 13 patients with HCM showed a large incidence of conduction system abnormalities. Ten out of 12 patients had a prolonged HV interval [191]. A further study on 155 HCM patients who underwent an EP study reports a high incidence of sinus node (66%) and His-

Purkinje HV node disturbances [192]. Bradycardia is exacerbated by Ca^{2+}-channel and ß-blockade used for the treatment of systolic or diastolic dysfunction or LVOT obstruction [17]. In young patients with AV-block specific subtypes of mutations should be suspected such as mutations in the gamma-subunit of AMP kinase (PRKAG2) [2;58;193] or desmin [194]. Cardiac amyloid or Fabry disease might be the cause of conduction disease in older individuals [2;60;195;196]. It is also a recognised feature in mitochondrial disease [197].

1.5.5. Thromboembolism

Cerebrovascular accidents, transient ischaemic attacks and peripheral emboli are common in HCM and associated with a high burden of morbidity and mortality [176]. In a cohort of 900 patients 51 (6%) experienced stroke or other vascular events. Forty-one of these patients died or were permanently disabled. An incidence of 0.8%/year was reported [176]. The prevalence of stroke in patients with HCM and AF was 21% in a cohort of 480 patients. Several predictors have been identified for thromboembolism. These include atrial fibrillation, age and NYHA class III and IV [176], gender, LA dimension, left ventricular end diastolic volume and fractional shortening [181]. Stroke was less common in anticoagulated patients as compared to antiplatelet therapy in two observational studies [176;177]. The role of anticoagulation with novel agents has not been evaluated.

1.5.6. Infective endocarditis

Infective endocarditis is a rare complication of HCM with an incidence in patients with LVOTO of 3.8 per 1000 person-years and the probability of endocarditis 4.3% at 10 years. It is usually seen in patients with LV outflow tract obstruction and LA dilatation. [198]. At the moment antibiotic prophylaxis is not generally recommended by the National

Institute of Clinical Excellence (NICE Guideline, CG64, March 2008). It should be considered for high-risk procedures in patients with prosthetic heart valves or prosthetic material used for valve repair, congenital heart disease or a history of previous endocarditis in accordance with the ESC/EACTS guidelines on the management of valvular heart disease [199].

1.6. Mortality

Most current series report an overall cardiovascular mortality of 1-2%/year. Sudden cardiac death (SCD), heart failure and thromboembolism are the main causes [200-202]. One study of 956 patients reports an annual rate of SCD of 1.02%, of heart failure of 0.55% and of and stroke 0.07% [200]. Earlier studies suggested higher rate of mortality between 3-6% [203;204]. The difference is most likely because of tertiary centre bias as patient with high risk patient are more likely to be referred to tertiary institutions [205]. Earlier recognition of disease due to systematic screening and improved treatment undoubtedly play a role as well [200].

1.6.1. Sudden cardiac death

There are several mechanism thought to responsible for SCD. Abnormal myocardial architecture, myocyte disarray and fibrosis lead to increased dispersion and abnormal intraventricular conduction. This creates the conditions for re-entry and arrhythmogenesis [100;206;207]. On a cellular level marked disorganisation of intercalated discs with localised abnormalities in desmosome organisation and abnormal gap junctions [208] in addition to altered myofilament Ca^{2+} sensitization have been found to be responsible for ventricular arrhythmia [209].

Certain subgroups of patients are at higher risk of SCD [210] and require consideration of implantation of a defibrillator (ICD) [17]. The main course of SCD is episodes of VF or sustained VT [211]. Conduction disease such as atrioventricular block, asystole and pulseless electrical activity can also occur [212-217]. A study of 16 patients, who had survived a cardiac arrest shows that these patients remain at risk for recurrence of their event [213]. Non-sustained ventricular tachycardia, maximal wall thickness ≥30mm, family history of SCD, unexplained syncope, and abnormal blood pressure response to exercise are historically used to estimate the relative risk and to guide implantation of an ICD [17]. Patients with multiple risk factors have an increased risk of sudden death [218]. One clinical approach was that the presence of two risk factors leads to consideration of ICD therapy [17]. This approach does not take the effect size of individual risk factors into consideration and the risk factors are considered as binary [219]. Some risk factors such as thickness of LV wall have also been reported to carry a continuous risk [220].

A recent multi-centre longitudinal cohort study of 3675 patients with HCM developed and validated a model to predict SCD [221], which is now incorporated into recent ESC guidelines [3]. The model utilises left atrial diameter, peak left ventricular outflow tract gradient and patient age in addition to the risk factors from previous guidelines to estimate and individualised absolute risk of SCD at 5 years. Abnormal blood pressure response is not included.

A large majority of data on sudden death stems from patient follow up following implantation of ICDs. An Australian study reports an appropriate intervention rate of the device of 11%/year [222]. A similar ICD intervention rate for secondary prevention occurred in 103 out of 502 patients. Out of 726 patients with HCM 45 (6.2%) had an

ICD. The annual appropriate ICD therapy rate was 11.1% for secondary prevention and 1.6% for primary prevention [223].

1.6.2. Heart Failure

Heart failure is the second most common cause of death in HCM and has been discussed in sections 1.5.1. and 1.5.2.. In a study of 106 patients with severe LVH over a follow-up of 92 +/- 50 months 13 patients had sudden cardiac deaths, 2 heart failure deaths and one patient underwent cardiac transplantation. Five-year survival from heart failure death or transplantation was 97.7% [224]. A further study of 293 consecutive HCM reports development of heart failure in 50 (17%) patients over a median follow-up of 6 years. Eighteen patients of these died or underwent transplantation [107].

1.6.3. Stroke related death

Cardiac thrombi causing cerebrovascular accidents, transient ischaemic attacks and peripheral embolism are common in HCM leading to a high burden of morbidity and mortality [16]. This is discussed in Section 1.5.5.

1.7. Rationale of research questions

AF and TE are very common comorbidities in patients with HCM. Unfortunately most data on these stem from observational studies. The large majority of these studies investigate small patient numbers and are poor in quality. The rationale of this work is to investigate the characteristics and predictors of AF and TE in the largest multicentre cohort assessed to date and to develop a risk prediction tool to guide treatment of patients. Exploratory analyses will also address the questions of anticoagulation, the

use of the CHA_2DS_2-VASc score and investigate the effect of AF on mortality and treatment with anti-arrhythmics.

2 THESIS OBJECTIVES

The aim of this thesis is to examine several clinical aspects of AF and TE in HCM using a large cohort of patients. The study cohort consisted of all consecutively evaluated patients with HCM, followed at seven European centres: (i) The Heart Hospital, London, UK, (ii) A Coruna University Hospital, A Coruna, Spain, (iii) Unit of Inherited Cardiovascular diseases, 1st Department of Cardiology, University of Athens, Greece, (iv) Institute of Cardiology, University of Bologna, Italy, (v) University Hospital Virgen de la Arrixaca, Murcia, Spain, (vi) Monaldi Hospital, Second University of Naples, Italy and (vii) Hospital Universitario Puerta del Hierro, Madrid, Spain.

a) Systematic review of atrial fibrillation (AF) and thromboembolism (TE) in patients with HCM and meta-analyse prevalence and incidence of AF and TE

Even though AF and TE are very common in HCM very little is known about predictors, treatment, morbidity and mortality. Estimates of incidence and prevalence also vary in the available literature. This analysis is presented in Chapter 3: Systematic review of atrial fibrillation and thromboembolism in patients with hypertrophic cardiomyopathy and meta-analysis of prevalence and incidence, page 44.

b) Assess prevalence, incidence and predictors of AF in a large multicentre cohort

c) Investigate the effect of AF on mortality

Most studies investigating predictors of AF are limited by the size of the cohort with cohorts of only a few hundred patients. The primary aim of this study was to investigate predictors of AF in a large multicentre cohort. Exploratory analyses were performed to investigate the effect of AF on mortality. This analysis is presented in Chapter 4: Predictors of atrial fibrillation in patients with hypertrophic cardiomyopathy, page 70.

d) Assess clinical predictors of thromboembolism and derive and validate a risk model for estimating the risk of thromboembolism in patients with and without documented atrial arrhythmia

e) Assess the performance of the CHA_2DS_2-VASc score

AF and TE are common complications of the disease and are associated with adverse clinical outcomes and reduced survival. However, HCM is a heterogeneous disorder with very variable clinical presentation and the absolute risk of TE – and by implication the likely benefit from treatment – in individual patients with different clinical characteristics is unknown.

The analysis of a risk model for estimating the risk of thromboembolic events in patients with and without documented atrial arrhythmia and an exploratory analysis to determine the performance of the CHA_2DS_2-VASc score is presented in Chapter 5: Prediction of thromboembolic risk in patients with hypertrophic cardiomyopathy (HCM Risk-CVA), page 87.

f) Assess the effect of therapy with vitamin K antagonists (VKA) in patients with AF and assess the bleeding risk in patients with HCM using the HAS-BLED score

g) Investigate effect of antiarrhythmic therapy on the development of AF

Current ESC and ACCF/AHA guidelines recommend anticoagulation in all patients with HCM and AF. The efficacy of vitamin K antagonists has not been proven in patients with HCM and AF in randomised controlled trials. The exploratory analyses to assess the outcome of therapy with vitamin K antagonists (VKA) in patients with AF and their bleeding risk and the effect of antiarrhythmic therapy on the prevention of AF are presented in Chapter 6: Outcome of anticoagulation and antiarrhythmic therapy in patients with HCM, page 110.

3 Systematic review of atrial fibrillation and thromboembolism in patients with hypertrophic cardiomyopathy and meta-analysis of prevalence and incidence

3.1. Aims

Many cohort studies have shown that atrial fibrillation (AF) is a common complication of the disease and that it is associated with adverse clinical outcomes. However, current guidelines for the management of AF lack detailed advice on the management of AF in patients with HCM because of an absence of adequately powered studies on pharmacological treatment strategies in this setting. The primary aim of this study was to perform a systematic review and meta-analysis of the published literature on atrial fibrillation and thromboembolism in patients with HCM to determine the feasibility of developing a disease specific algorithm for the management of AF.

3.2. Methods

The methodology and presentation of the review are based on the recommendations of the PRISMA statement [225].

3.2.1. Study selection

Two independent reviewers (OG and AA) performed the literature search and checked the eligibility of each study. Disagreement between the two reviewing authors was resolved by consensus with a third author (PE).

PubMed and Web of Science electronic databases were searched using the terms “hypertrophic cardiomyopathy”, “atrial fibrillation”, “stroke” and “thromboembolism” in title and abstract. Reviews, case reports and abstracts were excluded from the analysis. The reference lists of reviews, letters and editorials were scrutinised for additional papers. All searches were limited to ‘human’ and ‘English’. The last search was performed on 1st September 2012. Studies were eligible if they investigated atrial fibrillation and/or stroke or thromboembolism as primary or secondary outcome in a prospective or retrospective study.

The following variables were extracted from each study: number of patients, sex, mean age, predictors of AF and thromboembolism, prevalence of AF and thromboembolism, definitions of AF and stroke/thromboembolism and length of follow up (tables 1, 2). Where available the effect of antiarrhythmics, anticoagulants, and AF ablation on morbidity and mortality was investigated.

3.2.2. Meta-analysis

Studies reporting prevalence data for atrial fibrillation and thromboembolism were included in the meta-analysis. Not all studies provided standard error (SE) of the prevalence and so SE was calculated using the prevalence data and sample size in each study with the following formula:

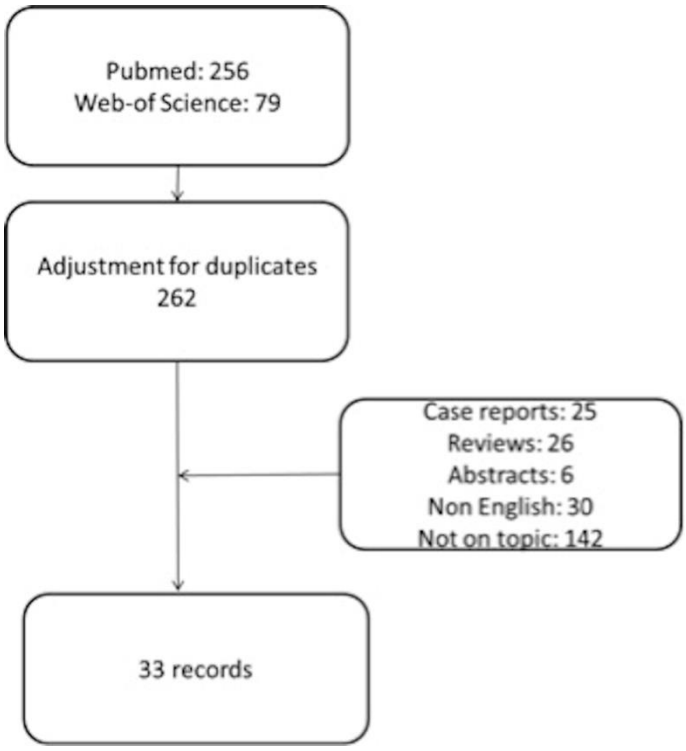
$$\text{SE (prevalence)} = \sqrt{\frac{prevalence(1-prevalence)}{sample\quad size}}$$

Assuming that the estimated values for prevalence follow a normal distribution we calculated 95% confidence interval for population prevalence. Incidence rate for each study was calculated using number of new cases and median follow-up time provided by the study. Standard error of incidence rate was calculated assuming Poisson distribution of number of new cases. A random effect meta-regression model [226] was then used to combine the prevalence data and to obtain the pooled (overall) prevalence and incidence for AF and thromboembolism separately. The overall prevalence was the weighted average of the prevalence across different studies, where weights were calculated using measures of precision (inverse of the variance of the prevalence). Intra- and inter-study variances were used in the calculation of precision. The intra-study variance was the variance of the prevalence obtained as above (square of SE) for each study. The inter-study variance, a parameter of the random effects meta-regression model, was estimated using method of moments [226]. The inter-study variance was used to adjust for the heterogeneity in prevalence between studies. Heterogeneity between studies was further assessed using I^2 statistic, which represents the proportion of total variability in the prevalence data attributable to the heterogeneity between the studies. The overall estimate, obtained using this method, takes more weight from the study with higher precision associated with smaller SE. A random effect meta-analysis was conducted to obtain an overall cut-off value of LA diameter for both SR and AF, using average median values of LA diameter from individual studies in patients with SR and AF. All these computations were conducted using Stata Statistical Software, version 11.

3.3. Results

Two hundred and fifty six articles in Pubmed and 79 in Web of Science fulfilled the search criteria. After adjustment for duplicates, 262 unique records remained. Following exclusions (figure 2), 33 records reporting data on 7381 patients remained (table 1) [134;176-184;201;202;227-247]. All studies were observational or used retrospective data collection.

Figure 2: Study selection process. The flow chart shows the study selection process



From Guttmann *et al* (2013) [16]

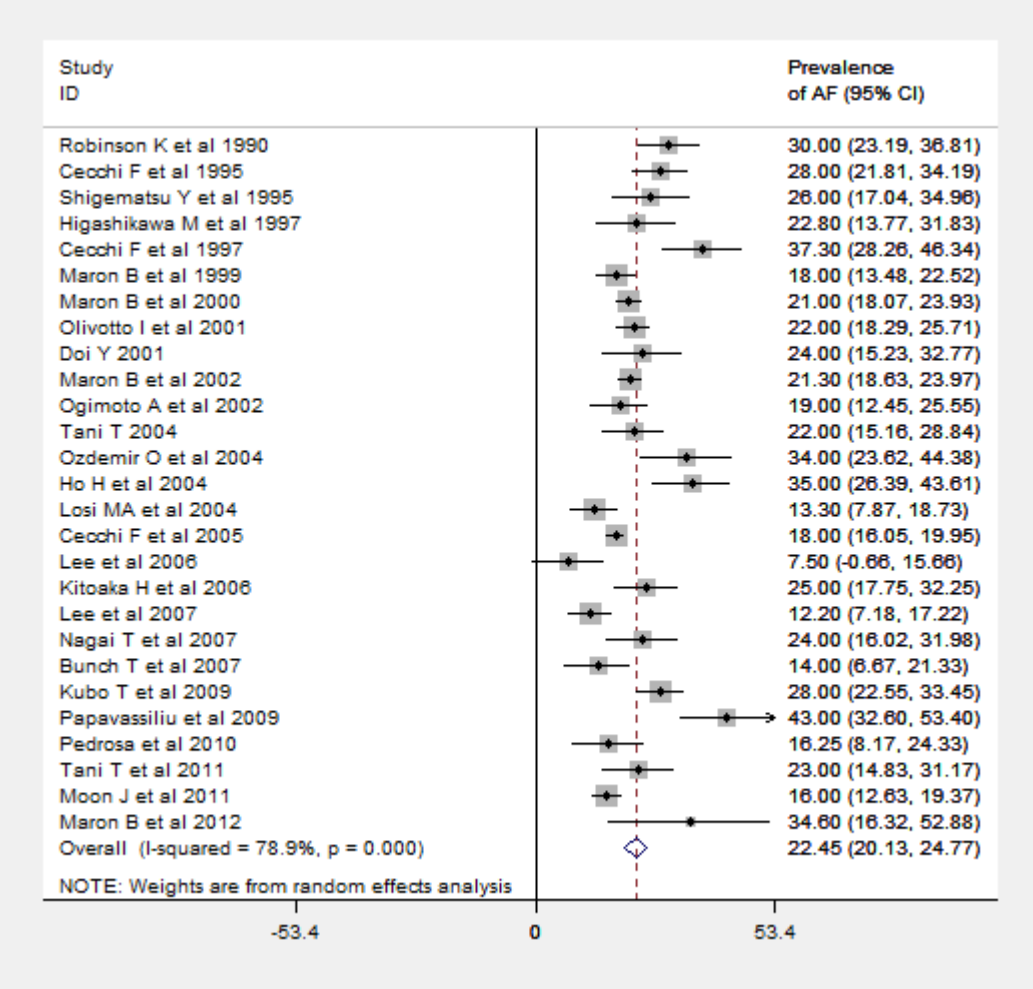
3.3.1. Incidence and prevalence of thromboembolism and AF

The definitions of AF varied between studies, but most included patients with paroxysmal and chronic AF. Six studies investigated only paroxysmal AF. Four studies defined paroxysmal AF by duration or symptoms (table 1).

The pooled overall prevalence of paroxysmal and permanent AF was 22.45 % (95% CI: 20.13-24.77, I^2= 78.9%, p<0.001), figure 3. The pooled overall prevalence of all thromboembolic complications in patients with HCM and AF was 27.09% (95% CI: 20.94-33.25, I^2= 61.4%, p<0.01), figure 4. The pooled overall incidence of paroxysmal and permanent AF was 3.08 % (95% CI: 2.63-3.54, I^2= 86.5%, p<0.001), figure 5. The reported overall incidence of AF in two studies was 2 and 2.6%, respectively [177;232]. The pooled overall incidence of all thromboembolic complications in patients with HCM and AF was 3.75% (95% CI: 2.88-4.61, I^2=37.9% (p=0.1), figure 6.

Figure 3: Prevalence of AF

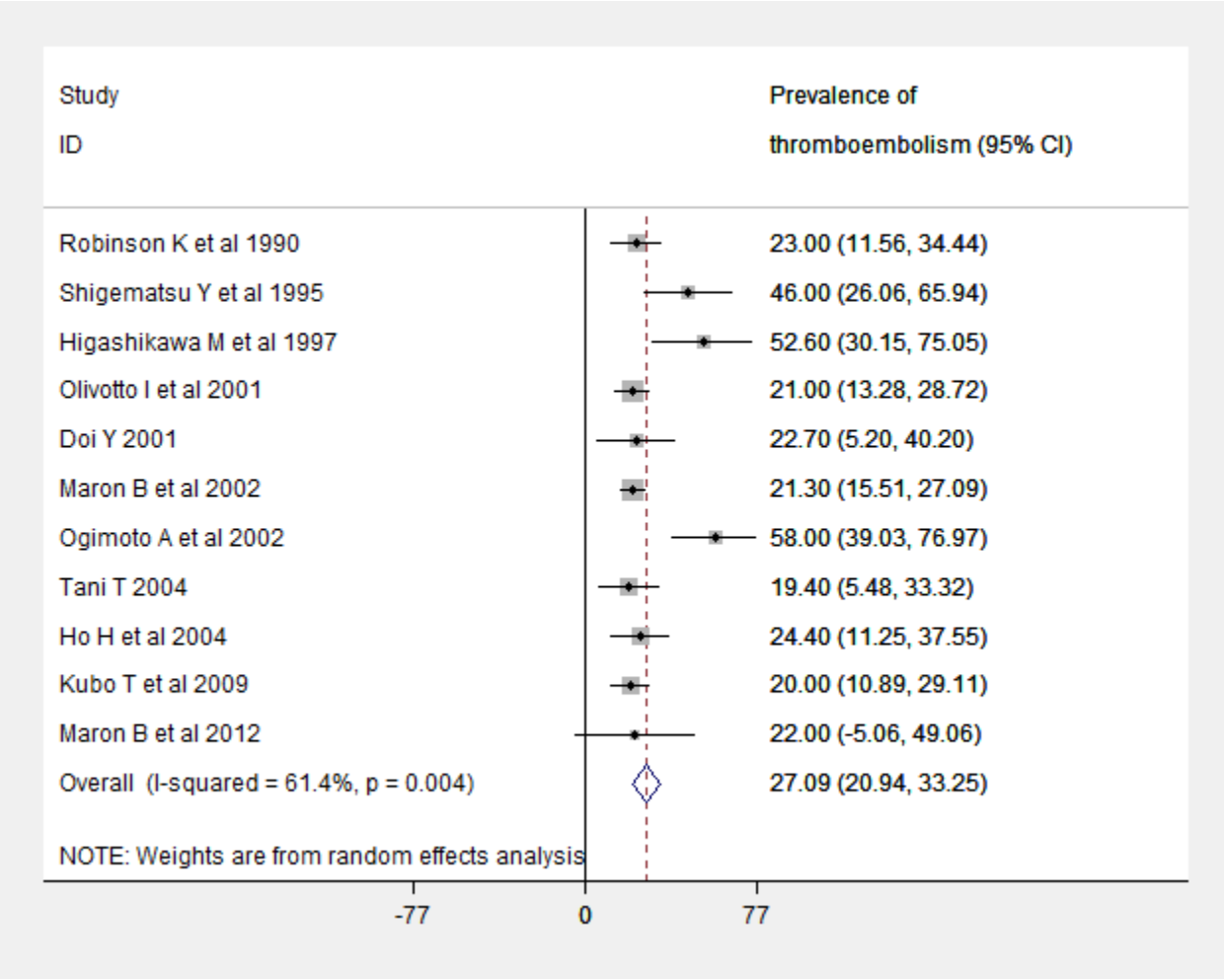
The forest plot from random effect meta-analysis showed study specific prevalence and the pooled (overall) prevalence of AF. The heterogeneity between the study was assessed by I^2 statistic estimated as, I^2=78.9%, p<0.001.The overall prevalence is 22.45%.



From Guttmann *et al* (2013) [16]

Figure 4: Prevalence of thromboembolism

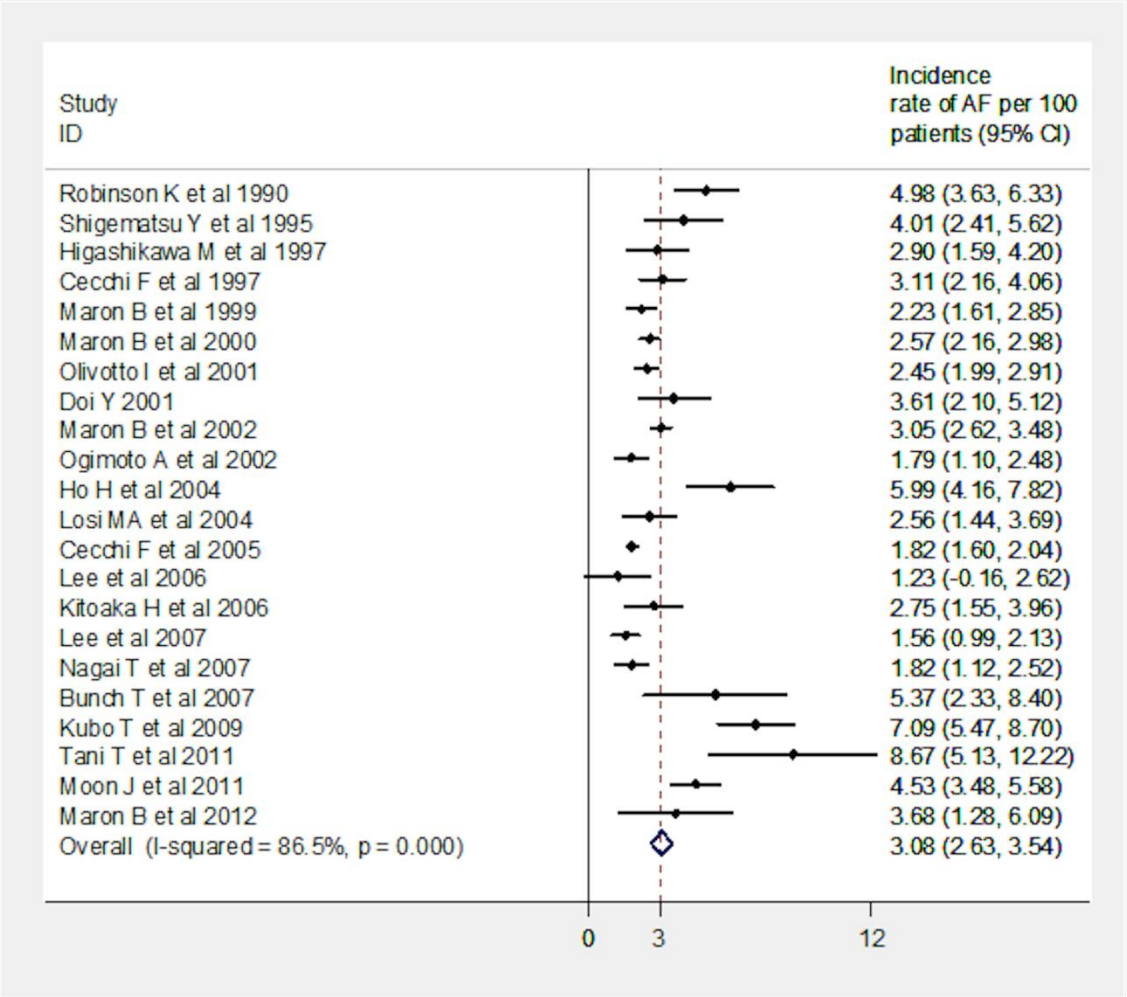
The forest plot from random effect meta-analysis showed study specific prevalence and the pooled (overall) prevalence of thromboembolism in patients with HCM and AF. The heterogeneity between the study was assessed by I^2 statistic estimated asI^2=61.4%, p<0.01.The overall prevalence is 27.09%.



From Guttmann *et al* (2013) [16]

Figure 5: Incidence of AF

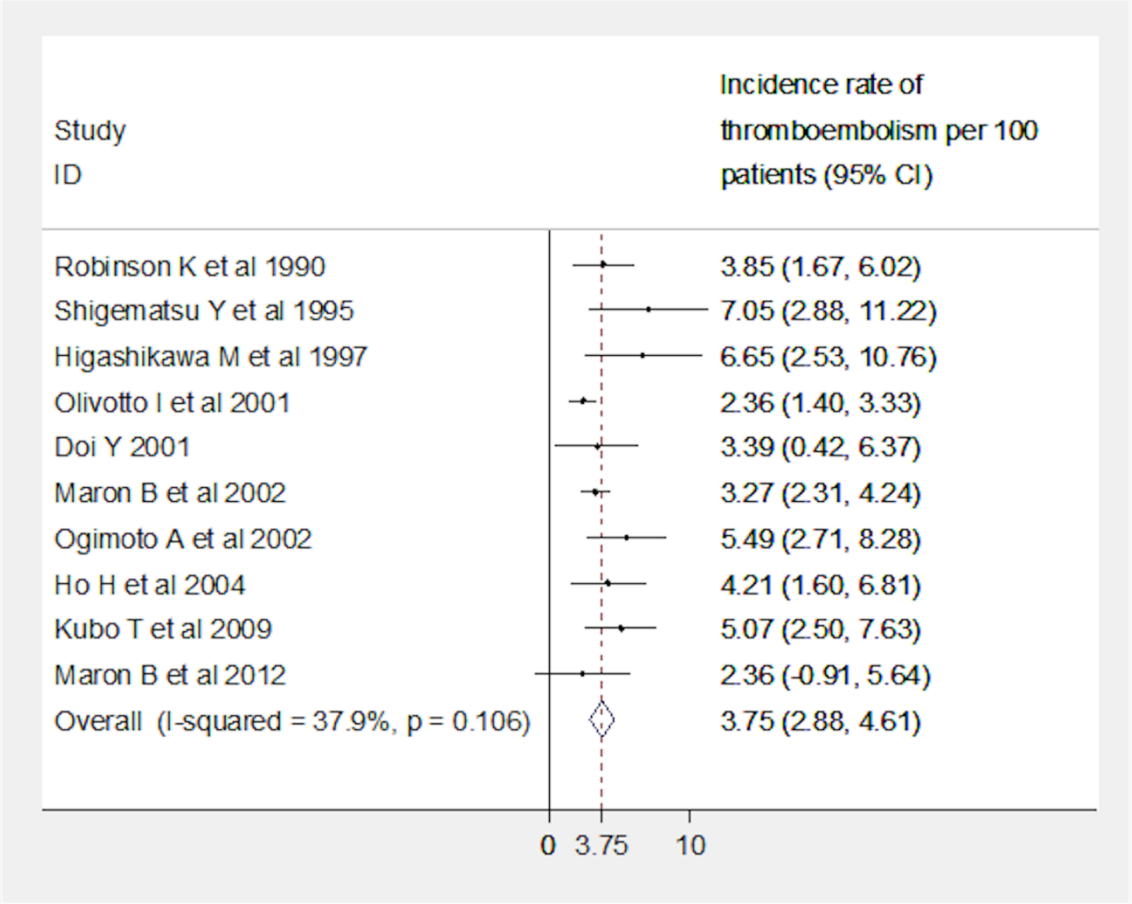
The forest plot from random effect meta-analysis showed study specific incidence and the pooled (overall) incidence of AF. The heterogeneity between the study was assessed by I^2 statistic estimated asI^2=86.5% p<0.01.The overall incidence rate is 3.08 per 100 patients per year.



From Guttmann *et al* (2013) [16]

Figure 6: Incidence of thromboembolism

The forest plot from random effect meta-analysis showed study specific incidence and the pooled (overall) incidence of thromboembolism in patients with HCM and AF. The heterogeneity between the study was assessed by I^2 statistic estimated as I^2=37.9%, p=0.1.The overall incidence rate is 3.75 per 100 patients per year.



From Guttmann *et al* (2013) [16]

3.3.2. Predictors of AF and thromboembolism

The lack of patient level data and heterogeneity between studies means that it is not possible to model clinical predictors of AF and thromboembolism. LA size and volume were associated with the development of AF in 15 studies and for the development of thromboembolism in 3 studies. Age was reported as a predictor of AF in 7 studies and of

thromboembolism in 2 studies. Other variables reported to be associated with an increased risk of AF and thromboembolism are shown in tables 1 and 2.

Table 1: Prevalence and predictors of AF in HCM

Study	PN	Male	MA	DT AF	AF Cases	Prev AF (%)	Definition of AF	Follow up
Robinson K et al, JACC 1990 [184]	174	NS	47	NS	52	30	pAF or established AF	0.5-24 years
Spirito P et al, Am J Cardiol 1992 [178]	127	NS	55	age, lower degree of LVH, LV cavity size	NS	NS	AF for>6 months	2 years
Cecchi F et al, JACC 1995 [179]	202	NS	41+-17	LA size, age	57	28	NS	NS
Shigematsu Y et al, JpnCirc J 1995 [228]	92	77	54	LA size, LV effective chamber size	24	26	NS	6.5 years
Higashikawa M et al, JpnCirc 1997 [233]	83	60	55.5+-7.8	age	19	22.8	NS	2889 +-1675 days
Cecchi F et al, Heart 1997 [229]	110	83	46+-15	p wave duration	41	37.3	AF on Holter monitor clinically evident episodes, excluded chronic AF at enrolment	12 years
Gruver EJ et al, Am J Cardiol 1999 [183]	24	NS	NS	Arg663His beta-cardiac myosin heavy chain mutation	NS	NS	NS	NS
Maron B et al, JAMA 1999 [201]	277	152	47	NS	50	18	NS	8.1 years
Maron B et	744	462	45+-20	NS	153	21	pAF and	8+-7

al, Circ 2000 [202]							chronic AF	years
Olivotto I et al, Circ 2001 [177]	480	292	45+-20	LA dimension, NYHA class III-IV, age	107	22	pAF or chronic AF, inclusion of AF at enrolment and follow up	9.1 +- 6.4 years
Doi Y, J Cardio 2001 [231]	91	62	51+-14	age, LA dimensions	22	24	pAF or chronic AF	6.7 +- 4.8 years
Yamaji K et al, Cardiovasc Pathol 2001 [234]	10	8	60+-22	LV fibrosis	NS	NS	NS	7 years
Maron B et al, JACC 2002 [176]	900	551	46+-20	NS	192	21.3	NS	7 +- 7 years
Ogimoto A et al, J Human Gen 2002 [182]	138	104	63+-31	LA dimension, ANP, ACE II genotype	26	19	NS	10.5 +- 7 years
Kose S et al, Clin Cardiol 2003 [236]	48	30	43 +- 11	LA diameter, p wave dispersion	NS	NS	pAF if>10mins	NS
Tani T, J Am Soc Echocardiogr 2004 [230]	141	96	61+-13	LA volume	31	22	pAF if symptomatic only, chronic AF excluded	NS
Ozdemir O et al, Int J Card 2004 [134]	80	45	53.1±11.1	p wave duration, LA diameter, LVOT gradient	27	34	AF duration > 30s	NS
Ho H et al, Am J Med 2004 [227]	118	62	54+-18	NS	41	35	NS	5.8+- 4.3
Losi MA et al, Am J Cardio 2004 [232]	150	91	41+-17	age, LA diameter, LA volume index, FS of LA	20	13.3	NS	5.2 +- 2.9 years
Cecchi F et al, Am	1677	1033	44+-19	NS	302	18	pAF or chronic AF, 263/1491	9.7+- 7.7

Heart J 2005 [245]							(18%) of AF during follow up	years
Lee et al, Cardiol 2006 [241]	40	30	56.1+-10.8	LA size	3	7.5	prevalence of pAF during follow up	72.2+-60.1 months
Lee et al, Clin Cardiol 2007 [246]	163	84	NS	NS	20	12.2	pAF during follow up, 20.3% of patients had AF during initial evaluation	5.3+-4.1 years
Kitoaka H et al, Circ J 2006 [243]	137	95	52 +-13	NS	29	25	22 patients in AF at initial evaluation, 29 patients developed during follow up, prevalence of AF (pAF/persistent AF) developed during follow up	11.4 +-5.7 years
Nagai T et al, Circ J 2007 [180]	110	86	53+-13	EDN2 A 985 allele, LA dimension, IV wall thickness, LV mass index	26	24	Prior pAF or persistent AF excluded	13+-7 years
Bunch T et al, Am J Cardiol 2007 [237]	86	57	56.6+-16.1	ST changes and PVC on ECG	12	14	NS	2.6+-2.8 years
Kubo T et al, Circ J 2009 [181]	261	173	64+-14	Age, NYHA III-IV, LVEDD, FS, LA dimension	74	28	pAF and persistent AF	4 years retrospective
Yang WI et al, J Am Soc Echocard 2009 [235]	81	51	57 ± 14	NS	NS	NS	NS	41 ± 17 months
Papavassili	87	54	58+-13	LA size, LGE	37	43	AF duration of	NS

u et al, J Cardiovasc Magn Reson 2009 [239]							1 hour	
Pedrosa et al, Chest 2010 [242]	80	39	47	LA diameter, severity of OSA	13	16.25	NS	NS
Shigematsu Y et al, J Cardiol 2011 [244]	88	71	65+-11	LA size, impaired LV function	NS	NS	NS	NS
Tani T et al, Cardiovascular Ultrasound 2011 [240]	102	73	61+-13	NS	23	23	pAF if symptomatic only, chronic AF excluded	30.8+-10 months
Moon J et al, Am J Cardiol. 2011 [238]	454	316	61+-11	NS	72	16	NS	43+-20 months
Maron B et al, AM J Cardiol 2012 [247]	26	8	92.2+-2	NS	9	34.6	NS	9.4+-7 years

From Guttmann *et al* (2013) [16]

ACE: angiotensin converting enzyme

ANP: atrial natriuretic peptide

DT: determinant

FS: fractional shortening

IV: interventricular wall

LA: left atrium

LGE: late gadolinium enhancement

LV: left ventricle

LVEDD: left ventricular end diastolic diameter

LVH: left ventricular hypertrophy

LVOT: left ventricular outflow tract

MA: mean age

NYHA: New York Heart Association Class

NS: not specified

OSA: obstructive sleep apnoea

pAF: paroxysmal atrial fibrillation

PN: patient number

PVC: premature ventricular contractions

Table 2: prevalence and predictors of thromboembolism in HCM

Study	PN	Male	MA	DT	CT	PT (%)	Definition of stroke	Follow up
Robinson K et al, JACC 1990 [184]	174	NS	47	NS	12	23	prevalence of systemic thromboembolism in HCM+AF population, including those at onset of AF and during long term follow up	0.5-24 years
Shigematsu Y et al, Jpn Circ J 1995 [228]	92	77	54	NS	11	46	incidence and prevalence of systemic thromboembolism in HCM+AF population	6.5 years
Higashikawa M et al, Jpn Circ 1997 [233]	83	60	55.5 +-7.8	AF, Age	10	52.6	prevalence of ischaemic stroke	2889 +-1675 days
Maron B et al, JAMA 1999 [201]	277	152	47	NS	11	4	stroke in total population	8.1 years
Maron B et al, Circ 2000 [202]	744	462	45+-20	NS	NS	NS	11 stroke related deaths	8+-7 years
Olivotto I et al, Circ 2001 [177]	480	292	45+-20	NS	23	21	prevalence of stroke in HCM AF patients	9.1 +-6.4 years
Doi Y, J Cardio 2001 [231]	91	62	51+-14	NS	5	22.7	prevalence of total embolic events in total HCM cohort 10.9%, prevalence of non-fatal stroke in HCM+AF group is 22.7%	6.7 +-4.8 years
Maron B	900	551	46+-	NYHA	44	21.3	5.6% prevalence of systemic	7 +- 7

et al, JACC 2002 [176]			20	III-V, AF, age			thromboembolism in total HCM population, 16.1% of strokes in HCM+AF, 21.3% of systemic thromboembolism in HCM+AF	years
Ogimoto A et al, J Human Gen 2002 [182]	138	104	63+-13	NS	15	58	thromboembolic events in the HCM+AF group	10.5 +-7 years
Tani T, J Am Soc Echocardiogr 2004 [230]	141	96	61+-13	NS	6	19.4	prevalence of stroke in the HCM+pAF group	NS
Ho H et al, Am J Med 2004 [227]	118	62	54+-18	NS	10	24.4	nonfatal stroke and fatal stroke associated with HCM+AF, 10/41	5.8+-4.3 years
Cecchi F et al, Am Heart J 2005 [245]	1677	1033	44+-10	NS	76	5.10	stroke or peripheral embolisation in HCM group, 76/1491 during follow up	9.7+-7.7 years
Lee et al, Cardiol 2006 [241]	40	30	56.1 +-10.8	NS	4	10	total population	72.2+-60.1 months
Kitaoka H et al, Circ J 2006 [243]	137	95	52 +-13	NS	NS	NS	6 deaths of stroke related to AF	11.4 +-5.7 years
Bunch T et al, Am J Cardiol 2007 [237]	86	57	56.6 +-16.1	NS	3	3.5	prevalence of stroke in total population	2.6+-2.8 years
Kubo T et al, Circ J 2009 [181]	261	173	64+-14	AF, gender, LVEDD, FS, LA	15	20	prevalence of embolic complications in the HCM+AF patients	4 years retrospective
Yang WI et al, J Am Soc Echocard 2009 [235]	81	51	57 ± 14	LA volume index	5	6	total stroke patient population	41 ± 17 months
Tani T et al, Cardiovascular Ultrasoun	102	73	61+-13	LAV	18	18	prevalence of stroke in total population	30.8+-10 months

d 2011 [240]								
Moon J et al, Am J Cardiol. 2011 [238]	454	316	61+-11	NS	26	6	prevalence of stroke in total population	43+-20 months
Maron B et al, AM J Cardiol 2012 [247]	26	8	92.2 +-2	NS	2	22%	prevalence of non-fatal stroke in HCM+AF	9.4+-7 years

From Guttmann *et al* (2013) [16]

AF: atrial fibrillation

CT: Cases of thromboembolism

DT: determinants of thromboembolism

FS: fractional shortening

HCM: hypertrophic cardiomyopathy

LAV: left atrial volume

LVEDD: left ventricular end diastolic diameter

MA: mean age

NYHA: New York Heart Association Class

pAF: paroxysmal atrial fibrillation

PN: patient number

Prev AF: prevalence of AF

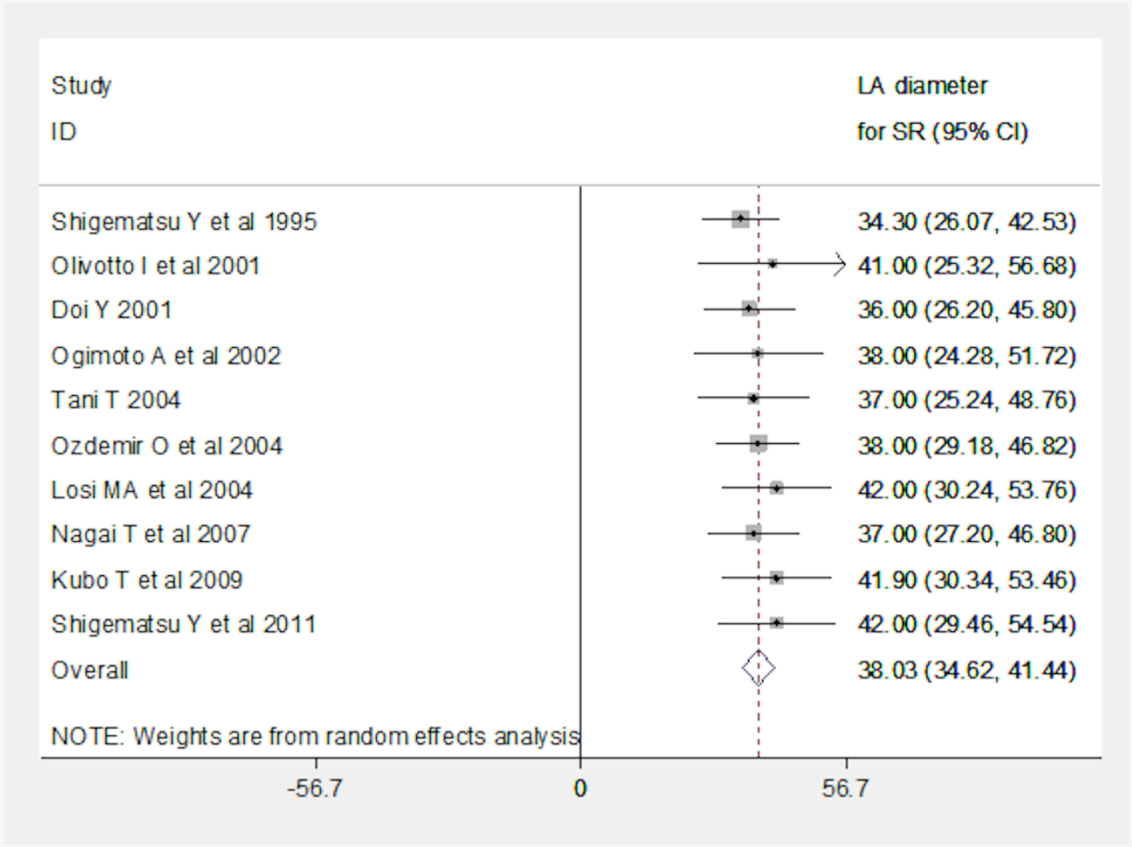
PT: prevalence of thromboembolism

The pooled overall LA diameter in patients with SR was 38.03 mm (95% CI: 34.62-41.44), figure 7. The heterogeneity between the studies was estimated as $I^2 = 0.02\%$ (p=0.95). The pooled overall LA diameter in patients with AF or paroxysmal AF was 45.37 mm (95% CI: 41.64-49.04), figure 8. The I^2 statistic was estimated as $I^2 = 0.01\%$ (p=0.96). One study reported an LA diameter of more than 40mm to be predictive of AF (p<0.01)[179]

and another an LA diameter of more than 42mm predictive of AF with a sensitivity of 96% and a specificity of 81% [134].

Figure 7: Left atrial diameter in patients with SR

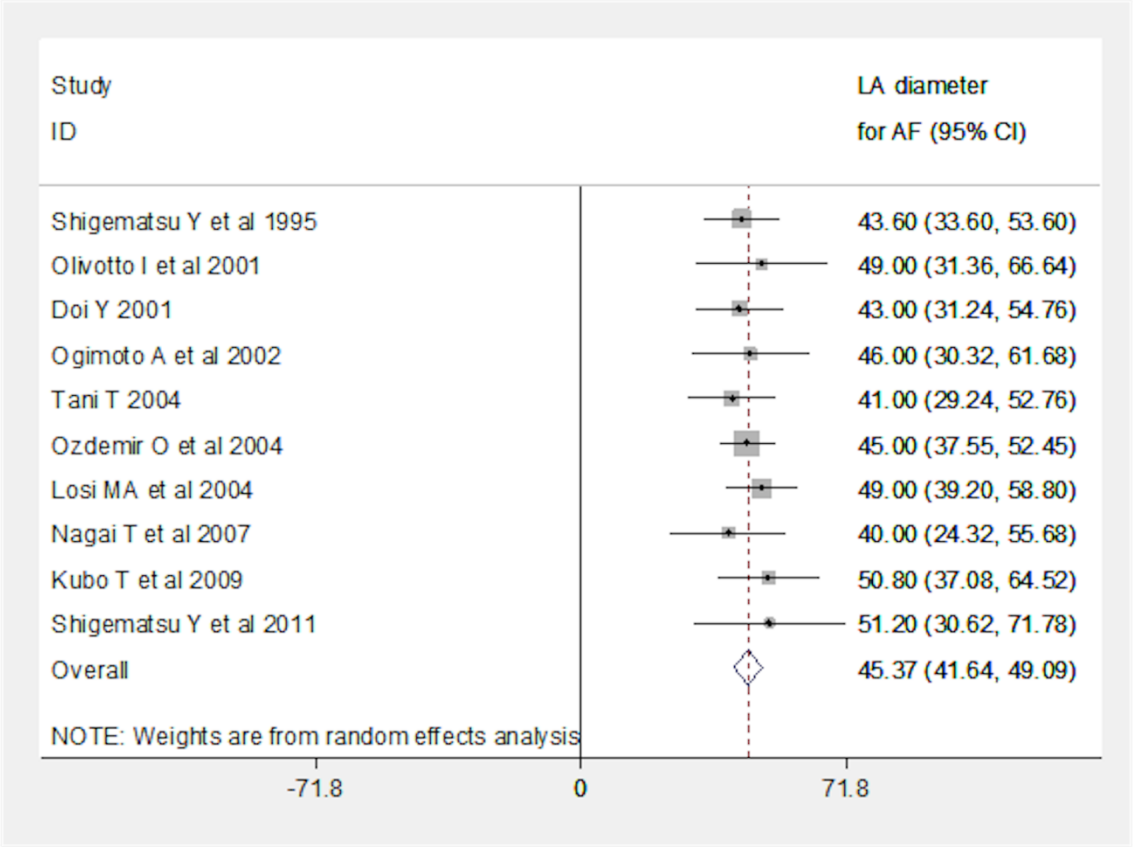
The forest plot from random effect meta-analysis showed study specific LA diameter for patients in SR and the pooled (overall) LA diameter. The heterogeneity between the study was assessed by I^2 statistic estimated as $I^2 = 0.02\%$, p=0.95. The overall cut-off point or diameter value is 38.03 in patients with SR.



From Guttmann *et al* (2013) [16]

Figure 8: LA diameter in AF

The forest plot from random effect meta-analysis showed study specific LA diameter for patients in AF or paroxysmal AF and the pooled (overall) LA diameter. The heterogeneity between the study was assessed by I^2 statistic estimated as $I^2 = 0.01\%$, p=0.96.The overall cut-off point or diameter value is 45.37 in patients with AF.



From Guttmann *et al* (2013) [16]

3.3.3. Mortality

A meta-analysis of mortality in the AF population was not possible due to limited data. Studies reporting statistically significant higher mortality in patients with AF compared to patients in sinus rhythm are presented in table 3.

Table 3: mortality data in available studies in whole population and in AF patients

Study	Patient Number	Male	mean age	Follow up	Mortality	mortality AF patients
Robinson K et al, JACC 1990 [184]	174	NS	47	0.5-24 years	NS	36.5% disease related death, 11.5% SD, 14% 5-year mortality, 29% 10-year mortality, 35% 15-year mortality
Cecchi F et al, JACC 1995 [179]	202	NS	41+-17		6% cardiovascular death related to HCM, 0.6% annual mortality for cardiovascular disease, 0.1% SD	impaired survival p<0.005, cumulative mortality rate after 15 years 24% (AF) vs 3% (SR)
Higashikawa M et al, Jpn Circ 1997 [233]	83	60	55.5+-7.8	2889 +- 1675 days	7.2 % CD	CD 5.3% (AF) vs 7.8% (SR)
Maron B et al, JAMA 1999 [201]	277	152	47	8.1 years	10.5% HRD, 1.3% annual HCM mortality, 0.7% SD , 6.5% 5 year mortality, 10.5% 10 year mortality, 16.8% 15 year mortality	NS
Maron B et al, Circ 2000 [202]	744	462		8+-7 years	12% HRD, 6% sudden and unexpected, 1.4% annual mortality for HCM related death 1.4% , 0.7% SD	NS
Olivotto I et al, Circ 2001 [177]	480	292	45+-20	9.1 +- 6.4 years	15.4% HCM related causes	35% HCM related causes, annual HCM mortality 3% in AF vs 1% SR
Doi Y, J Cardio	91	62	51+-14	6.7 +- 4.8	11% CD, 6% SD	32% cardiovascular death in AF group,

2001,J Cardiol [231]				years		13.6% SD, 3% 5- year mortality rate SR vs 34% AF (cardiovascular death)
Ho H et al, Am J Med 2004 [227]	118	62	54+-18	5.8+-4.3 years	1.6% annual cardiovascular mortality	NS
Cecchi F et al, Am Heart J 2005 [245]	1677	1033	44+-19	9.7+-7.7	1.3% annual all cause, 1% cardiovascular mortality, 0.4% SD	NS
Lee et al, Cardiol 2006 [241]	40	30	56.1+-10.8	72.2+-60.1 months	no mortality in apical HCM	NS
Lee et al, Clin Cardiol 2007 [246]	163	84		5.3+-4.1 years	2.8% annual all-cause mortality rate, 0.8% annual cardiovascular mortality rate, 0.2% SD	NS

Modified from Guttmann *et al* (2013) [16]

AF: atrial fibrillation

SR: sinus rhythm

HRD: HCM related death

CD: cardiac death

SD: sudden death

NS: not specified

3.3.3.4. Treatment of AF

We identified no randomised trials of antiarrhythmic drugs, oral anticoagulants or radiofrequency ablation. The results from observational studies on drug treatment and AF ablation are reported in tables 4 and 5.

Table 4: AF ablation in patients with HCM

Study	PN	Male	mean age	AF	RP (%)	SR	follow up	predictor of poor outcome
Di Donna P et al, Europace 2010 [248]	61	44	54+-13	pAF, recent & longstanding persAF	52	67% at of follow up	29+-16 months	LAV, NYHA
Bunch T et al, J Cardiovasc Electrophysiol 2008 [249]	33	25	51+-11	pAF, persAF, permAF		62% at 1 year	1.5+-1.2 years	persAF/cAF, LAV, advance diastolic disease
Gaita F et al, Am J Cardiol 2007 [187]	26	18	58+-11	pAF, permAF	19.20	64% at of follow up	19+-10 months	permAF
Kilicaslan F et al, Heart Rhythm 2006 [250]	27	NS	55+- 10	pAF, persAF, permAF	26	52% at of follow up	341 +-237 days	NS

From Guttmann *et al* (2013) [16]

cAF chronic AF

LAV: left atrial volume

NYHA: New York Heart Association class

NS: not specified

pAF: paroxysmal atrial fibrillation

persAF: persistent AF

permAF:permanent atrial fibrillation

PN: patient number

RP: redo procedures

Table 5: Studies of amiodarone treatment and anticoagulation in HCM patients

Study	Therapy	PN	Study design	Outcome
Robinson K et al, JACC 1990 [184]	A	174	45 of 52 patients with AF received conventional therapy alone (25) or followed by amiodarone, 7 amiodarone only	fewer alterations in drug therapy, fewer embolic episodes and more remained in SR, fewer DCCV attempts
Cecchi F et al, JACC 1995 [179]	A	202	63 patients to manage NSVT, cardioversion or control or prevent AF or recurrence of cardiac arrest	No sudden death
Olivotto I et al, Circ 2001 [177]	A	480	55.8% of patients with pAF	no difference of duration of SR and survival
Doi Y, J Cardio 2001 [231]	W	91	45% of patients with AF anticoagulated	42% of patients without warfarin vs 10% with warfarin experienced thromboembolism
Maron B et al, JACC 2002 [176]	W	900	43.2% patients with AF anticoagulated	cumulative incidence of thromboembolism among non-anticoagulated patients was twice that of patients anticoagulated (31% vs 18%)
Olivotto I et al, Circ 2001 [177]	W	480	55.1% patients with AF anticoagulated	stroke less common 10% vs non treated 39% $p<0.05$
Higashikawa M et al, Jpn Circ 1997 [233]	W	83	37% (7 out of 19) of HCM patients with AF treated with warfarin	Ischaemic strokes in 6 out of 7 warfarin treated HCM patients with AF, INR<2 in 5 cases

From Guttmann *et al* (2013) [16]

A: amiodarone

AF: atrial fibrillation

NSVT: non sustained ventricular tachycardia

pAF: paroxysmal atrial fibrillation

PN: patient number

SR: sinus rhythm

W: warfarin

3.4. Discussion

This review shows that studies on atrial fibrillation in HCM are very heterogeneous with respect to patient characteristics, follow up duration, inclusion criteria and endpoint definition. All are retrospective and observational in design and there are no randomised control trials of any treatment strategy. The data do show that AF occurs in about a fifth of patients with HCM, and strongly suggest that it is associated with adverse outcomes.

3.4.1. Data quality

Major limitations of existing data are the difference in age and sex distribution in the study populations and the small size (typically less than 150 patients) of most cohorts. There is also substantial variation in inclusion criteria and in the definition of AF. For example, some studies included patients who were in AF at the time of diagnosis [181;184] whereas others excluded patients who were in AF at enrolment and reported only on those individuals that developed AF during the follow up period.[229;232;241;246] Similarly, some reports included only patients with paroxysmal AF, but the definition of paroxysmal AF was highly variable in that some included AF only when associated with symptoms,[230;240] or when the AF was of a prespecified duration.[134;178;236;239] Finally, groups of investigators have published their cohorts in more than one study. It is impossible to discern the overlap in patient populations.[176;177;201;202;230;240;241;246] but the duplicate studies report different follow up duration and total population size. We therefore conclude that the overall estimate obtained by meta-analysis was not significantly affected [235;238;241].

3.4.2. Prevalence and incidence of thromboembolism

All studies showed that AF in patients with HCM is associated with thromboembolism. The prevalence figures were influenced by duration of follow up (the two studies reporting the

highest prevalence of thromboembolic events had significantly longer duration of follow up compared to the other studies [182;233]) and by the definition of thromboembolism. For example, some studies consider all thromboembolic events including cerebral and peripheral emboli in the reported prevalence of thromboembolic events [182;184;228] whereas others only report the prevalence of stroke [177;233].

3.4.3. Predictors of AF

The pathophysiological conditions that predispose to atrial arrhythmia in patients with HCM are poorly understood, but diastolic dysfunction and mitral regurgitation (usually in the context of outflow obstruction) are a major cause of increased atrial size and stretch. Small cross-sectional studies show associations with myocardial fibrosis detected by gadolinium enhanced cardiac magnetic resonance imaging and reduced hyperaemic myocardial blood flow determined using PET, but these phenomena are probable surrogates for disease severity rather than specific mechanisms or triggers for AF.[239;251] Several workers have postulated that patients with HCM have a primary atrial as well as a ventricular myopathy, but supportive evidence for this is scant. While heterogeneity between studies meant that it was not possible to perform a meta-analysis of all suggested predictors for AF and thromboembolism, the majority of reports examining this question found that LA size, LA volume, and age were independently associated with AF, suggesting that these should be considered when evaluating individual patients risk of developing AF.

There are no prospective randomised trials of any therapy for AF in HCM and, as this review shows, only limited observational data on pharmacological and non-

pharmacological treatment strategies.[177;179;184] Evidence that amiodarone therapy maintains sinus rhythm and reduces embolic episodes comes from a single study.[184] Data on warfarin use are similarly limited, but there are observational data reporting a lower incidence of stroke in patients treated with warfarin compared to those on antiplatelet therapy or no treatment.[176;177;231]

To date only small non-randomised studies have examined the efficacy of AF ablation in HCM, but the data accrued so far suggest that repeat procedures are frequently required and long term maintenance of sinus rhythm is achieved in no more than 50-60% of patients.[187;248-250]

3.5. Limitations

The review examines only those studies that investigated atrial fibrillation and/or stroke or thromboembolism as primary or secondary outcome, but we acknowledge that data on prevalence of AF are included in other observational cohort studies reporting on other aspects of the disease.

3.6. Conclusions

This review shows that the published literature is insufficient to create a robust clinical tool for the prediction of AF or thromboembolic risk. Nevertheless, most data suggest that once patients have AF, they have a high risk of thromboembolism and should be anticoagulated. More challenging is the management of patients with LA enlargement but no evidence for atrial arrhythmia. Given that LA diameter and volume are consistently shown to be independently associated with AF, patients with atrial

enlargement should as a minimum be monitored on a regular basis in order to detect AF. The role of prophylactic anticoagulation in this group should be evaluated in randomised prospective trials.

4 Predictors of atrial fibrillation in hypertrophic cardiomyopathy (HCM Risk-AF)

4.1. Aims

Most studies investigating predictors of AF are limited by the size of the cohort with cohorts of only a few hundred patients [177-179;184;201;233;244]. The primary aim of this study was to investigate predictors of AF in a large multicentre cohort. Exploratory analyses were performed to investigate the effect of AF on mortality.

4.2. Methods

4.2.1. Study design and overview

This study uses data from a retrospective, multicentre longitudinal cohort–the Hypertrophic Cardiomyopathy Outcome Investigators (www.HCMRisk.org) [167].

The study conforms to the principles of the Helsinki declaration. The sponsors of this study did not have a role in study design, data collection, analysis, and interpretation. O.G., M.P., R.O., and P.E. had access to all data and final responsibility to submit the article. The authors from each participating centre guarantee the integrity of data from their institution. All investigators have agreed to the article as written.

4.2.2. Study population and participating centres

The study cohort consisted of all consecutively evaluated patients with HCM, followed at seven participating European centres: (i) The Heart Hospital, London, UK, (ii) A Coruna University Hospital, A Coruna, Spain, (iii) Unit of Inherited Cardiovascular diseases, 1st Department of Cardiology, University of Athens, Greece, (iv) Institute of Cardiology, University of Bologna, Italy, (v) University Hospital Virgen de la Arrixaca, Murcia, Spain, (vi) Monaldi Hospital, Second University of Naples, Italy and (vii) Hospital Universitario Puerta del Hierro, Madrid, Spain. Some patients from this cohort are reported in other recently published studies [167;190;219;252-260].

Only adult patients (≥16 years of age) were studied. HCM was defined as a maximum left ventricular wall thickness ≥15 mm unexplained solely by loading conditions [261] or in accordance with published criteria for the diagnosis of disease in relatives of patients with unequivocal disease [20]. Patients with known inherited metabolic diseases or syndromic causes of HCM were excluded from the study. For the development of the predictors of AF, patients with AF at first evaluation were excluded from the analysis.

4.2.3. Patient assessment and data collection

All patients underwent clinical assessment, pedigree analysis, physical examination, resting and ambulatory electrocardiography and transthoracic echocardiography. Each participating centre collected data independently. Patients were reviewed every 6–12 months or earlier if there was a change in symptoms.

4.2.4. Clinical outcomes

The primary outcome was paroxysmal, permanent or persistent AF detected on ECG, Holter monitoring or device interrogation. The secondary outcome was all-cause and cardiovascular mortality (defined as sudden cardiac death, heart failure related death, stroke related death and other cardiac death).

4.2.5. Selection of predictors and coding

Following a review of the literature completed in September 2012 , predictors of AF that have been reported previously in patients with HCM were considered as candidate predictor variables [16]. Clinical parameters were used as pre-specified predictor variables only when associated with AF in at least one published study. All parameters were uniformly defined in all participating centres. In addition to these variables, predictors known to be associated with AF in the general population such as hypertension, diabetes mellitus and vascular disease were considered (table 6). All predictors are at baseline evaluation.

Table 6: Definition of pre-specified predictor variables assessed at baseline evaluation

Predictor variable	Definition	Coding
Sex	Male or female [181]	Binary, male/female
Age	Age at first evaluation in participating centres [176;233]	Continuous, years
AF	Physician reported detection of paroxysmal, permanent of persistent AF on ECG or Holter monitoring [176;233]	Binary, yes/no
NYHA	NYHA class at first evaluation [176]	Categorical, 1,2 and 3-4
LA	Anterior-posterior left atrial diameter determined by 2D echocardiography in the parasternal long axis or short-axis plane at time of first evaluation [181]	Continuous, mm
MWT	The greatest LV wall thickness measured at the level of the mitral valve, papillary muscles, and apex in the parasternal short-axis plane using 2-D echocardiography at time of evaluation [210]	Continuous, mm
FS	LV end-diastolic dimension-LV end-systolic dimension)/ LV end-diastolic dimension measured by M-Mode or 2D echocardiography at time of evaluation [181]	Continuous, %
LVOT max	The maximum LV outflow gradient determined at rest and with Valsalva provocation (irrespective of concurrent medical treatment) using pulsed and continuous wave Doppler from the apical three- and five-chamber views. Peak outflow tract gradients were determined using the modified Bernoulli equation: Gradient = $4V^2$, where V is the peak aortic outflow velocity [171]	Continuous, mmHg
Hypertension	Diagnosis of hypertension prior to first evaluation, as per CHA_2DS_2-VASc score [262;263]	Binary, yes/no
Diabetes	Diagnosis of diabetes prior to first evaluation, as per CHA_2DS_2-VASc score [262;263]	Binary, yes/no
Vascular disease	Myocardial infarction, complex aortic plaque and peripheral arterial disease, as per CHA_2DS_2-VASc score [262;263]	Binary, yes/no

AF: atrial fibrillation, NYHA: New York Heart Association Functional classification, LA: Left atrial size, MWT: Maximal wall thickness, FS: Fractional shortening, LVOT max: maximum LV outflow gradient

4.2.6. Sample size

A minimum of 10 AF events were required per coefficient estimated to ensure that the regression coefficients were estimated with adequate precision. The 740 AF endpoints observed in this cohort over a 10 year follow-up period allow the estimation of all regression coefficients (as shown in table 6) in a multivariable regression model and

variable selection from a set of relevant predictors of AF also adjusting for possible centre effects.

4.2.7. General statistical methods

STATA (version 12) and R (version 3.0) were used for the statistical analyses. For descriptive results, variables are expressed as mean ± standard deviation (SD), median and interquartile range (IQR) or counts and percentages as appropriate. The follow-up time for each patient was calculated from the date of their first evaluation at participating centres to the date of the relevant endpoint or to the date of their most recent evaluation. The annual event rate was calculated by dividing the number of patients reaching the endpoint by the total follow-up period for that endpoint. The Kaplan–Meier method was used to estimate the cumulative probability for the occurrence of an outcome.

4.2.8. Missing data

The characteristics of patients with missing information were compared to those of patients with complete information to investigate bias due to missing data. Logistic regression was used to identify the predictors of missingness. Data were assumed to be missing at random and values for the missing predictors were imputed using multiple imputation techniques based on chained equations [264]. The multiple imputation model included all predictors of missingness, the outcome, all pre-specified predictors of the risk model, and the estimate of the cumulative hazard function [265]. Rubin's rules were used to combine the estimates from 30 imputed data sets [266].

4.2.9. Univariate and multivariable analysis

Univariable and multivariable Cox regression models were fitted and tested for non-linearity of continuous predictors by inclusion of quadratic terms. The final fully adjusted Cox regression model for AF was developed using backwards elimination with a 15% significance level was used to select the final predictors [34]. The proportional hazards assumption was investigated using Schoenfeld residuals [35]. The entire cohort was used, making efficient use of the data [36]. A sensitivity analysis for centre effects was performed by including centre in the model.

4.2.10. Comparison of mortality in patients with AF and SR

Cardiovascular and non-cardiovascular mortality was compared in patients with AF and SR at first evaluation over a follow up period of ten years. Patients with AF at first evaluation were included for this analysis.

4.2.11. Relation of LA size to AF

A sub-analysis was performed to assess the relationship between risk of AF and LA size.

4.3. Results

4.3.1. Baseline clinical characteristics

The study cohort comprised 5104 patients; 197 patients, who were only seen once at baseline evaluation and an additional 659 patients with AF prior to first evaluation were

excluded for the analysis of AF events. The study cohort for the AF analysis consisted of 4248 patients. The baseline clinical characteristics are shown in table 7.

Table 7: Clinical characteristics of whole cohort and in patients with and without AF

	whole cohort			no AF			AF		
variable	**total**	**mean/n**	**SD/%**	**total**	**mean/n**	**SD/%**	**total**	**mean/n**	**SD/%**
Age	4244	47.73	16.3	3505	46.73	16.52	739	52.48	14.34
LA	4075	43.1	7.2	3355	42.12	6.8	720	47.69	7.26
MWT	4200	19.47	5.23	3461	19.33	5.27	739	20.14	5.02
FS	3862	0.41	0.09	3174	0.41	0.09	688	0.41	0.1
LVOT max	3634	32.67	41.49	2975	31.11	40.4	659	39.75	45.43
AF	4248	740	0.17						
Female	4247	1504	0.35	3507	1214	0.35	740	290	0.39
NYHA II	4069	1365	0.34	3356	1057	0.31	713	308	0.43
NYHA III,IV	4069	377	0.09	3356	269	0.08	713	108	0.15
Hypertension	4149	1171	0.28	3432	914	0.27	717	257	0.36
Diabetes	3500	225	0.06	2863	168	0.06	637	57	0.09
FH SCD	4117	995	0.24	3397	810	0.24	720	185	0.26
Vascular disease	3068	66	0.02	2431	41	0.02	637	25	0.04

SD: Standard deviation, n: Number, LA: Left atrial size, MWT: Maximal wall thickness, FS: Fractional shortening, LVOT max: maximum LV outflow gradient, AF: atrial fibrillation, NYHA: New York Heart Association Functional classification, FH SCD: Family history of sudden cardiac death

4.3.2. AF events during follow-up

During a follow up period of 22,743.04 patient years (median 5.4 years), 740 (17.4%) patients reached the primary endpoint within 10 years from first evaluation (57.8% paroxysmal and 42.2% permanent and persistent). The 5 and 10 year cumulative incidence rates of AF were 16.0% (95% CI 14.6%-17.5%) and 33.3% (95% CI 30.7%-36.1%), respectively. The clinical characteristics of patients with and without AF are shown in table 7.

4.3.3. Missing data

Missing data per variable are presented in table 8.

Table 8: Missing data per variable

Variable	n	Total	%
Sex	1	4,248	0.02
Age	4	4,248	0.09
NYHA	179	4,248	4.21
MWT	48	4,248	1.13
LA	173	4,248	4.07
LVEDD	194	4,248	4.57
LVESD	376	4,248	8.85
LVOT max	614	4,248	14.45
Hypertension	99	4,248	2.33
Diabetes	748	4,248	17.61
Vascular disease	1,180	4,248	27.78

n: Number, AF: atrial fibrillation, NYHA: New York Heart Association Functional classification, MWT: Maximal wall thickness, LA: Left atrial size, LVEDD: Left ventricular end-diastolic dimension, LVESD: left ventricular end-systolic dimension, LVOT max: maximum LV outflow gradient

4.3.4. Univariable and multivariable analyses

The models were developed based on the 740 events within the first 10 years of follow-up. Univariable analyses are shown in table 9. Only maximal LV wall thickness was found to have a nonlinear association with AF and so a quadratic term was included as a candidate predictor in the multivariable analysis.

Multivariable Cox regression revealed an association between AF and the following: female sex, LA diameter, NYHA class II, NYHA class III/IV, hypertension and vascular disease. There was a significant centre effect as part of a sensitivity analysis (shown in

table 9 and 10). The clinical characteristics according to centre are displayed in table 11.

Table 9: Univariable and multivariable analysis for predictors of AF in HCM

Univariable analysis				Multivariable analysis			
Predictor	**HR**	**p**	**CI**	**Predictor**	**HR**	**P**	**CI**
Female	1.27	<0.001	1.10 - 1.47	**Female**	1.34	<0.001	1.14 - 1.56
Age10	1.35	<0.001	1.29 - 1.41	**Age10**	1.23	<0.001	1.17 - 1.30
NYHA II	1.75	<0.001	1.49 - 2.05	**NYHA II**	1.29	<0.001	1.10 - 1.52
NYHA III,IV	2.96	<0.001	2.37 - 3.69	**NYHA III,IV**	1.66	<0.001	1.32 - 2.08
LA5	1.6	<0.001	1.53 - 1.68	**LA5**	1.1	<0.001	1.09 - 1.11
MWT	1.02	<0.001	1.01 - 1.03	**Hypertension**	1.17	0.06	0.99 - 1.38
FS	0.87	0.75	0.38 - 2.01	**Vascular disease**	1.4	0.1	0.94 - 2.09
LVOT max	1.01	<0.001	1.00 - 1.01				
LVEDD	1.02	<0.001	1.01 - 1.03				
LVESD	1.01	0.02	1.00 - 1.03				
Hypertension	1.72	<0.001	1.48 - 2.01				
Diabetes	1.52	<0.001	1.16 - 1.99				
Vascular disease	2.4	<0.001	1.61 - 3.58				
MWT1	1.21	<0.001	1.11 - 1.31				
MWT2	1	<0.001	0.99 - 1.00				

Age10: Hazard ratio for 10 year increments, NYHA: New York Heart Association Functional classification, LA5: Hazard ratio for left atrial size for 5mm increments, MWT: Maximal wall thickness, FS: Fractional shortening, LVOT max: maximum LV outflow gradient, LVEDD: Left ventricular end-diastolic dimension, LVESD: left ventricular end-systolic dimension, HR: Hazard ratio, p: p-value, CI: 95% confidence interval

Table 10: Multivariable analysis of AF predictors and sensitivity analysis for centre effect

variable	HR	P	CI
Female	1.34	<0.001	1.14 - 1.56
Age10	1.23	<0.001	1.17 - 1.30
NYHA II	1.29	<0.001	1.10 - 1.52
NYHA III,IV	1.66	<0.001	1.32 - 2.08
LA5	1.10	<0.001	1.09 - 1.11
Hypertension	1.17	0.06	0.99 - 1.38
Vascular disease	1.40	0.10	0.94 - 2.09
Athens	1.27	0.03	1.03 - 1.56
Bologna	0.69	0.01	0.52 - 0.92
Coruna	0.72	0.01	0.57 - 0.92
Madrid	1.65	0.01	1.16 - 2.34
Murcia	0.65	<0.001	0.48 - 0.87
Naples	0.44	0.01	0.25 - 0.81

Age10: Hazard ratio for 10 year increments, NYHA: New York Heart Association Functional classification, LA5: Hazard ratio for left atrial size for 5mm increments, HR: Hazard ratio, p: p-value, CI: 95% confidence interval.

Table 11: Clinical characteristics according to centre

Centre	variable	total	mean/n	(SD)/%	min	max
LONDON	**Age**	1928	44.75	15.45	16.03	88.37
	LA	1872	43.06	7.48	19.00	75.00
	MWT	1925	19.43	5.54	7.00	43.00
	FS	1842	0.42	0.09	0.10	0.82
	LVOT max	1612	27.01	38.04	1.00	260.00
	AF	1928	378	0.20		
	Female	1928	696	0.36		
	NYHA II	1816	702	0.39		
	NYHA III/IV	1816	156	0.09		
	Hypertension	1831	369	0.20		
	Diabetes	1218	56	0.05		
	FH SCD	1810	516	0.29		
	Vascular disease	1219	12	0.01		
ATHENS	**Age**	493	45.01	16.12	16.00	82.44
	LA	493	42.96	6.01	23.00	65.00
	MWT	493	18.06	4.03	12.00	37.00
	FS	493	0.40	0.07	0.07	0.64

	LVOT max	493	31.95	36.14	2.00	175.00
	AF	493	117	0.24		
	Female	492	141	0.29		
	NYHA II	493	158	0.32		
	NYHA III/IV	493	37	0.08		
	Hypertension	493	126	0.26		
	Diabetes	493	31	0.06		
	FH SCD	493	157	0.32		
	Vascular disease	493	7	0.01		
BOLOGNA	**Age**	439	49.52	15.69	17.16	90.26
	LA	433	44.19	7.62	20.00	75.00
	MWT	439	20.20	4.89	15.00	39.00
	FS	425	0.42	0.11	0.06	0.77
	LVOT max	439	35.07	49.04	4.00	237.00
	AF	439	55	0.13		
	Female	439	152	0.35		
	NYHA II	439	132	0.30		
	NYHA III/IV	439	37	0.08		
	Hypertension	439	123	0.28		
	Diabetes	439	23	0.05		
	FH SCD	439	69	0.16		
	Vascular disease	439	7	0.02		
CORUNA	**Age**	500	54.87	15.58	16.72	89.09
	LA	469	43.30	6.99	23.00	73.00
	MWT	468	20.18	5.10	12.00	42.00
	FS	456	0.42	0.09	0.14	0.70
	LVOT max	220	60.97	53.03	3.00	299.00
	AF	501	87	0.17		
	Female	501	175	0.35		
	NYHA II	487	164	0.34		
	NYHA III/IV	487	46	0.09		
	Hypertension	501	210	0.42		
	Diabetes	463	57	0.12		
	FH SCD	496	94	0.19		
	Vascular disease	501	5	0.01		
MADRID	**Age**	188	53.40	16.94	16.99	85.61
	LA	181	42.46	7.06	26.00	62.00
	MWT	186	20.76	5.43	7.00	41.00
	FS	173	0.42	0.12	0.08	0.74
	LVOT max	183	33.39	42.87	1.00	250.00
	AF	188	37	0.20		

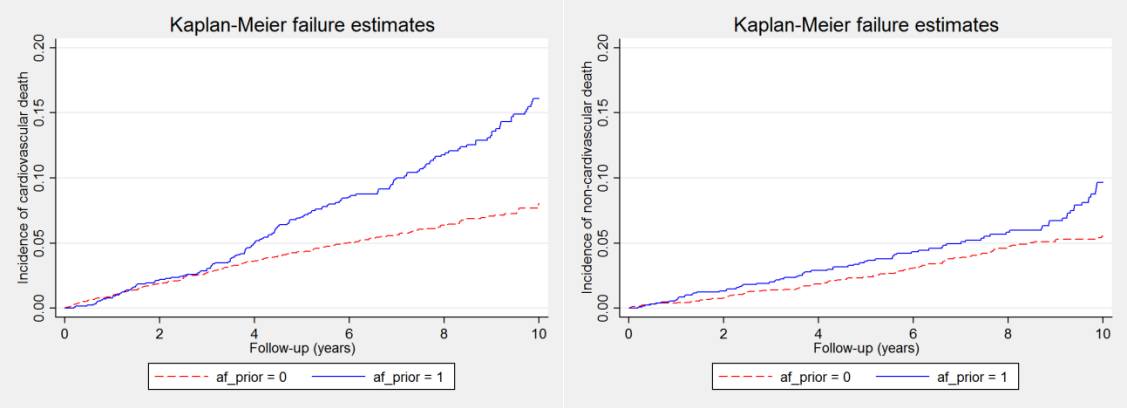
	Female	188	70	0.37		
	NYHA II	186	38	0.20		
	NYHA III/IV	186	10	0.05		
	Hypertension	188	82	0.44		
	Diabetes	188	23	0.12		
	FH SCD	186	32	0.17		
	Vascular disease	187	16	0.09		
MURCIA	**Age**	529	51.91	17.13	16.47	88.68
	LA	510	42.23	6.99	23.00	70.00
	MWT	521	18.96	5.13	8.00	43.00
	FS	460	0.39	0.10	0.05	0.72
	LVOT max	529	36.88	41.94	1.00	150.00
	AF	529	54	0.10		
	Female	529	201	0.38		
	NYHA II	524	141	0.27		
	NYHA III/IV	524	85	0.16		
	Hypertension	527	216	0.41		
	Diabetes	529	25	0.05		
	FH SCD	527	77	0.15		
	Vascular disease	59	8	0.14		
NAPLES	**Age**	167	44.58	15.03	16.31	77.24
	LA	117	44.38	7.11	25.00	66.00
	MWT	168	20.38	5.02	11.00	40.00
	FS	13	0.38	0.17	0.16	0.78
	LVOT max	158	31.72	26.05	7.00	135.00
	AF	170	12	0.07		
	Female	170	69	0.41		
	NYHA II	124	30	0.24		
	NYHA III/IV	124	6	0.05		
	Hypertension	170	45	0.26		
	Diabetes	170	10	0.06		
	FH SCD	166	50	0.30		
	Vascular disease	170	11	0.06		

SD: Standard deviation, n: Number, LA: Left atrial size, MWT: Maximal wall thickness, FS: Fractional shortening, LVOT max: maximum LV outflow gradient, AF: atrial fibrillation, NYHA: New York Heart Association Functional classification, FH SCD: Family history of sudden cardiac death

4.3.5. Comparison of mortality in patients with AF and SR

There were 4835 patients with follow up clinical data and recorded information on cause of death. Patients with unknown date (47 patients) or cause of death (25 patients) were excluded from this analysis. The prevalence of cardiovascular death was 8.38%; CI= [7.6%-9.2%] (405 out of 4835 patients) with an incidence of 6.66%; 95%; CI= [5.9 %-7.3%] (317 out of 4835 patients) at 10 year follow-up. The prevalence of non-cardiovascular death was 4.84%; 95%; CI= [4.2%-5.4%] (234 out of 4835 patients) with an incidence of 3.91%; 95%; CI= [3.4%-4.5%] (189 out of 4835 patients) at 10 year follow-up. The incidence of cardiovascular death was 4.92% in the SR group and 10.86% in the AF group (difference in proportions = 5.9%; 95% CI= [4.1%-7.8%]). The incidence of non-cardiovascular death was 3.15% in the SR group and 5.92% in the AF group (difference in proportions= 2.8%; 95% CI= [0.1%- 4.2%]). The Kaplan-Meier curves for cardiovascular and non-cardiovascular death in patients with AF and SR are displayed in Figure 9a. There is an increased mortality in patients with permanent/persistent AF compared with paroxysmal AF (difference in proportions = 8.1%; 95% CI= [4.5%-11.7%] (Figure 9b).

Figure 9: Kaplan-Meier failure estimates comparing cardiovascular (left) and non-cardiovascular mortality (right) in patients with AF and SR at baseline evaluation

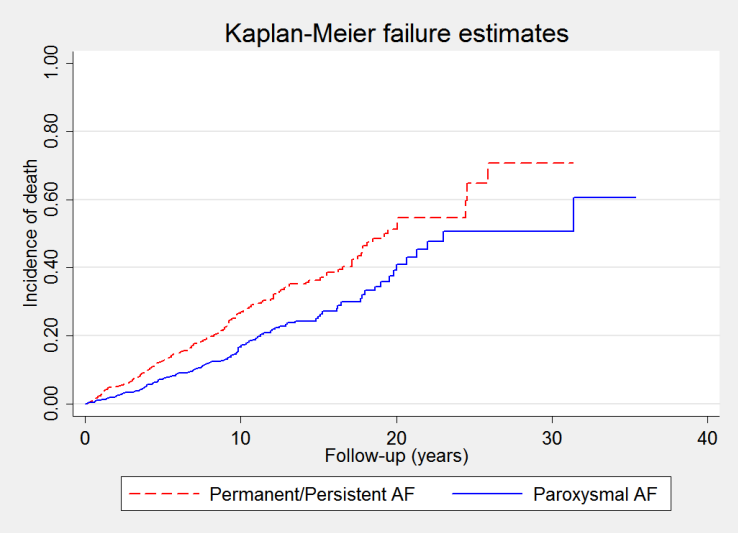


Breakdown of cardiovascular death:

SR group: sudden death: 96, heart failure related: 38, stroke related: 9, other cardiac: 29

AF group: sudden death: 38, heart failure related: 59, stroke related: 17, other cardiac: 31

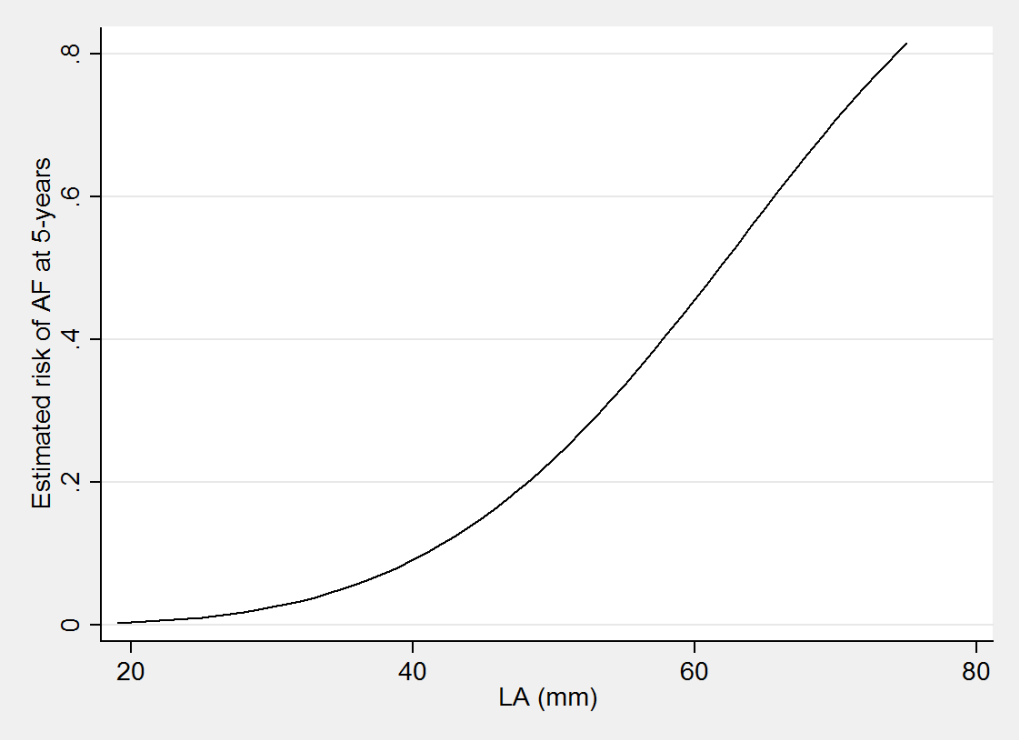
Figure 9b: Kaplan-Meier failure estimates comparing mortality (cardiovascular and non-cardiovascular) in patients with permanent/persistent AF compared with paroxysmal AF



4.3.6. Relation of LA size to AF

Figure 10 reveals the relationship between LA size and 5 year risk of AF. There was a linear relationship between LA size and AF risk above a diameter of 40 mm.

Figure 10: Relationship of LA size with risk of AF



4.4. Discussion

This study confirms the high prevalence and incidence of AF in this patient group and the association between atrial size, NYHA class and age [16;177;270] For the first time in a HCM population, we demonstrate an association with several other patient characteristics including sex, hypertension and vascular disease.

4.4.1. Prevalence, Incidence of AF and mortality

The prevalence and incidence of AF and mortality are in line with previous cohort studies in patients with HCM [16;177;179]. Evidence for the detrimental effect of AF on survival is limited in HCM [177;231] but in this study we show a significant association with cardiovascular mortality. Interestingly the survival curves for patients in SR and AF separate about 3 years into the follow up period with a higher proportion of heart failure and stroke related death in the AF group. The explanation for this delay is uncertain, but for patients who die of heart failure, it is possible that the onset of AF accelerates disease progression. Alternatively, AF may simply be a marker that identifies patients with advanced disease who are already on an accelerated trajectory to ventricular failure. There was also a trend towards increased mortality evident for non-cardiovascular mortality in the AF group. This is likely to represent a complex interplay between some miscoding of deaths, age at AF onset and the adverse consequences of cardiac disease and therapy on general health.

4.4.2. Significant centre effect in sensitivity analysis

The significant centre effect as part of a sensitivity analysis can be explained by the difference in some of the clinical characteristics of the participating cohorts including mean age, mean follow-up time and prevalence of atrial fibrillation. Further work is necessary to understand this, but it might reflect systematic differences in referral patterns.

4.4.3. Clinical implications

This study confirms the importance of atrial fibrillation in the natural history of HCM [271]. The findings in this and our previous study on stroke in HCM [272] suggest that patients with risk factors for AF should be undergo frequent ambulatory ECG monitoring to detect atrial arrhythmia and be considered for early prophylactic anticoagulation. The identification of associations with common cardiovascular risk factors such as vascular disease and hypertension is not by itself that surprising, but shows that management of these risk factors should be given equal priority to other disease-specific complications.

4.4.4. Limitations

The patient population in this study is large and diverse but the predictors identified in this study may not apply to patients with different characteristics. In particular, the study excludes paediatric patients (less than 16) and patients with metabolic or syndromic disorders in whom other disease characteristics may be influence the risk of AF development.

4.5. Conclusions

Readily available clinical parameters can be used to identify patients at high risk of AF who require more frequent monitoring and early anticoagulation. In the exploratory analysis AF was associated with increased cardiovascular and non-cardiovascular mortality.

5 Prediction of thromboembolic risk in patients with hypertrophic cardiomyopathy (HCM Risk-CVA)

5.1. Aims

AF and TE are common complications of the disease and are associated with adverse clinical outcomes and reduced survival [177;179;202;232;245;270]. However, HCM is a heterogeneous disorder with very variable clinical presentation and the absolute risk of TE – and by implication the likely benefit from treatment – in individual patients with different clinical characteristics is unknown [3;262;263;273].

The primary aim of this study was to derive and validate a risk model for estimating the risk of TE in patients with HCM. Exploratory analyses were performed to determine clinical predictors of TE and the performance of the CHA_2DS_2-VASc score [274].

5.2. Methods

5.2.1. Study design and overview

Data from a retrospective, multicentre longitudinal cohort – the Hypertrophic Cardiomyopathy Outcome Investigators (www.HCMRisk.org) [167] – were used in the development of the prognostic model.

The study conforms to the principles of the Helsinki declaration. The sponsors of this study did not have a role in study design, data collection, analysis, and interpretation. O.G., M.P., R.O., and P.E. had access to all data and final responsibility to submit the

article. The authors from each centre guarantee the integrity of data from their institution. All investigators have agreed to the article as written.

5.2.2. Study population and participating centres

The study cohort has been described in detail in Chapter 4.The study cohort consisted of all consecutively evaluated patients with HCM, followed at seven European centres: (i) The Heart Hospital, London, UK, (ii) A Coruna University Hospital, A Coruna, Spain, (iii) Unit of Inherited Cardiovascular diseases, 1st Department of Cardiology, University of Athens, Greece, (iv) Institute of Cardiology, University of Bologna, Italy, (v) University Hospital Virgen de la Arrixaca, Murcia, Spain, (vi) Monaldi Hospital, Second University of Naples, Italy and (vii) Hospital Universitario Puerta del Hierro, Madrid, Spain. Some patients from this cohort are reported in other recently published studies [167;190;219;252-260]. Only adult patients (≥16 years of age) were studied. HCM was defined as a maximum left ventricular wall thickness ≥15 mm unexplained solely by loading conditions [3] or in accordance with published criteria for the diagnosis of disease in relatives of patients with unequivocal disease [20]. Patients with known inherited metabolic diseases or syndromic causes of HCM were excluded from the study. Patients with a history of AF that had experienced TE prior to first evaluation at the centre were also excluded from the analysis.

5.2.3. Patient assessment and data collection

Patients were reviewed every 6–12 months or earlier if there was a change in symptoms. All patients underwent clinical assessment, pedigree analysis, physical examination, resting and ambulatory electrocardiography and transthoracic

echocardiography. Each centre collected data independently using the same methodology.

5.2.4. Clinical outcomes

The primary outcome was a thromboembolic event defined as a composite of cerebrovascular accident (CVA), transient ischaemic attack (TIA) or systemic peripheral embolus as defined in the CHA_2DS_2-VASc score. CVA was defined as a focal neurologic deficit of sudden onset as diagnosed by a neurologist, lasting more than 24h and caused by ischaemia. TIA was defined as a focal neurologic deficit of sudden onset as diagnosed by a neurologist, lasting less than 24 h. Peripheral embolism was defined as TE outside the brain, heart, eyes, and lungs [274].

5.2.5. Selection of predictors and coding

Following a review of the literature completed in September 2012 , predictors of TE that have been reported previously in patients with HCM were considered as candidate predictor variables [16]. Clinical parameters were used as pre-specified predictors only when associated with TE in at least one published study and were uniformly defined in all centres. In addition, predictors included in the CHA_2DS_2-VASc score and maximal LV wall thickness and peak left ventricular outflow tract (LVOT) gradient were considered for the analysis (table 12). Clinically plausible interactions between selected variables such as age and AF and age and vascular disease were also assessed. All predictors were assessed at baseline evaluation.

Table 12: Definition of pre-specified predictor variables assessed at baseline evaluation

Predictor variable	Definition	Coding
Sex	Male or female [181]	Binary, male/female
Age	Age at first evaluation in participating centres [176;233]	Continuous, years
VKA	Use of Vitamin K antagonist at first evaluation	Binary, yes/no
AF	Physician reported detection of paroxysmal, permanent of persistent AF on ECG or Holter monitoring [176;233]	Binary, yes/no
TE	Thromboembolism: CVA, TIA, peripheral embolus, as per CHA_2DS_2-VASc score [262;263]	Binary, yes/no
NYHA	NYHA class at first evaluation [176]	Categorical, 1,2 and 3-4
LA	Anterior-posterior left atrial diameter determined by 2D echocardiography in the parasternal long axis or short-axis plane at time of first evaluation [181]	Continuous, mm
MWT	The greatest LV wall thickness measured at the level of the mitral valve, papillary muscles, and apex in the parasternal short-axis plane using 2-D echocardiography at time of evaluation [210]	Continuous, mm
FS	LV end-diastolic dimension-LV end-systolic dimension)/ LV end-diastolic dimension measured by M-Mode or 2D echocardiography at time of evaluation [181]	Continuous, %
LVOT max	The maximum LV outflow gradient determined at rest and with Valsalva provocation (irrespective of concurrent medical treatment) using pulsed and continuous wave Doppler from the apical three- and five-chamber views. Peak outflow tract gradients were determined using the modified Bernoulli equation: Gradient = $4V^2$, where V is the peak aortic outflow velocity [171]	Continuous, mmHg
Hypertension	Diagnosis of hypertension prior to first evaluation, as per CHA_2DS_2-VASc score [262;263]	Binary, yes/no
Diabetes	Diagnosis of diabetes prior to first evaluation, as per CHA_2DS_2-VASc score [262;263]	Binary, yes/no
Vascular disease	Myocardial infarction, complex aortic plaque and peripheral arterial disease, as per CHA_2DS_2-VASc score [262;263]	Binary, yes/no
Heart failure	Heart failure, especially moderate to severe LV systolic dysfunction, defined arbitrarily as left ventricular ejection fraction (LVEF) <40% (calculated by FS), as per CHA_2DS_2-VASc score [262;263]	Binary, yes/no

VKA: Vitamin K antagonist, AF: atrial fibrillation, TE: thromboembolic event, NYHA: New York Heart Association Functional classification, LA: Left atrial size, MWT: Maximal wall thickness, FS: Fractional shortening, LVOT max: maximum LV outflow gradient

From Guttmann *et al* (2015) [272]

5.2.6. Sample size

A minimum of 10 thromboembolic events were required per coefficient estimated by the model to ensure that the regression coefficients of the model were estimated with adequate precision [275]. The 172 TE endpoints observed in this cohort over a 10 year follow-up period allow the estimation of up-to 17 regression coefficients with adequate precision and were sufficient for development of the risk model.

5.2.7. General statistical methods

STATA (version 12) and R (version 3.0) were used for the statistical analyses. For descriptive results, variables are expressed as mean ± standard deviation (SD), median and interquartile range (IQR) or counts and percentages as appropriate. The follow-up time for each patient was calculated from the date of their first evaluation at participating centres to the date of the study endpoint, death, heart transplantation, cerebral haemorrhage or to the date of their most recent evaluation. The annual event rate was calculated by dividing the number of patients reaching the endpoint by the total follow-up period for that endpoint. The Kaplan–Meier method was used to estimate the cumulative probability for the occurrence of an outcome.

5.2.8. Missing data

The characteristics of patients with missing information were compared to those of patients with complete information to investigate bias due to missing data. Logistic regression was used to identify the predictors of missingness. Data were assumed to be missing at random and values for the missing predictors were imputed using multiple imputation techniques based on chained equations [264]. The multiple imputation model included all predictors of missingness, the outcome, all pre-specified predictors of the

risk model, and the estimate of the cumulative hazard function [265]. Rubin's rules was used to combine the estimates from 30 imputed data sets [266].

5.2.9. Model development

All pre-specified predictors were candidates for inclusion in the final risk model. To account for potential non-linear relationships we considered the addition of quadratic terms for all continuous predictors. Due to sample size issues this was done in a pre-selection procedure where bi-variable models for each predictor and its quadratic term were fitted. Suspected interactions were also examined in a similar manner.

The model was developed based on the 172 events within the first 10 years of follow up. Backward elimination with a 15% significance level was used to select the predictors for the final risk model [267]. Centre was not included to allow the model to be used in patients from other centres. However, a sensitivity analysis for centre effect was performed by including centre in the model. The proportional hazards assumption required by the Cox model was investigated using Schoenfeld residuals [268]. The risk model was developed using the entire cohort.

5.2.10. Model validation

Bootstrapping was used to evaluate the performance of the model. This is the most efficient internal validation procedure as all aspects of the model development, including variable selection are validated [269]. Two hundred bootstrap samples were generated for each imputed dataset and the optimism-adjusted performance measures from the imputed datasets were combined using Rubin's rules [276]. The calibration slope was used to assess the degree of agreement between the observed and predicted hazards of thromboembolism [277]. A value close to 1 suggests good overall agreement. Graphical

comparisons of the observed and predicted thromboembolism at 5 years were performed. The C-index and D-statistic were used to measure how well the model discriminated between patients with high and low risk of thromboembolism [167;278-280]. A value of 0.5 for C-index indicates no discrimination and a value equal to 1 indicates perfect discrimination. The D-statistic can be interpreted as the log hazard ratio for having thromboembolism between the low and high risk groups of patients. A model with no discriminatory ability results in a value of 0 for D-statistic, with increasing values indicating greater separation.

5.2.11. Model presentation

The probability of TE at 5 years for an individual patient was calculated using the following equation, derived from the Cox proportional hazards model:

$$P_{\text{TE at 5 years}} = 1 - S_0(t)^{\exp(\text{Prognostic Index})}$$

where $S_0(t)$ is the average survival probability at time t (i.e. at 5 years), and the prognostic index is the sum of the products of the predictors and their coefficients.

5.2.12. Calculation of CHA_2DS_2-VASc score

The CHA_2DS_2-VASc score was calculated for a subset of patients with AF not treated with VKA at baseline [262;263;281]. The distribution of the score and number of events according to the score was determined.

5.2.13. Relation of patient characteristics to thromboembolic risk

Prespecified sub-analyses were performed to assess the relationship between five year risk of TE and age, vascular disease and LA size. Secondary analyses were performed in patients in SR who experienced TE and patients with AF who did not experience TE.

5.3. Results

5.3.1. Baseline clinical characteristics

During the study period 5104 patients were evaluated, of whom 197 were seen only once for baseline evaluation and were excluded from the analysis. Eighty six patients with a history of AF and TE prior to first evaluation were excluded. The final study cohort consisted of the remaining 4821 patients and the baseline clinical characteristics are shown in table 13. Table 14 displays the cohort characteristics according to centre.

Table 13: Clinical characteristics of whole cohort and in patients with and without thromboembolic endpoint (TE)

	Whole cohort		No TE		TE	
predictor	**total**	**mean (SD)/n(%)**	**total**	**mean (SD)/n(%)**	**total**	**mean (SD)/n(%)**
Age	4817	48.99 (16.40)	4645	48.74 (16.39)	172	55.73 (15.40)
LA	4627	43.97 (7.74)	4460	43.82 (7.68)	167	47.83 (8.37)
MWT	4768	19.44 (5.15)	4599	19.42 (5.18)	169	20.05 (4.16)
FS	4358	0.41 (0.10)	4198	0.41 (0.10)	160	0.40 (0.09)
LVOT max	4168	31.95 (40.94)	4023	31.85 (40.96)	145	34.72 (40.46)
Female	4820	1740 (36.10)	4648	1666 (35.84)	172	74 (43.02)
Prior TE	4821	80 (1.66)	4649	71 (1.53)	172	9 (5.23)
AF	4815	600 (12.46)	4643	552 (11.89)	172	48 (27.91)
VKA	4818	443 (9.20)	4646	410 (8.82)	172	33 (19.19)
NYHA II	4615	1584 (34.32)	4450	1519 (34.13)	165	65 (39.29)
NYHA III,IV	4615	494 (10.70)	4450	456 (10.24)	165	38 (23.03)
Vascular	3588	89 (2.48)	3438	79 (22.98)	150	10 (6.67)

disease						
Hypertension	4712	1414 (30.00)	4541	1354 (29.82)	171	60 (35.09)
Diabetes	4020	293 (7.29)	3868	279 (7.21)	152	14 (9.21)

SD: Standard deviation, n: Number, LA: Left atrial size, MWT: Maximal wall thickness, FS: Fractional shortening, LVOT max: maximum LV outflow gradient, TE: thromboembolic event, AF: atrial fibrillation, VKA: Vitamin K antagonist, NYHA: New York Heart Association Functional classification

Breakdown of AF in whole cohort: paroxysmal 314, persistent 102, permanent 181, not specified 3

From Guttmann *et al* (2015) [272]

Table 14: Cohort characteristics according to centre

Centre	Variable	total	mean(SD)/n	Min	max
LONDON	Age	2082	45.28 (15.45)	16.03	88.37
	LA	2026	43.65 (7.82)	19.00	75.00
	MWT	2079	19.36 (5.47)	7.00	43.00
	FS	1985	0.41 (0.09)	0.10	0.82
	LVOT max	1745	26.87 (37.98)	1.00	260.00
	Female	2082	748		
	Prior TE	2082	25		
	AF	2082	167		
	VKA	2082	103		
	NYHA II	1959	772		
	NYHA III,IV	1959	174		
	Vascular disease	1320	15		
	Hypertension	1977	405		
	Diabetes	1319	60		
ATHENS	Age	558	46.43 (16.24)	16.00	82.44
	LA	558	43.65 (6.37)	23.00	65.00
	MWT	558	18.11 (4.02)	12.00	37.00
	FS	558	0.40 (0.07)	0.07	0.64
	LVOT max	558	31.86 (36.18)	2.00	175.00
	Female	557	165		
	Prior TE	558	8		
	AF	558	75		
	VKA	558	42		
	NYHA II	558	182		
	NYHA III,IV	558	49		
	Vascular disease	558	9		

	Hypertension	558	148		
	Diabetes	558	37		
BOLOGNA	Age	516	50.88 (15.73)	17.16	90.26
	LA	510	45.48 (8.53)	20.00	85.00
	MWT	516	20.17 (4.89)	15.00	39.00
	FS	500	0.41 (0.11)	0.06	0.77
	LVOT max	516	33.97 (48.08)	4.00	237.00
	Female	516	184		
	Prior TE	516	5		
	AF	516	79		
	VKA	516	56		
	NYHA II	516	164		
	NYHA III,IV	516	51		
	Vascular disease	516	8		
	Hypertension	516	153		
	Diabetes	516	29		
CORUNA	Age	600	56.40 (15.34)	16.72	89.19
	LA	569	44.38 (7.79)	23.00	73.00
	MWT	568	20.05 (4.87)	12.00	42.00
	FS	547	0.41 (0.09)	0.05	0.72
	LVOT max	311	49.39 (50.31)	2.00	299.00
	Female	601	223		
	Prior TE	601	16		
	AF	601	102		
	VKA	601	101		
	NYHA II	587	198		
	NYHA III,IV	587	73		
	Vascular disease	601	7		
	Hypertension	601	260		
	Diabetes	563	77		
MADRID	Age	242	54.63 (16.52)	16.99	88.32
	LA	233	44.02 (7.78)	26.00	65.00
	MWT	237	20.51 (5.36)	7.00	41.00
	FS	221	0.41 (0.12)	0.08	0.74
	LVOT max	233	33.11 (41.01)	1.00	250.00
	Female	242	92		
	Prior TE	242	8		
	AF	237	53		
	VKA	242	53		
	NYHA II	240	58		
	NYHA III,IV	240	17		

	Vascular disease	241	20		
	Hypertension	242	108		
	Diabetes	242	31		
MURCIA	Age	614	53 (17.05)	16.47	90.26
	LA	594	43.28 (7.69)	23.00	81.00
	MWT	605	19.06 (5.09)	8.00	43.00
	FS	534	0.39 (0.10)	0.05	0.78
	LVOT max	613	35.90 (42.50)	1.00	205.00
	Female	614	241		
	Prior TE	614	15		
	AF	614	86		
	VKA	614	58		
	NYHA II	609	171		
	NYHA III,IV	609	122		
	Vascular disease	144	19		
	Hypertension	610	278		
	Diabetes	614	45		
NAPLES	Age	205	46.81 (15.95)	16.22	83.53
	LA	137	45.40 (7.81)	25.00	76.00
	MWT	205	20.32 (4.86)	11.00	40.00
	FS	13	0.38 (0.17)	0.16	0.78
	LVOT max	192	30.62 (24.93)	7.00	135.00
	Female	208	87		
	Prior TE	208	3		
	AF	207	38		
	VKA	205	30		
	NYHA II	146	39		
	NYHA III,IV	146	8		
	Vascular disease	208	11		
	Hypertension	208	62		
	Diabetes	208	14		

SD: Standard deviation, n: Number, LA: Left atrial size, MWT: Maximal wall thickness, FS: Fractional shortening, LVOT max: maximum LV outflow gradient, TE: thromboembolic event, AF: atrial fibrillation, VKA: Vitamin K antagonist, NYHA: New York Heart Association Functional classification, FH SCD: Family history of sudden cardiac death

5.3.2. Thromboembolic events during follow-up

During a follow up period of 28330.6 patient years (median 6.0 years, IQR= 3-9.7) 172 (3.6%) patients reached the primary endpoint within 10 years from first evaluation (105 CVA, 53 TIA and 14 peripheral emboli); 107 (2.2%) patients within the first 5 years. The

5 and 10 year cumulative incidences were 2.9% (95% CI 2.37%- 3.48%) and 6.4% (95% CI 5.42%-7.53%) respectively. The clinical characteristics of patients with and without TE are shown in table 13.

Patients in SR at first evaluation who developed TE during follow up were older (55.0 years vs 47.5 years; difference in means= 7.5 years; 95% CI= 4.60-10.42), had larger LA diameter (46.0 mm vs 43.0 mm; difference in means= 3.0 mm; 95% CI= 1.7-4.32) and were more symptomatic (NYHA III, IV) (14.4% vs 9.0%; difference in proportions= 0.054; 95% CI= 0.0099-0.1181) compared to patients who did not have an event. There was also a higher percentage of patients with vascular disease (5.7% vs 2.0%; difference in proportions= 0.037; 95% CI= 0.0074-0.0812) in the event cohort (Table 15). The mean age of patients with and without vascular disease was 62.4 years and 49.1 years respectively.

Table 15: Thromboembolic events in patients with sinus rhythm and AF at baseline evaluation

		SR	SR	AF	AF
	characteristic	**total**	**mean (SD)/n (%)**	**total**	**mean (SD)/n (%)**
TE no	**Age**	4087	47.49 (16.29)	552	57.87 (13.91)
	LA	3925	42.99 (7.15)	531	50.03 (8.60)
	MWT	4047	19.43 (5.26)	547	19.35 (4.51)
	FS	3719	0.41 (0.09)	475	0.38 (0.11)
	LVOT max	3502	32.45 (41.48)	516	27.70 (37.09)
	Female	4090	1436 (35.11)	552	225 (40.76)
	Prior TE	4091	71 (17.36)	552	0
	VKA	4089	85 (2.08)	552	323 (58.51)
	NYHA II	3921	1299 (33.13)	525	219 (41.71)
	NYHA III,IV	3921	353 (9.00)	525	103 (19.62)
	Hypertension	3995	1125 (28.16)	540	227 (42.04)
	Diabetes	3363	214 (6.36)	499	65 (13.03)
	Syncope	4063	602 (14.82)	541	85 (15.71)
	FH SCD	3966	957 (24.13)	539	111 (20.60)
	Vascular disease	2933	58 (1.98)	499	21 (4.21)

TE yes	**Age**	124	55.00 (15.48)	48	57.62 (15.20)
	LA	119	46.00 (7.82)	48	52.35 (8.04)
	MWT	121	20.38 (4.08)	48	19.23 (4.27)
	FS	114	0.41 (0.09)	46	0.38 (0.10)
	LVOT max	101	39.98 (41.33)	44	22.66 (36.00)
	Female	124	53 (42.74)	48	21 (43.75)
	Prior TE	124	9 (7.26)	48	0
	VKA	124	7 (5.64)	48	26 (54.17)
	NYHA II	118	54 (45.76)	47	11 (23.40)
	NYHA III,IV	118	17 (14.41)	47	21 (44.68)
	Hypertension	123	37 (30.08)	48	23 (47.92)
	Diabetes	108	9 (8.33)	44	5 (11.36)
	Syncope	124	17 (13.71)	47	6 (12.77)
	FH SCD	120	32 (26.67)	48	8 (16.67)
	Vascular disease	106	6 (5.66)	44	4 (9.10)

SD: Standard deviation, n: Number, LA: Left atrial size, MWT: Maximal wall thickness, FS: Fractional shortening, LVOT max: maximum LV outflow gradient, TE: thromboembolic event, AF: atrial fibrillation, VKA: Vitamin K antagonist, NYHA: New York Heart Association Functional classification, FH SCD: Family history of sudden cardiac death

From Guttmann *et al* (2015) [272]

5.3.3. Missing data

Missing data per variable are described in table 16.

Table 16: Missing data per variable

Predictor	n	Total	%
AF	6	4,821	0.12
VKA	3	4,821	0.06
Prior TE	0	4,821	0
Sex	1	4,821	0.02
Age	4	4,821	0.08
NYHA class	206	4,821	4.27
MWT	53	4,821	1.1
LA	194	4,821	4.02
LVEDD	218	4,821	4.52
LVESD	452	4,821	9.38
LVOT max	653	4,821	13.54
Syncope	43	4,821	0.89

FH SCD	142	4,821	2.95
Hypertension	109	4,821	2.26
Diabetes	801	4,821	16.61
Vascular disease	1,233	4,821	25.58

n: Number, AF: atrial fibrillation, VKA: Vitamin K antagonist, TE: thromboembolic event, NYHA: New York Heart Association Functional classification, MWT: Maximal wall thickness, LA: Left atrial size, LVEDD: Left ventricular end-diastolic dimension, LVESD: left ventricular end-systolic dimension, LVOT max: maximum LV outflow gradient, FH SCD: Family history of sudden cardiac death

From Guttmann *et al* (2015) [272]

5.3.4. Model Development

Univariable analyses are shown in table 17. Only maximal LV wall thickness was found to have a non-linear association with TE and so a quadratic term was included as a candidate for the final prognostic model. The interaction between AF and age was also found to be significant. There were 15 predictors (16 regression coefficients) candidates for the final model.

Age, AF, the interaction between age and AF, TE prior to first evaluation, NYHA class II, NYHA class III and IV, LA diameter, vascular disease, maximal LV wall thickness and (maximal LV wall thickness)^2 were included in the risk model. The estimates of the hazard ratios and the corresponding confidence intervals for the risk prediction model are shown in table 17. There was no significant centre effect as part of a sensitivity analysis (shown in table 18).

The risk of TE in 5 years for an individual HCM patient can be calculated from the following equation:

$P_{TE \text{ at } 5 \text{ years}} = 1 - 0.9999874^{\exp(\text{Prognostic Index})}$

,

where prognostic index=

0.030417476 *age (years) + 2.129977874 *af (yes=1/no=0) - 0.027069595 *age*af + 1.288557829 * TE prior (yes=1/no=0) + 0.224673046 *nyha classII (yes=1/no=0)+ 0.728180341 *nyha class III/IV (yes=1/no=0)+ 0.032251831 *la diam (mm) + 0.3735254 *mwt (mm) -0.008324216 *mwt^2 (mm) + 0.512492795 *vascular disease (yes=1/no=0)

Table 17: Exploratory univariable and multivariable analysis for predictors of thromboembolism in HCM

Univariable analysis					Multivariable analysis			
predictor	**HR**	**p**	**CI**		**predictor**	**HR**	**p**	**CI**
Sex	1.43	0.02	1.06 - 1.93		AGE	1.03	<0.001	1.02 - 1.04
AGE10	1.45	<0.001	1.31 - 1.60		AF	8.41	<0.001	1.95 - 36.35
AF	3	<0.001	2.15 - 4.19		age_af	0.97	0.03	0.95 - 1.00
prior TE	4.15	<0.001	2.12 - 8.13		prior TE	3.63	<0.001	1.81 - 7.29
NYHA II	1.61	0.01	1.14 - 2.29		NYHA II	1.25	0.21	0.88 - 1.78
NYHA III,IV	3.66	<0.001	2.44 - 5.48		NYHA III, IV	2.07	<0.001	1.35 - 3.17
LA5	1.36	<0.001	1.24 - 1.48		LA	1.03	<0.001	1.01 - 1.05
MWT	1.01	0.3	0.99 - 1.04		MWT	1.45	<0.001	1.12 - 1.88
FS	0.22	0.08	0.04 - 1.20		MWT^2	0.99	0.01	0.99 - 1.00
EF	0.3	0.08	0.08 - 1.16		Vascular disease	1.67	0.12	0.88 - 3.18
LVEDD	1	0.88	0.98 - 1.03					
LVESD	1.01	0.29	0.99 - 1.04					
LVOTmax	1	0.09	1.00 - 1.01					
Hypertension	1.46	0.02	1.06 - 1.99					
Diabetes	1.36	0.27	0.79 - 2.36					
Vascular disease	3.2	<0.001	1.68 -					

			6.07
MWT	1.67	<0.001	1.29 - 2.16
MWT^2	0.99	<0.001	0.98 - 0.99

Age10: Hazard ratio for 10 year increments, VKA: Vitamin K antagonist, AF: atrial fibrillation, TE: thromboembolic event NYHA: New York Heart Association Functional classification, LA5: Hazard ratio for left atrial size for 5mm increments, MWT: Maximal wall thickness, FS: Fractional shortening, EF: Ejection fraction, LVEDD: Left ventricular end-diastolic dimension, LVESD: left ventricular end-systolic dimension, LVOT max: maximum LV outflow gradient, HR: Hazard ratio, p: p-value, CI: 95% confidence interval, HR MI: Hazard ratio following multiple imputation, p MI: p-value following multiple imputation, CI MI: 95% confidence interval following multiple imputation

MWT and MWT^2 in the last two rows of the table adjust for MWT and its square term

From Guttmann *et al* (2015) [272]

Table 18: Thromboembolism risk prediction model and sensitivity analysis for centre effect

without centre				with centre		
predictor	HR	p	CI	HR	p	CI
AGE	1.03	<0.001	1.02 - 1.04	1.030	<0.001	1.02 - 1.04
AF	8.41	<0.001	1.95 - 36.35	8.740	<0.001	2.03 - 37.70
age_af	0.97	0.030	0.95 - 1.00	0.970	0.030	0.95 - 1.00
prior TE	3.63	<0.001	1.81 - 7.29	3.600	<0.001	1.78 - 7.28
NYHA II	1.25	0.210	0.88 - 1.78	1.230	0.250	0.86 - 1.75
NYHA III, IV	2.07	<0.001	1.35 - 3.17	2.020	<0.001	1.31 - 3.13
LA	1.03	<0.001	1.01 - 1.05	1.030	<0.001	1.01 - 1.05
MWT	1.45	<0.001	1.12 - 1.88	1.460	<0.001	1.13 - 1.89
MWT^2	0.99	0.010	0.99 - 1.00	0.990	0.010	0.99 - 1.00
Vascular disease	1.67	0.120	0.88 - 3.18	1.760	0.100	0.90 - 3.42
Athens				0.590	0.070	0.34 - 1.03
Bologna				0.880	0.660	0.50 - 1.55
Corunña				1.080	0.740	0.70 - 1.65
Madrid				0.660	0.380	0.26 - 1.67
Murcia				0.930	0.760	0.56 - 1.53
Naples				0.730	0.540	0.26 - 2.01

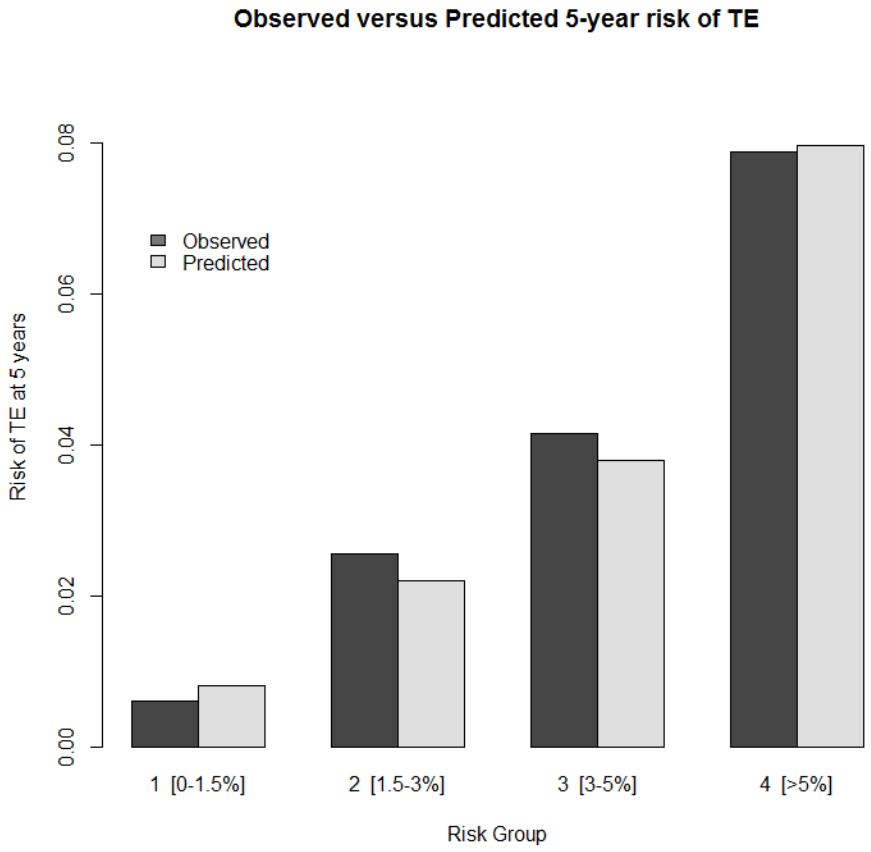
AF: atrial fibrillation, age_af: interaction between age and AF, TE: thromboembolic event, NYHA: New York Heart Association Functional classification, LA: Left atrial size, MWT: Maximal wall thickness, HR: Hazard ratio, p: p-value, CI: 95% confidence interval. Age: Hazard ratio for 10 year increments, LA: Hazard ratio for left atrial size for 5mm increments.

From Guttmann *et al* (2015) [272]

5.3.5. Model validation

Bootstrapping showed a good calibration slope of 0.91 (95% CI: 0.74, 1.08). Figure 11 illustrates a good agreement between the observed and predicted risk at exploratory thresholds of thromboembolic risk at 5 years. The C-index was 0.75 (95% CI: 0.70, 0.80) and the D-statistic was 1.30 (95% CI: 1.05, 1.56) indicating good discrimination.

Figure 11: Agreement between observed and predicted risk of at exploratory thresholds of thromboembolic risk at 5 years



Group 1: 2464 patients, Group 2: 1274, Group 3: 696, Group 4: 387

From Guttmann *et al* (2015) [272]

5.3.6. Comparison with conventional stroke prediction models

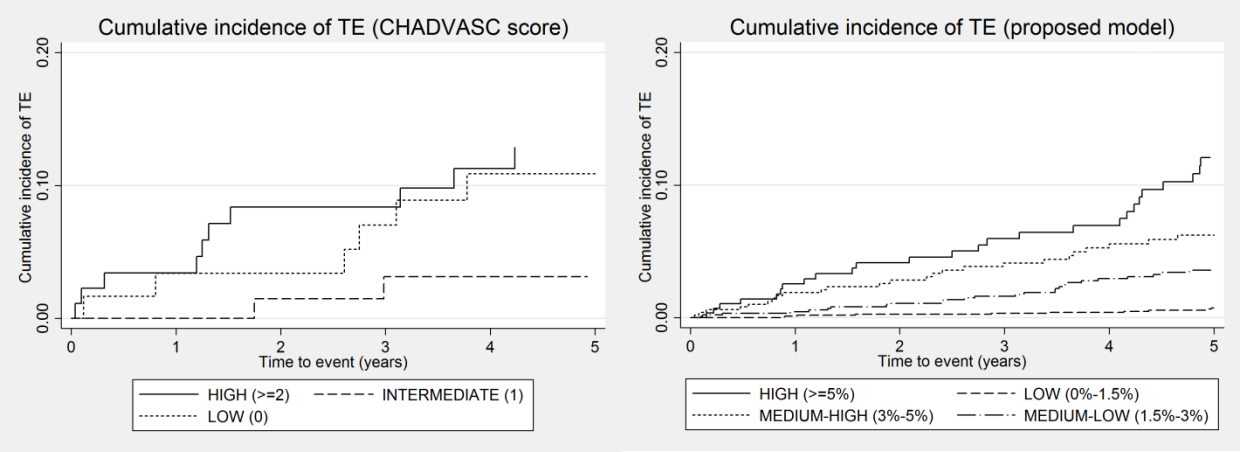
222 patients with complete data and AF not treated with VKA at baseline evaluation; of these, 61 (27.5%) had a CHA_2DS_2-VASc score of 0 and 19 (8.6%) a score between 4 and 6. No patient had a score of 7 to 9. Table 19 presents the prevalence of TE in patients according to CHA_2DS_2-VASc score. Figure 12 displays the cumulative incidence of TE according to CHA2DS2-VASc score groups and according to 5 year risk prediction model.

Table 19: Prevalence of thromboembolism according to CHA_2DS_2-VASc score in HCM patients with AF not treated with VKA.

CHA_2DS_2-VASc Score	TE no, n (%)	TE yes, n (%)
0	55 (90.16%)	6 (9.84%)
1	69 (95.83%)	3 (4.17%)
2	37 (88.10%)	5 (11.90%)
3	26 (92.86%)	2 (7.14%)
4	11 (73.33%)	4 (26.67%)
5	2 (66.67%)	1 (33.33%)
6	1 (100%)	0 (0%)
Total	201 (90.54%)	21 (9.46%)

TE: Thromboembolic events, n: Number
From Guttmann *et al* (2015) [272]

Figure 12: Kaplan-Meier failure estimates for cumulative incidence of TE. The left graph displays the cumulative incidence of TE according to CHA_2DS_2-VASc score groups (Low=0; 61 patients, Intermediate=1; 72 patients, High>2; 89 patients). The right graph displays the cumulative incidence of TE according to 5 year risk prediction model (Low= 0%-1.5%; 1643 patients, Medium-Low=1.5%-3%; 887 patients, Medium-High=3%-5%; 494 patients, High>5%; 287 patients)



From Guttmann *et al* (2015) [272]

4.3.7. Relationship of LA size to risk of thromboembolic events

Figure 13 shows the relationship between LA size and 5 year risk of TE. There appears to be a linear relationship up to about 45-50mm at which point the risk of TE rises exponentially with increasing LA diameter. In the cohort of patients in SR at first evaluation with a LA diameter more than 50 mm, the prevalence of a thromboembolic event was 4.7% (table 20). Patients with an event in this group were older (55.8 years vs 50.1 years) as compared to patients who did not experience an event. Patients in AF who did not develop a thromboembolic event had a smaller LA diameter (50.0 mm vs 52.3 mm) and were less symptomatic (NYHA III, IV) (19.6% vs 44.7%) compared to patients with an event. The characteristics are displayed in table 15.

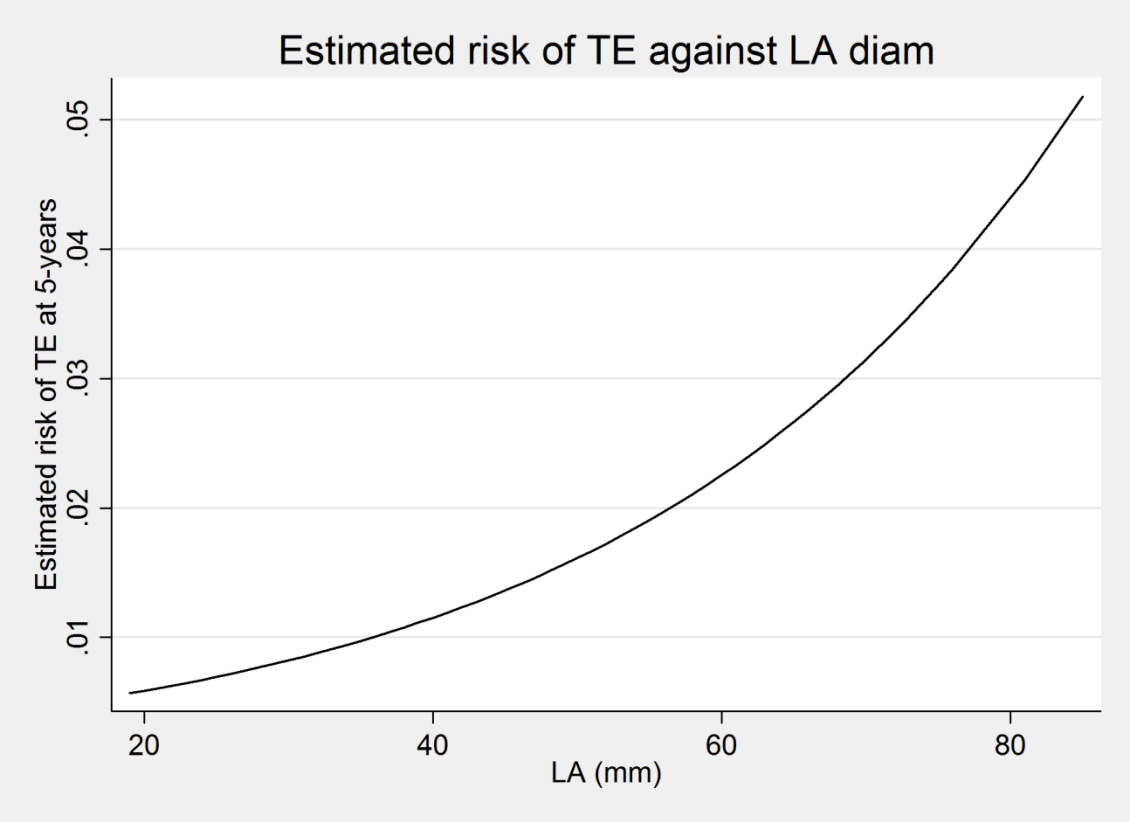
Table 20: Thromboembolic events in patients with sinus rhythm with LA>45 and sinus rhythm LA>50 at baseline evaluation

		SR, >45	SR, >45	SR, >50	SR, >50
	variable	**total**	**mean (SD)/n (%)**	**total**	**mean (SD)/n (%)**
TE no	**Age**	1499	50.45 (14.94)	728	50.15 (15.08)
	LA	1336	50.78 (4.40)	565	54.80 (3.86)
	MWT	1478	20.72 (5.19)	710	21.10 (5.37)
	FS	1291	0.41 (0.10)	567	0.41 (0.11)
	LVOT max	1275	42.68 (46.60)	608	45.12 (47.95)
	Female	1502	444 (29.56)	731	193 (26.40)
	Prior TE	1502	34 (2.26)	731	14 (1.92)
	VKA	1500	62 (4.13)	729	43 (5.90)
	NYHA II	1434	526 (36.68)	692	245 (35.40)
	NYHA III, IV	1434	192 (13.39)	692	118 (17.05)
	Hypertension	1462	465 (31.81)	711	227 (31.93)
	Diabetes	1223	102 (8.34)	593	48 (8.09)
	Syncope	1494	232 (15.53)	725	110 (15.17)
	FH SCD	1461	330 (22.59)	708	153 (21.61)
	Vascular disease	1114	22 (1.97)	543	9 (1.66)
TE yes	**Age**	62	56.20 (12.35)	34	55.75 (11.94)
	LA	57	52.12 (6.08)	29	56.03 (6.27)
	MWT	59	20.92 (4.35)	32	21.97 (4.98)
	FS	56	0.42 (0.09)	30	0.41 (0.10)
	LVOT max	51	42.96 (41.55)	29	41.76 (32.80)
	Female	62	24 (38.71)	34	11 (32.35)
	Prior TE	62	6 (9.68)	34	4 (11.76)
	VKA	62	6 (9.68)	34	5 (14.71)
	NYHA II	59	28 (47.46)	31	12 (38.71)
	NYHA III, IV	59	8 (13.56)	31	7 (22.58)
	Hypertension	61	19 (31.15)	34	11 (32.35)
	Diabetes	55	6 (10.01)	27	2 (7.41)
	Syncope	62	9 (14.52)	34	5 (14.71)
	FH SCD	59	14 (23.73)	32	9 (28.13)
	Vascular disease	55	5 (9.10)	28	4 (14.29)

SD: Standard deviation, n: Number, LA: Left atrial size, MWT: Maximal wall thickness, FS: Fractional shortening, LVOT max: maximum LV outflow gradient, TE: thromboembolic event, AF: atrial fibrillation, VKA: Vitamin K antagonist, NYHA: New York Heart Association Functional classification, FH SCD: Family history of sudden cardiac death

From Guttmann *et al* (2015) [272]

Figure 13: Relationship of LA size with risk of thromboembolism



From Guttmann *et al* (2015) [272]

5.4. Discussion

In this study we present the first validated model for TE prediction in a diverse population of adult patients with HCM. The study confirms the high risk of TE in patients with AF and the strong association between atrial size and thromboembolic risk [16;177;270]. It also demonstrates an association with several other patient characteristics including age, heart failure symptoms, and maximal LV wall thickness and, for the first time in this population, vascular disease.

5.4.1. CHA_2DS_2-VASc score in HCM

Clinical guidelines recommend the CHA_2DS_2-VASc score as means of stratifying patients with non-valvular AF for antithrombotic prophylaxis [262;263;281]. We show that this score has a relatively low predictive accuracy in patients with HCM that is probably explained by the lower prevalence of vascular risk factors. These data support recent consensus guidelines from the ESC that advise against the use of the CHA_2DS_2-VASc score in patients with HCM [3].

5.4.2. Implications for patients in SR

The risk of TE risk in patients who are in SR has not been examined in detail previously. Risk factors for TE in this group included advanced age, heart failure symptoms, increased LA diameter and vascular disease. While this study does not prove that patients in SR who have a high estimated risk of TE benefit from anticoagulation prior to the development of AF, it does support recent recommendations for frequent ambulatory ECG monitoring in patients with LA enlargement [3]. Irrespective of atrial rhythm, clinicians should also be alert to conventional vascular risk factors and treat them appropriately.

5.4.3. Limitations

The patient population in this study is large and diverse but the model should only be used in patients with similar characteristics. It is not validated in paediatric patients (less than 16) and in patients with metabolic or syndromic disorders.

A prospective external validation in a different cohort of patients would be ideal.

Ethnicity may have influenced the findings. Data on this was not available in this cohort.

The model includes patients who are treated with VKA according to current guidelines. Excluding these patients would exclude high risk patients and limit the statistical analysis.

5.5. Conclusions

The study shows that the risk of TE in patients with HCM can be identified using a small number of simple clinical features. LA size, in particular, should be monitored closely and the assessment and treatment of conventional vascular risk factors should be routine practice in older patients. The CHA2DS2-VASc score does not appear to correlate well with the clinical outcome in patients with HCM and should not be used to assess TE risk in this population.

6 Outcome of anticoagulation and antiarrhythmic therapy in patients with HCM

6.1. Aims

AF and TE are common complications of the disease and are associated with adverse clinical outcomes and reduced survival [177;179;202;232;245;270]. Current ESC and ACCF/AHA guidelines recommend anticoagulation in all patients with HCM and AF [262;263;273;282;283]. The efficacy of vitamin K antagonists has not been investigated in patients with HCM and AF in randomised controlled trials. Stroke was less common in anticoagulated patients as compared to antiplatelet therapy in two observational studies [176;177]. Further two small studies compared TE events in patients on and off warfarin [231;233]

The primary aim of this study was to assess the outcome of therapy with vitamin K antagonists (VKA) in patients with AF. The bleeding risk in patients with HCM was assessed using the HAS-BLED score in an exploratory analysis. Exploratory analyses were also performed to investigate the efficacy of antiarrhythmic therapy in the prevention of AF.

6.2. Methods

6.2.1. Study design and overview

Data from a retrospective, multicentre longitudinal cohort – the Hypertrophic Cardiomyopathy Outcome Investigators (www.HCMRisk.org) [167] – were used in the development of the prognostic model.

The study conforms to the principles of the Helsinki declaration. The sponsors of this study did not have a role in study design, data collection, analysis, and interpretation. O.G., M.P., R.O., and P.E. had access to all data and final responsibility to submit the article. The authors from each centre guarantee the integrity of data from their institution. All investigators have agreed to the article as written.

6.2.2. Study population and participating centres

The study cohort has been described in detail in Chapter 4 and 5.

Patients with a history of AF that had experienced TE prior to first evaluation at the centre were also excluded from the analysis.

6.2.3. Patient assessment and data collection

Patients were reviewed every 6–12 months or earlier if there was a change in symptoms. All patients underwent clinical assessment, pedigree analysis, physical examination, resting and ambulatory electrocardiography and transthoracic echocardiography. Each centre collected data independently using the same methodology.

6.2.4. Clinical outcomes

The primary outcome was a thromboembolic event defined as a composite of cerebrovascular accident (CVA), transient ischaemic attack (TIA) or systemic peripheral embolus as defined in the CHA_2DS_2-VASc score. CVA was defined as a focal neurologic deficit of sudden onset as diagnosed by a neurologist, lasting more than 24h and caused by ischaemia. TIA was defined as a focal neurologic deficit of sudden onset as diagnosed by a neurologist, lasting less than 24 h. Peripheral embolism was defined

as TE outside the brain, heart, eyes, and lungs [274]. For the analysis of the effect of antiarrhythmic therapy on the development of AF The outcome of interest was atrial fibrillation, which was defined as paroxysmal, permanent or persistent AF detected on ECG, Holter monitoring or device interrogation.

6.2.5. Clinical outcome of anticoagulation in patients with AF

The incidence of TE in patients with AF who and were not treated with anticoagulants was investigated using an intention to treat analysis. If a patient received treatment with VKA at any time prior to the event it was assumed that they continued on this medication for the duration of the study. The numbers of patients with and without TE that did or did not receive VKA were compared. The absolute risk reduction (ARR) and number needed to treat (NNT) were calculated for patients in AF at an exploratory threshold of 4% risk of thromboembolic event over 5 years.

6.2.6. HAS-BLED score in patients with HCM

To assess the bleeding risk of our population, we calculated the HAS-BLED score in HCM patients with AF [284].

6.2.7. Clinical outcome of antiarrhythmic therapy

An intention to treat analysis was used to investigate clinical outcomes in patients receiving β-adrenoreceptor blockers, non-dihydropyridine calcium channel antagonists, disopyramide and amiodarone. If a patient received treatment at any time prior to the event it was assumed that they continued on this medication until the endpoint of the study was reached or the end of follow up. The effect on development of AF was

investigated. The total number of patients in the AF group and SR groups on and off medication were compared and assessed for statistical significance.

6.3. Results

6.3.1. Baseline clinical characteristics

During the study period 5104 patients were evaluated, of whom 197 were seen only once for baseline evaluation and were excluded from the analysis of relation between anticoagulation and TE risk in patients with AF. Eighty six patients with a history of AF and TE prior to first evaluation were also excluded. The final study cohort for this analysis consisted of the remaining 4821 patients and the baseline clinical characteristics are shown in table 13.

For the analysis of the clinical outcomes in patients receiving antiarrhythmic therapy of the 5104 patients, 197 patients, who were only seen once at baseline evaluation and an additional 659 patients with AF prior to first evaluation were excluded for the analysis of AF events. The final study cohort therefore consisted of 4248 patients (baseline clinical characteristics are shown in table 7).

6.3.2. Relation between anticoagulation and TE risk in patients with AF

Table 21 presents an unadjusted exploratory analysis of the prevalence of TE over 10 years in patients with AF at first evaluation that did or did not receive VKA during the follow up period prior to TE; 12.4% of those not receiving VKA and 6.8% of patients of those who were receiving anticoagulation had a thromboembolic event. This corresponds to a relative risk reduction of 54.8% (CI 0.31-0.97, p=0.037) with VKA treatment. Figure 14 displays the Kaplan Meier curves comparing VKA and non VKA

groups. The absolute risk reduction (ARR) and number needed to treat (NNT) for patients in AF at an exploratory threshold of 4% risk of thromboembolic event over 5 years is 13% (95% CI, 2.1%-24%) and 7.7 respectively. These results should be interpreted with caution as the small numbers meant that a standard multivariable model adjusting for warfarin and the rest of the predictors was not appropriate. Instead we fitted a multivariable model as a sensitivity analysis using Lasso regression, a penalised regression method suitable for datasets with few events [285]. In this fully adjusted analysis, warfarin maintained its protective effect (results not shown). We also used propensity score analysis to further explore the indication for the effect of VKA on stroke. The propensity score for each patient was calculated as the predicted probability of receiving VKA. It is clinical practice in the participating centres to consider therapy with VKA in patients with significantly enlarged LA diameter. Indeed LA was the strongest balancing factor in the propensity score model to identify treatment allocation variables, while older age, history of diabetes and smaller fractional shortening were also associated with higher probability of receiving VKA. Subsequently a Cox regression model for the time to TE adjusting for VKA and the propensity score [286], showed that VKA maintained its protective effect (HR=0.41; 95% CI=(0.22-0.76)).

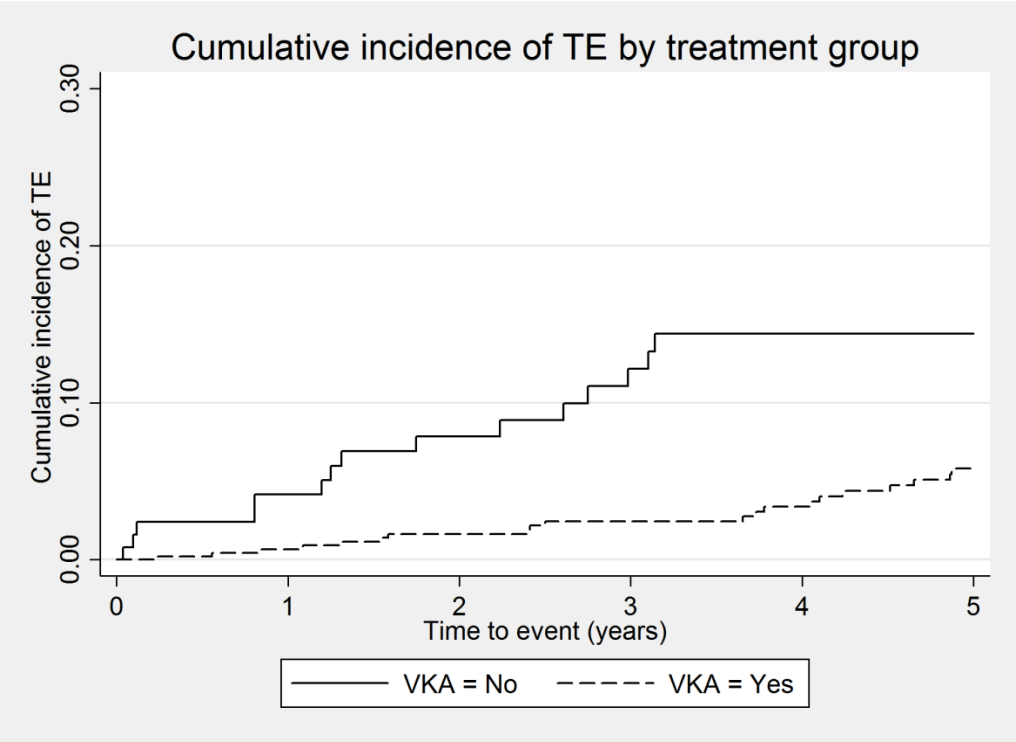
Table 21: Outcome of treatment with VKA prior to event in patients with AF at baseline evaluation with and without thromboembolism

AF	VKA	no TE	%	TE	%
	no	113	87.60	16	12.40
	yes	439	93.21	32	6.79
	Total	552	92.00	48	8.00

AF: Atrial fibrillation, VKA: Vitamin K antagonist, TE: Thromboembolic events
From Guttmann *et al* (2015) [272]

Figure 14: Kaplan-Meier failure estimates comparing thromboembolic events in VKA

and non VKA groups



From Guttmann *et al* (2015) [272]

6.3.3. Bleeding risk in patients with HCM

Calculation of the HAS-BLED score in a population of 536 patients with HCM and AF revealed a score of 0-2 in 500 patients (93.3%) with a score of 3-5 in 36 patients (6.7%). A score of ≥3 indicates 'high risk', leading to some caution and regular review of the patient following the initiation of VKA therapy (table 22) [262;263;284].

Table 22: Number of patients with HCM and AF and corresponding HASBLED score

HASBLED score	n	%
0	217	36.17
1	179	29.83
2	104	17.33
3	31	5.17
4	4	0.67
5	1	0.17

n: Number

6.3.4. Clinical outcome of antiarrhythmic therapy

The Kaplan-Meier survival curves for the development of AF over a ten year follow up period by treatment group are displayed in figure 15. To minimise the biasing effect of the intention to treat analysis the hazard ratios were also calculated for a follow up period of 1 year (table 23). In view of the HR of 2.07 of amiodarone the number of patients on amiodarone in the absence of a diagnosis of AF at first evaluation was also determined. Out of 311 patients receiving amiodarone at first evaluation only 23 had a prior diagnosis of AF. The Kaplan-Meier survival estimates for a follow up of one year for therapy with calcium channel blockers and disopyramide showed a trend towards a reduction in AF occurrence (figure 16).

Figure 15: Kaplan Meier survival estimates for development of AF over ten year follow up period in treatment and non-treatment group (beta-blocker, Ca-channel antagonist, disopyramide and amiodarone)

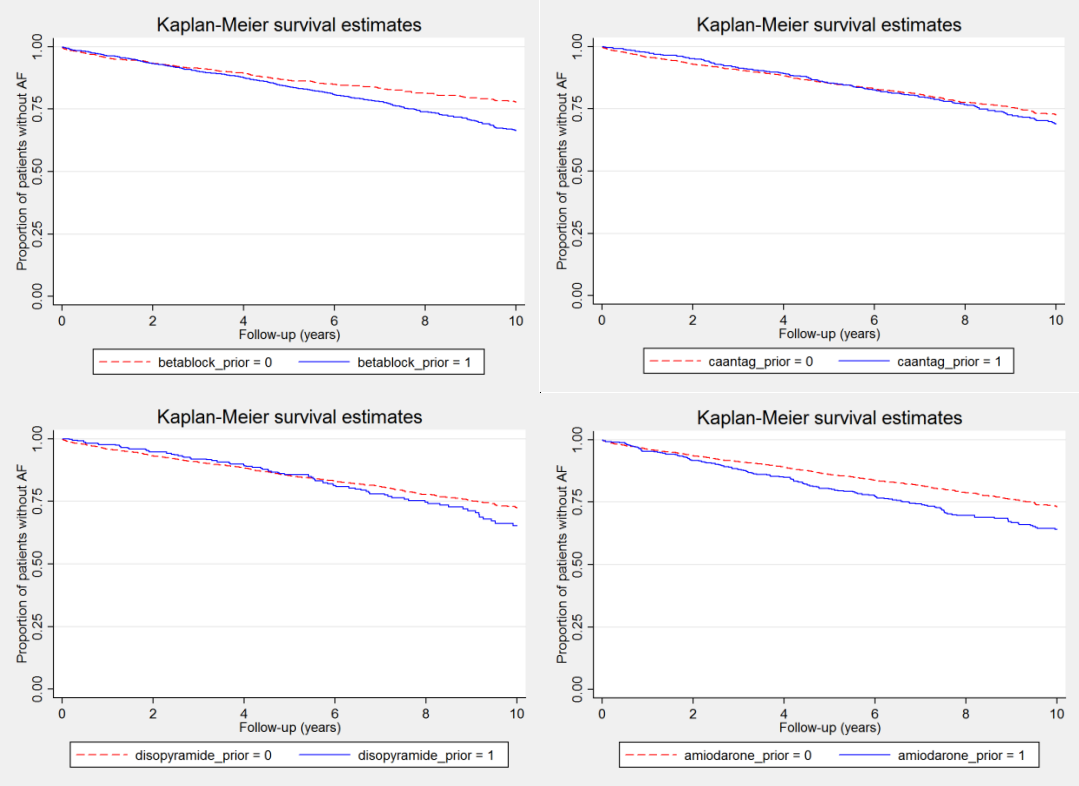
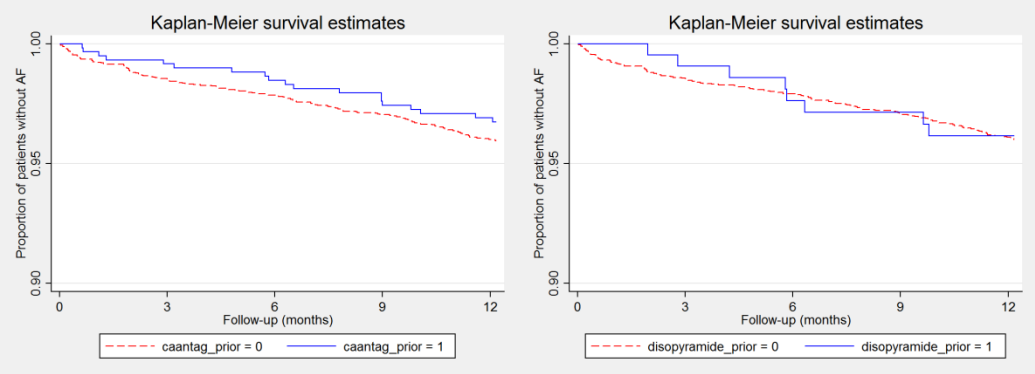


Table 23: 1 year hazard ratios for development of AF according to medication

Medication	HR	SE	p	CI
Beta-blocker	1.14	0.18	0.41	0.83-1.55
Ca-antagonist	0.80	0.20	0.36	0.49-1.29
amiodarone	2.17	0.47	<0.001	1.33-3.21
disopyramide	0.96	0.35	0.01	0.47-1.95

HR: Hazard ratio, p: p-value, CI: 95% confidence interval, SE: Standard error

Figure 16: Kaplan Meier survival estimates for development of AF over one year follow up period in treatment and non-treatment group (Ca-channel antagonist, left; disopyramide, right)



6.4. Discussion

6.4.1. Clinical outcome of anticoagulation in HCM patients

There are no prospective randomised trials of any therapy for AF in HCM including anticoagulation and only a very small number of observational studies comparing VKA with antiplatelet drugs [177;231;233;270]. In this study, an intention to treat analysis of VKA demonstrated a relative risk reduction for TE of 54.8% in anticoagulated patients who had AF at baseline evaluation, supporting current international guidelines for the use of VKA in HCM [3;273]. The intention to treat analysis was felt to be the most appropriate way of analysing data gathered retrospectively over such a long period, although we acknowledge that this methodology may have led to the inclusion of patients who had discontinued VKA or whose INR was sub-therapeutic at the time of a thromboembolic event. Current guidelines recommend anticoagulation with CHA_2DS_2-VASc score of 1 or above in patients with non-valvular AF, which corresponds to an adjusted stroke rate of 1.3% per year [262;263;274]. The absolute risk reduction (ARR) and number needed to treat (NNT) for patients in AF at an exploratory threshold of 4% risk of thromboembolic event

over 5 years are 13% and 7.7 respectively. The NNT in non-valvular AF is 12 for secondary 37 for primary prevention [287].

6.4.2. Use of HAS-BLED score in HCM

The use of the HAS-BLED score to assess the bleeding risk with VKA revealed a high risk value in only 6.8% of the cohort, reflecting the fact that HCM cohorts are generally younger with less conventional risk factors for bleeding.

6.4.3. Effect of antiarrhythmic on AF development

There are no prospective randomised trials of medical therapy for AF in HCM and only a very small number of observational studies assessing efficacy of antiarrhythmic therapy in maintenance of sinus rhythm or suppression of supraventricular tachycardia [288;289]. In this study, an intention to treat analysis of β-adrenoreceptor blockers, calcium channel antagonists and disopyramide demonstrated no significant effect of these therapies in prevention of AF. The retrospective nature of the analysis means that interpretation of these findings should be cautious; however, we sought to minimise the biasing effect of this analysis by also calculating the hazard ratios for a follow up period of 1 year which demonstrated findings consistent with those at 10 years.

Contrary to expectations, amiodarone therapy appeared to have detrimental effect on the development of AF with a hazard ratio of 2.07. However, a large proportion of patients on amiodarone did not have a history of AF at first evaluation and we speculate that the apparent negative effect of the drug might be explained by its use to prevent sudden cardiac death in patients with non-sustained ventricular tachycardia (NSVT) as

this group of patients may have more myocardial fibrosis and advanced disease and thus be more prone to AF [290-292].

6.4.4. Clinical implications

In this study, an intention to treat analysis of VKA demonstrated a relative risk reduction for TE in anticoagulated patients who had AF, supporting current international guidelines for the use of VKA in HCM [3;273]. In spite of its limitations, the intention to treat analysis for antiarrhythmic therapy suggests that rhythm control strategies for patients with AF and HCM are suboptimal and require revaluation in prospective randomised trials.

6.5. Conclusions

Exploratory analyses show for the first time evidence for a reduction of TE with VKA treatment. HCM patients appear to be a good target for therapy with anticoagulation with less conventional risk factors for bleeding. There was no evidence for a beneficial effect of antiarrhythmic therapy on the development of atrial arrhythmia.

7 CONCLUSIONS

7.1 Significance of AF and thromboembolism in HCM

It has been clear since the early descriptions of hypertrophic cardiomyopathy that AF and thromboembolism are common [15]. In this thesis I have shown how important AF and stroke are when looking after patients with HCM. A large proportion of patients with AF suffer from symptoms secondary to AF and the related mortality is very high. The analyses shown here represent the most comprehensive review of current literature in this area with a meta-analysis and systematic analysis of a large population of patients with HCM. I have shown that previous studies on AF and thromboembolism in HCM are very heterogeneous in terms of cohort size, definitions of disease and follow up durations. In addition I have collated, assessed and investigated the largest population of patients with HCM to date from seven centres in Europe. The analyses reveal that about a fifth of patients are affected by AF and that a very large proportion of these patients will suffer from thromboembolic complications.

AF affects 1–2% of the general population and the prevalence of AF increases with age, from 0.5% at 40–50 years, to 5–15% at 80 years [293;294]. AF and stroke in HCM are different though. As seen in this work the patient population are younger with a mean age of 49 years. A large proportion of these patients is asymptomatic and also has a low vascular risk factor profile, which can at least partially be explained by the mean age of the population.

Nevertheless the incidence and prevalence of thromboembolism is striking and the implication in terms of morbidity and mortality enormous. I have demonstrated the

impact of AF in these patients by demonstrating the statistically significant difference in incidence of cardiovascular death comparing patients in the sinus rhythm and in AF.

7.2 Predictors of AF and thromboembolism

The good news is that I have shown that clinicians can predict AF and stroke by using simple readily available clinical parameters such as age, sex, LA dimension, thickness of the left ventricular wall and symptomatic status such as NYHA class and general risk factors such as hypertension and vascular disease. Again these risk factors are at least partially different from the general population and reflect the difference in the disease processes in HCM as compared to the general population.

This is underlined by the fact that I have shown that the CHA_2DS_2-VASc score, which is used as a means of stratifying patients with non-valvular AF for antithrombotic prophylaxis, has a relatively low predictive accuracy in patients with HCM. This is probably explained by the lower prevalence of vascular risk factors [262;263;281]. The data shows that this score performs poorly in patients with HCM and that it should not be used to assess TE risk in this population.

Instead I was able to develop a risk stratification model to predict an individual patient's risk of thromboembolism over 5 years using these clinical parameters. The model revealed satisfactory performance and will guide management of these patients and decision making with regards to therapy with anticoagulation.

7.3 Medical therapy in patients with HCM

It is not enough to predict a thromboembolic event. Clinicians must be able to treat patients satisfactorily and avoid this complication. Using an intention to treat analysis I was able to provide exploratory analyses on anticoagulation that show for the first time evidence for a reduction of TE with VKA treatment in a unique and large cohort of patients. VKA treatment was associated with a significant relative risk reduction in HCM patients with AF.

Medical therapy has not proven to be effective in preventing or at least delaying the development of AF in HCM in the first place. To investigate this, I utilised an intention to treat analysis to assess the use of commonly used antiarrhythmic drugs in this disease, i.e. β-adrenoreceptor blockers, calcium channel antagonists, disopyramide and amiodarone. This did not demonstrate evidence for the efficacy of antiarrhythmic therapy in development of atrial fibrillation.

7.4 Future directions

There are several research questions that have become clear and evident to me during the course of this project. A prospective study on AF and thromboembolism in HCM patient could be an idea for the future. Setting this up could prove to be very challenging. This is in view of the event rate and therefore a very long follow up time to achieve a meaningful prognostic model for statistical analysis. There are some clinical parameters that would be most interesting to additionally include at that point. LA

volume or area and LV mass would be some of these. Similarly cardiac MR data could then be incorporated and could also include assessment of atrial fibrosis. Arterial blood pressure as a continuous variable would also be of some interest in determining the relationship with AF. To be more certain that of the diagnosis of HCM, it would also have been helpful to collect the distribution of hypertrophy.

Diagnosing AF and thromboembolism especially transient ischaemic attacks can be challenging in clinical practice. This is especially the case in retrospective data collection. A prospective study could potentially improve the system of detecting AF and thromboembolism and would therefore improve the power of the statistical analysis.

Another topic for future assessment is medical therapy. Many patients remain hesitant with regards to treatment with vitamin K antagonists but prefer therapy with newer agents. Due to current guidelines randomised controlled studies on anticoagulation in HCM are unlikely in the future, but investigating vitamin K antagonists versus novel agents of anticoagulation would be a desired approach for a randomised control studies in this area.

My work did not demonstrate evidence for the efficacy of antiarrhythmic therapy in development of atrial arrhythmia, but there is a clear lack of randomised controlled trials in this area. If medical therapy turns out to not be successful in preventing the recurrence or the development of atrial fibrillation in the first place substrate modification would be an interesting field of work for the future. Data on electrophysiological ablative therapy is limited but reveals suboptimal results to date. Long term maintenance of sinus rhythm is achieved in no more than 50-60% of patients [187;248-250]. Early treatment of LVOTO even in the absence of symptoms is another pathway worth considering in

this respect as well. The rational for this approach would be to intervene early prior to development of atrial dilatation and scarring.

A large proportion of patients with HCM have enlarged atrial dimensions but no clinical evidence of atrial fibrillation when reviewed in clinic. These patients are at high risk of developing of AF and therefore thromboembolism as I have shown in this thesis. Addressing this important question of the efficacy and risk of aggressive and early use of anticoagulation in patients is therefore crucial. Some groups also advocate the use of aspirin in these patients in the absence of atrial fibrillation. There is no evidence available to support this approach. A study into the effectiveness of thromboembolic prophylaxis of this approach would be of high clinical relevance.

Finally inclusion of further centres especially centres from outside of Europe for external validation of the proposed prognostic model is necessary and would be very useful. Further data from centres outside of Europe to ensure availability of data from further ethnic groups is crucial.

7.5 Limitations

There are some limitations of this work that I would like to point out. All the studies in this thesis were carried out on a cohort of HCM patients based at seven collaborating centres in Italy, Spain, Greece and the UK. Referral bias is therefore an important limitation to consider. The collaborating centres are mostly tertiary referral centres. More severely affected and therefore the referred patients might have therefore been included into the study. It is also important to note that some of the associations identified are too weak to be predictive.

Some of the limitations are due to retrospective nature of the data collection. This meant a limitation of clinical parameters that could be examined, which are those that were historically collected by investigators at the international centres. The left atrial area or volume for example as a measure of LA size would have been preferable rather than left atrial diameter. The same is true for maximal wall thickness of a single myocardial segment rather than the total LV mass. A prospective external validation in a different cohort of patients would hence be ideal.

The inter-observer variability data for assessment of LA size and the lack of data on bleeding rates are also an important limitations.

Ethnicity may have influenced the findings. Data on this were not available in this cohort.

A further important point is that the patient population in this study is large and diverse but the model should only be used in patients with similar characteristics. It is not validated in paediatric patients (less than 16) and in patients with metabolic or syndromic disorders.

In this study, an intention to treat analysis was utilised in investigating the effect of vitamin K antagonists and antiarrhythmic medication. The intention to treat analysis was felt to be the most appropriate way of analysing data gathered retrospectively over such a long period. This may have led to the inclusion of patients who had discontinued medication or whose INR was sub-therapeutic at the time of a thromboembolic event.

AF in HCM can be difficult to diagnose especially in patients with paroxysmal episodes of arrhythmia. A similar difficulty applies to the detection of TIA in clinical practice. This is a limitation common to all studies of AF and thromboembolism. A prospective study

with these as primary outcomes would be a way around this and has been discussed above.

The prognostic model includes patients who are treated with VKA according to current guidelines. Excluding these patients would exclude high risk patients and limit the statistical analysis.

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9 APPENDIX

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9.2 Supervision

Primary supervisor: Prof Perry M. Elliott, Professor of Inherited Cardiac Diseases, UCL

Secondary supervisor: Prof William McKenna, Professor of Cardiology, UCL

9.2 Location of research

The research was carried out at The Heart Hospital, University College London Hospital NHS Trust.

9.3 Ethics

Patients at A Coruña University Hospital (Spain), 1st Department of Cardiology, University of Athens (Greece), University Hospital Virgen de la Arrixaca (Spain), and Monaldi Hospital (Italy) provided written informed consent. The data collection at The Heart Hospital (UK) and Hospital Universitario Puerta de Hierro (Spain) have been

approved by the appropriate ethics committee. The ethics committee at the Institute of Cardiology at the University of Bologna (Italy) were informed, but approval was not required under local research governance arrangements.

9.4 Personal contributions

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9.6 Publication arising from research activities

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Guttmann OP, Pavlou M, O'Mahony C, Monserrat L, Anastasakis A, Rapezzi C, Biagini E, Gimeno JR, Limongelli G, Garcia-Pavia P, McKenna WJ, Omar RZ, Elliott PM; Hypertrophic Cardiomyopathy Outcomes Investigators. Prediction of thrombo-embolic risk in patients with hypertrophic cardiomyopathy (HCM Risk-CVA). Eur J Heart Fail. 2015 Jul 16. doi: 10.1002/ejhf.316.

Lopes LR, Syrris P, **Guttmann OP**, O'Mahony C, Tang HC, Jenkins S, Hubank M, Monserrat L, McKenna WJ, Plagnol V, Elliott PM. Novel genotype–phenotype associations demonstrated by high-throughput sequencing in patients with hypertrophic cardiomyopathy. Heart. 2015 Feb;101(4):294-301.

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9.7 Word count

The word count including footnotes, tables and figures but excluding bibliography, appendices and supporting data is 25041.