Hereditary haemorrhagic telangiectasia:

novel cardiopulmonary insights

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Hereditaire hemorragische teleangiëctasieën:
nieuwe cardiopulmonale inzichten

(met een samenvatting in het Nederlands)

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CHAPTER 1

General introduction

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VMM Vorselaars

HEREDITARY HAEMORRHAGIC TELANGIECTASIA

Background

Hereditary haemorrhagic telangiectasia (HHT), or Rendu-Osler-Weber (ROW) syndrome, is a genetic vascular disorder [1]. The estimated worldwide prevalence is at least one in 5000 individuals, however large regional variance exists [2,3]. HHT is characterised by abnormal direct artery-to-vein communications [1]. These abnormal vascular structures range from small telangiectasia (dilated microvessels in skin and mucous membranes) to large arteriovenous malformations (AVMs) which occur predominantly in the liver, brain and lungs [4], but can theoretically grow in every organ (figure 1). These AVMs cause shunting and carry the risk for paradoxical embolism and haemorrhage and are therefore associated with significant morbidity and potential severe complications. If untreated, life expectancy is significantly lower in HHT patients compared to their partners, but prevention of HHT complications with screening programs could improve life expectancy [5,6].

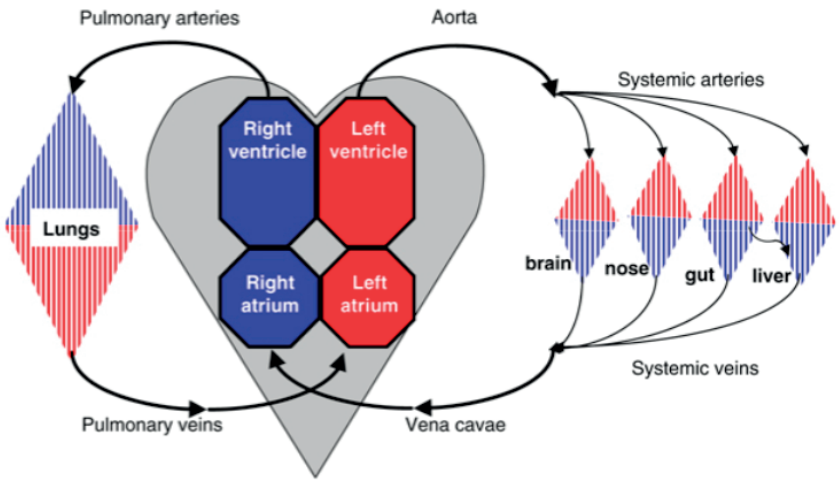

Figure 1. Schematic of systemic and pulmonary circulations showing capillary beds in which telangiectasia or arteriovenous malformations occur.
Adapted from Govani et al.[88], with permission of the publisher.

Molecular genetics

HHT has an autosomal dominant pattern of inheritance with two main subtypes (including over 80% of all HHT patients); HHT type 1 (HHT1) and HHT type 2 (HHT2). HHT1 results from mutations in the *ENG* gene encoding the protein endoglin (cytogenetic location 9q34.11; OMIM187300) [7]. HHT2 results from mutations in the *activin receptor-like kinase (ACVRL1)* gene, encoding the protein ALK-1 (cytogenetic location 12q13.13; OMIM600376) [8]. A third disease-causing mutation has been found in the *SMAD4* gene (cytogenetic location 18q21.2; OMIM175050), causing a combination of the juvenile

polyposis syndrome and HHT [9]. Two more rare types have been found on chromosome 5 (HHT type 3; OMIM601101) and 7 (HHT type 4; OMIM610655) [10,11]. None of the mutations of the *ENG* and *ACVRL1* genes prevail, and all types of mutations have been reported (including missense, nonsense, deletions, insertions and splice site). Most families with HHT have a unique mutation (more than 900 mutations are described on www.hhtmutation.org).

Pathogenesis

The pathogenesis of HHT is still controversial and the precise sequence of events remains to be determined. However, haploinsufficiency with an inadequate level of protein products for normal function, seems to be an important underlying cause of HHT [12].

Endoglin, ALK-1 and SMAD4 proteins are vascular endothelial receptors of the transforming growth factor β (TGF-β) family [13,14]. TGF-β signalling is necessary for formation and maintenance of normal blood vessels. During the normal development of vessels TGF-β signals via the endoglin, ALK-1 and other TGF-β receptors in a complicated positive feedback loop to enhance endothelial cell-pericyte contact, which promotes vascular stability [15] .

In HHT, signalling by the endoglin and ALK-1 receptor complex is reduced, leading to decreased TGF-β activation and thereby breakdown of endothelial cell-pericyte contacts [16]. The interrupted cell contact results in abnormal angiogenesis and the formation of fragile vessels with a high bleeding tendency, which explains the typical clinical features of HHT.

A characteristic finding is that different affected members within the same family mutation display highly different phenotypes, ranging from non-penetrance to extensive visceral AVMs and/or severe epistaxis. This phenotypic variability suggests that other genes or environmental factors might also influence the HHT phenotype.

Clinical aspects

The clinical diagnosis of HHT is based on the Curaçao criteria (table 1) [1,17]. Three criteria suffice for a definitive diagnosis of HHT, two criteria are considered as 'possible or suspected' HHT and one or no criterion makes the clinical diagnosis 'unlikely'. Importantly, the clinical presentation of HHT is age dependent and varies among patients. In addition, a genotype-phenotype relationship exists with pulmonary arteriovenous malformations (PAVMs) and cerebral arteriovenous malformations (CAVMs) more prevalent in HHT1 and hepatic arteriovenous malformations (HAVMs) more prevalent in HHT2 [18].

Table 1. Clinical Curaçao criteria

Criteria	Description	Prevalence	
		HHT type 1	*HHT type 2*
Epistaxis	Spontaneous and recurrent	93% [25]	94% [25]
Telangiectasia	Multiple at characteristic sites: lips, oral cavity, fingers, nose	92%[25]	89% [25]
Visceral lesions	CAVM	10%[46]	1% [18,46]
	HAVM	32-78%* [1,85-87]	
	PAVM	61%** 91%*** [21]	14%** 53%*** [21]
	GI-telangiectasia	72%**** [18,46]	66%**** [18,46]
Family history	First-degree relative with HHT		

*HHT, hereditary haemorrhagic telangiectasia; CAVM, cerebral arteriovenous malformation; HAVM, hepatic arteriovenous malformation; PAVM, pulmonary arteriovenous malformation; GI: gastrointestinal. *Depending on screening technique and more prevalent in HHT type 2. **PAVM on chest computed tomography. ***Pulmonary right-to-left shunt on transthoracic contrast echocardiogram. ****Endoscopic screening only in patients with unexplained anaemia.*

PART I: PULMONARY SHUNTING IN HEREDITARY HAEMORRHAGIC TELANGIECTASIA

Background

PAVMs are low-resistance, high-flow abnormal vascular structures (figure 2) that most often connect a pulmonary artery directly to a pulmonary vein. PAVMs thus bypass the normal pulmonary capillary network, which results in a permanent right-to-left shunt (RLS) [19]. Up to 94% of PAVMs are associated with HHT [20]. The prevalence of pulmonary RLS on transthoracic contrast echocardiography (TTCE) is 91% and 53% in HHT1 and HHT2, respectively, corresponding with a prevalence of macroscopic PAVMs detectable on chest computed tomography (CT) of 61% in HHT1 and 14% in HHT2 [21].

Most non-HHT related pulmonary RLS are small physiologic connections, idiopathic (mostly solitary PAVMs) or secondary to hepatopulmonary syndrome [22,23].

PAVM related complications

PAVMs reduce the filtering capacity of the pulmonary capillary bed, therefore the presence of PAVMs predisposes to complications from paradoxical systemic embolisation of both thrombotic and septic origin, including stroke (3-14%) and brain abscess (1-13%) [24,25]. However, only moderate (pulmonary RLS grade 2; see subchapter describing TTCE) and large shunts (pulmonary RLS grade 3) are associated with an increased risk of cerebral complications (OR 4.78; 95% CI 1.14-20.0; P=0.03 and OR 10.4; 95% CI 2.4-45.3; P=0.002, respectively) [25]. Other less common complications are haemoptysis (1-3%) and spontaneous haemothorax (0-3%) due to rupture of the fragile vascular structure

[1,24,25]. PAVMs may also result in hypoxaemia, dyspnoea and cyanosis depending on the degree of RLS, as blood flows directly from the pulmonary artery to pulmonary vein, bypassing the capillary-alveolar barrier without effective gas exchange. Furthermore, there is an association with aura accompanied migraine [26,27]. However, the severe neurologic complications may be the presenting manifestation of PAVMs in otherwise asymptomatic patients.

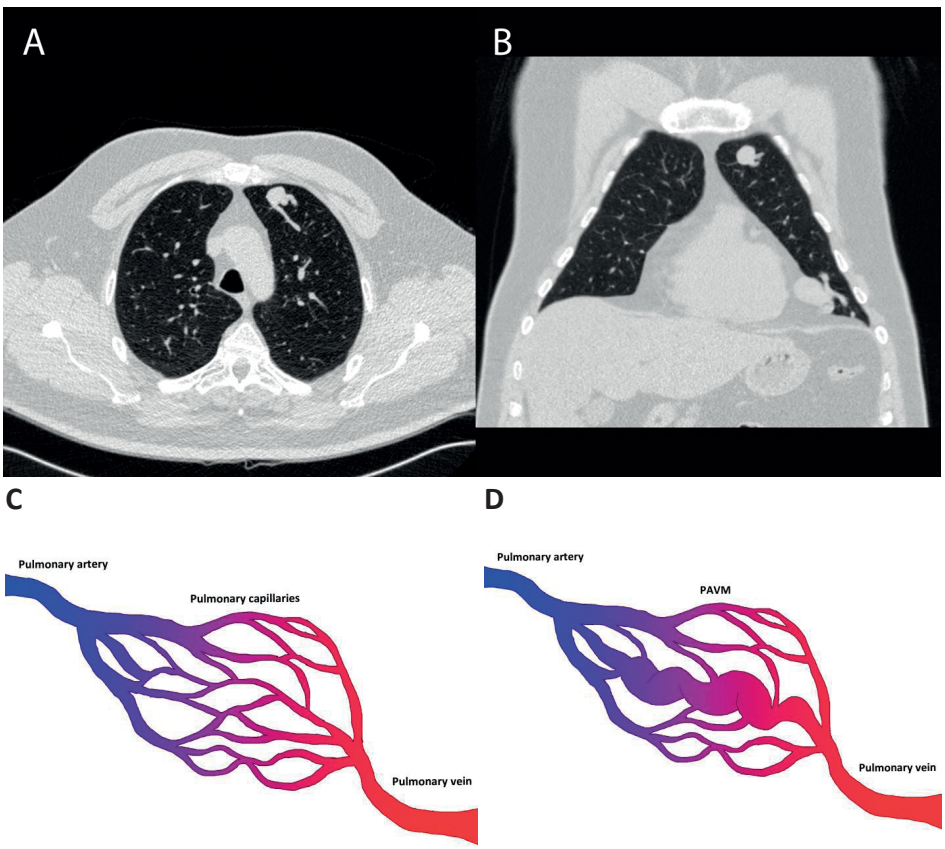

Figure 2. Pulmonary arteriovenous malformations

A. Chest CT (axial view) showing large PAVM in the left upper lobe.

B. Chest CT (coronal view) showing large PAVM in the left upper lobe and second PAVM in the lingular segment.

C. Schematic image of normal pulmonary network.

D. Schematic image of PAVM.

PAVM, pulmonary arteriovenous malformation.

Screening for PAVMs

Because of the high prevalence of PAVMs in HHT, the associated life threatening and debilitating complications and the effective and safe options for treatment, the international guidelines recommend screening for PAVMs in all persons with suspected or confirmed HHT [1].

Transthoracic contrast echocardiography

Rationale

The rationale of TTCE is based on the permeability of the pulmonary capillary network and the difference in density between gas-contained microbubbles and the surrounding blood [28,29]. The capillary network normally measures 8 to 10 µm in diameter, and therefore the injected microbubbles with a mean diameter of 27 µm, will be trapped in de pulmonary circulation. If PAVMs are present, the filtering capacity of the capillary network will be diminished and microbubbles will pass the pulmonary filter and appear in the left side of the heart [30].

Technique

The contrast contains 8mL physiologic saline solution, 1mL blood and 1mL air. The agitated saline (containing microbubbles) is created by reverse flushing between two syringes. With 2-D Doppler echocardiography the four-chamber view is projected and 5mL agitated saline is injected in preferably the right ante-cubital vein. This procedure should be repeated while performing a Valsalva manoeuvre [21,25,31,32].

Shunt origin and quantification

RLS visualised with TTCE can have cardiac or pulmonary origin. For the differentiation between these two, visualisation of the pulmonary veins is essential. All microbubbles visualised through a pulmonary vein should be classified as pulmonary RLS and all RLS visualised through the interatrial septum as cardiac RLS (e.g. patent foramen ovale). When the pulmonary vein cannot be visualised, RLS interpretation is usually based on the delay in cardiac cycles after which the microbubbles appear in the left side of the heart. TTCE has been variably defined as positive for pulmonary RLS based on a delay of more than three [33] or four [31,32,34] cardiac cycles.

There are two different systems for RLS quantification described in the literature. Barzilai and colleagues proposed a 4-point grading system, representing minimal, moderate and extensive opacification without or with outlining of the endocardium (grade 1-4 respectively) [35]. To offer a more objective interpretation, other HHT centres (including ours) use a 3-point grading system [21,31,36]. This grading system is based on the maximum number of microbubbles counted in the left ventricle in one still-frame. With this system, a pulmonary RLS can be graded as 1 (maximum of 29 microbubbles), 2 (30-100 micro-

bubbles) or 3 (>100 microbubbles), meaning that both the grade 3 and grade 4 RLS as described by Barzilai *et al*. are included in the grade 3 pulmonary RLS (figure 3a-d). A high inter-observer agreement with a Kappa coefficient of 0.85 up to 0.94 has been reported for this 3-point RLS quantification system [36,37]. However, reliability of TTCE depends on reproducibility in a single patient, which has never been examined yet.

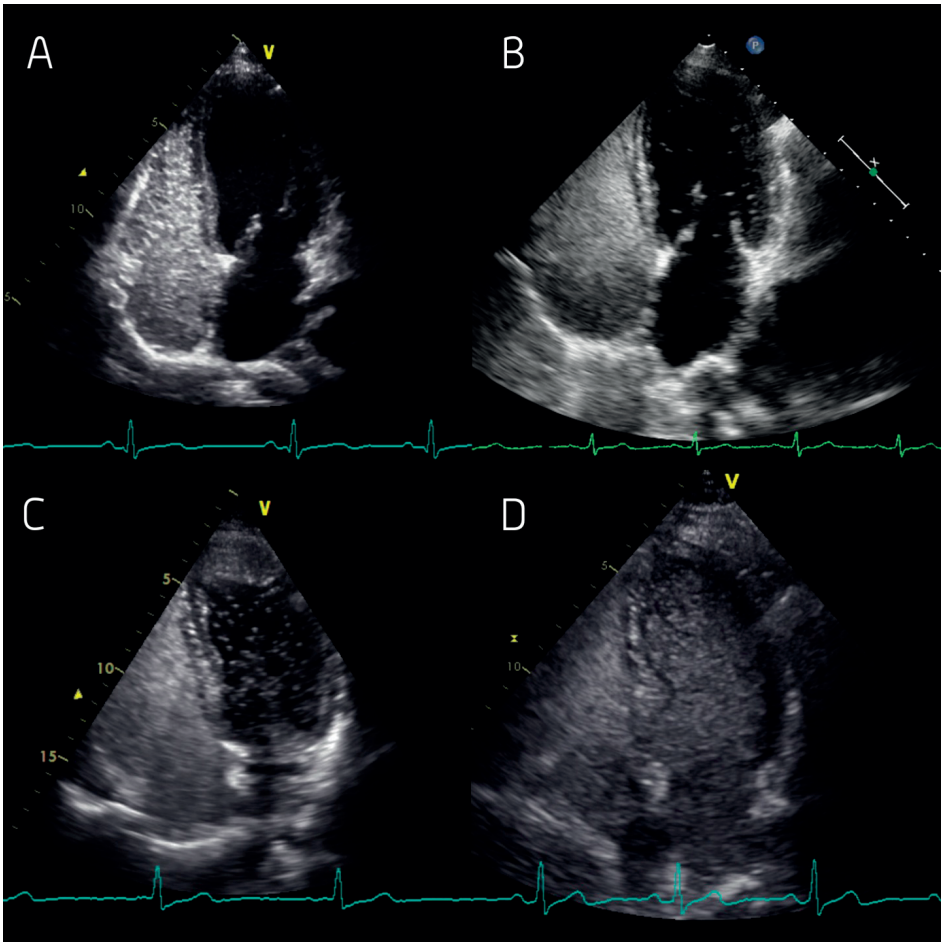

Figure 3. Pulmonary right-to-left shunt (RLS) on transthoracic contrast echocardiogram.
A. No pulmonary RLS. B. Pulmonary RLS grade 1. C. Pulmonary RLS grade 2. D. Pulmonary RLS grade 3.

Other diagnostic approaches

Chest CT has an excellent sensitivity and specificity for diagnosing treatable PAVMs and is therefore generally considered as the gold standard investigation for pre-embolisation diagnosing of PAVMs. Chest CT accurately estimates the PAVM feeding artery diameter on pulmonary angiography and gives information about the location, type (simple or complex)

and amount of PAVMs [34,38]. Although chest CT will detect lesions far below the size for which embolisation is feasible, chest CT remains negative in approximately 55% and 8% of patients with a pulmonary RLS grade 2 and 3 on TTCE, respectively [21]. Therefore, nowadays chest CT is no longer part of the standard first line screening algorithm of PAVMs. According to the current guidelines, all positive screening tests (positive TTCE, independent of the RLS grade) should be confirmed with an unenhanced multidetector chest CT. However, based on recent literature, it seems reasonable to withheld CT in patients with a pulmonary RLS grade 1 on TTCE as this RLS grade is not associated with neurologic complications nor treatable PAVMs [21,25]. Radiation exposure, which is the most important disadvantage of CT in this mostly young population, can be diminished by using low-dose CT techniques without contrast [39,40].

Large studies investigating the value of different diagnostics tests in patients screened for HHT, revealed that chest X-ray, blood gas analysis and shunt fraction measurement (with ^{99m}TC radionuclide scanning) lack sensitivity for the presence of PAVMs [2,31,34]. The diagnostic value of shunt fraction measurement with 100% oxygen method is demonstrated in this thesis.

Magnetic resonance imaging is a promising new diagnostic technique for the detection of PAVMs. A major advantage compared to chest CT is the avoidance of radiation while the anatomy of the PAVMs can still be demonstrated. However, at this moment, large comparative prospective studies are lacking and the lower spatial resolution compared to CT is currently a major limiting factor [41-45].

Follow-up

Although the exact pathogenesis of growth or formation of PAVMs is not completely understood, it is known that HHT has an age dependent penetrance [46]. Theoretically, the high flow through PAVMs, due to the relatively low resistance compared to the capillary network can result in growth of PAVMs. Other potential factors include female hormones and increase in cardiac output (e.g. due to HAVMs, anaemia or pregnancy) [1,47]. Studies including patients with PAVMs treated with embolisation showed growth in 11-18% of patients [48,49]. However, studies on the natural growth of PAVMs in patients with no or only small PAVMs are lacking. In the current guideline for HHT [1], the recommendations for follow-up of PAVMs are therefore scarce and based on small series or expert opinion. Patients with negative initial TTCE are advised to repeat screening every 5-10 years and more often after puberty or pregnancy. In patients with small untreated PAVMs or microscopic PAVMs (positive TTCE but negative chest CT), follow-up is advised every 1-5 years with chest CT on a case-by-case basis. After treatment, follow-up with chest CT is advised after 6 months and thereafter approximately 3 years to discover recanalization or reperfusion of PAVMs [1].

Treatment

In order to reduce the risk of neurologic complications, patients can be safely treated with transcatheter embolotherapy, an endovascular intervention that occludes the feeding artery of the PAVM with vascular plugs or coils [26,48-50]. A feeding artery diameter of 3 mm or greater is generally accepted for embolisation, although nowadays PAVMs with a feeding artery diameter as low as 2 mm are considered feasible for embolisation [1]. This therapeutic embolisation procedure should be performed by a highly experienced team in a dedicated HHT centre and is described in children and adults [51,52]. As embolisation closes a low resistance pathway for pulmonary blood flow, effect on haemodynamics such as pulmonary resistance and cardiac output should be expected. Haemodynamic changes may be important, especially when other cardiovascular diseases such as pulmonary hypertension (PH) exists.

All patients with PAVMs are advised to use antibiotic prophylaxis for procedures with risk of bacteraemia [1].

PART II: CARDIOVASCULAR ASPECTS OF HEREDITARY HAEMORRHAGIC TELANGIECTASIA

Pulmonary hypertension

PH, defined by an increased pulmonary artery pressure of ≥ 25 mmHg, is increasingly recognised as an important complication of HHT. There are two potential mechanisms that could explain the presence of PH in patients with HHT.

The HHT-related gene mutations in *ENG* and *ACVRL1* predispose to the development of heritable pulmonary arterial hypertension (HPAH) [53-58]. In HPAH, proliferation of endothelial cells and vascular smooth muscle cells will reduce the intraluminal space of the pulmonary arterioles, thereby increasing the arterial pressure. Although both mutations are named in the international guidelines for PH, less than 50 cases are described in literature and only a few observational studies exist [53-55,57,59-61,61-68].

HAVMs are associated with post-capillary PH and therefore this is mostly present in HHT2. The high pulmonary blood flow, which is due to the high cardiac output will lead to an increase of pulmonary pressure eventually leading to right ventricular failure. In HHT severe anaemia could contribute to this high output state [69,70].

Although the above described mechanisms are most common in HHT, all other forms of PH can exist in HHT. Pre-capillary PH may be the result of chronic thromboembolic PH (CTEPH) since HHT patients may encounter an increased thrombotic risk and post-capillary PH may be the result of diastolic dysfunction of the left ventricle [71,72]. Differentiation between all forms of PH is essential, since both entities are associated with severe morbidity and mortality with different treatment options.

Aortopathy in HHT

The TGF-β pathway is important for vascular integrity. Mutations in *SMAD3*, *FBN1, TGFβR1* and *TGFβR2*, all part of this pathway, are linked to dilation of the thoracic aorta (known pathogenic mutations for familial thoracic aortic aneurysms and dissection, Marfan syndrome and Loeys-Dietz syndrome respectively). These aneurysms can lead to life-threatening complications including aorta dissection or rupture with an extremely high mortality rate [73-75]. Due to the shared pathway, thoracic aortopathy could be expected in all HHT patients. However, this association is only described in a few case series of *SMAD4*-associated HHT patients [76-79].

Treatment of other cardiovascular diseases in patients with HHT

Many patients with HHT require the use of oral anticoagulation or antiplatelet therapy for additional cardiovascular diseases such as atrial fibrillation (AF) or myocardial infarction. The current HHT guidelines adhere no absolute contraindication when visceral sources for life-threatening bleeding (e.g., due to PAVMs or CAVMs) are excluded [1]. Although there are patients that tolerate both therapies, in many patients increase of epistaxis leads to a decrease in quality of life [80-82]. Therapeutic options without anticoagulation are the desired solution for HHT patients. However, since HHT patients may have an increased thrombotic risk a tailor-made approach is necessary [72].

Left atrial appendage closure is a low risk procedure that proved to be non-inferior to anti-coagulation with regard to the prevention of stroke, systemic embolism and cardiovascular death in large studies including patients with AF [83,84]. Therefore, this therapeutic option may also be useful in HHT patients with AF.

AIMS OF THIS THESIS

The main goals of this thesis are to describe the diagnostic value of TTCE to screen for PAVMs and show how this diagnostic tool can be used in the follow-up op patients with and without PAVMs. The second aim of this thesis is to describe the broad phenotype of HHT in which many severe cardiovascular complications are present.

This thesis tries to answer the following questions:

1. Should we accept all pulmonary RLS as a new Curaçao criterion to increase the sensitivity of the clinical criteria for HHT?
2. Is there a roll for less invasive diagnostic tools such as pulmonary shunt fraction measurement in the evaluation of PAVMs?
3. What is the reproducibility of TTCE?
4. How can TTCE be used in the follow-up of patients with (suspected) PAVMs?

5. Is there a change in pulmonary RLS size and thereby PAVM growth in time?
6. What are the haemodynamic consequences of embolisation of PAVMs?
7. Is there an association between the *SMAD4* gene mutation and dilation of the thoracic aorta and are *ENG* and *ACVRL1* mutation carriers involved as well?
8. How important is the association between HHT and PH and which subgroups can be identified?
9. How can we handle patients with HHT and AF, is left atrial appendage closure a solution for bleeding complications due to oral anticoagulation?

OUTLINE OF THIS THESIS

Part I: Pulmonary shunting in hereditary haemorrhagic telangiectasia

- Chapter 2 answers the question whether we should accept the presence of any pulmonary RLS on TTCE as a new Curaçao criterion to diagnose patients with HHT.
- In chapter 3 we describe the diagnostic accuracy of pulmonary shunt measurement using the 100% oxygen method in detecting pulmonary RLS compared to RLS quantification with TTCE.
- Chapter 4 shows the reproducibility of RLS quantification with TTCE in patients with (suspected) HHT.
- In chapter 5, data is presented on the long-term follow-up of pulmonary RLS with TTCE, showing increase in RLS size after 5 years but no significant PAVMs in patients without pulmonary RLS at screening.
- Chapter 6 analyses the direct haemodynamic consequences of PAVM embolisation with vascular plugs or coils.

Part II: Cardiovascular aspects of hereditary haemorrhagic telangiectasia

- Chapter 7.1-7.3 demonstrate the association between the *SMAD4* gene mutation and an increased risk of aortic dilation compared to *ENG* and *ACVLR1* mutation carriers and non-HHT controls.
- Chapter 8.1-8.3 confirm the presence of different forms of PH as complication of HHT with both original data and a structured overview of the literature.
- In chapter 9 the data of five HHT patients are described showing evidence for left atrial appendage closure as stroke prevention in patients with HHT and AF who are intolerant to anticoagulation.
- In chapter 10 the results of this thesis are summarized and discussed.

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PART I

Pulmonary shunting in hereditary haemorrhagic telangiectasia

CHAPTER 2

Role of transthoracic contrast echocardiography in the clinical diagnosis of hereditary haemorrhagic telangiectasia

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ABSTRACT

Background: Hereditary haemorrhagic telangiectasia (HHT) can be diagnosed according to the four clinical Curaçao criteria, including the presence of pulmonary arteriovenous malformations (PAVMs). In the past few years, transthoracic contrast echocardiography (TTCE) replaced chest high-resolution CT (HRCT) imaging for the screening of PAVMs. The objective of this study was to determine whether the presence of any pulmonary shunt on TTCE can be accepted as a new clinical Curaçao criterion in diagnosing HHT.

Methods: Between 2004 and 2012, we included 487 first-degree relatives of known HHT-causing mutation carriers who underwent both TTCE and chest HRCT imaging to screen for PAVMs. A quantitative three-point grading scale was used to differentiate among minimal, moderate, or extensive pulmonary shunt on TTCE (grade 1-3). Genetic testing was performed in all people and considered the gold standard for the diagnosis of HHT.

Results: Chest HRCT imaging demonstrated PAVMs in 114 of 218 patients (52.3%) with a pulmonary shunt on TTCE. The addition of any pulmonary shunt on TTCE to the current clinical Curaçao criteria increased the number of positive criteria in 92 of 487 individuals (18.9%), which increased the sensitivity in diagnosing HHT from 88% to 94% at the expense of a decreased specificity from 74% to 70%. Accepting only pulmonary shunt grades ≥ 2 on TTCE as a diagnostic criterion for HHT enhanced the number of positive criteria in 30 (6.2%) individuals, which led to an increased sensitivity of 90% with no decrease in specificity (74%).

Conclusions: The addition of only pulmonary shunt grades ≥ 2 on TTCE to the current clinical Curaçao criteria increases its sensitivity without affecting specificity in the diagnosis of HHT.

INTRODUCTION

Hereditary haemorrhagic telangiectasia (HHT) is an autosomal-dominant inherited disorder characterised by vascular abnormalities varying from small telangiectases in skin and mucosal membranes to large arteriovenous malformations (AVMs) predominantly in the brain, liver, and lungs [1]. There are mainly two types of HHT that correspond with gene mutations coding for endoglin (HHT1) and ALK1 (HHT2) [2,3]. A third disease-causing mutation has been shown in *SMAD4*, which causes a combined syndrome of juvenile polyposis and HHT [4]. HHT commonly presents with epistaxis and anaemia but carries the risk of more severe complications when visceral AVMs are involved. Most feared are intracranial haemorrhages from ruptured cerebral AVMs and cerebral ischemic events or brain abscesses from paradoxical embolisations through pulmonary AVMs (PAVMs) [5,6]. Because effective treatment options can prevent these severe complications, early and accurate recognition of HHT is important. The clinical diagnosis of HHT is established according to the four Curaçao criteria, which comprise spontaneous and recurrent epistaxis, telangiectases at characteristic sites, a first-degree relative with HHT, and the presence of visceral AVMs [7]. Three criteria suffice for a definite diagnosis of HHT, two criteria are considered as possible HHT, and one or no criterion makes the diagnosis unlikely. Screening for PAVMs traditionally has been done with chest high-resolution computed tomography (HRCT) imaging [8,9], but the past few years demonstrated that transthoracic contrast echocardiography (TTCE) has an excellent sensitivity, negative predictive value (NPV), and wide availability with lower risks and costs; therefore, TTCE is now advised in the international guidelines as the first-line screening technique for the detection of PAVMs [10-14]. However, to our knowledge, the exact role of a pulmonary shunt on TTCE as a potential new diagnostic criterion for HHT has never been evaluated. Therefore, the aim of the present large prospective study was to determine whether the presence of any pulmonary shunt on TTCE can be accepted as a clinical Curaçao criterion in diagnosing HHT.

METHODS

Study Population

From May 2004 until October 2012, 872 consecutive people aged > 15 years were screened for HHT as family members of index patients or in cases of clinical symptoms suggesting HHT. Both TTCE and chest HRCT scan were performed in 799 of 872 screened people (91.6%) to detect potential PAVMs. Patients with previously treated PAVMs were not included in the analysis. From February 2011, a subsequent chest HRCT scan was withheld in the absence of a pulmonary shunt on TTCE [10,13,15]. Because validation of the clinical Curaçao criteria is strongly influenced by the composition of the study population, we selected a highly uniform group of first-degree family members of patients with genetically proven HHT1 or HHT2. This prevented patients with clinically diagnosed HHT

in whom genetic testing could not detect a disease-causing mutation from being falsely classified as HHT negative. In addition, we excluded all index patients with HHT because they might be more symptomatic than their subsequently screened relatives, which would have introduced a bias in the study. Therefore, the present study included 487 first-degree relatives of patients with HHT who all underwent genetic testing and screening for PAVMs with both TTCE and chest HRCT scan. Genetic testing was performed as described earlier [16] and used as the gold standard for the presence or absence of HHT. The selection of the study population is illustrated in figure 1. A complete history and physical examination was performed in all individuals by a pulmonologist and otorhinolaryngologist with dedicated expertise in HHT. The clinical diagnosis of HHT was established according to the current Curaçao criteria. Epistaxis had to be both spontaneous and recurrent to fulfill a criterion. At least three telangiectases had to be present at characteristic sites (lips, oral cavity, nose, fingers) to satisfy a clinical criterion. Screening for gastrointestinal telangiectases and hepatic AVMs was performed only when suggested by history, physical examination, or blood test results. Patients who were given a definite diagnosis of HHT were offered a brain magnetic resonance imaging scan to exclude cerebral AVMs. All participants provided informed consent, and the study was approved by the hospital review board (LTME/Z-12.41).

Transthoracic Contrast Echocardiography

TTCE was performed as previously described [6]. Two experienced cardiologists with dedicated expertise in HHT interpreted the shunt on TTCE. In case of right-to-left shunting, visualisation of shunt origin was pursued in every TTCE. All shunts visualised through a pulmonary vein were classified as pulmonary shunts. On the occasion of poor visualisation of shunt origin, we used a delay of four cardiac cycles to distinguish between a pulmonary or cardiac shunt. TTCE results were considered positive for a pulmonary shunt if microbubbles appeared in the left atrium after four cardiac cycles [13,14,16]. Right-to-left shunts within four cardiac cycles with poor visualisation of shunt origin were classified as indeterminate shunts (n = 7) and excluded from further analysis. Opacification of the left ventricle was graded as 1 (maximum of 29 microbubbles), 2 (30-100 microbubbles), or 3 (> 100 microbubbles). This division was based on the maximum number of microbubbles in the left ventricle counted in one still frame, which was confirmed by checking the five frames before and after the chosen frame. A good κ coefficient of 0.85 was found for interobserver agreement regarding pulmonary shunt grade on TTCE in previous studies [14,15,17]. In cases of disagreement on presence, quantity, or timing of microbubbles in the left ventricle, the TTCE was reviewed again by both cardiologists together to agree on the final determination. A patent foramen ovale was diagnosed only after a positive Valsalva manoeuvre without a spontaneous right-to-left shunt.

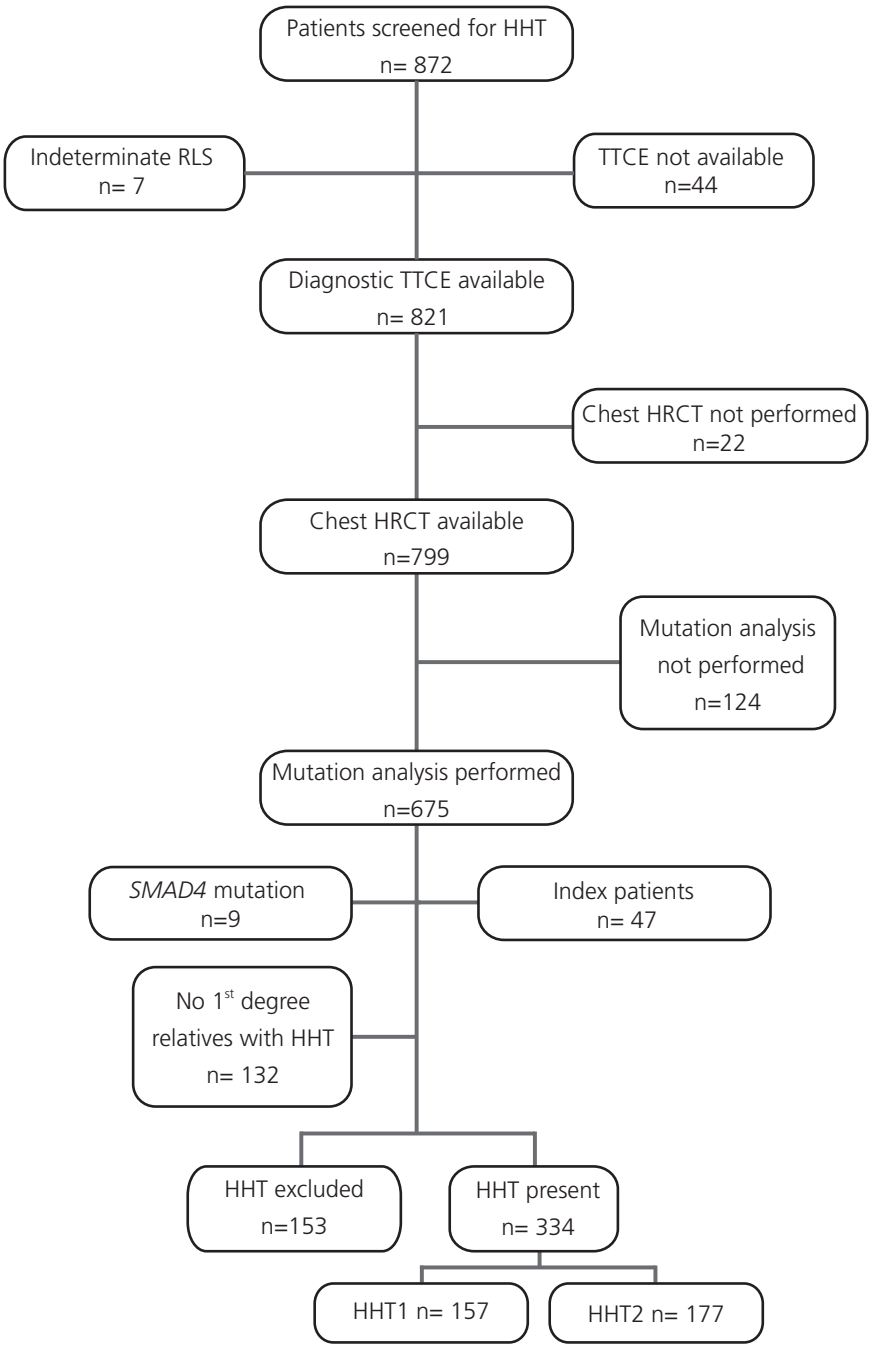

Figure 1. Selection of study population

List of abbreviations: HHT, hereditary haemorrhagic telangiectasia; RLS, right-to-left shunt; n, number; HRCT, high-resolution computed tomography.

Chest HRCT Scan

Chest HRCT scanning (Koninklijke Philips NV) was performed without contrast with the use of the single breath-hold technique and a slice thickness of 1 mm. Identification of PAVMs was based on the presence of a nodular opacity with both an afferent and an efferent vessel. The HRCT scans were evaluated by two independent observers (a radiologist and an experienced pulmonologist), both blinded to the TTCE results. When both observers disagreed, the chest HRCT images were considered positive for a PAVM, and angiography of the pulmonary artery was performed given the impact of complications from a potentially missed treatable PAVM.

Statistical Analysis

Descriptive statistics were used to describe patient characteristics. Continuous variables with normal distribution were presented as mean ± SD. Sensitivity was expressed as the number of patients with clinically confirmed HHT (three or more Curaçao criteria) divided by the number of patients with a confirmed HHT-causing mutation on genetic testing. Specificity was expressed as the number of patients with an unlikely clinical diagnosis (no or one Curaçao criterion) divided by the number of individuals in whom genetic testing excluded the known HHT family mutation. Positive predictive values (PPVs) and NPVs were subsequently calculated with their 95% CIs. Statistical analysis were performed with SPSS, version 17.0 (IBM Corporation) software.

RESULTS

Study Population

The selected study population comprised 487 people (mean age, 42.2 ± 14.7 years, 56.3% women) in whom genetic testing confirmed HHT1 in 157 (32.2%), HHT2 in 177 (36.3%), and no HHT in 153 (31.4%) (table 1).

TTCE and Chest HRCT Imaging

TTCE documented a pulmonary shunt in 134 patients (85.4%) with HHT1, 75 (42.4%) with HHT2, and nine (5.9%) without HHT (table 1). All nine individuals without HHT had a pulmonary shunt grade 1 on TTCE. The distribution of the three pulmonary shunt grades differed between both HHT subtypes. Grade 3 shunts tended to be more frequent in HHT1 (53.7% of all shunts in HHT1), whereas grade 1 shunts were predominant in HHT2 (64.0% of all shunts in HHT2). Of the 136 individuals with a pulmonary shunt grade 1 and 2 on TTCE, only five (3.7%) had a maximum number of microbubbles close to the cutoff point between these two shunt grades (e.g. five of 136 had between 27 and 32 bubbles in the left ventricle). The mean number of left-sided microbubbles was 10 and 49 for a pulmonary shunt grade 1 and 2, respectively on TTCE. Chest HRCT scan confirmed the presence

of PAVMs in 114 of 218 patients (52.3%) with a pulmonary shunt on TTCE, whereas all patients with a PAVM on chest HRCT scan demonstrated a pulmonary shunt on TTCE.

Table 1. Clinical characteristics of the study population.

	HHT1	HHT2	No HHT
Total, n	157	177	153
Age (years ± SD)	41.6±15.4	46.0±14.0	38.3±13.7
Female, n (%)	96 (61.1)	95 (53.7)	83 (54.2)
Male, n (%)	61 (38.9)	82 (46.3)	70 (45.8)
HHT criteria, n (%)			
First degree family member with HHT, n (%)	157 (100)	177 (100)	153 (100)
Epistaxis	146 (93.0)	167 (94.4)	22 (14.4)
Telangiectases	145 (92.4)	158 (89.3)	20 (13.1)
Visceral AVM[a]	107 (68.2)	42 (23.7)	1 (0.7)
Pulmonary AVM (chest HRCT scan)	97 (61.8)	17 (9.6)	0
Cerebral AVM [b]	13 (8.3)	1 (0.6)	n/a
Hepatic AVM [c]	4 (2.5)	19 (10.7)	1 (0.7)
GI telangiectases [d]	17 (10.8)	11 (6.2)	n/a
Clinical diagnosis, n (%) [e]			
Definite	145 (92.4)	149 (84.2)	3 (2.0)
Possible	12 (7.6)	28 (15.8)	37 (24.2)
Unlikely	0	0	113 (73.8)
Pulmonary RLS on TTCE, n (%)	134 (85.4)	75 (42.4)	9 (5.9)
Grade 1	25 (18.7)	48 (64.0)	9 (100)
Grade 2	37 (27.6)	17 (22.7)	0
Grade 3	72 (53.7)	10 (13.3)	0

Data are presented as mean ± SD or n (%). List of abbreviations: HHT, hereditary haemorrhagic telangiectasia; n, number; SD, standard deviation; AVM, arteriovenous malformation; HRCT, high-resolution computed tomography; GI, gastrointestinal; n/a, not applicable. [a]29 persons with two different localisations of visceral AVMs and one person with three different visceral localisations of AVMs. [b]Screening performed in 174 patients. [c]Screening performed in 53 patients. [d]Screening performed in 36 patients. [e]According to the current clinical Curaçao criteria (using chest HRCT for the presence of PAVMs).

The Presence of a Pulmonary Shunt on TTCE as a Clinical Curaçao Criterion for Diagnosing HHT

According to the current clinical Curaçao criteria (use of chest HRCT scan to detect the presence of PAVMs), HHT was definite in 294 patients (88.0%) and possible in 40 (12.0%) with a genetically proven HHT-causing mutation (table 1). There were no patients with

genetically proven HHT in whom the current criteria suggested HHT to be unlikely. Genetic testing excluded HHT in three individuals (1.9%) given a definite clinical diagnosis on the basis of the current criteria. All three had a first-degree relative with HHT, frequent and spontaneous epistaxis, and at least three telangiectasia on the characteristic sites but no visceral AVMs. The first-degree family mutations of these individuals with negative DNA findings were clearly pathogenic. The sensitivity, specificity, PPV, and NPV of the current clinical Curaçao criteria for diagnosing HHT were 88.0% (95% CI, 0.84-0.91), 73.9% (95% CI, 0.66-0.80), 99.0% (95% CI, 0.97-0.99), and 100% (95% CI, 0.97-1.0), respectively (table 2).

Table 2. Diagnosis of HHT according to current clinical Curaçao criteria without TTCE.

Clinical Diagnosis	HHT Mutation	No HHT Mutation	Total
Definite	294 (88.0)	3 (1.9)	297
Possible	40 (12.0)	37 (24.2)	77
Unlikely	0	113 (73.9)	113
Total	334	153	487

Data are presented as n (%) unless otherwise indicated. Calculated sensitivity, 88.0% (95% CI, 0.84-0.91); specificity, 73.9% (95% CI, 0.66-0.80); positive predictive value; 99.0% (95% CI, 0.97-0.99) for a definite diagnosis of HHT; negative predictive value, 100% (95% CI, 0.97-1.0) for an unlikely diagnosis of HHT. List of abbreviations: HHT, hereditary haemorrhagic telangiectasia; TTCE, transthoracic contrast echocardiography.

Accepting the presence of any pulmonary shunt on TTCE as a clinical Curaçao criterion enhanced the number of positive criteria in 92 of 487 individuals (18.9%), which actually changed the clinical diagnosis in 30 of them (32.6%). The remaining 62 (67.4%) changed from three to four positive criteria, which had no clinical consequences because three Curaçao criteria already suffice for a definite diagnosis of HHT (table 3). This increased criteria sensitivity from 88.0% (95% CI, 0.84-0.91) to 94.3% (95% CI, 0.91-0.96) at the expense of a decreased specificity from 73.9% (95% CI, 0.66-0.80) to 69.9% (95% CI, 0.62-0.77) (table 4). This decline in specificity was completely related to the presence of grade 1 pulmonary shunts in individuals without HHT; all nine of the 153 people (5.9%) without HHT incorrectly given a diagnosis of possible or definite HHT showed a pulmonary shunt grade 1 on TTCE. The range of microbubbles counted in the left side of the heart in these nine individuals was between three and 19, which illustrates that there was no doubt about the presence of a pulmonary shunt grade 1 (and not grade 2) on TTCE. In contrast, genetic testing confirmed the presence of HHT in all individuals with a pulmonary shunt grade ≥ 2 on TTCE. Therefore, we also determined the diagnostic accuracy of the clinical Curaçao criteria with the addition of only pulmonary shunt grades ≥ 2 on TTCE as a positive criterion. This strategy enhanced the number of positive criteria in 30 of 487 individuals (6.2%), which correctly changed the clinical diagnosis in seven of 30 (23.3%)

and prevented additional incorrect clinical diagnoses of HHT, with no difference between HHT1 and HHT2 (table 5). Accepting the presence of only pulmonary shunt grades ≥ 2 on TTCE as a positive clinical Curaçao criterion actually improved the overall diagnostic accuracy of the current criteria, resulting in a sensitivity, specificity, PPV, and NPV of 90.1% (95% CI, 0.86-0.93), 73.9% (95% CI, 0.66-0.80), 99.0% (95% CI, 0.97-0.99), and 100% (95% CI, 0.97-1.0), respectively, in diagnosing HHT (table 6).

Table 3. Changes in the number of positive Curaçao criteria with the addition of any pulmonary shunt on TTCE

No. Criteria	HHT1	HHT2	No HHT
0→1	n/a	n/a	n/a
1→2	0	0	6 (3.9)
2→3	6 (3.8)	15 (8.4)	3 (2.0)
3→4	28 (17.8)	34 (19.2)	0
Total	34 (21.7)	49 (27.7)	9 (5.9)

Data are presented as n (%). List of abbreviations: No, number; HHT, hereditary haemorrhagic telangiectasia; TTCE, transthoracic contrast echocardiography.

Table 4. Diagnosis of HHT with the addition of any pulmonary shunt on TTCE to the current clinical Curaçao criteria

Clinical Diagnosis	HHT Mutation	No HHT Mutation	Total
Definite	315 (94.3)	6 (3.9)	321
Possible	19 (5.7)	40 (26.1)	59
Unlikely	0	107 (69.9)	107
Total	334	153	487

Data are presented as n (%) unless otherwise indicated. Calculated sensitivity, 94.3% (95% CI, 0.91-0.96); specificity, 69.9% (95% CI, 0.62-0.77); positive predictive value, 98.1% (95% CI, 0.96-0.99) for a definite diagnosis of HHT; negative predictive value, 100% (95% CI, 0.97-1.0) for an unlikely diagnosis of HHT. List of abbreviations: HHT, hereditary haemorrhagic telangiectasia; TTCE, transthoracic contrast echocardiography.

Table 5. Changes in the number of positive Curaçao criteria with the addition of only pulmonary shunt grades ≥ 2 on TTCE

No. Criteria	HHT1	HHT2	No HHT
0→1	n/a	n/a	n/a
1→2	0	0	0
2→3	3 (1.9)	4 (2.3)	0
3→4	13 (8.3)	10 (5.6)	0
Total	16 (10.2)	14 (7.9)	0

Data are presented as n (%). List of abbreviations: No, number; HHT, hereditary haemorrhagic telangiectasia; TTCE, transthoracic contrast echocardiography.

Table 6. Diagnosis of HHT with the addition of only pulmonary shunt grades ≥ 2 on TTCE to the current clinical Curaçao Criteria

Clinical Diagnosis	HHT Mutation	No HHT Mutation	Total
Definite	301 (90.1)	3 (1.9)	304
Possible	33 (9.9)	37 (24.2)	70
Unlikely	0	113 (73.9)	113
Total	334	153	487

Data are presented as n (%) unless otherwise indicated. Calculated sensitivity, 90.1% (95% CI, 0.86-0.93); specificity, 73.9% (95% CI, 0.66-0.80); positive predictive value, 99.0% (95% CI, 0.97-0.99) for a definite diagnosis of HHT; negative predictive value, 100% (95% CI, 0.97-1.0) for an unlikely diagnosis of HHT. List of abbreviations: HHT, hereditary haemorrhagic telangiectasia; TTCE, transthoracic contrast echocardiography.

DISCUSSION

To our knowledge, this large prospective study is the first to evaluate the role of a pulmonary shunt on TTCE in the clinical Curaçao criteria for diagnosing HHT. The results demonstrate that the addition of only pulmonary shunt grades ≥ 2 on TTCE to the current Curaçao criteria increases its sensitivity without affecting specificity, suggesting that a pulmonary shunt grade 1 on TTCE should not be accepted as a diagnostic criterion for HHT.

The characteristic feature of vascular pathology in HHT is the presence of direct artery-to-vein communications, which carries the risk of complications from shunting and haemorrhage. HHT has an estimated prevalence of 1 in 5,000 individuals [18], but many do not receive a diagnosis and are at risk for potential preventable complications [19,20]. Although genetic testing for an HHT-causing mutation has been improved and has become more widely available in the past few years, mutation analysis per se is not always sufficient in the diagnosis of HHT. Previous studies described a mutation detection rate of 72% to 93% in patients with clinically confirmed HHT [21-24], and underuse of genetic testing has been reported in relatives at risk for HHT [25]. Therefore, an accurate clinical evaluation remains essential in all persons with suspected HHT. The original clinical Curaçao criteria were designed in the year 2000 as a consensus statement and consist of spontaneous and recurrent epistaxis, telangiectases at characteristic sites, a first-degree relative with HHT, and the presence of visceral AVMs [7]. Our group previously demonstrated that these criteria already have a good diagnostic performance compared with genetic testing [26]. However, further improvement of the criteria remains desirable, as was already recognised by the authors of the original clinical Curaçao criteria [8,9]. Although TTCE replaced chest HRCT scanning in the past few years as the first-line screening technique for detecting PAVMs [10], it remains unknown whether a pulmonary shunt on TTCE can stand on its own as a (new) Curaçao criterion in the clinical diagnosis of HHT. This question can be of special

importance because the present study confirms that chest HRCT scanning demonstrates a PAVM in only 52.3% of patients with a pulmonary shunt on TTCE.

In the present study, the addition of any pulmonary shunt on TTCE to the current clinical Curaçao criteria resulted in an increased sensitivity from 88% to 94%, but decreased specificity from 74% to 70%. Although a pulmonary shunt grade 1 on TTCE was present in 73 of 334 patients (21.9%) with HHT and correctly changed the clinical diagnosis from possible to definite HHT in 14 (4.2%), this small pulmonary shunt was also found in nine individuals (5.9%) without HHT and falsely changed the clinical diagnosis from possible to definite in three (2.0%). This finding suggests a limited diagnostic significance of a pulmonary shunt grade 1 on TTCE. The presence of small pulmonary shunts on TTCE has been previously described in around 6% of healthy subjects [14,16] and may represent a normal variant in the general population in the absence of an underlying patent foramen ovale (excluded in all people with a pulmonary shunt grade 1 without HHT in the present study). Woods *et al*. [27] described a relatively high prevalence of small pulmonary right-to-left shunts on TTCE (28%) in a healthy volunteer population compared with the 5.9% found in the present study. A potential explanation for this discrepancy might be that the study by Woods *et al*. could have been biased toward volunteers with migraines (because the study advertisements described a research study investigating shunts and migraines), a population in which a higher prevalence of pulmonary shunts has been reported [27,28]. This potential advertisement-based bias might also explain the relatively high prevalence of individuals with a patent foramen ovale (38%) and migraine (40%) compared with the percentage in other studies (15%-27% and 6%-18%, respectively) [27,29-31]. However, because we cannot think of other plausible explanations for this different finding, we should consider that small pulmonary shunts on TTCE might be more common in healthy subjects than previously appreciated. Therefore, we believe that only pulmonary shunt grades ≥ 2 on TTCE should be accepted as a positive clinical Curaçao criterion in diagnosing HHT. With this strategy, the sensitivity of the current clinical criteria is increased to 90% without decreasing specificity.

This study had several limitations. First, the study included a selected group of first-degree family members of patients with genetically proven HHT1 or HHT2 (who all fulfilled already one Curaçao criterion), and caution is needed in extrapolating the results to less selected screening populations. However, it can be expected that the diagnostic significance of the pulmonary shunt grade on TTCE is even higher in patients with no known first-degree family member with HHT. Second, the study population was screened in an HHT clinic with specific expertise in TTCE, and it remains uncertain whether the results also apply to a general population in hospitals without this specific expertise. However, screening for PAVMs with TTCE is preferably performed in specialised HHT clinics to achieve the accuracy reported in the literature. Third, the clinical presentation of HHT is age dependent and validity of the clinical Curaçao criteria ideally should be stratified for different age categories

[32]. The present study was not adequately powered for this purpose, and caution should be taken in extrapolating the results to young people with a pulmonary shunt grade 1 on initial TTCE and those aged < 15 years because this last age category was not included in the present study. Fourth, routine screening for liver, cerebral, or gastrointestinal AVMs was absent in the study. This might have caused an underappreciation of the current clinical Curaçao criteria but does reflect a more realistic representation of the screening algorithm for HHT in daily practice. Finally, the present study is a single-centre investigation, and different results may be found by a different set of investigators.

CONCLUSION

The addition of only pulmonary shunt grades ≥ 2 on TTCE to the current clinical Curaçao criteria further increases its sensitivity without affecting specificity in diagnosing HHT. A pulmonary shunt grade 1 on TTCE should not be accepted as a diagnostic criterion for HHT.

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CHAPTER 3

Pulmonary shunt fraction measurement compared to contrast echocardiography in hereditary haemorrhagic telangiectasia patients: time to abandon the 100% oxygen method?

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ABSTRACT

Background: The presence of pulmonary right-to-left shunting (RLS) is associated with severe neurological complications from paradoxical embolisation in patients with hereditary haemorrhagic telangiectasia (HHT) and screening is warranted. Pulmonary shunt fraction measurement with the 100% oxygen method can be used for the detection and quantification of functional pulmonary RLS, although transthoracic contrast echocardiography (TTCE) has emerged as the gold standard over the last few years.

Objective: The aim of this study was to determine the true diagnostic accuracy of the established 100% oxygen method in detecting pulmonary RLS, as compared to TTCE.

Methods: We analysed 628 persons screened for HHT between 2004 and 2010, all of whom underwent TTCE. A quantitative 3-point grading scale was used to differentiate between minimal, moderate or extensive pulmonary RLS on TTCE (grade 1–3, respectively). Additional shunt fraction measurement with the 100% oxygen method was pursued in cases of pO_2 <13 or <12 kPa in patients younger or older than 30 years, respectively. A shunt fraction >5% was considered pathological.

Results: Both TTCE and the 100% oxygen method were performed in 210 subjects. Although the presence of a pathological shunt fraction correlated with an increased pulmonary shunt grade on TTCE, the 100% oxygen method confirmed a >5% shunt fraction in only 51% of patients with pulmonary RLS on TTCE (14, 20 and 72% for grade 1, 2 and 3, respectively).

Conclusion: Pulmonary shunt fraction measurement with the 100% oxygen method is not a useful screening technique for the detection of pulmonary RLS in HHT as its sensitivity is too low and large pulmonary shunts on TTCE may remain undetected using this method.

INTRODUCTION

Pulmonary arteriovenous malformations (PAVMs) are thin-walled abnormal vessels replacing normal capillaries between the pulmonary arterial and venous circulation [1]. A PAVM causes permanent pulmonary right-to-left shunting (RLS), thereby bypassing the pulmonary capillary filter. Consequently, both emboli of thrombotic and septic origin may directly reach the systemic circulation, causing potential severe neurological complications, such as ischemic strokes or brain abscesses [2]. Gas exchange may also be compromised in the presence of pulmonary RLS, resulting in hypoxaemia and dyspnoea. The majority of PAVMs (70–90%) are associated with hereditary haemorrhagic telangiectasia (HHT) [3,4]. HHT is an autosomal dominant inherited disorder, characterised by vascular abnormalities varying from small telangiectasias in skin and mucosal membranes to large arteriovenous malformations, predominantly in the brain, liver and lungs. There are mainly two types of HHT, corresponding with gene mutations coding for endoglin (HHT1) and ALK1 (HHT2) [5,6]. Pulmonary RLS has been reported in 91% of HHT1 and in 53% of HHT2 patients [7]. Due to this high prevalence coupled with its potential severe complications and the existence of effective treatment options with transcatheter embolotherapy, screening for pulmonary RLS is recommended in all patients with possible or confirmed HHT [8-10]. Pulmonary shunt fraction measurement with the 100% oxygen method has long been performed as the initial screening technique, based on alveolar-arterial oxygen differences after breathing 100% oxygen [3], but during the last few years transthoracic contrast echocardiography (TTCE) has evolved as the recommended first-line screening technique for the detection of pulmonary RLS [9-11]. However, the 100% oxygen method has never been directly compared to TTCE and is still in use for the detection and quantification of pulmonary RLS and analysis of unexplained hypoxaemia. Therefore, the present large observational study determined the true diagnostic accuracy of pulmonary shunt fraction measurement with the established 100% oxygen method in detecting functional pulmonary RLS compared to TTCE as the modern gold standard.

METHODS

Study population

Between May 2004 and December 2010, 669 subjects above 15 years of age were screened for HHT in the St. Antonius Hospital, Nieuwegein, The Netherlands. Subjects were screened in a 1-day protocol, as family members of index patients or in case of clinical symptoms suggesting HHT. A complete history and physical examination were performed by a pulmonologist with dedicated expertise in HHT. The clinical diagnosis of HHT was established according to the Curaçao criteria [12]. These criteria consist of spontaneous and recurrent epistaxis, telangiectasia at characteristic sites, visceral arteriovenous malformations and a first-degree relative with HHT. Genetic testing for the HHT-causing gene mutation was offered to all

screened subjects and performed as published previously [13]. A definite diagnosis of HHT was established in the case of three or more clinical Curaçao criteria, or when genetic testing identified the HHT-causing mutation. The diagnosis of HHT was 'possible' in patients with two clinical Curaçao criteria without genetic testing or if no mutation had been found in the family. The diagnosis of HHT was 'unlikely' in the presence of less than two clinical Curaçao criteria without genetic testing or if no mutation had been found in the family. HHT was rejected when genetic testing excluded the known HHT-causing family mutation. The study was approved by the institutional review board (LTME/Z-12.41).

Pulmonary shunt fraction measurement with the established 100% oxygen method

The arterial partial oxygen pressure (pO_2, kPa) was measured at rest, breathing room air in a semi-recumbent position. An additional shunt fraction measurement with the 100% oxygen method was pursued in cases with a pO_2 <13 or <12 kPa in patients younger or older than 30 years, respectively. Patients breathed 100% oxygen from a Douglas bag, via a closely fitting mouthpiece and two-way valve, while wearing a nose clip and taking a deep inspiration every minute. An arterial blood sample was obtained after breathing 100% oxygen for 30 min and the samples were cooled on ice and analysed immediately for oxygen and carbon dioxide tensions. The pulmonary shunt fraction was calculated using the established classical equation: $Q_s/Q_t = (C_{c,O2} - C_{a,O2})/(C_{c,O2} - C_{v,O2})$, in which Q_s/Q_t is the RLS as a fraction of the cardiac output, $C_{c,O2}$ is the oxygen content at the end of the pulmonary capillary, $C_{a,O2}$ is the oxygen content of arterial blood and $C_{v,O2}$ is the oxygen content of mixed venous blood [14]. The $C_{v,O2}$ was defined as the $C_{a,O2}$ – 4.4 ml O_2/100 ml blood [15]. Since the total blood oxygen content is composed of dissolved O_2 plus HbO_2 and the solubility of oxygen in blood is 0.0225 ml/100 ml/kPa, the oxygen content was calculated as follows: oxygen content (ml O_2 /100 ml blood) = (0.0225 × pO_2) + (2.24 × haemoglobin × SaO_2/100), where pO_2 is the partial oxygen pressure (kPa), haemoglobin is expressed in mmol/l and SaO_2 is the arterial oxygen saturation (%). The haemoglobin oxygen saturation at the end of the pulmonary capillary is assumed to be 100%. The partial pressure of carbon dioxide (pCO_2) is assumed to equal the alveolar partial pressure of oxygen (pO_2) and can be calculated as follows: pO_2 = barometric pressure (101.3 kPa) – pCO_2 – alveolar saturated water vapour pressure ($P_{A,H2O}$). $P_{A,H2O}$ is 6.3 kPa at a body temperature of 37°C. Using the 100% oxygen method, a pulmonary shunt fraction of >5% was considered pathological [3,15]. Patients with previously treated PAVMs were not included in our analysis.

Transthoracic contrast echocardiography

TTCE was performed by placing an intravenous line in the right ante-cubital vein to which two 10 ml syringes were connected, one filled with an 8 ml physiologic saline solution and the other with 1 ml of air. Subsequently, 1 ml blood was drawn in the airfilled syringe and

mixed with the saline-filled syringe by reverse flushing between both syringes, creating agitated saline (microbubbles). The patient was positioned in the left lateral position and 5 ml of fresh agitated saline was injected within 3 s while projecting the 4-chamber apical view, with and without a Valsalva manoeuvre. TTCE was performed by a constant group of three trained echocardiographers. Interpretation of RLS was performed by two cardiologists with dedicated expertise in both TTCE and HHT, who were unaware of the patients' medical history or prior shunt fraction measurement. In the case of RLS, visualisation of shunt origin was pursued in every TTCE. All RLS visualised through a pulmonary vein was classified as pulmonary RLS. On the occasion of poor visualisation of shunt origin, we used a delay of 4 cardiac cycles to distinguish pulmonary from cardiac RLS, in which TTCE was considered positive for pulmonary RLS if microbubbles appeared in the left atrium after 4 cardiac cycles, as published previously [11,16,17]. This delay in the appearance of microbubbles in the left atrium was measured in the number of cardiac cycles after the first appearance of microbubbles in the right atrium. Opacification of the left ventricle was quantitatively graded as 1 (a maximum of 29 microbubbles in the left ventricle), 2 (30–100 microbubbles) or 3 (>100 microbubbles). This division was based on the maximum number of microbubbles in the left ventricle counted in one still frame. A good κ coefficient of 0.85 up to 0.94 was found for inter-observer agreement concerning this pulmonary shunt grade on TTCE in previous studies [17,18]. RLS within 4 cardiac cycles with poor visualisation of shunt origin was classified as indeterminate shunting and excluded from further analysis. A patent foramen ovale was diagnosed only after a positive Valsalva manoeuvre, without spontaneous RLS. The presence of an atrial septum defect was routinely excluded in all RLS using colour Doppler or potential negative contrast in the right atrium [19].

Chest computed tomography

Chest CT images were routinely obtained on a 16+ multi-detector CT scanner (Philips Medical Systems), with a dedicated high-resolution algorithm and maximum slice thickness of 1 mm. Identification of PAVMs on chest CT was based on the presence of a nodular opacity with both an afferent and efferent vessel. Chest CT scans were evaluated by two independent observers highly experienced in evaluating PAVMs (a radiologist and a pulmonologist), both blinded to the results from TTCE and shunt fraction measurement. When the observers disagreed, chest CT was considered positive for a PAVM and additional angiography of the pulmonary artery was performed given the impact of potential complications from a missed treatable PAVM.

Statistics

Descriptive statistics were used to describe patient characteristics. Continuous variables with a normal distribution were presented as the mean ± standard deviation. A median range was used when normal distribution was absent. Sensitivity, specificity, positive pre-

dictive value and negative predictive value with their 95% confidence intervals (CI), and the area under the receiver operating characteristic (ROC) curve were determined for shunt fraction measurement with the 100% oxygen method compared to TTCE as the gold standard. Statistical analyses were performed using the statistical software application SPSS (version 17.0; SPSS Inc., Chicago, Ill., USA).

RESULTS

Study population

Out of the 669 individuals screened for HHT, a diagnostic TTCE was available in 628 (93.9%). Arterial blood gas analysis was performed in 614 out of these 628 subjects (97.8%). Based on the previous described pO_2 values, 210 persons (34.2%) underwent additional pulmonary shunt fraction measurement with the 100% oxygen method and were included for further analysis (figure 1). Out of these 210 persons, the presence of HHT was definite in 135 patients (64.3%), possible in 17 (8.1%), unlikely in 19 (9.0%) and excluded in 39 (18.6%). Genetic testing was performed in 188 out of the 210 patients

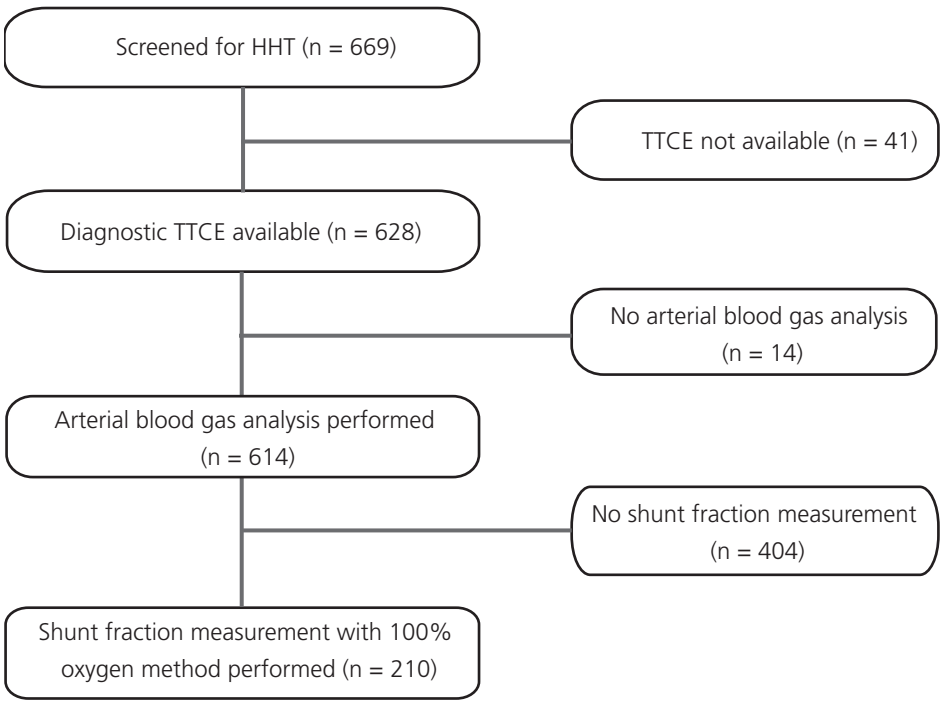

Figure 1. Flow chart of the selected study population.
HHT, hereditary haemorrhagic telangiectasia; n, number; TTCE, transthoracic contrast echocardiography.

(89.5%), which identified 62 (29.5%) with HHT1 and 62 (29.5%) with HHT2. The baseline characteristics of our final study population are described in table 1.

Table 1. Baseline characteristics of the study population analysed with both TTCE and the 100% oxygen method

Patients	210
Female	128 (61.0)
Age, years	46.8± 15.1
HHT	
Definite	135 (64.3)
HHT 1	62 (29.5)
HHT 2	62 (29.5)
Possible	17 (8.1)
Unlikely	19 (9.0)
Excluded	39 (18.6)
Pulmonary shunt on TTCE	
Grade 1	22 (10.5)
Grade 2	15 (7.1)
Grade 3	61 (29.0)
No pulmonary shunt on TTCE	112 (53.3)
PFO	10 (4.8)
100% oxygen method	
Shunt fraction ≤5%	144 (68.6)
Shunt fraction >5%	66 (31.4)
PAVM on chest CT	
Yes	66 (31.4)
No	141 (67.1)

Data are presented as number with percentages in parentheses, or the mean ± standard deviation. HHT, hereditary haemorrhagic telangiectasia; PFO, patent foramen ovale.

Pulmonary shunt fraction measurement with the 100% oxygen method compared to TTCE

Pulmonary RLS on TTCE was present in 98 out of the 210 patients (46.7%). A pulmonary shunt grade 1 was diagnosed in 22 patients (22.4%), grade 2 in 15 (15.3%) and grade 3 in 61 (62.2%). The 100% oxygen method documented a pathological shunt fraction of >5% in 13.6, 20.0 and 72.1% of the patients with a pulmonary shunt grade 1, 2 or 3 on TTCE, respectively (figure 2). The mean pulmonary shunt fraction in patients with pulmonary shunt grades 1, 2 and 3 on TTCE was 2.2, 3.8 and 10.1%, respectively (figure 3). Using TTCE as the gold standard for the detection of functional pulmonary RLS, the 100% oxygen method had a sensitivity of 51% (95% CI 0.41-0.61) and a specificity of 86% (95%

CI 0.78-0.91; table 2a), with an area under the ROC curve of 0.74 (95% CI 0.67-0.81). The diagnostic accuracy of the 100% oxygen method in detecting only moderate to large pulmonary shunts on TTCE (grades 2 and 3) slightly increased to a sensitivity of 62% (95% CI 0.51-0.72) and a specificity of 86% (95% CI 0.79-0.9; table 2b), with an area under the ROC curve of 0.82 (95% CI 0.75-0.88).

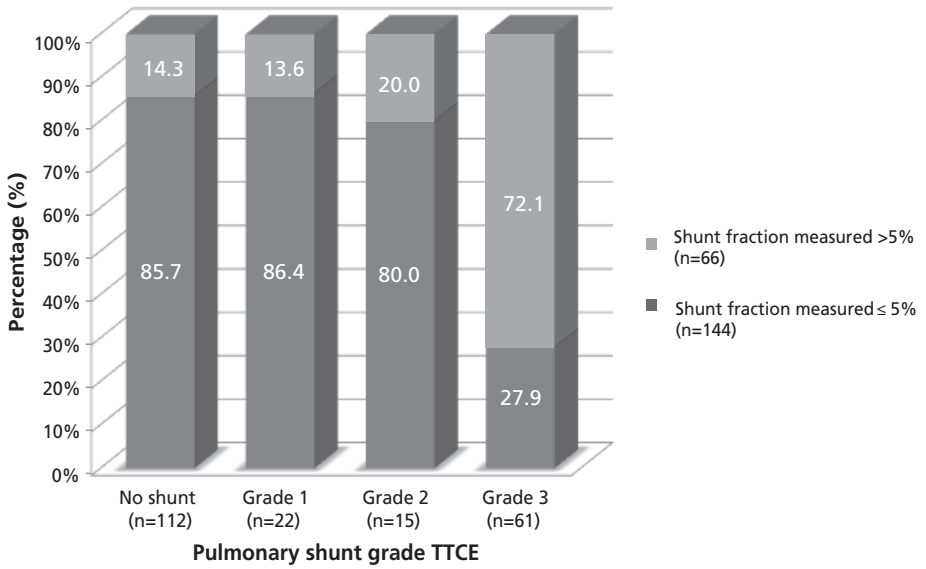

Figure 2. Pulmonary shunt fraction measurement with the 100% oxygen method compared to different pulmonary shunt grades on TTCE (n = 210).
TTCE, transthoracic contrast echocardiography; n, number.

Table 2. Pulmonary shunt fraction measurement using the 100% oxygen method compared to TTCE

a *Diagnostic accuracy of the 100% oxygen method in detecting any pulmonary shunt on TTCE*

	Shunt fraction ≤5%	Shunt fraction >5%	Total
No pulmonary shunt on TTCE	96	16	112
Any pulmonary shunt on TTCE	48	50	98
Total	144	66	210

Sensitivity 51% (0.41 – 0.61), specificity 86% (95% CI 0.78 – 0.91), positive predictive value 76% (95% CI 0.64– 0.84), negative predictive value 67% (95% CI 0.58 – 0.74).

b *Diagnostic accuracy of the 100% oxygen method in detecting only pulmonary shunt grades 2 and 3 on TTCE*

	Shunt fraction ≤5%	Shunt fraction >5%	Total
No or grade 1 pulmonary shunt on TTCE	115	19	134
Only pulmonary shunt grade 2 or 3 on TTCE	29	47	76
Total	144	66	210

Sensitivity 62% (95% CI 0.51 – 0.72), specificity 86% (95% CI 0.79 – 0.9), positive predictive value 71% (95% CI 0.59 – 0.81), negative predictive value 80% (95% CI 0.73 – 0.86).

TTCE, transthoracic contrast echocardiography.

The 100% oxygen method revealed a pathological shunt fraction of >5% in 14.3% of subjects without any RLS on TTCE. A mean shunt fraction of 9.1% was found in these false-positive cases. Chest CT demonstrated regions with atelectasis and/or pulmonary fibrosis in 53.3% of these persons, but no alternative explanation was found in the other 46.7% of false-positive cases. TTCE showed a patent foramen ovale in 10 out of the 210 patients (4.8%), where the 100% oxygen method revealed a pathological shunt fraction of >5% in only 3 of these patients. No atrial septum defect was documented. Chest CT was performed in 207 out of the 210 patients (98.6%) with both TTCE and shunt fraction measurement. Although already selected by a lower pO_2, chest CT confirmed the presence

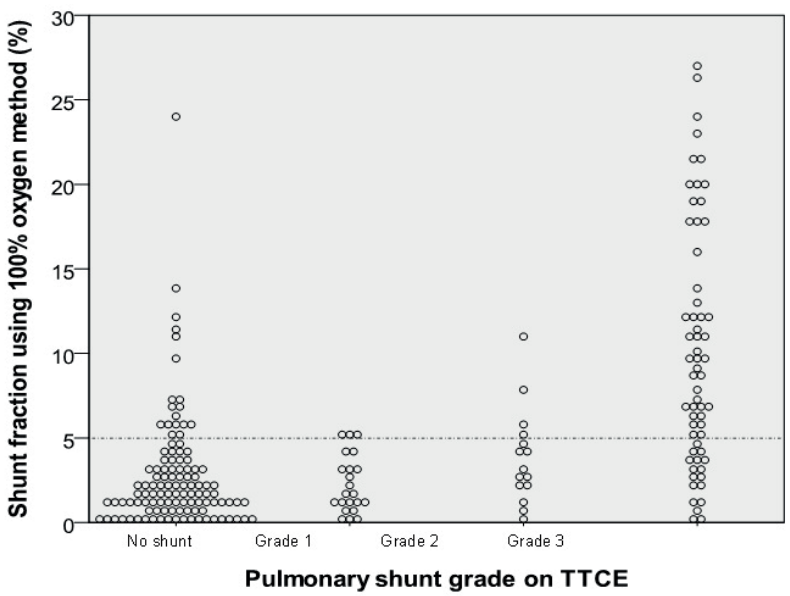

Figure 3. Dot plot demonstrating the shunt fractions measured with the 100% oxygen method compared to graded TTCE as the gold standard (n = 210).
TTCE, transthoracic contrast echocardiografie

of PAVMs in 66 out of 98 patients (67.3%) with pulmonary RLS on TTCE. Pulmonary shunt fraction measurement with the 100% oxygen method identified a pathological shunt fraction in 46 out of 66 patients with a PAVM on chest CT (69.7%). The 100% oxygen method did not reveal an abnormal shunt fraction in the other 41.7% of patients with visible PAVMs on chest CT.

DISCUSSION

To our knowledge, this is the first large study evaluating the true diagnostic accuracy of pulmonary shunt fraction measurement with the 100% oxygen method in detecting functional pulmonary RLS compared to TTCE as the modern gold standard. Our results firmly indicate that pulmonary shunt fraction measurement with the 100% oxygen method is not a reliable test to screen for PAVMs in patients with HHT.

Screening for PAVMs is warranted in all patients with possible or confirmed HHT, given the high risk of neurological complications that may be prevented by transcatheter embolotherapy. Arterial blood gas analysis and additional pulmonary shunt fraction measurement with the 100% oxygen method have been used as non-invasive screening tests for the detection of functional pulmonary RLS in these subjects. Under normal conditions, the fraction of cardiac output that shunts from right-to-left (i.e. the shunt fraction) is ≤5 %. A pathological shunt fraction of >5% has been reported in up to 97.5% of patients with PAVMs prior to transcatheter embolotherapy and the 100% oxygen method has long been assumed to be accurate enough for the detection of clinically important pulmonary RLS [20-25]. However, a retrospective study of 105 individuals screened for HHT by Cottin *et al*. [26] previously suggested a markedly lower sensitivity (up to 68%) of the 100% oxygen method in detecting pulmonary RLS compared to anatomical-based tests like chest CT and pulmonary angiography. Over the last few years, TTCE has evolved as the first-line screening technique for the detection of functional pulmonary RLS based on its excellent sensitivity, high negative predictive value and wide availability with low risks and costs [9,11,17,27-29]. However, studies directly comparing the 100% oxygen method to TTCE are lacking. The current analysis was warranted to reveal the true diagnostic accuracy of pulmonary shunt fraction measurement with the 100% oxygen method as we know that chest CT confirms the presence of PAVMs in only 45% of patients with pulmonary RLS on TTCE [7]. The present study demonstrates that the 100% oxygen method detects a pathological shunt fraction of >5% in only 51% of patients with any pulmonary RLS on TTCE. The majority of patients with a pulmonary shunt grade 1 or 2 on TTCE remain undetected using the 100% oxygen method (86.4 and 80.0%, respectively). Even in patients with a large, pulmonary shunt grade 3 on TTCE, the 100% oxygen method fails to detect an abnormal shunt fraction in 27.9% of cases. Our group recently described that only pulmonary shunt grades 2 and 3 on TTCE have clinical implications, as neurological

complications due to paradoxical embolisations are encountered in up to 14.2% of these shunts and transcatheter embolotherapy of PAVMs is indicated in 52.5% of these patients [2,7,30]. However, the present study reveals that the 100% oxygen method fails to detect a pathological shunt fraction of >5% in 38.2% of patients with a pulmonary shunt grade 2 or 3 on TTCE. Furthermore, the current international guideline on the diagnosis and management of HHT recommends the use of antibiotic prophylaxis before procedures with risk of bacteraemia in patients with any documented pulmonary RLS in order to prevent the occurrence of brain abscesses [9]. The current study illustrates that up to 49% of these patients will not be identified using shunt fraction measurements with the 100% oxygen method.

On the other hand, we documented a pathological shunt fraction of >5% in 14.3% of individuals without any RLS on TTCE. Additional chest CT imaging showed regions with atelectasis and/or pulmonary fibrosis in 53.3% of these persons, but no alternative explanation for the increased shunt fraction was found in the remaining 46.7% (true false positives). Besides the fact that a small degree of physiologic RLS normally takes place via the bronchiolar system of the lung perfusion and Thebesian veins, there may be several sources of error resulting in false-positive or negative findings with the 100% oxygen method. The detection of pulmonary RLS using TTCE is based on the true anatomical and functional shunt, while the 100% oxygen method measures the alveolar-arterial oxygen difference, which is then converted into a shunt magnitude. The 100% oxygen method will be influenced by the presence of multiple small PAVMs, where oxygen uptake may still take place and the actual RLS is underestimated. Furthermore, the arteriovenous difference in oxygen content is not routinely measured in the established 100% oxygen method and deviations from the assumed value could therefore result in deviations from the calculated shunt fraction. Measurement of the actual individual arteriovenous difference would improve the accuracy of the 100% oxygen method, but is not feasible in daily practice because of its invasive character. In addition, an accurate test can only be obtained when the patient truly receives 100% oxygen. A small leak in the oxygen delivery system will overestimate the degree of shunt fraction by lowering the true alveolar partial oxygen pressure. Similarly, breathing 100% oxygen for an inadequate period of time may result in an overestimation of the shunt fraction, owing to inadequate denitrogenation of poorly ventilated alveoli (which was not the case in the present study). It has also been reported that breathing 100% oxygen can occasionally cause a small amount of pulmonary RLS (up to 11%) in healthy subjects, due to complete denitrogenation and micro-atelectasis [31]. Taking a deep inspiration every minute helps to prevent this 100% oxygen related micro-atelectasis [20]. Further study limitations are the fact that additional shunt fraction measurement was not routinely performed in all 669 screened persons and that the 100% oxygen method was tested in a population at high pre-test risk for PAVMs (HHT). Therefore, our results do not automatically support generalisation to all other aetiologies of pulmonary shunting.

CONCLUSION

Pulmonary shunt fraction measurement with the 100% oxygen method is not a useful screening technique for the detection of pulmonary RLS in HHT. The reasons for this are that its sensitivity is too low and a large proportion of clinically important pulmonary RLS remains undetected.

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CHAPTER 4

Reproducibility of right-to-left shunt quantification using transthoracic contrast echocardiography in hereditary haemorrhagic telangiectasia

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ABSTRACT

Aim: Transthoracic contrast echocardiography (TTCE) is recommended for screening of pulmonary arteriovenous malformations (PAVMs) in hereditary haemorrhagic telangiectasia (HHT). Shunt quantification is used to find treatable PAVMs. So far, there has been no study investigating the reproducibility of this diagnostic test. Therefore, this study aimed to describe inter-observer and inter-injection variability of TTCE.

***Methods*:** We conducted a prospective single centre study. All consecutive persons screened for presence of PAVMs in association with HHT in 2015 were included. The videos of two contrast injections per patient were separated and reviewed by two cardiologists blinded for patient data. Pulmonary right-to-left shunts (RLS) were graded using a three-grade scale. Inter-observer and inter-injection agreement was calculated with κ statistics for the presence and grade of pulmonary RLS.

***Results*:** 107 persons (accounting for 214 injections) were included (49.5% male, mean age 45.0 ± 16.6 years). Pulmonary RLS was present in 136 (63.6%) and 131 (61.2%) injections for observer 1 and 2 respectively. Inter-injection agreement for the presence of pulmonary RLS was 0.96 (95% CI 0.9-1.0) and 0.98 (95% CI 0.94-1.00) for observer 1 and 2 respectively. Inter-injection agreement for pulmonary RLS grade was 0.96 (95% CI 0.93-0.99) and 0.95 (95% CI 0.92-0.98) respectively. There was disagreement in RLS grade between the contrast injections in 11 patients (10.3%). Inter-observer variability for presence and grade of pulmonary RLS was 0.95 (95% CI 0.91-0.99) and 0.97 (95% CI 0.95-0.99) respectively.

***Conclusion*:** TTCE has an excellent inter-injection and inter-observer agreement for both the presence and grade of pulmonary RLS.

INTRODUCTION

Transthoracic contrast echocardiography (TTCE) is used to screen for the presence of pulmonary arteriovenous malformations (PAVMs). Over 90% of PAVMs are associated with hereditary haemorrhagic telangiectasia (HHT), an inheritable disease characterised by abnormal artery-to-vein connections in the brain, liver or lungs [1,2]. The majority of cases is caused by mutations in the *ENG* and *ACVRL1* gene, leading to HHT type 1 and HHT type 2 respectively. A more rare mutation is located on the *SMAD4* gene. PAVMs are frequently described in all subgroups but more prevalent in HHT type 1. PAVMs are associated with severe neurologic complications in up to 21% of patients, such as brain abscesses and ischemic stroke [3]. Furthermore, PAVMs can result in hypoxemia, haemoptysis and migraine. Most patients however remain asymptomatic before the development of complications, making screening for PAVMs extremely important in all patients with or suspected for HHT [1,4-7].

TTCE represents a functional measurement in which PAVMs are visualised as a pulmonary right-to-left shunt (RLS). Pulmonary RLS grade on TTCE is a good predictor for the presence of treatable PAVMs on chest computed tomography (CT) [8]. Importantly, only moderate to large RLS seem to have clinical implications [3,8,9]. The international HHT guidelines recommend TTCE as first-choice screenings test for PAVMs. However, the reliability of TTCE is dependent on the reproducibility in the individual patient. Although the inter-observer variability is already described is some studies, there has been no research evaluating the reproducibility of TTCE. Therefore, we aimed to evaluate both the inter-injection and inter-observer variability of TTCE in this prospective single centre study.

MATERIAL AND METHODS

Study population

All consecutive persons screened for the presence of HHT and all consecutive HHT patients visiting the outpatient clinic for a regular 5 year follow-up at the Dutch HHT centre between February and November 2015 were included. The clinical diagnosis was established according to the Curaçao criteria, which consist of spontaneous and recurrent epistaxis, telangiectases at characteristic sites, visceral arteriovenous malformations, and a first-degree relative with HHT [1]. Genetic testing was offered to all included patients. A definite diagnosis of HHT was established in case of three or more Curaçao criteria, or when genetic testing identified the HHT-causing gene mutation (e.g. *ENG, ACVRL1* or *SMAD4*).

Patients were excluded if TTCE was not complete (e.g. due to intravenous line failure or image storage problems). The study was approved by the local ethics committee (R&D/Z14.059).

Transthoracic contrast echocardiography

All TTCEs were performed according to the local clinical protocol. TTCEs were conducted on a Philips IE33 ultrasound instrument (S5-1 transducer; Philips Medical Systems, Best, The Netherlands) or a General Electronic Vivid S6 ultrasound instrument (3S transducer; General Electronic Healthcare, Wauwatosa, The United States).

An intravenous line was inserted in the right antecubital vein, 1mL blood was drawn and 8mL physiological saline solution and 1mL air was added. A second syringe was connected by a bi-directional luer-lock system. In total 10mL agitated saline (containing microbubbles) was created by reverse flushing between the connected syringes [3,8-10]. The patient was positioned in the left lateral position and 5mL of agitated saline was injected at rest while projecting the four-chamber apical view. After all microbubbles dissolved, this procedure was repeated. All TTCE videos were blinded for patient data, numbered and stored in a database. The observers viewed all individual contrast injections in random order to minimise the possibility of bias. Two independent cardiologists (observer 1: VV and observer 2: SV) quantified each shunt.

All RLS that clearly originated out of the pulmonary vein were classified as pulmonary RLS, and all RLS appearing through the septum as cardiac RLS. If shunt origin was not visible, a delay of four cardiac cycles was used to distinguish between a pulmonary and cardiac shunt. The TTCE was considered positive for a pulmonary RLS if microbubbles appeared in the left atrium after four or more cardiac cycles. The pulmonary RLS was graded based on the maximum number of microbubbles present in the left ventricle in one still frame. RLS was graded as 1, 2 or 3 corresponding with 1-29, 30-100 and over 100 microbubbles respectively (figure 1 and video 1-3) [3,8,10-12].

The technical quality of the studies was reviewed. Image quality was described as good, sufficient or insufficient. Quantity of contrast in the right ventricle (RV) was described as sufficient when opacification of the RV was densely filled (with endocardial lining) [13].

Chest computer tomography

Chest CT was performed in all patients with a pulmonary RLS grade ≥2 in at least one of the injections [3,14]. CTs were performed with a ≥16-detector CT scanner (Philips Medical Systems, Best, the Netherlands) with a high-resolution algorithm and slice thickness of 1 mm. All CT images were evaluated by an interventional radiologist and pulmonologist unaware of the TTCE results.

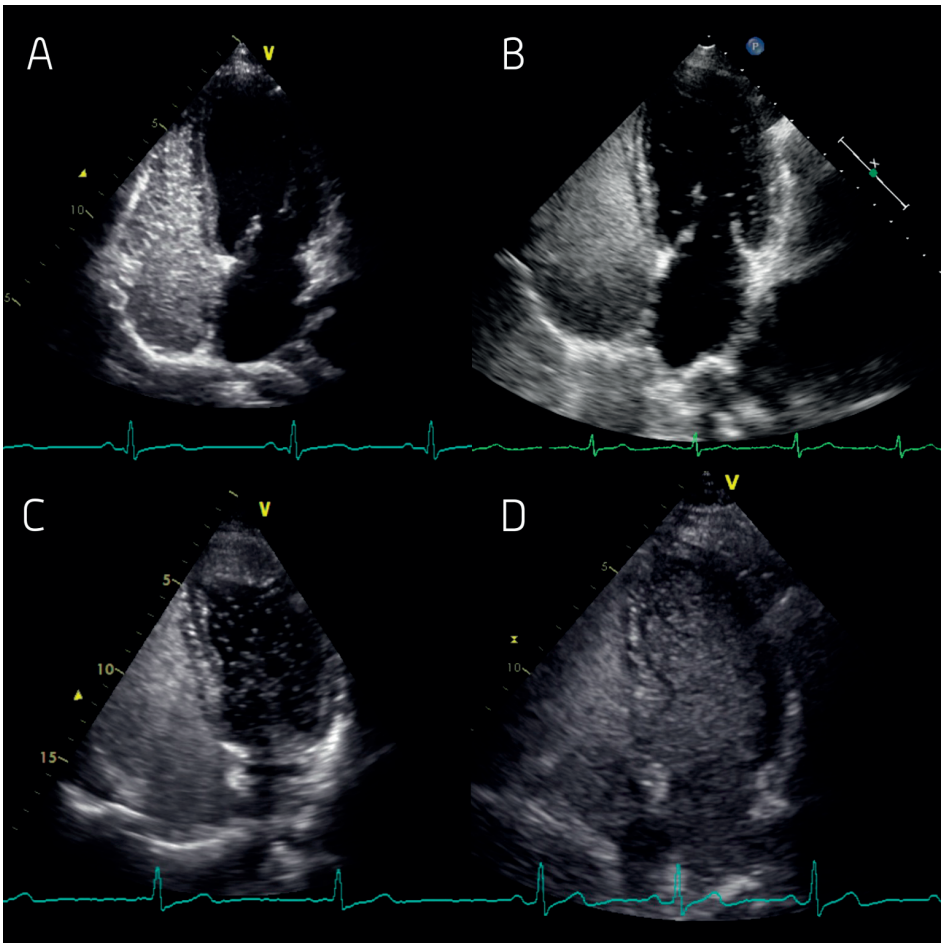

Figure 1. Pulmonary right-to-left shunt (RLS) on transthoracic contrast echocardiogram
A. No pulmonary RLS. B. Pulmonary RLS grade 1. C. Pulmonary RLS grade 2. D. Pulmonary RLS grade 3.

Statistical analysis

Descriptive statistics were used to describe patients' characteristics. Continuous variables were reported as mean ± standard deviation. Proportions were given by numbers and corresponding percentages. Inter-injection agreement was measured for the presence and grade of pulmonary RLS between the two contrast injections at rest in one patient. Inter-observer variability was measured for the presence and grade of pulmonary RLS between observer 1 and observer 2. Cohen's unweighted kappa coefficient (with 95% confidence intervals (CI)) was used for nominal characteristics and Cohen's weighted kappa coefficient (with 95% CI) was used for ordinal characteristics. Level of agreement was described according to Landis and Koch [15]. Odds ratio (OR) with 95% CI was calculated by performing a binominal logistic regression analyses to describe predictors for inter-injection

differences. Statistics were performed using a statistical software package (SPSS, version 22; SPSS Inc., Chicago and R (www.r-project.org, version 3.1.2)).

RESULTS

Between February and November 2015, TTCE was performed on 110 patients. After excluding 3 patients (image storage problem N=2, intravenous line failure N=1), 107 patients (49.5% male, mean age 45.0 ± 16.6 years) were included for further analysis (table 1). Image quality of TTCE was good, sufficient and insufficient in 97 (90.7%), 9 (8.4%) and 1 (0.9%) patient respectively. Quantity of contrast opacification of the RV was sufficient in 197 contrast injections (92.1%). A pulmonary RLS was present in 136 (63.6%) and 131 (61.2%) injections for observer 1 and 2 respectively. A cardiac RLS at rest was present in 3 patients (2.8%) for both observers.

Table 1 Baseline characteristics

Number	107
Age (years)	45.0 ± 16.6
Male	53 (49.5%)
Time of TTCE	
Screening for HHT£	57 (53.3%)
Follow-up of pulmonary RLS¶	50 (46.7%)
Definite HHT*	77 (72.0%)
HHT type	
Type 1	32 (29.9%)
Type 2	36 (33.6%)
SMAD4	6 (5.6%)
Unknown	3 (2.8%)

*Data are presented as number (%) or mean ± standard deviation. £ TTCE made to screen for pulmonary RLS. ¶ TTCE made at regular 5 year follow-up. * Based on genetic testing or clinical criteria [1]*

Inter-injection agreement

Inter-injection agreement (table 2 and 3) for the presence of pulmonary RLS was κ coefficient 0.96; 95% CI 0.90-1.00 (observer 1) and κ coefficient 0.98; 95% CI 0.94-1.00 (observer 2). Inter-injection agreement for pulmonary RLS grade was 0.96; 95% CI 0.93-0.99 (observer 1) and κ coefficient 0.95; 95% 0.92-0.98 (observer 2). There was disagreement in RLS grade between first and second contrast injection in 8 and 9 patients for observer 1 and 2 respectively. This included 11 patients (10.3%) in total, technical quality of the studies showed good image quality in 8 of these patients (72.7%) and difference in contrast opacification of the RV in 6 patients (54.4%). Disagreement between RLS grade 1 and 2 occurred in 3 and 5 patients respectively (5 patients (4.7%) in total). CT was made in all 5

patients and showed a very small PAVM in 1 patient with no possibility for percutaneous treatment. Difference between two injections was never more than one grade. Quantity of contrast in the RV was a predictor for inter-injection disagreement (OR 6.6; 95% CI 1.5-29.8, p=0.01).

Table 2 Overview agreement

	Presence of pulmonary RLS	Pulmonary RLS grade
Inter-injection agreement (1)		
Kappa	0.96 (0.90-1.00)	0.96 (0.93-0.99)
Absolute agreement	105 (98.1%)	99 (92.5%)
Inter-injection agreement (2)		
Kappa	0.98 (0.94-1.00)	0.95 (0.92-0.98)
Absolute agreement	106 (99.1%)	98 (91.6%)
Inter-observer agreement		
Kappa	0.95 (0.91-0.99)	0.97 (0.95-0.99)
Absolute agreement	209 (97.7%)	203 (94.9%)

Absolute agreement is described as number with percentage. Other agreements are described as kappa with 95% confidence interval. (1): observer 1; (2): observer 2. RLS: right-to-left shunt.

Inter-observer agreement

Inter-observer agreement (table 2 and 3) for pulmonary RLS presence and grade was κ coefficient 0.95; 95% CI 0.91-0.99 and κ coefficient 0.97; 95% CI 0.95-0.99 respectively.

Complications

During placement of intravenous line, one patient (0.9%) experienced vagal symptoms and an irregular heartrate. Rhythm on TTCE showed atrial fibrillation, which was confirmed with a 12-leads electrocardiogram. During TTCE there were no major complications. One patient (0.9%), with a RLS grade 2, reported dizziness the first hour after TTCE, with spontaneous full recovery.

DISCUSSION

This is the first study describing the reproducibility of the detection and quantification of pulmonary RLS on TTCE in patients screened for the presence of PAVM in association with HHT. We found a high level of agreement for the detection and grading of a pulmonary RLS in an individual patient (κ coefficient 0.95-0.98).

The use of TTCE to detect intra-pulmonary RLS was firstly described in 1976 [16]. The technique for TTCE is based on the permeability of the pulmonary capillary network and the difference in density between gas-contained microbubbles and the surrounding blood

Table 3 Inter-injection and inter-observer agreement

Inter-injection agreement, observer 1						
		Injection 1				
		No RLS	*RLS grade 1*	*RLS grade 2*	*RLS grade 3*	*Total*
Injection 2	*No RLS*	38	1	0	0	39
	RLS grade 1	1	39	0	0	40
	RLS grade 2	0	3	14	0	17
	RLS grade 3	0	0	3	8	11
	Total	39	43	17	8	107
Inter-injection agreement, observer 2						
		Injection 1				
		No RLS	*RLS grade 1*	*RLS grade 2*	*RLS grade 3*	*Total*
Injection 2	*No RLS*	41	0	0	0	41
	RLS grade 1	1	38	0	0	39
	RLS grade 2	0	5	12	1	18
	RLS grade 3	0	0	2	7	9
	Total	42	43	14	8	107
Inter-observer agreement						
		Observer 1				
		No RLS	*RLS grade 1*	*RLS grade 2*	*RLS grade 3*	*Total*
Observer 2	*No RLS*	78	5	0	0	83
	RLS grade 1	0	78	4	0	82
	RLS grade 2	0	0	30	2	32
	RLS grade 3	0	0	0	17	17
	Total	78	83	34	19	214

Data are described as number. RLS: right-to-left shunt.

[17,18]. The capillary network normally measures 8 to 10 µm in diameter, and therefore the injected microbubbles with a mean diameter of 27 µm, will be trapped in the pulmonary circulation. If PAVMs are present, the filtering capacity of the capillary network will be diminished and microbubbles will pass the pulmonary filter and appear in the left side of the heart [9].

Clinical implications of the use of TTCE in HHT have emerged in the last few years. Gazzaniga *et al*. described an association between pulmonary RLS grade and the occurrence of complications (haemoptysis, cerebral abscesses and stroke) [11]. This was confirmed in a large multicentre study including over 1000 patients, which described pulmonary RLS grade 2 and 3 as independent predictors for the prevalence of a cerebral ischemic event or brain abscess (OR 4.78; p= 0.03 and OR 10.4, p= 0.002) [3]. The pulmonary RLS grade also predicts the size of PAVMs on CT and the subsequent feasibility of embolisation [8]. This suggests that only moderate and large RLS have clinical implications. Therefore, CT can be

safely withheld in patients with a grade 1 pulmonary RLS reducing radiation exposure for many HHT patients [8,19]. Within 5 years, no treatable PAVMs develop in patients without pulmonary RLS at screening. However, increase in pulmonary RLS occurs in approximately 18%, leading to the need for embolisation in 12% of patients with initially non-treatable PAVMs at screening [20].

Although the above data already described the importance of TTCE, reliability of TTCE is based on the inter-observer variability and reproducibility in the individual patient. Many studies already described a high inter-observer agreement (κ coefficient 0.85-0.94) for pulmonary RLS detection; therefore our results are in line with the previous [11,12,21]. This is the first study describing the inter-injection agreement for pulmonary RLS quantification since none of the previous studies consecutively performed multiple injections in one single patient. Although not completely comparable, repeated injections for the diagnosis of a cardiac RLS have shown to increase the detection of RLS due to a high number of false negative injections [13,22]. Differences in quantification of cardiac RLS were mainly based on the technique of provocation, insufficient contrast in the RV and poor image quality. The inter-observer and intra-observer agreements for the detection of a cardiac RLS seem much lower (0.77 and 0.82 respectively) in comparison to those found for pulmonary RLS [23]. This may be explained by the different mechanisms of both shunts. In contrast to cardiac RLS, pulmonary RLS represents a persistent shunt and therefore no provocation is required to visualise the RLS. However, our study confirms the need for sufficient contrast opacification of the RV to obtain a reliable result, as it is shown to be a predictor for inter-injection disagreement (OR 6.6; 95% CI 1.5-29.8, p=0.01). This confirms the uttermost importance of adequately producing and injecting the agitated saline. Obtaining a good acoustic window is essential for reliable RLS quantification [24]. Performance of a second contrast injection should therefore be recommended when any doubt on RLS grade exists. TTCE may also be used to diagnose pulmonary RLS related to hepatopulmonary syndrome. Although there are pathophysiological differences, the results of our study may be translated to these patients.

Although this study showed an almost perfect level of inter-injection agreement, it should be emphasised that there was disagreement between the first and second contrast injection in 11 patients (10.3%). This might have clinical implications when treatable PAVMs are missed. Since CT is withheld in patients with a pulmonary RLS <2, quantification of a RLS grade 1 instead of grade 2 might have consequences. In this study, this occurred in 5 patients (4.7%). In none of these patients treatable PAVMs where found on CT. This reinforces our previous described recommendation to exclude RLS grade 1 from further analyses with CT.

Some clinicians have concerns regarding the safety of the injection of contrast, especially in patients with a RLS. However, the microbubbles in the injected agitated saline are very small and implode easily. Multiple recent studies in patients with and without HHT confirm

the safety of TTCE describing only minor and self- resolving side effects such as dizziness and migraine in 0-2% [11,19,25,26]. In line with previous studies, our study showed no severe side effects.

At this moment TTCE is the cornerstone in the diagnostic evaluation of pulmonary RLS in the context of HHT. However, chest CT remains the gold standard for pre- and post-embolisation evaluation of PAVMs since it provides essential information on the PAVM anatomy (localisation, complexity, size of feeding arteries and aneurysmal sac). Magnetic resonance imaging is also used for PAVM detection. A big advantage compared to chest CT is the avoidance of radiation while the anatomy of the PAVMs can still be demonstrated. Although some studies show promising results, large comparative prospective studies are lacking and the lower spatial resolution compared to CT is currently a major limiting factor [27-29].

Limitations

First, this is a single centre study performed in a tertiary referral hospital highly experienced in HHT and TTCE, therefore the results may not apply to other centres. Secondly, the level of agreement could be influenced by the presence of only a few patients with a large pulmonary RLS. Thirdly, the number of cardiac RLS might be underestimated because we only studied the contrast injections at rest focussing on the presence of pulmonary RLS. This resulted in a prevalence of 3% compared to 23% in previous studies [30]. Because we used the four beat rule in case shunt origin could not be visualised, the risk for false positive pulmonary RLS is small.

CONCLUSION

TTCE has an excellent inter-observer and inter-injection agreement for both the presence and grading of pulmonary RLS.

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SUPPLEMENTARY MATERIAL:

The following supplementary material is available online - Movie Clips (Windows media player):

Video 1 Pulmonary right-to-left shunt grade 1. Transthoracic contrast echocardiogram, apical 4chamber view.

Video 2 Pulmonary right-to-left shunt grade 2. Transthoracic contrast echocardiogram, apical 4chamber view.

Video 3 Pulmonary right-to-left shunt grade 3. Transthoracic contrast echocardiogram, apical 4chamber view.

CHAPTER 5.1

Follow-up of pulmonary right-to-left shunt in hereditary haemorrhagic telangiectasia

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ABSTRACT

Background: Pulmonary arteriovenous malformations (PAVMs) are associated with severe neurological complications in hereditary haemorrhagic telangiectasia (HHT). Transthoracic contrast echocardiography (TTCE) is recommended for screening of pulmonary right-to-left shunts (RLS). Although growth of PAVMs is shown in two small studies, no prior study on follow-up with TTCE exist.

Methods: All HHT patients underwent a second TTCE five years after initial screening. Patients with a history of PAVM embolisation were excluded. Pulmonary RLS grade on TTCE after 5 years was compared to the grade at screening.

Results: 200 patients (53.5% female, mean age at screening 44.7 ± 14.1 years) were included. Increase in RLS grade occurred in 36 (18%) patients, of whom 6 (17%) underwent embolisation. The change in RLS grade between screening and follow-up was not more than one grade. Of patients with nontreatable pulmonary RLS at screening (n=113), 14 (12.4%) underwent embolisation. In patients without pulmonary RLS at initial screening (n=87), no treatable PAVMs developed during follow-up.

Conclusion: Within 5 years, no treatable PAVMs developed in HHT patients without pulmonary RLS at initial screening. Increase in pulmonary RLS grade occurred in 18% of patients, and never increased by more than one grade. Of patients with non-treatable pulmonary RLS at initial screening, 12% underwent embolisation.

INTRODUCTION

Pulmonary arteriovenous malformations (PAVMs) are abnormal vessels that replace the normal capillaries between the pulmonary arterial and venous circulation.[1] Up to 90% of PAVMs are associated with hereditary haemorrhagic telangiectasia (HHT), a rare autosomal dominant inherited disorder.[2-4] HHT is characterised by the presence of direct artery-to-vein communications, which vary from small telangiectasia (dilated microvessels) in skin and mucous membranes to large arteriovenous malformations, predominantly localised in the brain, liver and lungs.[1,5] HHT consist of two main types, HHT type 1 and HHT type 2, which are caused by mutations in the *ENG* and *ACVRL1* gene respectively.[6,7]

PAVMs bypass the normal pulmonary capillary filter and result in a permanent anatomic right-to-left shunt (RLS). PAVMs are therefore associated with severe neurologic complications due to paradoxical emboli of both thrombotic and septic origin.[1] Depending on HHT type, the prevalence of a pulmonary RLS is 35%-85%.[1,8] In order to reduce the risk of neurologic complications patients can be safely treated with transcatheter embolotherapy, an endovascular intervention that occludes the feeding artery of the PAVM with a vascular plug or coils.[9,10]

Transthoracic contrast echocardiography (TTCE) has an excellent sensitivity and negative predictive value for the presence of PAVMs with low risks and costs. It is therefore recommended as the first-line screening technique for PAVMs in all persons with suspected HHT.[3,8,11,12] Importantly, two recently published studies of Velthuis and colleagues [1,13] demonstrated that small pulmonary RLS are not associated with neurological complications and that the pulmonary RLS on TTCE predicts the size of PAVMs on chest computed tomography (CT) and their feasibility for subsequent transcatheter embolisation.

Unfortunately, the recommendations for follow-up of pulmonary RLS are mainly based on expert opinion and there is no literature on the long-term follow-up with TTCE. However, there are a few small studies, which included patients after embolisation, that demonstrate growth of PAVMs despite embolisation.[9,10] Besides, HHT is known for its age-related penetrance. Therefore we hypothesised that in HHT patients without treatable PAVMs at screening, increase in pulmonary RLS may occur during follow-up. We present the first study on the 5 year follow-up of pulmonary RLS with TTCE in HHT patients.

METHODS

Study population

All patients with a definite HHT diagnosis who were screened for PAVMs with TTCE between September 2004 and June 2010 were invited for 5 year follow-up at the St. Antonius Hospital (Nieuwegein, the Netherlands), an HHT centre of excellence. The HHT diagnosis was based on genetic testing or presence of three or more clinical criteria[3], which consist of spontaneous and recurrent epistaxis, telangiectasia at characteristic sites, visceral lesions

and a first-degree relative with HHT. Patients with history of PAVM embolisation were excluded. The study was approved by the institutional medical ethics committee (R&D/ Z14.059).

Contrast echocardiography

TTCE at screening was performed with a Philips Sonos 7500 ultrasound instrument and a S3 transducer or a Philips IE33 ultrasound instrument and a S5-1 transducer (Philips Medical Systems, Best, The Netherlands). The TTCE at follow-up was performed with a Philips IE33 ultrasound instrument and a S5-1 transducer or a General Electronic Vivid S6 ultrasound instrument and a 3S transducer (General Electronic Healthcare, Wauwatosa, The United States).

An intravenous line was placed in the right antecubital vein to which two 10mL syringes were connected. One syringe was filled with 8mL physiological saline solution and the other with 1mL air. Subsequently, 1mL blood was drawn into the air-filled syringe and mixed with the saline by reverse flushing between both syringes, creating agitated saline which contains microbubbles.[1,13,14]

All TTCE's were performed by echocardiographers trained for contrast echocardiography. The patient was positioned in the left lateral position and 5mL agitated saline was injected within 3 s while projecting the four-chamber apical view. When possible the pulmonary veins were visualised. This procedure was repeated with a Valsalva manoeuvre. All shunts visualised through a pulmonary vein were classified as pulmonary RLS and all shunts through the septum as patent foramen ovale. When shunt origin was not visible, a delay of four cardiac cycles was used to distinguish between a pulmonary and a cardiac shunt. The TTCE was considered positive for a pulmonary RLS if microbubbles appeared in the left atrium after four or more cardiac cycles.[1,8,11,13] The RLS grade was based on the maximum number of microbubbles counted in the left side of the heart in one still frame. The RLS was graded as 1 (1-29 microbubbles), 2 (30–100 microbubbles) or 3 (>100 microbubbles), as described previously.[11,12,15]

Shunt interpretation was performed by two independent cardiologists with expertise in HHT who were blinded for the individual patient characteristics. In case of disagreement on the presence, origin or grade of the RLS, the TTCE was reviewed again by both cardiologists together until final agreement was reached. When the quality of the TTCE was to poor for shunt interpretation, the patient was excluded from further analysis.

PAVM diagnosis and embolisation

At follow-up, chest CT was advised in all patients with a pulmonary RLS grade ≥ 2 [1,13] and was performed with a ≥ 16-detector CT scanner (Philips Medical Systems, Best, the

Netherlands) with a dedicated high-resolution algorithm and maximum slice thickness of 1 mm.

All chest CT images were evaluated by an interventional radiologist and pulmonologist with expertise in HHT and were discussed in a multidisciplinary consensus meeting in which the radiologists and pulmonologists were unaware of the results of TTCE. All PAVMs with a feeding artery diameter of ≥ 3 mm were considered accessible for transcatheter embolotherapy. For PAVMs with a smaller diameter, feasibility for embolisation was based on the anatomy and location of the PAVM. Increase in PAVM was defined as increase of PAVM feeding artery and/or presence of new PAVM. When more than one PAVM was present, the feeding artery of the largest PAVM was measured.

Statistical analysis

Descriptive statistics were used to describe patients characteristics. Continuous variables were reported as mean ± SD. Proportions were given by numbers and corresponding percentages. Cohen's κ coefficient was calculated to assess inter-observer and inter-injection (between two repeating contrast injections in one particular patient) agreements. Statistical analysis were performed using a statistical software package (SPSS, version 22; SPSS Inc., Chicago, IL, USA).

RESULTS

Study population

Between September 2004 and June 2010 screening with TTCE was performed in 351 HHT patients. 148 patients were excluded because of prior embolisation (n=80), death (n=9) or other reasons ((N=59), e.g. follow-up in other hospitals, or follow-up without TTCE). Between February 2010 and June 2015 follow-up using TTCE was performed in 203 patients. Three patients were excluded because the quality of the TTCE at follow-up was not sufficient for RLS gradation. The patient selection is summarised in figure 1.

200 patients (53.5% female, mean age at screening 44.7 ± 14.1 years) were included for further analysis. HHT type 1, HHT type 2 and SMAD4 were found in 66 (33.0%), 130 (65.0%) and 2 (1.0%) patients, respectively. In two (1.0%) patients the HHT type was unknown. The mean follow-up time was 5.6 ± 0.9 years. In 37 patients macroscopic PAVMs were visualised on chest CT, of whom none were embolised (too small n=36, declined embolisation n=1). Patient characteristics are summarised in table 1, characteristics of PAVMs at screening are described in table 2 and figure 2.

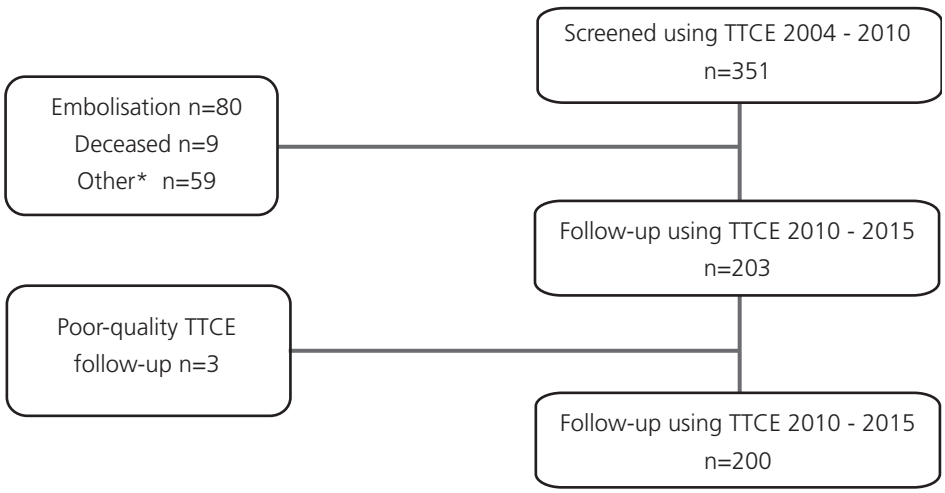

Figure 1. Patient selection

*TTCE, transthoracic contrast echocardiogram; n, number. * e.g. loss to follow-up, follow-up without TTCE.*

Pulmonary RLS on TTCE

A pulmonary RLS was present in 113 (56.5%) patients at screening and 130 (65.0%) patients at follow-up (figure 3). A change in RLS grade was seen in 51 (25.5%) patients. In 36 (18.0%) patients (55.6% HHT type 2) an increase in RLS grade was seen; in 10 (27.8%) of these patients there was an increase from pulmonary RLS grade 1 to grade 2. The change in RLS between screening and follow-up was never more than one grade. The other results are summarised in figure 4.In 30 consecutive patients inter-injection agreement (κ coefficient 0.95) and inter-observer agreement (κ coefficient 0.92) were calculated.

PAVMs and embolisation

At follow-up, chest CT was performed in 52 out of 55 patients with a pulmonary RLS grade ≥ 2. Three patients declined chest CT. In 37 out of these 52 patients (71.2%) a PAVM was seen on chest CT and embolisation could be performed in 14 of these patients (26.9%). In the subgroup of patients without pulmonary RLS at screening (n=87), no treatable PAVMs developed during follow-up. In the subgroup of patients with non-treatable pulmonary RLS at screening (n=113), 14 (12.4%) underwent embolisation during follow-up. In the total cohort, increase of feeding artery diameter and/or new PAVMs was present in 21 patients (10.5%) (figure 2 and table 3). The flowchart for embolisation is described in figure 5.

Table 1. Baseline characteristics

Subjects	200
Age at screening (years)	44.7 ± 14.1
Sex	
Male	93 (46.5%)
Female	107 (53.5%)
HHT type[#]	
HHT type 1	66 (33.0%)
HHT type 2	130 (65.0%)
SMAD4	2 (1.0%)
Unknown	2 (1.0%)
Follow-up time (years)	
Mean±SD	5.6 ± 0.9
Median (range)	5.3 [4.0-10.2]

Data are presented as number (%), mean ± standard deviation or median [range]. HHT, hereditary haemorrhagic telangiectasia. ***#:*** *No DNA analysis in six patients with definite HHT according to the Curacao criteria and a known family mutation.*

Table 2. Presence of one or more PAVMs on chest CT at screening

	PAVM	No PAVM
Total	37 (18.9%)	159 (81.1%)
Subgroup		
No RLS	0 (0%)	85 (100.0%)
Grade 1	8 (13.6%)	51 (86.4%)
Grade 2	12 (36.4%)	21 (63.6%)
Grade 3	17 (89.5%)	2 (10.5%)

Presence of one or more PAVMs on chest CT at screening. n=196. PAVM, pulmonary arteriovenous malformation; RLS, right-to-left shunt; CT, chest computed tomography.

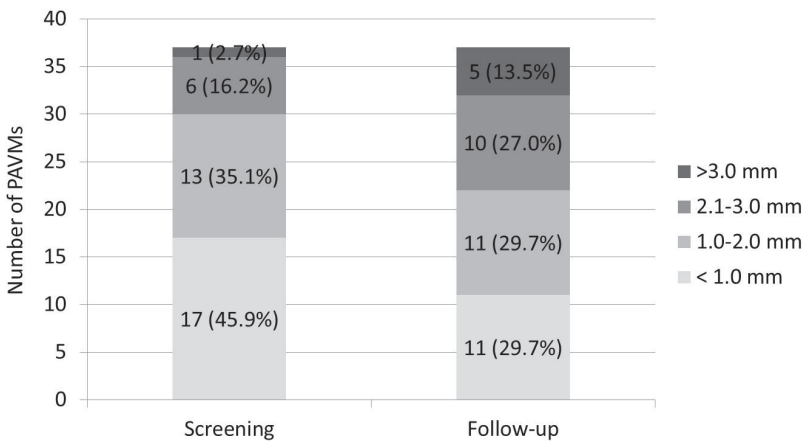

Figure 2. Diameter feeding artery at screening and follow-up

Diameter of feeding artery at screening and follow-up. When more than one pulmonary arteriovenous malformation (PAVM) is present, the feeding artery of the largest PAVM is described.

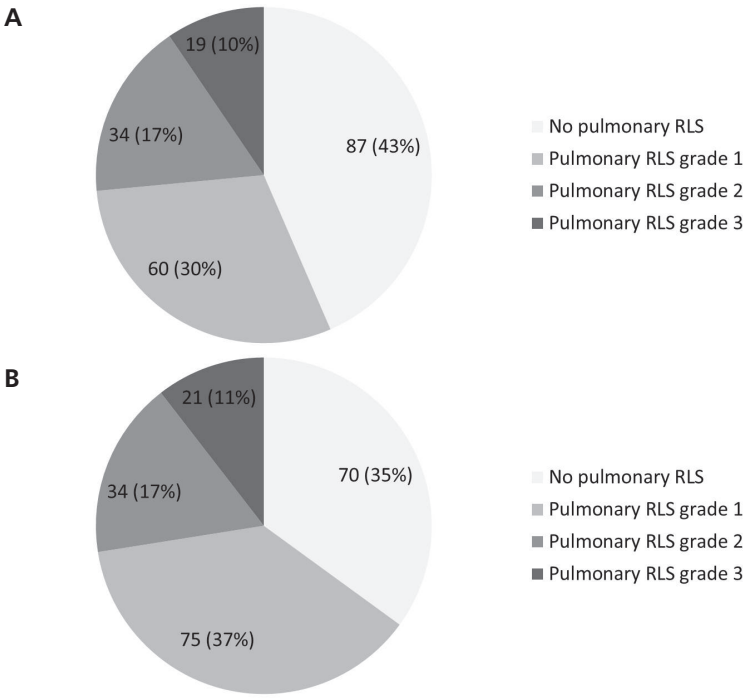

Figure 3. Pulmonary right-to-left shunt at screening and follow-up
A) Screening and B) follow-up. RLS, right-to-left shunt.

Table 3. Changes in PAVMs on chest CT

	Patients	PAVM screening[#]	PAVM FU[+]	Increase in PAVM	Embolisation
Total	200	37[+]	37	21	14
RLS increase					
No RLS to grade 1	22	0	NA	NA	NA
Grade 1 to grade 2	10	5	5*	5	3
Grade 2 to grade 3	4	3	4	4	3
RLS decrease					
Grade 1 to no RLS	5	0	NA	NA	NA
Grade 2 to grade 1	8	0	NA	NA	NA
Grade 3 to grade 2	2	2	2	0	0
RLS no change					
No RLS	65	0	NA	NA	NA
grade 1	45	3	NA	NA	NA
grade 2	22	9	9	2	2
grade 3	17	15	17	10	6

*Presence of PAVMs on chest CT at screening and follow-up, increase in PAVM and embolisation. Subgroups are stratified according to pulmonary RLS grade and change during follow-up. Increase in PAVM is defined as increase of feeding artery of largest PAVM and/or presence of new PAVMs. Data are presented as n. PAVM, pulmonary arteriovenous malformation; FU, follow-up; RLS, right-to-left shunt. [#]: Chest computed tomography (CT) advised in all patients, [+]: Chest CT advised if pulmonary RLS grade ≥ 2, *:Chest CT performed in 8 patients.*

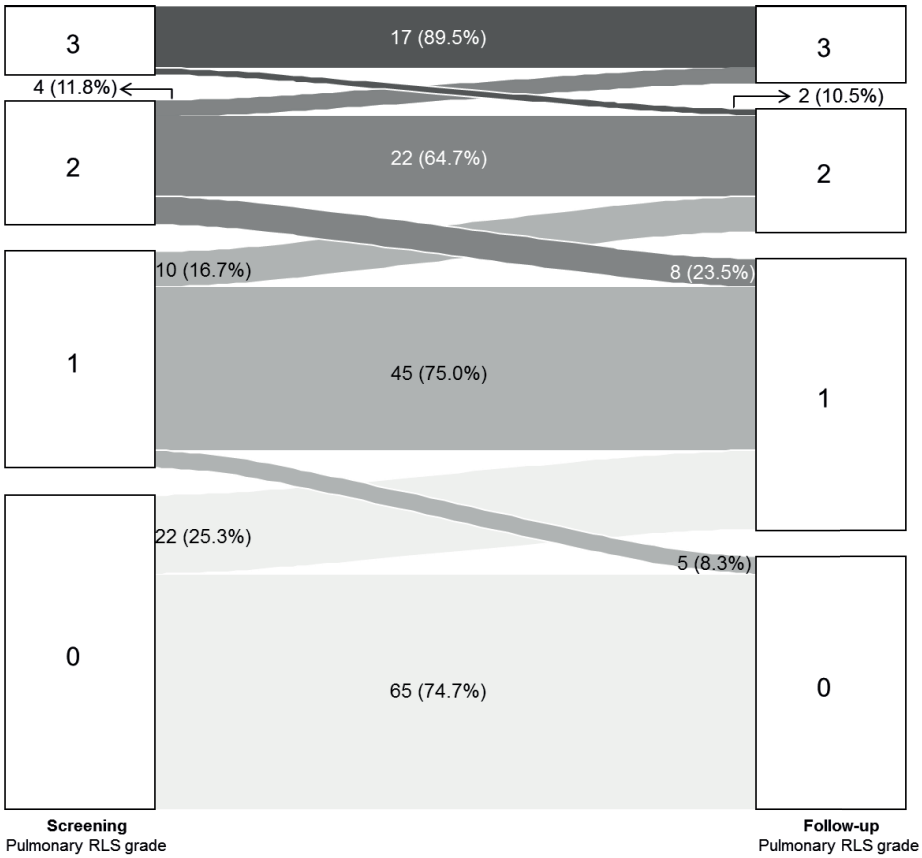

Figure 4. Change in pulmonary right-to-left shunt
RLS, right-to-left shunt.

Complications

Between initial screening and follow-up an ischaemic stroke was documented in one patient. This patient had no pulmonary RLS and had a history of atrial fibrillation. In another patient an old infarction was found incidentally on cerebral magnetic resonance imaging; this patient did have a pulmonary RLS grade 2 and macroscopic PAVMs which were treated with embolisation subsequently. TTCE caused no complications.

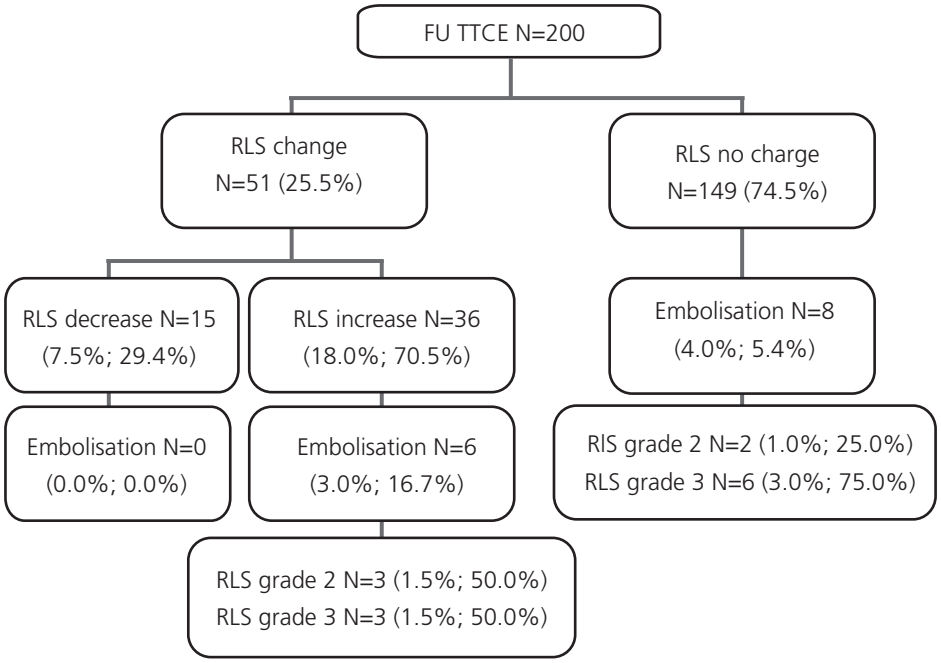

Figure 5. Flowchart pulmonary right-to-left shunt and embolisation
FU, follow-up; TTCE, transthoracic contrast echocardiography; RLS, right-to-left shunt at follow-up. Data are presented as n (% total cohort; % subgroup).

DISCUSSION

This is the first study using TTCE for the long-term follow-up of pulmonary RLS in patients with HHT. An important finding of this study is that in patients with no pulmonary RLS at screening, no treatable PAVMs developed within 5 years. However, importantly, during follow-up, increase in pulmonary RLS grade occurred in a substantial number of patients (18%). This increase was present in patients with and without RLS at screening, although it increased never more than one grade. In patients with non-treatable pulmonary RLS at screening, 12% underwent embolisation during follow-up. Therefore this study demonstrates that repeated screening with TTCE might be necessary in all HHT patients.

Growth of PAVMs has been described in a few studies. Mager *et al.* [9] described the long-term follow-up of 112 patients after embolisation; recanalisation occurred in 13% and growth of PAVMs in 11%. No new PAVMs developed during follow-up. Pollak *et al.* [10] described 155 patients with PAVMs who underwent embolisation and found growth of small non-embolised PAVMs in 18%. However, since both studies included only patients treated with embolotherapy, follow-up was performed with chest CT instead of TTCE. Therefore these studies are not comparable to our current study.

In the current guideline for HHT [3], the recommendations for follow-up of PAVMs are scarce and based on small series or expert opinion. Patients with negative initial TTCE are advised to repeat screening every 5-10 years and more often after puberty or pregnancy. In patients with small untreated PAVMs or microscopic PAVMs (positive TTCE but negative chest CT), follow-up is advised every 1-5 years with chest CT on a case-by-case basis.[3]
The use of chest CT for the follow-up of HHT patients has a few limitations. Firstly, chest CT remains negative in ~55% and ~8% of patients with a pulmonary RLS grade 2 and 3 on TTCE, respectively.[13] Therefore, follow-up with chest CT may result in many unrecognised moderate to large pulmonary RLS in patients that still have a risk of paradoxical cerebral complications.[13] Second, follow-up with chest CT causes radiation exposure in this (mainly) young population. In contrast, TTCE has an excellent sensitivity and negative predictive value for the presence of PAVMs and a very low incidence of minor and all self-resolving side-effects.[12,16,17] Moreover, recently published findings of our centre demonstrated a good correlation between pulmonary RLS grade and the probability of detecting PAVMs on chest CT and the subsequent feasibility for transcatheter embolotherapy.[1,13] Patients with a grade 1 RLS do not have PAVMs that are large enough for embolisation and these RLS are not associated with neurologic complications.[1,13] For follow-up of pulmonary RLS, the reproducibility of TTCE is uttermost important. Although the high inter-observer variability has already been described in several studies (κ coefficient 0.85-0.94) [8,12,15], no previous reports describe the reproducibility of TTCE in one particular patient. In our experience inter-injection agreement in a single patient is high (κ coefficient 0.95). Surprisingly, a decrease in pulmonary RLS grade was seen in 15 (7.5%) patients. Most patients showed only a mild decrease in number of microbubbles which could be explained by a difference in amount of contrast in the right ventricle, quality of the TTCE or haemodynamic differences. However, as written above, the reproducibility of contrast injection seems excellent. Other explanations for this decrease in microbubbles could be fibrosis or thrombosis of small PAVMs.
In this study, all patients treated with embolisation after screening were excluded, since TTCE remains positive in 90% of these patients.[18] As a result, the majority of the patients in our study had no or a small RLS (73%) at screening. The result of these selection bias is the inability to extrapolate these present findings to patients with larger shunts.
Increase in pulmonary RLS can be due to both increase in (diffuse) microscopic PAVMs or growth of macroscopic PAVMs, which are visible on chest CT. However, the exact pathogenesis of growth of PAVMs is not completely understood. Theoretically, the high flow through PAVMs, due to the relatively low resistance compared to the capillary network, can result in growth of PAVMs. Furthermore, it is known that HHT has an age dependent penetrance and that the prevalence of PAVMs depends on the genotype. Other factors such as female hormones (*e.g.* during pregnancy) have shown to influence other characteristics of HHT such as epistaxis [3] and may thus be possibly related to the growth of PAVMs as well.

In addition, an increase in cardiac output, due to hepatic arteriovenous malformations, pregnancy or anaemia, might theoretically cause growth of PAVMs. However in this study, these factors seem to had little influence on the difference in shunt grade, since pregnancy occurred in only one patient, HAVMs were present in two patients and no patients had severe anaemia (defined as haemoglobin ≤ 6.0 mmol·L^{-1}) at time of follow-up. Therefore, future larger studies are necessary to find predictors for increase in pulmonary RLS size in order to develop a tailor-made approach for each individual HHT patient.

Interestingly, 12% of patients with non-treatable PAVMs at screening underwent embolisation during follow-up. This supports the concept of possible growth of PAVMs in HHT patients and provides justification for the recommendation to repeat screening in HHT patients with small or microscopic PAVMs. Moreover, in the subgroup of patients with no pulmonary RLS and a pulmonary RLS grade 1, increase was described in 25% and 17% respectively. This implies that development of PAVMs can occur in all HHT patients. Therefore, repeated screening for PAVMs should be performed in every HHT patient. More research is necessary to determine the optimal time interval for different patient groups.

This study presents some limitations. First, not all TTCE's were made with the same ultrasound machine, which could lead to difference in quality and interpretation of the RLS grade. Although, a very good inter-observer agreement (κ coefficient of 0.92) and inter-injection agreement (κ coefficient 0.95) was found. Second, this is a single-centre study in a hospital with high experience with both PAVMs and TTCE, therefore it is not known if the results apply for patients screened in other hospitals. A prospective multicentre validation study will be of major importance to confirm our data. Third, information on other cardiac parameters (e.g. valvular heart disease, left ventricular function, right ventricular systolic pressure, heart rate and cardiac output) that might have influenced the pulmonary shunt grading is absent.

On the basis of the results of this study, we recommend follow-up of patients with a pulmonary RLS every 5 years using TTCE. In patients with no pulmonary RLS at screening a conservative management strategy with an interval of >5 years might be safe.

CONCLUSION

Within 5 years, no treatable PAVMs were found in HHT patients without pulmonary RLS at initial screening. Increase in pulmonary RLS grade occurred in 18%, both in patients with and without pulmonary RLS at screening, and increased never more than one grade. In the subgroup of patients with non-treatable pulmonary RLS at initial screening, 12% underwent embolisation.

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CHAPTER 5.2

How to follow-up patients with hereditary haemorrhagic telangiectasia and suspected pulmonary arteriovenous malformations

Editorial; European Respiratory Journal 2016;47:1618-1621*

T Chinet

Pulmonary arteriovenous malformations (PAVMs) are abnormal vascular structures that connect one or several pulmonary arteries to one or several pulmonary veins without interposition of a capillary bed, resulting in a right-to-left shunt (RLS) [1]. Over 80% of PAVMs are associated with the genetic disease hereditary haemorrhagic telangiectasia (HHT). The vascular malformations predispose patients to severe complications due to paradoxical systemic emboli of thrombotic or septic origin, such as stroke and brain abscess [2-5]. The treatment of choice for PAVMs is transcatheter embolotherapy, although concerns are emerging regarding the long-term consequences of this procedure, especially in children [6].

Because of the risk of severe complications, international guidelines recommend screening patients with HHT for the presence of PAVMs at the time of initial clinical evaluation and also after puberty, after pregnancy, within 5 years preceding a planned pregnancy, and otherwise every 5–10 years [7]. Transthoracic contrast echocardiography (TTCE) is the first-line screening technique for the detection of PAVMs in HHT [7]. In expert hands, it has excellent sensitivity and negative predictive value for the presence of PAVMs in children and adults. Several grading scales are used in different HHT centres to quantify the pulmonary RLS size [6-8]. Microbubbles of air in agitated saline may expose patients with RLS to the risk of cerebral air emboli inducing migraine, blurred vision, numbness and paraesthesia, but these symptoms resolve quickly without residual side effects [9,10]. To minimise this risk, many groups perform a chest radiograph prior to TTCE to detect large-size PAVMs, which are likely to carry the highest risk of complications from air bubbles. TTCE is therefore a safe diagnostic tool and its benefits undoubtedly outweigh its potential minor risks. One must bear in mind that TTCE does not detect PAVMs but rather pulmonary RLS. Chest computed tomography (CT) is considered the gold standard diagnostic tool for PAVMs as it enables visualisation of the vascular malformations (except microscopic ones) and provides essential information on their characteristics (shape, location, size of the feeding arteries, size of the aneurismal sac, etc.). Recent studies have reported that, in patients with HHT, the grade of pulmonary RLS on TTCE predicts the size of PAVMs on chest CT and the feasibility of subsequent transcatheter embolotherapy [7]. The absence of, or a small, pulmonary RLS (i.e. a low grade on TTCE) is associated with no risk of neurological complications and with PAVMs too small for embolisation [5,7].

The natural history of PAVMs is not well known. There is some evidence that PAVMs may grow not only in children but also in adults, spontaneously or after transcatheter embolotherapy of other larger PAVMs in the same patient [11]. However, in adults, it is not clear whether it is only growth of previously small PAVMs that occurs, or whether de novo PAVMs may also appear. This point is important, because if truly new fistulas can develop in adult patients, a negative initial screening, even using a highly sensitive test, cannot rule out the development of PAVMs in the future, necessitating repeated screenings during the lifetime. The possible growth of PAVMs during adulthood implies repeated follow-up evaluations to

detect when the PAVMs become large enough to be embolised (based on a diameter of the feeding arteries of >2–3 mm). However, the precise method and the appropriate screening interval are not agreed upon. Practices differ from one centre to another.

In this issue of the *European Respiratory Journal*, VORSELAARS *et al.* [12] retrospectively reviewed change in RLS evaluated using TTCE at 5 years in 200 HHT patients. At the initial screening, all patients but one had no RLS or RLS but a non-treatable PAVM. This study found that an increase in pulmonary RLS grade occurred in 18% of patients (both with and without RLS at screening), while a decrease was observed in 7.5%. 55 patients had an RLS grade ⩾2 on follow-up and had a chest CT. 14 of these patients underwent embolotherapy.

This work contributes noteworthy findings and clues for future investigation. First, it confirms that over a period of 5 years, PAVMs may grow in a significant percentage of HHT patients who did not have previous embolotherapy. When chest CT was repeated 5 years after screening in patients with RLS grade ⩾2, the diameter of the feeding artery of the largest PAVM exceeded 2.1 mm in 15 patients versus in seven at baseline. This resulted in embolotherapy in 14 patients, although it is not clear whether this procedure was performed only because the PAVMs grew or because the authors' experience with embolotherapy had improved in recent years, with improved technical ability to occlude smaller PAVMs (i.e. with a feeding artery of 2–3 mm). This study therefore provides further justification for the recommendation to follow-up HHT patients who have non-treatable PAVMs. Secondly, this study indicates that RLS may appear during follow-up in HHT adult patients with no pulmonary RLS at the initial screening. Although a grade 1 RLS in a patient with a previously grade 0 RLS is difficult to interpret, it is certainly unwise not to rescreen patients who were initially negative, especially because 10 patients with a grade 1 RLS at screening had a grade 2 RLS 5 years later.

Thirdly, this study found that the increase in RLS is never more than one grade over 5 years. Therefore, follow-up in patients with no pulmonary RLS at screening could theoretically be safely deferred to more than 5 years, although this remains to be confirmed in other cohorts. Fourthly, this study promotes the use of TTCE rather than chest CT in the screening and follow-up of HHT patients with low-grade RLS. Patients with HHT, including children and young adults, are exposed to a significant cumulative radiation dose from diagnostic and therapeutic interventions, which are associated with harmful effects in the long term [13]. Recently, MATHEWS *et al*. [14] studied cancer risk in 680 211 people exposed to CT scans in childhood or adolescence. Overall, the cancer incidence during a mean follow-up of 9.5 years was 24% greater in exposed than in non-exposed people. Chest CT to detect PAVMs can safely be avoided in patients with an absence of, or grade 1, RLS on TTCE [8,10,15].

There are still numerous areas of uncertainty. TTCE lacks specificity and has a low positive predictive value for the presence of PAVMs. In this study, 23 HHT patients with a grade

2 or 3 RLS had no PAVM on chest CT at the initial screening, which is consistent with the finding that 6–28% of the normal population has a positive TTCE [15-17]. This can reflect true false positive results or microscopic PAVMs that are too small to be detected with chest CT. In any case, an increase in RLS could be due to the development of PAVMs or to other unknown and possibly unrelated factors. A chest CT is clearly required at least in the case of a grade ⩾2 RLS. The reproducibility of TTCE in a particular patient is not clear, meaning that comparisons between screening and follow-up results must be treated with caution. Surprisingly, a decrease in pulmonary RLS grade was seen in 7.5% of patients. The authors evaluated the reproducibility of TTCE per patient by performing two separate contrast injections in 30 patients, and found good agreement between the assigned grades. However, this evaluation was conducted during the same procedure, in a small number of patients, in an experienced centre, and needs to be further defined in a larger cohort of patients and in various centres.

This was a single-centre study, performed by a team with a high level of expertise in TTCE and PAVMs. As outlined by the authors, a prospective multi-centre validation study is required to confirm these results. TTCE has no value for the follow-up of patients with known untreated macroscopic PAVMs and a grade ⩾2 RLS. However, the optimal method for the follow-up of patients with macroscopic PAVMs and a grade 1 RLS is still uncertain. In this study, out of eight of these patients, five progressed from grade 1 to grade 2 RLS and therefore had a chest CT (three underwent embolotherapy); three remained at grade 1 RLS and did not have a chest CT. This group of patients needs to be investigated further. Finally, TTCE cannot be used to follow patients with PAVMs who have undergone previous embolisation, because it remains positive in almost all of them, and chest CT is required to evaluate the late complications of embolization such as reperfusion [11,18-20].

At this point, what should the follow-up strategy be for clinicians in charge of HHT patients? We obviously need to adapt our protocols to particular groups of patients according to the results of the initial screening procedure. Patients with no RLS can safely be followed-up using TTCE: a chest CT is not necessary to screen for PAVMs in these patients. Whether an interval of more than 5 years before the next evaluation of these patients is safe requires more data. Patients with a grade 2 or 3 RLS on initial screening or during follow-up should definitely undergo a chest CT. More research is necessary for patients with a grade 1 RLS. Some centres will perform a chest CT, while others will not, until further data are available. The optimal interval between evaluations for patients with RLS on TTCE, or with PAVMs on chest CT, remains to be determined and is very likely to differ according to the patient's age, the grade of RLS, the characteristics of PAVMs on CT, previous embolisations, etc. Again, more research is necessary. The follow-up of patients with treated PAVMs cannot rely on TTCE [10]. International guidelines indicate that chest CT should be performed within 6–12 months after embolisation and then approximately every 3 years to detect reperfusion of treated PAVMs and growth of untreated PAVMs. However, there has been no comparative

study to determine the optimal method to detect reperfusion of PAVMs and the optimal interval. Interestingly, a recent paper reported that reperfusion of occluded PAVMs could be predicted by a diameter of the draining vein of 2.5 mm or more on unenhanced chest CT [21]. The use of unenhanced chest CT rather than contrast-enhanced CT might limit the risk of paradoxical emboli due to venous injection of the contrast agent in these patients. Follow-up with chest CT in patients with PAVMs is not satisfactory because, as discussed above, it exposes often young patients to the harmful effects of large amounts of radiation. New magnetic resonance imaging techniques might prove useful in the future.

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CHAPTER 6.1

Direct haemodynamic effects of pulmonary arteriovenous malformation embolisation

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ABSTRACT

Background: Transcatheter embolisation is widely used to close pulmonary arteriovenous malformations (PAVMs) in patients with hereditary haemorrhagic telangiectasia (HHT). Data on the direct cardiovascular haemodynamic changes induced by this treatment are scarce.

Objectives: We investigated the direct haemodynamic effects of transcatheter embolisation of PAVMs, using non-invasive finger pressure measurements.

Methods: During the procedure, blood pressure, heart rate (HR), stroke volume (SV), cardiac output (CO), total peripheral resistance (TPR) and delta pressure/delta time (dP/dt) were continuously monitored using a Finometer®. Potential changes in these haemodynamic parameters were calculated from the pressure registrations using Modelflow® methodology. Absolute and relative changes were calculated and compared using the paired sample t-test.

Results: The present study includes 29 HHT patients (mean age 39 ± 15 years, 11 men) who underwent transcatheter embolotherapy of PAVMs. The total number of embolisations was 72 (mean per patient 2.5). Directly after PAVM closure, SV and CO decreased significantly by –11.9 % ($p = 0.01$) and –9.5 % ($p = 0.01$) respectively, without a significant change in HR (1.8 %). Mean arterial blood pressure increased by 4.1 % ($p = 0.02$), while the TPR and dP/dt did not increase significantly (5.8 % and 0.2 %, respectively).

Conclusions: Significant haemodynamic changes occur directly after transcatheter embolisation of PAVMs, amongst which a decrease in stroke volume and cardiac output are most important.

INTRODUCTION

Hereditary haemorrhagic telangiectasia (HHT) is an autosomal dominant inherited disease characterised by vascular malformations, ranging from small telangiectases in skin and mucosal membranes to large visceral arteriovenous malformations (AVMs) predominantly localised in the lungs, brain and liver [1-3]. Pulmonary arteriovenous malformations (PAVMs) are abnormally dilated vessels between pulmonary arteries and veins that cause a permanent extra-cardiac right-to-left shunt, which carries the risk of cerebral paradoxical embolisation of both thrombotic and septic origin [2]. Transcatheter embolisation of PAVMs can be safely performed, in order to prevent these potentially severe neurological complications, such as ischaemic stroke or cerebral abscess [3,4].

Currently, there are no data regarding the potential haemodynamic changes occurring directly after PAVM embolisation. Therefore, the present study investigated the direct haemodynamic effects of PAVM embolisation, using noninvasive finger pressure measurements.

METHODS

Patient population

Between 2008 and 2010, we included 29 patients who underwent transcatheter embolisation of PAVMs in the St. Antonius Hospital Nieuwegein, which is a national HHT referral centre in the Netherlands. All patients provided informed consent.

Transcatheter embolotherapy of PAVMs

A PAVM was defined as a direct communication between a pulmonary artery and a pulmonary vein, bypassing the pulmonary capillary filter [5], and was diagnosed using transthoracic contrast echocardiography (TTCE) and subsequent chest computed tomography (CT). Before and after PAVM embolisation, the right-to-left shunt fraction was measured using the 100 % oxygen method as previously described [6]. All patients were discussed in a multidisciplinary team including a pulmonologist and interventional radiologist. PAVMs with a feeding artery diameter of 2–3 mm or greater were found suitable for embolisation therapy [7]. The procedure (Fig. 1a–c) was performed under local anaesthesia (lidocaine 1%). Percutaneous access was derived through the right femoral vein and a six French sheath was inserted. The interventional radiologist selected the PAVM closure device, based on the diameter and anatomy of the PAVM. The most preferred closure device was the Amplatzer® vascular plug (AGA Medical, Golden Valley, MN, USA) (Fig. 1d). Plugs with a diameter of 4–12 mm were used. If PAVM closure with a plug was not possible, detachable coils (Boston Scientific, Natick, Ma) were used. The embolic material was implanted under fluoroscopic guidance, with a maximum contrast volume of 300 ml (Xenetix; Iobitrol, Guerbet, Villepinte, France). Within 24 h after embolisation, a chest X-ray was performed. No standard medication was given.

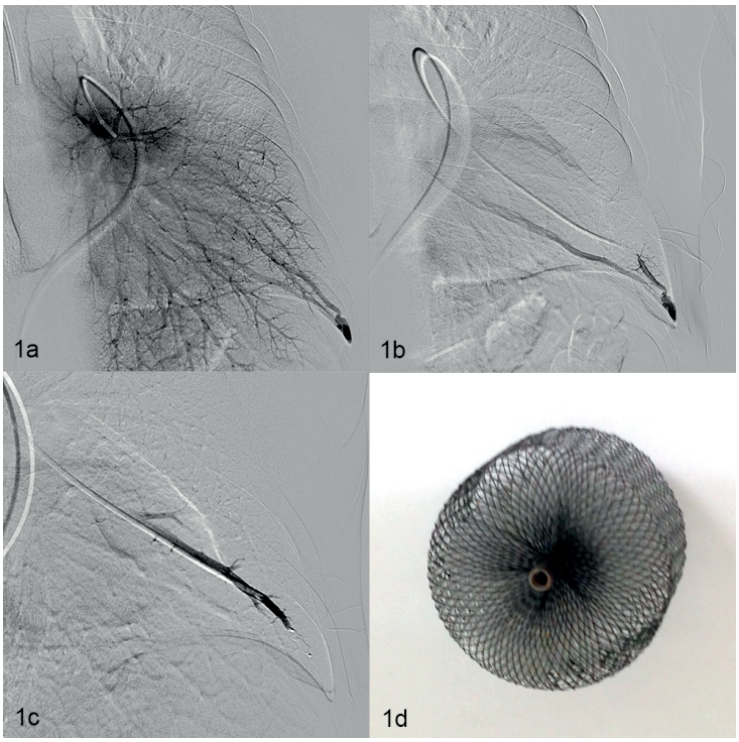

Figure 1

a Pulmonary angiogram of pulmonary arteriovenous malformation in the left lower lobe.
b Selective angiogram of pulmonary arteriovenous malformation in the left lower lobe.
c Embolisation of pulmonary arteriovenous malformation in the left lower lobe with an Amplatzer® vascular plug.
d Amplatzer® vascular plug.

Haemodynamic changes after PAVM embolisation, using non-invasive finger pressure measurements

During the transcatheter embolisation of PAVMs, arterial pressure was measured on a finger of the left hand using a Finometer® device (FMS, Finapres Medical Systems, Amsterdam, the Netherlands). The Finometer® measures blood pressure by a combination of the volume clamp method of Penaz and the 'Physiocal' criteria developed by Wesseling [8-10]. The hand was kept at heart level and a cuff was wrapped around the same arm for individual blood pressure calibration using the return-to-flow calibration [8,11]. Because of potential distortion of the measurements at the time of PAVM closure, parameters were recorded after stabilization of the finger pressure signal during a blanking period of 1 min immediately before and after placement of the first and the last plug.

Data registration and analysis

During the PAVM embolisation, finger pressure measurements with the associated event marks were monitored and digitally stored. Review of these data was performed using BeatScope® software. Five minute averages of systolic (SBP), diastolic (DBP) and mean blood pressure (MBP), heart rate (HR), stroke volume (SV), cardiac output (CO), total peripheral resistance (TPR) and delta pressure/delta time (dP/dt) were calculated after an electronic calibration procedure. SV and CO (CO is the product of SV and HR) were calculated from the finger pressure wave using the Modelflow® methodology .[12] The cardiac index (CI) was calculated from the CO and the body surface area. TPR was defined as MBP divided by CO [8]. Return-to-flow calibration, using the arm cuff, was used for calibration of the blood pressure. There was no calibration with invasive determinations for the CO, SV and TPR. Both absolute values and absolute and relative changes (delta absolute and delta percent) are presented.

Statistical analysis

The statistics were performed using SPSS version 17.0 for Windows (SPSS Inc., Chicago, IL, USA). Descriptive statistics were used to describe patient characteristics. Continuous variables with normal distribution were presented as mean ±SD. Differences within groups were analysed performing paired samples t-tests. A significance level of $p < 0.05$ was considered significant.

RESULTS

Patient population

A total of 29 HHT patients (62% female, mean age 39.2 ± 15.3 years) were included, in which 72 PAVMs were embolised (mean per patient 2.5). An Amplatzer® plug was used in 54 cases and a detachable coil in the remaining 18 cases. The baseline characteristics are presented in tables 1 and 2.

Haemodynamic changes using non-invasive finger pressure measurements

Directly after PAVM embolisation the SV and CO decreased significantly: –6.4 ± 13.0 ml (range –45.9 to 17.9 ml; –11.9%, p = 0.01) and –0.4 ± 0.8 l/min (range –3.0 to 1.58 l/min; –9.5%, p = 0.01). As expected, the CI decreased as well (range –1.5 to 0.9 l/min/m^2; –9.5%, p = 0.01). DBP and MBP increased significantly by 5.2 ± 10.3 mmHg (range –9.0 to 32.9 mmHg; 5.9%, p = 0.01) for DBP and 5.7 ± 12.1 mmHg (range –12.7 to 32.6 mmHg; 4.1 %, p = 0.02) for MBP, respectively. There was no significant change in SBP (4.0 ± 16.3 mmHg (range –25.5 to 38.5 mmHg; 1.7 %, p = 0.20). The dP/dt did not change significantly: 2.1 ± 290.0 mmHg/s (range –750.0 to 848.2 mmHg/s; 0.2%, p = 0.97). There was a correlation between the delta dP/dt and the SBP (Pearson coefficient r = 0.73, r^2 =

0.53, $p < 0.0001$). HR and TPR increased, but this appeared to be non-significant: 1.6 ± 7.4 beats/min (range –17.4 to 21.2 beats/min; 1.8%, $p = 0.24$) and 0.1 ± 0.5 Woods units (range –1.62 to 1.39 Woods units; 5.8%, $p = 0.16$). These data are summarised in Table 3.

Table 1. Baseline characteristics of patients

Total	29
Gender	
Male	11 (37.9)
Female	18 (62.1)
Age (years)	39.2 ± 15.3
BMI (kg/m^2)	24.0 ± 5.0
BSA (m^2)	1.9 ± 0.2
HHT	
Definite	28 (96.6)
Type 1	19 (65.5)
Type 2	2 (6.9)
Type unknown	8 (27.6)
SaO2 (%)	
Before procedure	95.5 ± 3.5
After procedure	98.4 ± 2.3
Shunt fraction (%)	
Before procedure	13.6 ± 8.3
After procedure	4.9 ± 6.6

BMI, body mass index; BSA, body surface area; Kg, kilogram; kg/m^2, kilogram per square meter; HHT, hereditary haemorrhagic telangiectasia; SaO_2, saturation level of oxygen in haemoglobin, PAVM, pulmonary arteriovenous malformation. All characteristics are written in number with (percentage) or mean with standard deviation.

Table 2. Baseline characteristics of embolisation procedure

Treated PAVMs	1 PAVM	9 (31.0)
	2 PAVMs	9 (31.0)
	3 PAVMs	2 (6.9)
	4 PAVMs	8 (27.6)
	> 4 PAVMs	1 (3.4)
Closure device	Amplatzer plug	54 (75.0)
	Coil	18 (25.0)
Plug diameter *	< 4 (mm)	1 (1.9)
	4 (mm)	13 (24.1)
	6 (mm)	13 (24.1)
	8 (mm)	9 (16.7)
	10 (mm)	10 (18.5)
	> 10 (mm)	6 (11.1)
	Not known	2 (3.7)

*PAVM, pulmonary arteriovenous malformation; Mm, millimetre. All characteristics are written in number with (percentage). * diameter coils are not know.*

Table 3. Haemodynamic measurements before and after embolisation with absolute and relative changes

	Before ± SD	After ± SD	Delta absolute	Delta percent	P
SBP (mmHg)	144.9 ± 25.3	148.9 ± 28.4	4.0	1.7	0.20
DPB (mmHg)	85.5 ± 11.5	90.7 ± 16.3	5.2	5.9	0.01
MBP (mmHg)	108.0 ± 16.0	113.7 ± 19.9	5.7	4.1	0.02
HR (beats/min)	78.8 ± 14.6	80.4 ± 13.9	1.6	1.8	0.24
SV (ml)	70.9 ± 20.9	64.5 ± 19.3	-6.4	-11.9	0.01
CO (l/min)	5.5 ± 1.6	5.1 ± 1.4	-0.4	-9.5	0.01
CI (l/min/m^2)	3.0 ± 0.8	2.8 ± 0.8	-0.2	-9.5	0.01
TPR (woods units)	1.4 ± 0.5	1.5 ± 0.5	0.1	5.8	0.16
dP/dt (mmHg/sec)	1233.7 ± 463.8	1235.8 ± 481.7	2.1	0.2	0.97

SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; HR, heart rate; SV, stroke volume; CO, cardiac output; CI, cardiac index; TPR, total peripheral resistance; dP/dt, delta pressure/delta time; SD, standard deviation; min, minutes; ml, millilitres; l/min, litres per minute; mmHg, millimetres of mercury; sec, second.

DISCUSSION

To our knowledge, this is the first study describing the occurrence of significant haemodynamic changes directly after transcatheter embolisation of PAVMs. Using the Finometer® and Modelflow® methodology, our study accurately recorded beat-to-beat non-invasive finger pressure measurements and thereby the immediate haemodynamic changes after PAVM embolisation, amongst which a decrease in SV and CO were most important. Only one case report previously documented haemodynamic changes 4 months after PAVM embolisation with a marked reduction in CO of –5.1 l/min (41%) [13]. This seems to be in line with the results in our current study in 29 HHT patients who all underwent transcatheter embolisation of PAVMs. However, we found a less pronounced decrease in CO, which can be explained by the smaller right-to-left shunts in our study population, with a mean shunt fraction before closure of 14% versus 31% described by Andrivet *et al*. [13] It is possible that the CO may further decrease over time as a result of additional thrombosis of the plug or coil in the PAVM feeding artery. However, the long-term haemodynamic changes after PAVM embolisation remain hard to predict, since new PAVMs may occur and existing PAVMs may grow, so this is still subject for larger studies in the future.

The haemodynamic responses after PAVM embolisation may differ between HHT patients, which can be related to difference in number and size of PAVMs at baseline [2]. In the present study, nine patients underwent embolisation of at least 4 PAVMs in one session, whereas less PAVMs were embolised in the remaining 20 patients. Furthermore, a total of 16 PAVMs were treated with a large plug (diameter ≥10 mm), whereas 14 PAVMs could be treated with smaller endovascular plugs (diameter ≤4 mm). Unfortunately, we could

not find a significant association between plug size (size of the PAVM) and the changes in haemodynamic parameters. Furthermore, the haemodynamic response can be influenced by a different prevalence of underlying hepatic arteriovenous malformations (HAVMs) in different HHT subtypes [5,14,15]. HAVMs may cause a hyperdynamic circulation with high CO [7]. In our cohort, only one patient had a history of HAVMs, which might be an underestimation, since screening for HAVMs was only performed when clinically or biochemically suspected. Clinically significant HAVMs seemed to be absent in the present study, as a hyperdynamic circulation with an abnormal high CI at baseline was not documented in the present study (mean CI within the normal range of 3.0±0.8 l/min/m^2). The amount of microscopic PAVMs under the detection limit of chest CT may further influence the shunt percentage and different haemodynamic responses in HHT patients.

Due to the decrease in pulmonary right-to-left shunt from 14% to 5% after PAVM embolisation, a consequent decrease in preload and SV can be expected (mean change –12% in the current study). As there was no change in HR, the CO decreased as well (–9.5%). The MBP increased by 4 % after PAVM embolisation, which is probably due to the non-significant increase in TPR (6%), as the MBP is a product of TPR and CO. As blood pressure is inversely related to indoor temperature [16], the fall in ambient temperature during the procedure may have caused further vasoconstriction and thereby an increase in blood pressure. In a previous study about the association between brachial pulse dP/dt and other haemodynamic parameters in a chronic haemodialysis population, a Pearson coefficient of $r = 0.6$ ($r^2 = 0.36$, $p < 0.001$) was reported for the correlation between the delta blood pressure and the delta dP/dt [17]. This is in line with the results found in our study ($r = 0.73$, $r^2 = 0.53$, $p < 0.0001$) and demonstrates that the dP/dt is responsible for more than 50% of variance in the blood pressure. Because of the significant increase in MBP and decrease in CO, we also expected a significant increase in TPR. A possible explanation might be an increase in central venous pressure due to the embolisation. Unfortunately, no measurements of the right atrium pressure were performed during the embolisation procedure. A potential clinical implication of our findings might be associated with the presence of pulmonary hypertension (PH) in HHT [18,19]. PH can occur both as gene-related pulmonary arterial hypertension and as a response to high output due to HAVMs [14]. As PAVMs are abnormally dilated vessels between pulmonary arteries and veins they provide low resistance pathways for the pulmonary blood flow and one may therefore expect an elevation in pulmonary artery pressure (PAP) after transcatheter closure. Surprisingly, in a prior study by Shovlin *et al*.[14] there was no described increase in PAP after transcatheter embolisation of PAVMs. It was suggested that this might be caused by other haemodynamic changes, for example recruitment of the pulmonary vasculature or a decrease in CO, although this has been previously suggested in only one case report [13]. We now present the first study that confirms the decrease in CO after embolisation in a larger population of HHT patients, which may indeed provide a potential explanation

for the absent increase in PAP after embolisation. Furthermore, PAVM-related hypoxaemia can induce pulmonary vasoconstriction with a concomitant increase in pulmonary vascular resistance (PVR). In our study there was indeed an increase in saturation after embolisation of PAVMs (Table 1) with probably an decrease in pulmonary vasoconstriction and PVR.

Study limitations

First, our study is limited by its small sample, which may have influenced the results. Second, the Modelflow® model does not seem to be accurate in measuring absolute values of SV, CO and TPR. The differences between the uncalibrated model and invasive determinations (measured with the thermodilution method) in individual patients are usually small, but can be substantial and unreliable in some [8,20]. However, the Modelflow® methodology is an accurate model to compare changes in haemodynamics within one patient and the British Hypertension Society has recommended the Finometer® for measurements in the clinical set-up as well as for research purposes [8,21].

CONCLUSION

The present study shows that significant haemodynamic changes occur directly after embolisation of pulmonary arteriovenous malformations, amongst which a decrease in stroke volume and cardiac output are most important. This may especially provide additional insights into the haemodynamic responses after PAVM embolisation in HHT patients prone to PH.

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CHAPTER 6.2

Pulmonary arteriovenous malformations: haemodynamics and shunt closure

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The first description of pulmonary arteriovenous malformations (PAVM) was made already in 1897 by Churton [1]. PAVM are abnormal vascular structures that connect the pulmonary arterial bed directly to the pulmonary venous bed, thereby bypassing the pulmonary capillaries and resulting in an intrapulmonary shunt which, depending on number and size of the PAVM, may result in arterial desaturation and paradoxical systemic embolisation [2]. Because the PAVM are thin-walled vascular structures, they are prone to rupture, resulting in haemoptysis. A major step forward in the treatment of PAVM was the use of percutaneous transcatheter embolisation, using femoral venous access [3]. This procedure is safe and reduces the risk of paradoxical thromboembolisation and haemoptysis. Moreover, by reducing shunt flow, arterial oxygen saturation increases.

Although percutaneous closure of PAVM has been performed for years, and a surprisingly small effect on pulmonary haemodynamics has been shown [4], the study by Vorselaars and co-workers in the present issue of the Netherlands Heart Journal is the first to investigate the immediate consequences of PAVM closure on systemic haemodynamics [5]. Using Finapres technology, which derives changes in stroke volume and systemic pressure from the pressure wave form as measured on a finger, they describe an overall decrease in stroke volume and cardiac output that corresponds in magnitude with the overall decrease in shunt fraction. This decrease in stroke volume is similar to the decrease in stroke volume, as measured directly by right heart catheterisation, observed in a case study at 4 months follow-up after PAVM [6]. It was speculated that such a decrease in stroke volume helped to explain the absence of an increase in pulmonary artery pressures [4], despite the fact that pulmonary vascular resistance should obviously increase as a consequence of the closure of the low resistance PAVM.

The mechanism behind the observed decrease in stroke volume is unclear. It could be speculated that shunt closure and the accompanying increase in pulmonary vascular resistance result in a slight increase in right ventricular afterload, which limits right ventricular output. It is, however, more likely to assume that oxygen is regulated to fulfil the oxygen demand of peripheral tissues; shunt closure increases the oxygen content of arterial blood and hence for the same oxygen delivery, in the presence of improved oxygenation, less flow is required. The latter explanation is in accordance with the observation that the shunt fraction prior to embolisation (14 %) is similar in magnitude to the decrease in cardiac output (10 %) [5].

As pulmonary artery pressure increases during exercise, the driving pressure for the shunt flow increases, resulting in an increased shunt flow, which is accompanied by augmented arterial desaturation during exercise [7-9]. However, exercise capacity is surprisingly well-maintained in patients with PAVM, potentially due to the capability of the right heart to deal with volume overload. Indeed, although most patients report an increased exercise capacity and quality of life following shunt closure, an objective increase in exercise capacity is not found in all patients [10]. It is possible that changes in pulmonary and systemic

haemodynamics upon shunt closure, which are relatively small under resting conditions, are exacerbated during exercise. Thus, while the study by Vorselaars in the present issue of the Netherlands Heart Journal provides an important observation in resting patients [5], a comprehensive evaluation of shunt flow, pulmonary and systemic haemodynamics and oxygen saturation during exercise prior to and following shunt closure would be of great benefit to enhance our understanding of the implications of shunt closure on exercise capacity.

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PART II

Cardiovascular aspects of hereditary haemorrhagic telangiectasia

CHAPTER 7.1

SMAD4 gene mutation increases the risk of aortic dilation in patients with hereditary haemorrhagic telangiectasia

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ABSTRACT

Background: Mutations in the genes *ENG*, *ACVRL1* and *SMAD4* that are part of the transforming growth factor-beta signalling pathway cause hereditary haemorrhagic telangiectasia (HHT). Mutations in non-HHT genes within this same pathway have been found to associate with aortic dilation. Therefore, we investigated the presence of aortic dilation in a large cohort of HHT patients as compared to non-HHT controls.

Methods*:* Chest computed tomography of consecutive HHT patients (*ENG*, *ACVRL1* and *SMAD4* mutation carriers) and non-HHT controls were reviewed. Aortic root dilation was defined as a z-score > 1.96. Ascending and descending aorta dimensions were corrected for age, gender and body surface area.

Results*:* In total 178 subjects (57.3% female, mean age 43.9±14.9 years) were included (32 *SMAD4,* 47 *ENG*, 50 *ACVRL1* mutation carriers and 49 non-HHT controls). Aortopathy was present in a total of 42 subjects (24% of total). Aortic root dilatation was found in 31% of *SMAD4*, 2% of *ENG*, 6% of *ACVRL1* mutation carriers, and 4% in non-HHT controls ($p < 0.001$). The aortic root diameter was 36.3 ± 5.2 mm in *SMAD4* versus 32.7 ± 3.9 mm in the non-*SMAD4* group ($p = 0.001$). *SMAD4* was an independent predictor for increased aortic root (β-coefficient 3.5, $p < 0.001$) and ascending aorta diameter (β-coefficient 1.6, $p = 0.04$).

Conclusions*: SMAD4* gene mutation in HHT patients is independently associated with a higher risk of aortic root and ascending aortic dilation as compared to other HHT patients and non-HHT controls.

INTRODUCTION

Hereditary haemorrhagic telangiectasia (HHT) is an autosomal dominant inherited disease, characterised by epistaxis, telangiectasia and arteriovenous malformations (AVMs) most often found in the lungs, liver and brain [1]. Most commonly, HHT is caused by a mutation in the *ENG* (OMIM 187300) or *activin receptor-like Kinase 1 (ACVRL1)* (OMIM 600376) gene, causing HHT type 1 (HHT1) and HHT type 2 (HHT2) respectively [2,3]. In 1-3% of HHT patients, mutations in the *SMAD4* gene (OMIM 175050) are found instead [4]. This mutation can lead to a combined syndrome of HHT and familial juvenile polyposis syndrome (JPS) with both AVMs and multiple gastrointestinal polyps with increased risk of cancer in the gastrointestinal tract.

ENG, ACVRL1 and *SMAD4* genes all play a role in the transforming growth factor-beta (TGF-β) signalling pathway, which is essential for vascular integrity and angiogenic remodelling [5,6]. Interestingly, mutations in other genes within that same pathway are known to cause thoracic aortopathy. This includes mutations in *SMAD3* (familial thoracic aortic aneurysms and dissection), *FBN1* (Marfan syndrome), *TGFβR1* and *TGFβR2* (Loeys-Dietz syndrome). Most patients with thoracic aortic aneurysms are asymptomatic, but these aneurysms can lead to life-threatening complications including aortic dissection or rupture with a high mortality rate [7-11].

Given the shared pathway, thoracic aortopathy could be expected in HHT patients. There are a few case reports and small case series that report dilation or aneurysms of the thoracic aorta in individual HHT patients, particularly in patients with a *SMAD4* gene mutation [7,12-19]. However, the risk of aortopathy or its potential complications has not been previously explored in a large HHT cohort. The aim of this study is to assess the prevalence of aortic dilation in patients affected by HHT caused by a *SMAD4* mutation compared to HHT patients with a disease causing mutation elsewhere and HHT negative controls.

METHODS

Study population

Patients were retrospectively recruited from The Dutch HHT Centre at St. Antonius Hospital (Nieuwegein, the Netherlands) and The Toronto HHT Centre at St. Michael's Hospital (University of Toronto, Toronto, Canada). All consecutive patients with a *SMAD4* gene mutation and available chest computed tomography (CT) who visited the out-patient clinic between 2008 and 2015 were included from both hospitals (CT not available N = 2). The *ENG* (HHT1) and *ACVRL1* (HHT2) mutation carriers and HHT-negative controls were included from St. Antonius Hospital. These included all consecutive subjects screened for HHT with available genetics and chest CT between 2009 and 2010 (CT not available N = 0). Subjects were classified as HHT negative if genetic testing excluded the known HHT-causing family mutation. Patients who underwent HHT screening, with negative diagnostic

testing for *ENG* and *ACVRL1*, but no known family mutation were excluded from this study. This prevented patients with clinically diagnosed HHT in whom genetic testing could not detect a disease-causing mutation from being falsely classified as HHT negative. The study was approved by the local ethics committee of both hospitals (R&D/Z14.059 and REB # 15-325).

Chest CT measurement

All included patients underwent chest CT. The chest CTs were performed with a 256 slice IDT scanner (Philips Healthcare, the Netherlands) or a 64 slice VCT scanner (General Electronic Healthcare, Wauwatosa, The United States). All chest CTs where non-contrast enhanced high resolution CT scans with a slice thickness of 1-1.25 mm. Measurements were performed in the transversal plain at the level of the aortic root, the ascending aorta and the descending aorta. The last two were measured at the level of the right pulmonary artery. The short axis from outer to outer wall was measured at all three levels. Measurements were performed by two interventional radiologists experienced in aortic imaging, blinded to the patient data.

The primary objective at the time of scanning was the detection of pulmonary arteriovenous malformations. Therefore, scans were not contrast enhanced or electrocardiographic triggered. All CTs were performed as part of routine HHT testing, therefore the included patients were not exposed to additional radiation.

Aortic dilation

Aortopathy was defined as dilation at any part of the thoracic aorta. Z-scores (describing the relationship to the known mean for a group) indexed for body surface area (BSA) were calculated for the aortic root. Aortic root dilation was defined as an aortic root z-score > 1.96 or calculated according the reference values corrected for age, gender and BSA [20,21]. Dilation of the ascending and descending aorta was calculated according the reference values corrected for age, gender and BSA [20,22]. BSA was calculated according to Dubois and Dubois [23].

Statistical analysis

Descriptive statistics were used to evaluate patient characteristics. Continuous variables were reported as mean ± standard deviation (SD). Differences between groups were analysed with analysis of variance (ANOVA) or Student's *t* test for continues variables and Chi-squared test for nominal variables. Linear regression analysis was used to determine predictors for aorta diameter (presented as β-coefficient). Logistic regression analysis was

used to determine predictors for an increased z-score (presented as odds ratio (OR) with 95% confidence interval (CI)). For multivariate adjustment, variables were chosen based on clinical relevance and included age, gender, BSA and gene mutation. Statistics were performed using a statistical software package (SPSS, version 24; SPSS Inc., Chicago).

RESULTS

Study population

In total, 178 subjects (57.3% female, 43.9 ± 14.9 years of age) were included (table 1). This included a total of 129 HHT patients (32 *SMAD4* (18.0%), 47 *ENG* (26.4%), 50 *ACVRL1* (28.1%) mutation carriers) and 49 non-HHT controls (27.5%). Clinical signs of JPS were present in 84% of *SMAD4* mutation carriers. There were no statistically significant differences in baseline characteristics (age, gender, BSA, clinical criteria, presence of hypertension and absolute aorta diameter) between *SMAD4* mutation carriers of both hospitals (18 from Nieuwegein and 14 from Toronto).

Aortopathy

Aortopathy was present in 42 (23.6%) patients (*SMAD4* 15/32 (46.9%), *ENG* 7/47 (14.9%), *ACVRL1* 10/50 (20.0%), non-HHT controls 10/49 (20.4%), p = 0.007; table 1). Absolute diameter of the aortic root was significantly higher in *SMAD4* mutation carriers compared to all other groups (*SMAD4* 36.3 ± 5.2 mm, *ENG* 32.6 ± 3.8 mm, *ACVRL1* 33.7 ± 3.6 mm, non-HHT controls 31.8 ± 4.0 mm, p < 0.001). Aortic root dilation was present in 31.3% of *SMAD4* mutation carriers compared to 2.3%, 6.0% and 0% in *ENG*, *ACVRL1* and non-HHT controls respectively (p < 0.001). Within the non-*SMAD4* groups there were no significant differences besides the absolute aortic root diameter (*ACVRL1* vs non-HHT p=0.017). All other results are described in tables 1 and 2 and figure 1.

After adjusting for age, gender and BSA, *SMAD4* was an independent predictor of increased aortic root and ascending aorta diameter (β-coefficient 3.5, p < 0.001 and β-coefficient 1.6, p = 0.04 respectively; table 2). *SMAD4* mutation (OR 10.5; 95% CI 2.1-51.8) and male gender (OR 4.5; 95% CI 1.4-14.7) were predictors for aortic root dilation (z-score > 1.96) (table 3).

Table 1. Baseline characteristics and presence of aortic dilation

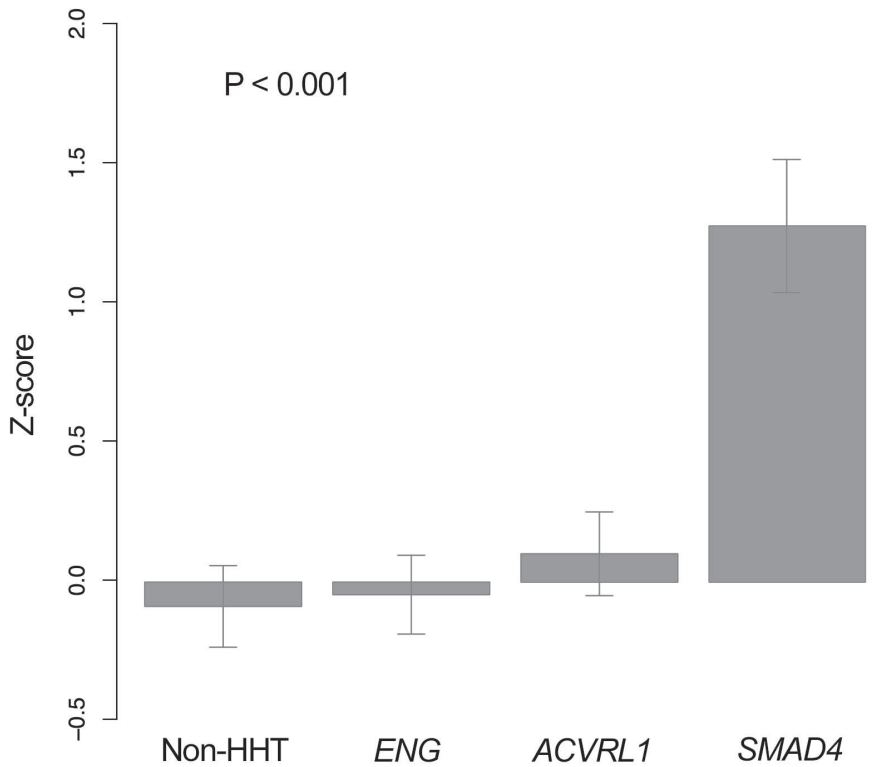
	Total	HHT (N = 129)			Non-HHT (N = 49)	p- value
		SMAD4	*ENG*	*ACVRL1*		
Number	178	32 (18.0%)	47 (26.4%)	50 (28.1%)	49 (27.5%)	
Age (years)	43.9±14.9	41.9±14.2	43.5±16.0	47.8±15.1	41.8±13.8	0.175
Female gender	102 (57.3%)	14 (43.8%)	27 (57.4%)	26 (52.0%)	35 (71.4%)	0.073
BSA (m^2)	1.90±0.21	1.92±0.24	1.89±0.23	1.94±0.20	1.86±0.20	0.248
Clinical criteria						<0.001
Definite	104 (58.4%)	21 (65.6%)	45 (95.7%)	37 (74.0%)	1 (2.0%)	
Possible	31 (17.4%)	9 (28.1%)	2 (4.3%)	13 (26.0%)	7 (14.3%)	
Unlikely	43 (24.2%)	2 (6.3%)	0 (0.0%)	0 (0.0%)	41 (83.7%)	
Systolic BP (mmHg)	132.0±16.9	122.9±19.7	133.9±15.7	136.7±15.0	131.2±16.1	0.005
Diastolic BP (mmHg)	78.3±10.0	71.0±13.5	79.9±9.4	80.7±8.7	78.3±7.3	<0.001
Hypertension*	25 (15.2%)	4 (13.3%)	5 (11.9%)	10 (21.7%)	6 (12.8%)	0.535
Smoking[£]	29 (17.4%)	1 (3.6%)	8 (19.0%)	5 (10.4%)	15 (30.6%)	0.010
Aorta diameter (mm)						
Aortic root	33.4±4.3	36.3±5.2	32.6±3.8	33.7±3.6	31.8±4.0	<0.001
Ascending Aorta	31.5±5.1	32.7±5.3	30.6±5.6	32.4±4.6	30.7±4.8	0.096
Descending Aorta	23.4±3.7	23.7±4.4	23.0±4.1	24.0±3.3	23.0±3.3	0.461
Aortopathy	42 (23.6%)	15 (46.9%)	7 (14.9%)	10 (20.0%)	10 (20.4%)	0.007
Aortic root						
Above UL[†]	14 (8.0%)	10 (31.3%)	1 (2.3%)	3 (6.0%)	0 (0%)	<0.001
Z-score >1.96[¶]	16 (9.2%)	10 (31.3%)	1 (2.3%)	3 (6.0%)	2 (4.2%)	<0.001
Mean Z-score	0.23±1.18	1.28±1.35	-0.05±0.94	0.10±1.06	-0.09±1.01	<0.001
Ascending aorta						
Above UL[†]	23 (13.1%)	7 (21.9%)	4 (8.7%)	7 (14.0%)	5 (10.4%)	0.348
Descending aorta						
Above UL[¥]	25 (15.2%)	5 (15.6%)	5 (10.6%)	9 (18.0%)	6 (12.2%)	0.730

*Continuous values are mean ± SD. Categorical values are n (%). HHT, hereditary haemorrhagic telangiectasia; BSA, body surface area; m^2. square meters; BP, blood pressure; mmHg, millimetres of mercury; mm, millimetres; UL, upper limit. * Defined as grade II hypertension. [33] [£] N = 167 [†]According Campens et al.[20] [¶]According Devereux et al. [21] [¥] According Rogers et al.[22]*

Table 2. Multivariate predictors of aortic diameter

	Aortic root		Ascending aorta		Descending aorta	
	β-*coefficient*	*p-value*	β-*coefficient*	*p-value*	β-*coefficient*	*p-value*
Age (years)	0.1	<0.001	0.2	<0.001	0.2	<0.001
BSA (m2)	6.5	<0.001	6.1	<0.001	5.3	<0.001
Gender						
Male	2.0	<0.001	0.3	0.6	1.4	<0.001
Female	Ref		Ref		Ref	
Subgroup						
SMAD4	3.5	<0.001	1.6	0.04	-0.1	0.9
ENG	-0.1	0.98	-0.8	0.2	-0.7	0.06
ACVRL1	0.2	0.7	-0.1	0.9	-0.8	0.03
Non-HHT	Ref		Ref		Ref	

BSA, body surface area; m^2, square meters; HHT, hereditary haemorrhagic telangiectasia.



Figuur 1. Mean Z-score aortic root

Aortic root Z-score described as mean with SEM. HHT, hereditary hemorrhagic telangiectasia; SEM, standard error of the mean.

Table 3. Predictors for aortic root dilation

	Z-score ≤ 1.96 (N = 158)	Z-score > 1.96 (N = 16)	OR [95% CI]*	p-value
Age (years)	44.0±15.2	46.6±11.8	1.0 [0.98-1.05]	0.5
BSA (m2)	1.90±0.21	1.96±0.21	3.3 [0.3-34.2]	0.3
Gender				
Male	63 (39.9%)	12 (75.0%)	4.5 [1.4-14.7]	0.01
Female	95 (60.1%)	4 (25.0%)	Ref	
Subgroup				
SMAD4	22 (68.8%)	10 (31.3%)	10.5 [2.1-51.8]	0.004
ENG[∞]	43 (97.7%)	1 (2.3%)	0.5 [0.5-6.1]	0.6
ACVRL1	47 (94.0%)	3 (6.0%)	1.5 [2.3-9.1]	0.7
Non-HHT[¶]	46 (95.8%)	2 (4.2%)	Ref	

BSA, body surface are; m², square meters; HHT, hereditary haemorrhagic telangiectasia.
** Univariate analysis, [∞]N = 44, [¶]N = 48*

At time of measurement there were no complications due to aortopathy observed. None of the patients required intervention since maximum diameter found was 45.7 mm, 45.9 mm and 34.0 mm for the aortic root, ascending and descending aorta respectively (recommendations for intervention for the aortic root or ascending aorta ≥ 50 mm, descending aorta ≥ 55 mm in Marfan patients) [24].

DISCUSSION

Presence of aortic aneurysms and other cardiac manifestations are previous described in patients with JPS and the combined JPS-HHT syndrome. However, to our knowledge, this is the first dedicated study investigating the presence of thoracic aortic dilation in patients with *SMAD4* gene mutation associated with HHT compared to patients with other mutations causing HHT and non-HHT control patients. The main finding of this study is that there is an increased risk of aortic dilation and especially dilation of the aortic root in *SMAD4* mutation carriers compared to *ENG* or *ACVRL1* associated HHT and non-HHT controls.

The association between HHT and aortopathy was suggested by Thomas and Muggia, who described it as early as 1964 when a case was published of a patient with clinical characteristics of HHT (although HHT causing mutation was unknown) and a thoracic aortic aneurysm leading to death [14,15]. More recently, 2 HHT patients, without classic risk factors for aneurysm, presented with an aortic root dilation and a descending artery aneurysm with type B aortic dissection. Genetic testing in these patients revealed an *ACVRL1* mutation in one of them [13,17]. Surprisingly, in our study no significant dilation of the descending aorta was found.

A previous retrospective single centre study of 26 HHT patients (including 16 *SMAD4* patients) described a high prevalence of aortopathy in the *SMAD4* related patients (6 patients (38%)) [12]. Although a different imaging modality (echocardiography) and thereby different measurements were performed in that study, these results seem comparable to our study. In none of the other HHT patients, aortopathy was described. Unfortunately, very few patients with *ACVRL* or *ENG* associated HHT (1 and 5 respectively) were included, therefore conclusions for these groups could not be drawn [12]. In our current study, approximately 100 *ENG* and *ACVRL1* patients were included showing no increased prevalence of aortic dilation compared to 50 non-HHT control family members. In fact, a *SMAD4* mutation seems the only relevant and discriminant factor associated with aortic dilation in these patients.

Besides aortic involvement, other forms of connective tissue disease may also be present in *SMAD4* patients. Andrabi et al. described a paediatric *SMAD4* patient with both aortopathy and mitral valve prolapse at young age with the same features in 3 family members [16]. Presence of mitral valve prolapse was also noticed in another small study on the phenotype of JPS (including 31 *SMAD4* mutation carriers). This study confirms the broad clinical features of *SMAD4* mutation carriers with clinically JPS or the combined JPS-HHT syndrome. Cardiac manifestations were present in 12% (including 7% mitral valve prolapse) and aneurysms (although none of the thoracic aorta) described in 5% [25]. Although the relation between mitral valve prolapse and *SMAD4* was not investigated extensively, this relation was not found in our study.

The pathophysiological mechanism of *SMAD4* mutations leading to aortopathy is not completely clear. All involved gene mutations play an important role in the extremely complex TGF-β pathway. Increased TGF-β stimulation has shown to increase the aortic dimension in Marfan mouse models via elevated levels of nuclear Smad2, possibly in HHT patients aortic aneurysms originate from this same mechanism [26-28]. In Marfan patients, aortic enlargement is generally more pronounced at the aortic root. In our study, this pattern is mimicked in the *SMAD4* mutation carriers. *SMAD4* may impair the resolution phase of angiogenesis creating a less robust vessel wall [28-30]. It is unclear why patients with *ENG* and *ACVRL* gene mutations, which disturb the same TGF-β pathway more upstream, are less affected by aortopathy. Possibly normal function of *SMAD4* more downstream neutralizes the effects caused by mutation in *ENG* or *ACVRL1* resulting in no or a less pronounced impairment of angiogenesis. This genetic effect is present in *SMAD4* associated JPS patients with and without the clinical characteristics of HHT.

Most patients with aortic dilation remain asymptomatic before catastrophic disease presentations occur such as rupture or dissection. Therefore, identification of patients at risk is important to diminish mortality [8]. The risk of aorta dissection or rupture increases rapidly after exceeding a diameter of 60 mm, guidelines for intervention recommend earlier intervention in patients with familial aortic disease. Although none of the patients in this study

met the criterion for intervention [24], *SMAD4* patients are at increased risk for aortic dilation and thereby its possible catastrophic complications. At this stage, no exact recommendations for treatment and follow-up could be given. However, the ESC guidelines on the diagnosis and treatment of aortic disease recommend treating all patients with Marfanoid like connective tissue disease as Marfan patients. Therefore, intervention should be considered at a diameter of 50 mm [24,31].

Both larger observational studies and subsequent studies to look at the growth rate of aortic aneurysms are required to assess the risk of serious complications as a result of aortic dilatation in *SMAD4* mutation carriers. Following the American College of Gastroenterology who advises yearly cardiovascular screening in all JPS *SMAD4* mutation carriers, we believe it is important to systematically screen all HHT patients with a *SMAD4* mutation for aortic root and ascending dilatation on a regular interval [32]. Since the exact consequences for the patients are not explored yet, we recommend to use the routinely performed HHT screening imaging to monitor aortic dimensions, thereby limiting the additional risks for the patients.

Limitations

The present investigation is a retrospective analysis, with all inherent limitations. This resulted in the use of non-electrocardiographic triggered CT techniques due to other diagnostic proposes of the CT. This could have led to over- and underestimation since the aorta is not a round structure, especially when tortuous aneurysms exist [24], besides aortic stiffness was not measured. Since the CTs were not contrast enhanced measurement of aortic diameter was performed form outer to outer wall. Therefore the aortic dimension could be slightly over-estimated compared to leading edge measurement on echocardiography. Second, only one chest CT was used for measurement and the number of patients included in this study was too low to show significant complications. Therefore future prospective research should provide serial measurements and include more patients. Furthermore, there may be a survivor bias because patients who died of aortic rupture would not have made it to our clinic and are therefore not included. Last, genetic testing for other mutations in the TGF-β pathway was not performed and consequently it has not been explicitly ruled out that the included patients did not also suffer from a connective tissue disease. However, we expect there is only a minimal effect of these limitations since we used a control group.

CONCLUSION AND RECCOMENDATION

SMAD4 gene mutation in HHT patients independently increases the risk of aortic root and ascending aortic dilation as compared to other HHT patients and healthy control patients. Further research should assess the risk of complications in this population.

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CHAPTER 7.2

SMAD4 gene mutation and risk of aortic dilation: Lessons from hereditary haemorrhagic telangiectasia

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Hereditary haemorrhagic telangiectasia (HHT, historically called Osler-Weber-Rendu syndrome) is an autosomal dominant disorder characterised by vascular malformations [1]. Classic manifestations include mucocutaneous telangiectasia, epistaxis, and gastrointestinal bleeding leading to iron deficiency anaemia. Potentially life-threatening manifestations may also develop, such as pulmonary, hepatic, and cerebral arteriovenous malformations. More than 600 different HHT-causing mutations have been described; however, three major disease genes can be identified: the *ENG* gene, encoding for the protein endoglin (OMIM # 187300); *ACVRL1*, encoding for activin receptor-like kinase 1 (OMIM # 600376), and SMAD4, whose mutations account for approximately 1% of HHT cases (OMIM # 175050) [1]. Of note, causative mutations in these *ENG*, *ACVRL1* and *SMAD4* genes all affect the transforming growth factor-beta (TGF-β) signaling pathway. Specifically, *ENG* and *ACVRL1* encode for receptors found on the surface of vascular endothelial cells, whereas *SMAD4* is a downstream mediator shared by both signal transduction cascades [2]. The TGF-β signaling pathway is essential for vascular integrity and angiogenic remodeling, and abnormal TGF-β signaling causes vascular dysfunction and malformation in experimental models of Marfan syndrome [3]. It is thus not unexpected that mutations along the TGF-β pathway causing HHT may also predispose to the development of thoracic aortopathy in general.

However, prior to the article by Vorselaars *et al*., published in this issue of the *International Journal of Cardiology,* there were only few, small studies documenting thoracic aortopathy in HHT patients, particularly in patients with a *SMAD4* gene mutation [4]. This new study is the largest to systematically explore the risk of developing thoracic aortopathy in HHT patients, and the first dedicated study comparing the frequency of thoracic aortic dilation in HHT patients with *SMAD4* as opposed to different gene mutations. Thanks to a collaborative effort between the Dutch HHT Centre at St. Antonius Hospital (Nieuwegein, the Netherlands) and the Toronto HHT Centre at St. Michael's Hospital (Toronto, Canada), Vorselaars and colleagues evaluated chest computed tomography images from 129 HHT patients (32 *SMAD4*, 47 *ENG*, 50 *ACVRL1* mutation carriers) and 49 non-HHT controls. The main finding of this study is a markedly increased risk of aortic root dilation among HHT patients; the most unexpected finding is that this risk can be almost entirely ascribed to the subgroup of *SMAD4* mutation carriers. Specifically, aortic root dilation was found in 31% of *SMAD4*, 2% of *ENG*, 6% of *ACVRL1* mutation carriers, and in 4% of non-HHT controls. Furthermore, *SMAD4* was an independent predictor of increased aortic root and ascending aorta diameter: the aortic root diameter was 36.3 ± 5.2 mm in SMAD4 versus 32.7 ± 3.9 mm in the non-*SMAD4* group.

This study corroborates existing evidence that different pathogenic gene mutations affect disease phenotype in HHT patients in general. For example, pulmonary and cerebral arteriovenous malformations are more common in HHT patients harboring mutations in the *ENG* gene [5] , whereas mutations in *SMAD4* can lead to the development of a combined syndrome of HHT and familial juvenile polyposis [1], characterised by both vascular malfor-

mation and multiple gastrointestinal polyps with increased risk of cancer development. In the study by Vorselaars and colleagues, the vast majority of *SMAD4* mutation carriers had indeed clinical signs of juvenile polyposis-HHT overlap syndrome.

Aortic aortopathy is not an uncommon manifestation of juvenile polyposis, and yearly screening for aortic dilation is already recommended for juvenile polyposis patients by the American College of Gastroenterology, albeit based on limited evidence [6]. This study by Vorselaars *et al*. suggests that the *SMAD4* mutation is specifically associated with aortic dilation regardless of the clinical diagnosis, and provides solid rationale for systematically screening all juvenile polyposis, HHT, or juvenile polyposis-HHT overlap patients with a *SMAD4* mutation for aortic dilation on a regular interval. One could argue that clinicians should minimize the significant radiation exposure that follows repeated imaging studies, chiefly by restricting screening to individuals for which the results will affect evidence-based management. In these regards, guidelines on the treatment of aortic disease recommend that patients with Marfanoid manifestations due to a connective tissue disease other than Marfan syndrome be managed as Marfan syndrome patients, thus receiving surgery when the maximal aortic diameter exceeds 45 or 50 mm (depending on family history of aortic dissection), or when the aneurysm is rapidly dilating (at a rate higher than 3 mm/year) [7]. In the study cohort described by Vorselaars *et al*. the aortic diameter in HHT patients was well below this recommended threshold for surgical management, and patients neither met the criterion for intervention nor developed complications. However, information on the evolution of aortic aneurysm in HHT patients carrying mutations in *SMAD4* is scant. In analogy with other connective tissue diseases sharing the same pathophysiologic pathway characterised by abnormal TGF-beta signaling, it seems prudent to expect accelerated aortic dilation growth rates until more precise information is available. Thus, although no exact recommendation for management could be given, regular follow-up of HHT patients carrying *SMAD4* mutations seems particularly reasonable. Whether this can be effectively achieved with echocardiography or magnetic resonance as opposed to radiation-based techniques can be debated.

Finally, the study by Vorselaars and colleagues has implications for personalized approaches to the management of HHT patients. At present, gene testing is not mandatory to establish a diagnosis of HHT. However, not only the identification of a pathogenic sequence variant in *ENG*, *ACVRL1*, or *SMAD4* is of great aid in assessing the diagnosis, but it is also becoming progressively more evident that different pathogenic mutations are often associated with distinct phenotypic manifestations. An approach combining clinical diagnosis, genetic profiling, and patient-tailored radiologic screening seems within reach for HHT patients. However, one should also be mindful that all classical disease features can be observed in any HHT patient, regardless of different disease-causing genes, and that current gene testing strategies cannot identify all possible mutations, or may conversely reveal sequence variants that are not disease-causing. For these reasons, in addition to those discussed

above, the article by Vorselaars *et al*. suggests that screening for radiologic signs of aortic dilation should be considered in all patients with a diagnosis of HHT.

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CHAPTER 7.3

Correspondence: Thoracic Aorta Dilation in Patients with Hereditary Hemorrhagic Telangiectasia Due to *SMAD4* Gene Mutation

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Correspondence to: Heald B, Rigelsky C, Moran R, LaGuardia L, O'Malley M, Burke CA, Zahka K. Prevalence of Thoracic Aortopathy in Patients with Juvenile Polyposis Syndrome-Hereditary Hemorrhagic Telangiectasia due to SMAD4. 2015. Am J Med Genet Part A 9999A:1–5.

TO THE EDITOR, with great interest, we have read the article by Heald *et al.* entitled "Prevalence of thoracic aortopathy in patients with juvenile polyposis syndrome-hereditary haemorrhagic telangiectasia due to *SMAD4*". In this retrospective chart review the authors describe a high prevalence (38%) of aortopathy in patients with combined syndrome of juvenile polyposis (JPS) and hereditary hemorrhagic telangiectasia (HHT) due to *SMAD4* mutations [1].

There are a few case reports and small case series that describe patients with *SMAD4* mutation and aortopathy; however, the prevalence has never been investigated systematically. Therefore, this study is an exceptional contribution to the knowledge of this specific population. Nonetheless, we have some comments that we would like to discuss.

First, transthoracic echocardiography (TTE) is used for the measurement of the different aorta dimensions, including the aortic annulus and root, the sinotubular junction, and ascending aorta. However, only the dimensions of the aortic root are mentioned in the results. Although TTE is an excellent imaging modality for measurement of the most proximal part of the thoracic aorta, the current guidelines recommend that all patients with a mutation associated with aortic disease or a family history of aortic disease, should have the entire thoracic aorta imaged by an appropriate imaging technique [2]. Therefore, computed tomography (CT) seems a more suitable imaging technique in this population [3,4]. At least 3 out of the 6 patients with aortopathy have pulmonary arteriovenous malformations and should have undergone a chest CT [5]. Since differences between CT and TTE can exist, we are curious about the measurements of the aorta on CT.

Second, the aorta dimension depends on age, sex, and body size of the patient [4,6]. Besides the gene mutations, some other factors might contribute to aortic dilation as well, e.g. smoking and arterial hypertension [4,6]. These characteristics have not been mentioned in the study but could have influenced the data.

Third, the 2-dimensional echocardiograms were reviewed by only one (pediatric) cardiologist. Although a good interobserver variability was found in a study of Campens *et al*., the interobserver variability in this study is not known [7].

Both *ENG* and *ACVRL1* (pathogenic mutations for HHT type 1 and HHT type 2 respectively), and *SMAD4* encode members of the transforming growth factor-beta (TGF-β) pathway, which is important for the vascular integrity and angiogenic remodeling [8,9]. The pathophysiology of aortopathy in patients with *SMAD4* mutations is not completely revealed. Mutations in other important genes encoding proteins (e.g. *SMAD3*, *FBN1*, *TGFβR1* and *TGFβR2*), however, lead to perturbations in the TGF-β pathway and are associated with aortopathy (familiar thoracic aortic aneurysms and dissection, Marfan syndrome and Loeys-Dietz syndrome respectively) [4,10].

The authors state correctly that no conclusions could be drawn for the patients with an *ACVRL1* or *ENG* mutation (since only 1 and 5 patients were included, respectively). Although there are no prior reports on aortopathy or its potential complications in large cohorts of

HHT patients, these mutations are part of the TGF-β pathway and might potentially also lead to aortopathy. Therefore, new studies should also include enough HHT patients with these mutations.

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CHAPTER 8.1

Pulmonary hypertension in a large cohort of hereditary haemorrhagic telangiectasia

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ABSTRACT

Background: Hereditary hemorrhagic telangiectasia (HHT) is a vascular disorder, characterized by arteriovenous malformations in the brain, liver and lungs. Pulmonary hypertension (PH) is increasingly recognized as a severe complication of HHT. However, there are no studies describing the prevalence of PH in HHT compared to HHT negative controls.

Objectives: To assess the estimated prevalence of PH in patients with HHT as compared to HHT negative controls.

Methods: All consecutive subjects screened for HHT with available genetic testing and echocardiographic based peak tricuspid regurgitation velocity (TRV) measurement were included. Increased probability PH was defined as TRV > 2.8 m/s.

Results: In 578 subjects, both echocardiography and genetic testing were available. A reliable TRV was measured in 383 (66.3%). Of whom 127 HHT type 1 (HHT1), 150 HHT type 2 (HHT2) and 106 non-HHT controls, with a mean TRV of 2.3±0.4 m/s, 2.4±0.5m/s, and 2.2±0.3m/s, respectively ($p=0.008$ and $p<0.001$ vs. controls). Increased probability PH was found in 42 subjects (8.7% in HHT1, 18.0% in HHT2 and 3.8% in non-HHT controls). HHT2 and hepatic arteriovenous malformations (HAVMs) were the most important predictors for increased probability PH (OR 5.6; $p=0.002$ and OR 11.3; $p<0.001$, respectively). Heritable pulmonary arterial hypertension (HPAH) was diagnosed in 2 patients (0.7%) and only found in HHT2 (1.3%).

Conclusion: The estimated prevalence of PH is higher in HHT as compared to non-HHT controls. This increase is especially present in HHT2 and mainly associated with the presence of HAVMs. HPAH appears to be rare in HHT patients, and was only diagnosed in HHT2.

INTRODUCTION

Hereditary haemorrhagic telangiectasia (HHT) is an autosomal dominant inherited disorder, characterised by abnormal artery-to-vein communications varying from small telangiectasia in skin and mucosal membranes, to large arteriovenous malformations (AVMs), predominantly in the brain, liver and lungs [1-4].

HHT consists of two main subtypes, HHT type 1 (HHT1) and type 2 (HHT2), both resulting from different gene mutations in two genes encoding for TGFβ receptors [2,5,6]. HHT1 results from mutations in the *ENG* gene on chromosome 9 encoding the protein endoglin [6], whereas HHT2 results from mutations in the *activin receptor-like kinase 1 (ACVRL1)* gene on chromosome 12 encoding the protein ALK-1 [7]. Pulmonary hypertension (PH) is increasingly recognised as a potential important complication of HHT, as it is associated with poor prognosis and might lead to progressive right-sided heart failure and premature death [8-10].

There are two potential mechanisms that could explain the presence of PH in patients with HHT. PH may result from a high pulmonary blood flow, which is due to the high cardiac output (CO) state associated with hepatic AVM (HAVMs) [11-13]. Furthermore HHT-related gene mutations in *ACVRL1* or *ENG* potentially predispose to the development of heritable pulmonary arterial hypertension (HPAH) with a normal or low CO [14-19].

Currently, transthoracic contrast echocardiography (TTCE) is recommended in all HHT patients for the detection of pulmonary AVMs (PAVMs), which could also be used for PH screening [2]. Previous studies describing the association between PH and HHT suffer from major limitations, including a small sample size, inclusion of patients with known history of PH in whom symptoms of HHT were present and not differentiating between HHT types [14-22]. In the current study, we report the estimated prevalence of all cause PH in a large well-defined cohort of patients with HHT as compared to HHT negative controls.

METHODS

Study population

All consecutive subjects, older than 15 years of age, who were screened for HHT between May 2004 and October 2012 at St. Antonius Hospital (designated by the international HHT foundation as HHT centre of excellence), were eligible for inclusion. The clinical diagnosis of HHT was established according to the Curaçao criteria [1]. Genetic testing for the HHT-causing mutation was offered to all screened subjects and performed as previously described [23]. Subjects were included for further analysis if definite mutation analysis was available, and classified as HHT1, HHT2 and non-HHT controls. Subjects were included in the control group when genetic testing for the known HHT-causing family mutation (i.e. the mutation present in a family member) was negative. Subjects who underwent HHT screening, with negative genetic testing for *ENG* and *ACVRL1*, but no known family muta-

tion were excluded from this study. This prevented patients with clinically diagnosed HHT in whom genetic testing could not detect a disease-causing mutation from being falsely classified as HHT negative. Subjects without a reliable peak tricuspid regurgitation velocity (TRV) measurement on TTCE were excluded [24,25].

Screening for the presence of PAVMs was routinely performed with TTCE and/or chest computed tomography as previously described [26,27]. Screening for HAVMs was only performed in case of suspected HAVMs by medical history, physical examination or blood test results (abnormal gamma-glutamyl transpeptidase or alkaline phosphatase). All subjects provided informed consent and the study was approved by the institutional review board of the St. Antonius hospital (R&D/ Z13.040).

Screening and diagnosis of PH

A commercially available Philips IE33 ultrasound instrument and a S5-1 transducer (Philips Medical Systems, Best, The Netherlands) were used for cardiac imaging. Peak TRV was measured with continuous wave Doppler. Intermediate probability PH was defined as TRV > 2.8 m/s and ≤ 3.4 m/s and high probability PH as TRV > 3.4 m/s, according to the international guidelines for the diagnosis and treatment of PH [9]. The term increased probability was used to describe both intermediate probability PH and high probability PH. Atrial dimensions and area, right ventricular (RV) dimension, CO and left ventricular ejection fraction were obtained from the apical 4 chamber view. Two independent cardiologists, who were blinded for other characteristics, with experience in both HHT and PH reviewed all TTCEs.

PH was defined as a mean pulmonary artery pressure (PAP) of ≥ 25 mmHg at rest obtained by right heart catheterization (RHC) [9]. During RHC, we measured systolic, diastolic and mean PAP, right atrial pressure, pulmonary artery wedge pressure (PAWP), RV pressure, pulmonary vascular resistance (PVR), CO, cardiac index (CI) and saturation in different compartments. All patients classified as high probability PH were discussed in a multidisciplinary meeting. Classification and indication for RHC was performed according to the international guidelines [9,28].

Statistical analysis

Descriptive statistics were used to describe patient characteristics. Continuous variables were reported as mean ± standard deviation. Proportions were given by numbers and corresponding percentages. Differences between groups were analysed by independent Student's *t* test for continues variables and Chi-squared test or Fisher's exact test for nominal variables. Univariate statistical analysis with logistic regression were used to identify predictors for increased probability PH. Odds ratios (ORs) with their 95% confidence intervals (CIs) were calculated, for continuous variables ORs were calculated per unit of

measurement. Statistics were performed using a statistical software package (SPSS, version 22; SPSS Inc., Chicago).

RESULTS

Study population

Between May 2004 and October 2012, in a total of 658 subjects screened for HHT mutation analysis was available (figure 1). Out of these 658 subjects an adequate TTCE was made in 578 subjects (87.8%). In 27 subjects no TTCE was made due to failure of placement of the intravenous line or patient refusal. In 53 subjects, the TTCE quality was too poor for interpretation. A reliable TRV was measured in 383 subjects (66.3%), of whom 127 HHT1 (61.4% female, mean age 44.2 ± 15.6 years), 150 HHT2 (59.3% female, mean age 48.9 ± 14.2 years) and 106 non-HHT controls (62.3% female, mean age 38.6 ± 12.8 years). The mean TRV was higher in HHT1 (2.3 ± 0.4 m/s, $p = 0.008$) and HHT2 (2.4 ± 0.5 m/s, $p < 0.001$) patients as compared to the non-HHT controls (2.2 ± 0.3 m/s). Characteristics of our study population are listed in table 1.

Increased probability PH

Increased probability PH was found in 42 subjects (11.0%), 11 (8.7%) HHT1, 27 (18.0%) HHT2 and 4 (3.8%) non-HHT controls (HHT versus non-HHT p=0.005; table 2). Within these groups HAVMs were found in 9.1%, 44.4% and 0% respectively. The mean CO was 3.9 ± 1.0 L/min, 5.1 ± 1.6 L/min and 4.4 ± 0.9 L/min respectively. In patients with HAVMs, mean CO was 5.8 L/min as compared to 4.1 L/min in those without HAVMs.

Predictors of PH

The presence of *ACVRL1* mutation and HAVMs were the most important predictors for increased probability PH in univariate analysis (OR 5.6: 95% CI 1.9-16.5; p=0.002 and OR 11.3: 95% CI 4.8-26.7; $p < 0.001$ respectively). Other predictors were age, haemoglobin levels, left atrial (LA) area and partial oxygen pressure (table 3).

High probability PH

In HHT1, 2 patients (1.6%) were classified as high probability PH. Both patients were older than 65 years and classified as PH due to left heart disease (world health organization (WHO) class II). Patient 1 had mitral valve regurgitation and diastolic dysfunction of the left ventricle. Patient 2 had both systolic and diastolic dysfunction of the left ventricle. Both had normal liver function and CO.

In HHT2, 6 patients (4.0%) were classified as high probability PH of whom 4 underwent RHC (patients 3, 4, 5, 6). Patient 1 and 2 were classified as PH due to diastolic dysfunction of the left ventricle (WHO class II). Two patients (1.3%, patient 3 and 4) were diagnosed with HPAH.

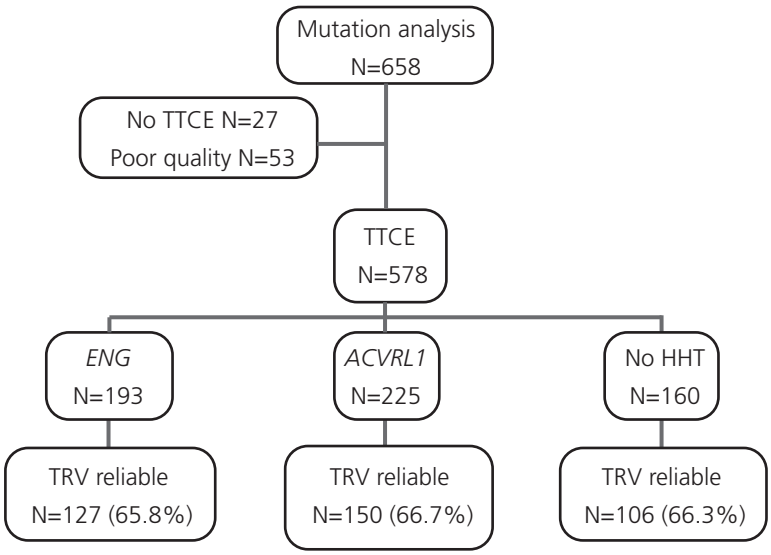

Figure 1. Flowchart of patient selection.
*N, Number; ACVRL1, activin receptor-like kinase 1; TTCE, transthoracic contrast echocardiogram; TRV, tricuspid regurgitation velocity. * Patients are only included when the family mutation is known.*

Patient 3 had a mutation known for HPAH (*ACVRL1*;7:DelG1042stop) and HAVMs (RHC: PAP 75/30/45 mmHg, PAWP 15 mmHg, PVR 3.4 Wood units (WU), CI 3.7 L/min/m2). Patient 4 had no HAVMs and no signs of left heart disease on echocardiogram (RHC: PAP 90/26/45 mmHg, PAWP 19 mmHg, PVR 5.6 WU, CI 2.4 L/min/m2). Pulmonary embolism was excluded with ventilation/perfusion lung scan in both patients. Despite treatment with PAH specific medication, both patients died due to right heart failure. Patient 5 was classified as high output PH based on HAVMs (PAP 63/26/40 mmHg, PAWP 13 mmHg, PVR 2.0 WU; CI 6.4 L/min/m2). In patient 6 no PH was found (RHC: PAP 30/15/20 mmHg, PAWP 14 mmHg, PVR 0.4 WU, CI 8.7 L/min/m2), however, this patient showed a high CO due to HAVMs.
In the non-HHT subgroup, none of the controls were classified as high probability PH.

DISCUSSION

To our knowledge, this is the first large study reporting the estimated prevalence of PH in patients with genotyped confirmed HHT as compared to HHT negative controls. Our study demonstrates that the estimated prevalence of PH is increased in HHT as compared to HHT negative controls. This increase is most prominent in HHT2 and mainly results from the high CO state associated with HAVMs. However, HPAH is rare in HHT patients.
The prevalence of PH in HHT is only described in a few studies. A recently published study with 504 HHT patients showed a prevalence of PH of 5.6% and HPAH of 2.4%.

Table 1. Patient characteristics

	HHT1	HHT2	Controls	p-value (HHT1-controls)	p-value (HHT2-controls)
Patients, N	127	150	106		
Age (years)	44.2±15.6	48.9±14.2	38.6±12.8	0.002	<0.001
Sex, N					
Male	49 (38.6%)	61 (40.7%)	40 (37.7%)	0.9	0.7
Clinical diagnosis¶				<0.001	<0.001
Definite	117 (92.9%)	123 (82.0%)	0 (0%)		
Possible	9 (7.1%)	25 (16.7%)	34 (32.1%)		
Unlikely	0 (0%)	2 (1.3%)	72 (67.9%)		
Blood pressure (mmHg)					
Systolic	131.0±16.0	133.8±16.0	128.6±14.9	0.2	0.01
Diastolic	77.2±7.8	77.7±8.2	76.7±7.1	0.6	0.3
Other					
Saturation (%)	97.1±2.2	97.5±1.3	97.8±1.0	0.003	0.08
PaO2 (kPa)	11.6±2.0	12.1±1.5	12.5±1.5	0.001	0.04
Hb (mmol/L)	8.5±1.4	8.2±1.4	8.6±0.8	0.4	0.01
Hepatic involvement, N					
Abnormal GGT/ALP	4 (3.1%)	26 (17.3%)	0 (0%)	0.07	<0.001
*HAVM**	4 (3.1%)	22 (14.7%)	0 (0.0%)	0.07	<0.001
Pulmonary involvement, N					
TTCE: Pulmonary RLS	110 (86.6%)	58 (38.7%)	11 (10.4%)	<0.001	<0.001
Chest CT: PAVM	78 (61.4%)	15 (10.0%)	0 (0.0%)	<0.001	0.003
Echocardiography					
Peak TRV (m/s)	2.3±0.4	2.4±0.5	2.2±0.3	0.008	<0.001
RVSP (mmHg)	27.0±7.4	28.8±9.6	24.6±5.5	0.006	<0.001
RVDd (cm)	3.6±0.5	3.9±0.6	3.7±0.4	0.7	<0.001
RA area (cm2)	14.8±4.7	17.2±4.7	14.8±3.3	0.99	<0.001
LA area (cm2)	15.5±4.2	18.3±4.3	15.6±3.3	0.9	<0.001
LVEF (%)	59.6±8.9	64.1±6.6	61.8±8.3	0.08	0.04

*Characteristics are written in N (number) with percentage (%) or mean with standard deviation. HHT, hereditary haemorrhagic telangiectasia; mmHg, millimetre of mercury; PaO2, partial oxygen pressure; kPa; kilopascal; Hb, haemoglobin; mmol/L, millimoles per Liter; GGT, gamma-glutamyl transpeptidase; ALP, alkaline phosphatase; HAVM, hepatic arteriovenous malformation; TTCE, transthoracic contrast echocardiogram; RLS, right-to-left shunt; CT, computed tomography; PAVM, pulmonary arteriovenous malformations; TRV, tricuspid regurgitation velocity; m/s, meter per second; RVSP, right ventricular systolic pressure; RVDd, Right ventricular diastolic diameter; cm, centimetre; cm2, square centimetre; RA, right atrium; LA, left atrium; LVEF, left ventricular ejection fraction. ¶ Based on the Curaçao criteria[2] * Abdominal ultrasound/ CT only performed when history, physical examination or blood test results were suggestive for HAVMs.*

Table 2. Characteristics of patients with increased probability PH

	HHT1	HHT2	Controls
Patients, N	11 (8.7%)	27 (18.0%)	4 (3.8%)
Age (years)	55.2±16.3	57.9±14.2	41.6±18.0
Sex, N			
Male	4 (36.4%)	7 (25.9%)	1 (25.0%)
Blood pressure (mmHg)			
Systolic	129.4±15.0	132.9±16.3	135.0±21.8
Diastolic	74.5±8.8	75.8±7.0	83.3±11.5
Other			
Saturation (%)	97.3±0.9	96.8±2.2	96.3±1.2
PaO2 (kPa)	11.2±.2.0	11.5±1.3	10.0±1.1
Hb (mmol/L)	7.6±1.6	7.4 ±1.1	8.2±1.3
Hepatic involvement, N			
Abnormal GGT/ALP	1 (9.1%)	13 (48.1%)	0 (0.0%)
*HAVM**	1 (9.1%)	12 (44.4%)	0 (0.0%)
Pulmonary involvement, N			
TTCE: Pulmonary RLS	10 (90.9%)	13 (48.1%)	1 (25.0%)
Chest CT: PAVM	6 (54.4%)	3 (11.1%)	0 (0.0%)
Echocardiography			
TRV (m/s)	3.0±0.4	3.1±0.4	2.8±0.1
RVSP (mmHg)	42.2±9.9	43.9±10.5	38.3±2.5
RVDd (cm)	3.6±0.9	4.2±0.7	3.8±0.2
RA area (cm2)	17.5±10.2	19.6±6.3	16.3±1.4
LA area (cm2)	18.3±8.1	21.0±5.1	18.3±3.0
LVEF (%)	58.3±12.4	63.8±8.6	62.0±7.4
CO (L/min)	3.9±1.0	5.1±1.6	4.4±0.9

*Characteristics are written in N (number) with percentage (%) or mean with standard deviation. HHT, hereditary haemorrhagic telangiectasia; mmHg, millimetre of mercury; PaO2, partial oxygen pressure; kPa, kilopascal; Hb, Haemoglobin; mmol/L, millimoles per Liter; GGT, gamma-glutamyl transpeptidase; ALP, alkaline phosphatase; HAVM, hepatic arteriovenous malformation; TTCE, transthoracic contrast echocardiography; RLS, right-to-left shunt; CT, computed tomography; PAVM, pulmonary arteriovenous malformation; TRV, tricuspid regurgitation velocity; m/s, meter per second; RVSP, right ventricular systolic pressure; RVDd, right ventricular diastolic diameter; cm, centimetre; cm2, square centimetre; RA, right atrium; LA, left atrium; LVEF, left ventricular ejection fraction; CO, cardiac output; L/min, Litre per minute. * Abdominal ultrasound/ CT only performed when history, physical examination or blood test results were suggestive for HAVMs.*

Unfortunately, in this study genetic testing was only available in 8 patients [22]. Two other small studies using echocardiography also reported the estimated prevalence of PH in HHT patients [20,21]. Olivieri *et al*. reported an elevated echocardiography based right ventricular systolic pressure (RVSP) in 9 (20%) out of 44 HHT patients (22 *ACVRL1*, 3 *ENG*, 19 unknown mutation). In 7 out of these 9 subjects an *ACVRL1* gene mutation was found. Three patients were family members of patients with HPAH, one patient had a mitral valve stenosis and all others had HAVMs [20]. Sopena and colleagues found a high estimated prevalence (31%) of PH in 29 hospitalized patients with HHT with a mean RVSP of 73 ± 17.0 mmHg measured by echocardiography. In 67% of these patients HAVMs were found [21]. In contrast, in our current study increased probability PH was found in 14% of the HHT patients (13.7% in overall HHT group; 8.7% in HHT1 and 18.0% in HHT2). Therefore our results are not similar to the last two studies. The lower estimated prevalence as compared to the previous studies may be due to inclusion at time of screening, when most patients are asymptomatic.

In HHT, PH can be categorised in two distinct types. Firstly, PH as the result of a high pulmonary blood flow that accompanies the high CO state associated with HAVMs [11,13,29]. In patients with HAVMs, shunting of blood from the hepatic arteries and/or portal veins to the hepatic veins results in a hyperdynamic state, in which the CO can be elevated up to three fold [30]. Exercise testing in healthy persons revealed that an increase in CO leads to elevation in PAP (increase in mean PAP up to 0.5 to 3.0 mmHg/L/min) [31]. This implicates that a high CO state of 10 L/min will result in a mean PAP up to 30 mmHg without the presence of pulmonary arteriopathy. In HHT, a multifactorial cascade will eventually lead to high CO failure. First, the increase in CO will be compensated by dilation of the pulmonary arteries with normal pulmonary pressures. An increase in LA pressure will predispose patients for atrial fibrillation (due to enlargement of the LA) and diastolic dysfunction of the left ventricle. Increased LA pressure and impaired pulmonary vasodilatation will eventually result in PH. The combination of volume and pressure overload leads to RV dilation, decreased RV systolic function and subsequent right heart failure. Especially in HHT, severe bleeding (e.g. epistaxis or gastro-intestinal bleeding) and anaemia, which many patients experience, may trigger this cascade due to increase in CO [11,29,30]. This hypothesis was confirmed in our study, as haemoglobin was a predictor for increased probability PH (OR 0.6: 95% CI 0.5-0.7).

HAVMs are typically seen in HHT2, however HAVMs in patients with HHT1 have also been reported [23,32]. Our study supports this pattern, as HAVMs were found in 3.1% of HHT1 and 15.3% of HHT2. Interestingly, HAVMs were diagnosed in 44.4% in the subgroup of HHT2 with increased probability PH, which resulted in an increased CO. Moreover, HAVMs were an important predictor for increased probability PH (OR 11.3: 95% CI 4.8-26.7). Secondly, in HHT, pre-capillary HPAH due to the HHT-related gene mutations in *ENG* or especially *ACVRL1* might develop [14-19]. Another TGFβ receptor, *bone morphogenetic*

Table 3. Predictors for increased probability PH

	TRV ≤ 2.8 m/s	TRV > 2.8 m/s	Univariate analysis (OR [95% CI])	p-value
Patients, N	341 (89.0%)	42 (11.0%)		
Age (years)	43.3±14.3	55.6±15.4	1.1 [1.0-1.1]	<0.001
Sex, N				
Male	138 (40.5%)	12 (28.6%)	1.7 [0.8-3.4]	0.1
Mutation, N				0.002
ENG	116 (34.0%)	11 (26.2%)	2.4 [0.7-7.8]	0.1
ACVRL1	123 (36.1%)	27 (64.3%)	5.6 [1.9-16.5]	0.002
Control	102 (29.9%)	4 (9.5%)	Reference	
Blood pressure (mmHg)				
Systolic	131.3±15.8	132.1±15.9	1.0 [1.0-1.0]	0.8
Diastolic	77.4±7.7	76.0±8.0	1.0 [0.9-1.0]	0.3
Other				
Saturation (%)	97.5± 1.6	96.9±1.9	0.8 [0.7-1.0]	0.05
PaO2 (kPa)	12.1±1.7	11.3±1.5	0.8 [0.6-0.9]	0.01
Hb (mmol/L)	8.5±1.2	7.6±1.2	0.6 [0.5-0.7]	<0.001
Hepatic involvement, N				
Abnormal GGT/ALP	16 (4.8%)	14 (33.3%)	9.9 [4.4-22.4]	<0.001
*HAVM**	13 (3.8%)	13 (31.0%)	11.3 [4.8-26.7]	<0.001
Pulmonary involvement, N				
TTCE: Pulmonary RLS	155 (45.5%)	24 (57.1%)	1.6 [0.8-3.0]	0.2
Chest CT: PAVM	84 (24.6%)	9 (21.4%)	0.8 [0.4-1.8]	0.6
Echocardiography				
LA area (cm2)	16.2±3.8	20.0±5.9	1.2 [1.1-1.3]	<0.001
LVEF (%)	61.8±8.0	62.2±9.6	1.0 [1.0-1.1]	0.8

*Characteristics are written in number (N) with percentage (%) or mean with standard deviation. TRV, tricuspid regurgitation velocity; m/s, meter per second; ACVRL1, activin receptor-like kinase 1; mmHg, millimetre of mercury; PaO2, partial oxygen pressure; kPa, kilopascal; Hb, Haemoglobin; mmol/L, millimoles per Liter; GGT, gamma-glutamyl transpeptidase; ALP, alkaline phosphatase; US, ultrasound; HAVM, hepatic arteriovenous malformation; TTCE, transthoracic contrast echocardiography; RLS, right-to-left shunt; CT, computed tomography; PAVM, pulmonary arteriovenous malformation; cm2, square centimetre; LA, left atrium; LVEF, left ventricular ejection fraction. * Abdominal ultrasound/ CT only performed when history, physical examination or blood test results were suggestive for HAVMs.*

protein receptor type II (*BMPRII*), was already known for its association with HPAH [33]. In 2001, it was demonstrated that different mutations in *ACVRL1* predispose patients for the development of HPAH [14]. This association was confirmed in a few case series describing the presence of HPAH in patients with an *ACVRL1* mutation and clinical features of HHT [8,14,15,17-19]. Trembath *et al*. described that mutations in *ACVRL1* may lead to both occlusion of the pulmonary arteries, resulting in HPAH, together with vascular dilatation,

manifesting as AVMs in HHT [14]. Importantly, HPAH patients with an *ACVRL1* mutation are diagnosed at a younger age and have worse prognosis than other patients with HPAH [8]. *ENG* mutations have also been identified in patients with both HHT and HPAH, although this is described less frequently [16,17,34].

Although HPAH is rare in HHT, in this study 0.7% of HHT patients (1.3% of HHT2 patients) screened by Doppler echocardiography were found to have PH clinically attributable to HPAH. Therefore, the estimated prevalence of HPAH in HHT patients seems increased as compared to the general population (HPAH prevalence 5.9 to 50 per one million persons) [9,35,36]. We found no HPAH in HHT1. However, for this very rare condition, even our large study might be too small.

Other etiologies of PH [9,28,37] might also be present in HHT patients (e.g. PH due to diastolic dysfunction of the left ventricle or chronic thromboembolic pulmonary hypertension (CTEPH)). Since we used a control group it is less likely that these factors contributed to the difference in TRV between the groups. However, given the substantial age and LA area difference between patients with and without increased probability, diastolic dysfunction of the LV could have contributed to the increase in TRV found in HHT. Alternatively, high CO could also lead to increased LA pressure resulting in enlargement of the LA, which makes patients more prone for diastolic dysfunction. The prevalence of left heart failure increases with age, with a low prevalence in patients under 65 years of age (prevalence 0.9% in the general population aged 55-64 years) [38]. The mean age of HHT patients with increased probability PH is approximately 55 years in our study, therefore it seems less likely that isolated diastolic dysfunction of the left ventricle could have had significant influence on the prevalence of PH [39,40]. We did not rule out CTEPH in all patients with an increased TRV and there is some evidence that HHT patients have a higher coagulability [41]. However, CTEPH was excluded in patients diagnosed with HPAH and there is no literature describing an increased prevalence of CTEPH in HHT.

Because of the potentially fatal prognosis, the non-specific symptoms and the increased estimated prevalence of PH in HHT, we recommend to refer all HHT patients to an HHT centre of excellence.

Study limitations

This study has some limitations. First, we could only measure a reliable TRV in 66%, it is possible that we missed some subjects with a high TRV. However, other parameters, such as the RV dimension and RA dimensions (mean RV dimension 3.7 ± 0.5 cm and RA area 15.4 ± 4.1 cm^2), that could suggest PH were normal in all excluded subjects [42]. Therefore, this subgroup could be classified as low probability PH [9]. Second, invasive hemodynamic measurements were only performed in a few patients, as supported by the guidelines [9]. Therefore, it is possible that the estimated amount of PH is overrated and does not reflect the true proportion of patients. Third, it is possible that we missed some

patients with HAVMs, because imaging (Doppler ultrasound) was not performed in all patients. Importantly, most HAVMs are small and do not lead to symptoms or abnormal biomarkers [32]. High morbidity and mortality is only associated with symptomatic HAVMs. Hence, the international guidelines recommend screening for HAVMs with Doppler ultrasound only when medical history, physical examination or blood test results are suggestive [2]. However, this could have led to bias toward the presence of HAVMs in HHT2 and PH patients. Fourth, since no mutation is found in *ENG* or *ACVRL1* in approximately 2% of HHT patients, this study does not apply for this small subgroup [23]. Last, no multivariable analysis was performed. Future studies including more patients might provide more insight into this subject by providing adjusted associations.

CONCLUSION

The estimated prevalence of PH is increased in patients with HHT as compared to non-HHT controls. The estimated prevalence of PH is particularly high in patients with HHT2 and results from the high CO state due to the presence of HAVMs. HPAH appears to be rare in HHT patients (0.7%), and was only diagnosed in HHT2 (1.3%).

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CHAPTER 8.2

Pulmonary hypertension in hereditary haemorrhagic telangiectasia

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ABSTRACT

Hereditary haemorrhagic telangiectasia (HHT) is an autosomal dominant inherited disorder characterised by vascular malformations in predominantly the brain, liver and lungs. Pulmonary hypertension (PH) is increasingly recognised as a severe complication of HHT. PH may be categorised into two distinct types in patients with HHT. Post-capillary PH most often results from a high pulmonary blood flow that accompanies the high cardiac output state associated with liver arteriovenous malformations. Less frequently, the HHT-related gene mutations in ENG or ACVRL1 appear to predispose patients with HHT to develop pre-capillary pulmonary arterial hypertension (PAH). Differentiation between both forms of PH by right heart catheterisation is essential, since both entities are associated with severe morbidity and mortality with different treatment options. Therefore all HHT patients should be referred to an HHT centre.

HEREDITARY HAEMORRHAGIC TELANGIECTASIA

Hereditary haemorrhagic telangiectasia (HHT), also known as Rendu-Osler-Weber syndrome, is an autosomal dominant inherited disorder with late onset penetrance (nearly 97% at the age of 60 years) characterised by vascular malformations with an estimated prevalence of 1:5000 individuals [1,2]. The abnormal vascular structures in HHT range from small telangiectasia of the skin and mucosal membranes to arteriovenous malformations (AVMs) in predominantly the brain, liver and lungs [3,4].

Genetics and pathogenesis

HHT consist of two main subtypes, HHT type 1 and HHT type 2, which results from mutations in the *ENG* gene on chromosome 9, encoding the protein endoglin and from mutations in the *activin receptor-like kinase (ACVRL1)* gene on chromosome 12, encoding the protein ALK-1 respectively.[5,6] A third disease-causing mutation has been found in the *SMAD4* gene, causing a combination of the juvenile polyposis syndrome and HHT [7]. Most HHT families have a unique mutation and many types of mutations have been described. The exact pathogenesis of HHT is still unclear. However, hypoxia or local hemodynamic changes could act as a possible triggers promoting tissue inflammation or endothelial cell injury [8,9]. Both endoglin, ALK-1 and SMAD4 proteins are endothelial receptors of the transforming growth factor β (TGF-β) superfamily. All three proteins cooperate in the TGF-β/ALK-1 signalling pathway, which is involved in angiogenesis. In HHT, most vessels are normal, but the mutations in *ACVRL1* and *ENG* result in abnormal angiogenetic responses and lead to the formation of abnormal ateriovenous connections, ranging from small telangiectases that bleed easily, to large arteriovenous malformations, that can occur in every organ, but especially in the lungs, liver and brain [10,11] .

Diagnosis

The clinical diagnosis can be based on the four Curaçao criteria [1], which consist of: (1) Spontaneous, recurrent epistaxis; (2) Multiple telangiectasia at characteristic sites (lips, oral cavity, fingers, nose); (3) Visceral lesions (gastrointestinal telangiectasia, pulmonary, hepatic, cerebral or spinal AVMs); and (4) A first degree relative with HHT. Three criteria suffice for a definitive diagnosis of HHT, two criteria are considered as 'possible' HHT, and one or no criterion makes the diagnosis 'unlikely'. The positive predictive value for a definite clinical diagnosis and the negative predictive value for an unlikely diagnosis are excellent (100% and 97.7% respectively), when compared with DNA testing [12]. However, HHT has an age dependent penetrance and the clinical presentation varies among patients [1]. Therefore genetic testing has emerged as an important tool to help make the diagnosis in paediatric patients and younger adults with a 'possible' clinical diagnosis [12].

PULMONARY HYPERTENSION

Pulmonary hypertension (PH) is a haemodynamic and pathophysiological condition defined as an increase in mean pulmonary arterial pressure (mPAP) of equal to or more than 25 mmHg as assessed by right heart catheterisation (RHC) [13]. PH is a progressive disease of many origins, affecting more than 100 million people worldwide [14]. The elevated pressure in the pulmonary circulation can lead to various symptoms including limited exercise capacity and dyspnoea on exertion. The chronic elevated pressure may ultimately result in right-sided heart failure and premature death [13]. Depending on the origin, PH can be divided into two main groups; pre- and post capillary PH. Patients with pre-capillary PH are characterised by a mPAP ≥ 25 mmHg, pulmonary artery wedge pressure (PAWP)≤ 15 mmHg, and elevated pulmonary vascular resistance (PVR) (> 3 Wood units) [15]. Pre-capillary PH can be further divided in different clinical groups, based on pathophysiological mechanisms, clinical presentation and therapeutic options (table 1) [13].

Transthoracic echocardiography is the cornerstone for screening in all patients suspected of PH. Typically, a dilatation of the right ventricle with septal flattening (also called D-sign) and an increase in right ventricular systolic pressure (RVSP) (sum of right ventricle-right atrium pressure gradient and estimated pressure in the right atrium based on the dimension and collapse of the inferior caval vein) (figure 1) [13,16].

PULMONARY HYPERTENSION AND HEREDITARY HAEMORRHAGIC TELANGIECTASIA

PH is increasingly recognised as an important complication of HHT. HHT associated PH can occur by several mechanisms. Most often, post-capillary PH may develop as a consequence of a hyperkinetic state resulting in heart failure associated with high cardiac output (CO) due to hepatic arteriovenous malformations (HAVMs) (figure 2) [17], while less frequently, precapillary PH can be related to pulmonary arterial hypertension (PAH) characterised by remodeling of small pulmonary arteries with broadly similar histologic lesions than observed in idiopathic PAH [17]. The HHT-related gene mutations (*ENG* or *ACVRL1*) appear to predispose for the development of PAH [9,18-22]. Various studies found a high estimated prevalence of PH in HHT when screening with echocardiography [23,24]. An elevated RVSP on echocardiography was found in 9 (20.5%) out of 44 HHT patients (22 *ACVRL1*, 3 *ENG*, 19 unknown mutation), in 7 out of these 9 subjects an *ACVRL1* gene was found [23]. Sopena *et al*. [24] found a high estimated prevalence of PH (31%) in 29 hospitalised patients with HHT with a mean estimated RVSP of 73 ± 17.0 mmHg measured with echocardiography. HAVMs were documented in 67% of these patients. However, large observational studies including consecutive HHT patients are lacking.

Table 1. Updated classification of pulmonary hypertension

1. Pulmonary arterial hypertension
   - 1.1 Idiopathic PAH
   - 1.2 Hereditable PAH
   - 1.2.1 *BMPR2*
   - 1.2.2 *ACVRL1*, *ENG* (with or without hereditary haemorrhagic telangiectasia), *SMAD9*, *CAV1*, *KCNK3*
   - 1.2.3 Unknown
   - 1.3 Drug and toxin induced
   - 1.4 Associated with:
     - 1.4.1 Connective tissue diseases
     - 1.4.2 HIV infection
     - 1.4.3 Portal hypertension
     - 1.4.4 Congenital heart diseases
     - 1.4.5 Schistosomiasis

1′ Pulmonary veno-occlusive disease and/or pulmonary capillary haemangiomatosis

1″ Persistent pulmonary hypertension of the newborn (PPHN)

2. Pulmonary hypertension due to left heart disease
   - 2.1 Left ventricular systolic dysfunction
   - 2.2 Left ventricular diastolic dysfunction
   - 2.3 Valvular disease
   - 2.4 Congenital/ acquired left heart inflow/outflow tract obstruction and congenital cardiomyopathies

3. Pulmonary hypertension due to lung diseases and/or hypoxia
   - 3.1 Chronic obstructive pulmonary disease
   - 3.2 Interstitial lung disease
   - 3.3 Other pulmonary diseases with mixed restrictive and obstructive pattern
   - 3.4 Sleep-disordered breathing
   - 3.5 Alveolar hypoventilation disorders
   - 3.6 Chronic exposure to high altitude
   - 3.7 Developmental abnormalities

4. Chronic thromboembolic pulmonary hypertension (CTEPH)

5. PH with unclear and/or multifactorial mechanisms
   - 5.1 Haematological disorders: chronic haemolytic anaemia, myeloproliferative disorders, splenectomy
   - 5.2 Systemic disorders: sarcoidosis, pulmonary histiocytosis, lymphangioleiomyomatosis,
   - 5.3 Metabolic disorders: glycogen storage disease, Gaucher disease, thyroid disorders
   - 5.4 Others: tumoural obstruction, fibrosing mediastinitis, chronic renal failure, segmental PH

PAH, pulmonary arterial hypertension; BMPR2, bone morphogenetic protein receptor type 2; ACVRL1, activin receptor-like kinase; CAV1, caveolin-1; HIV: human immunodeficiency virus. Adapted from Simonneau et al.[46], with permission of the publisher.

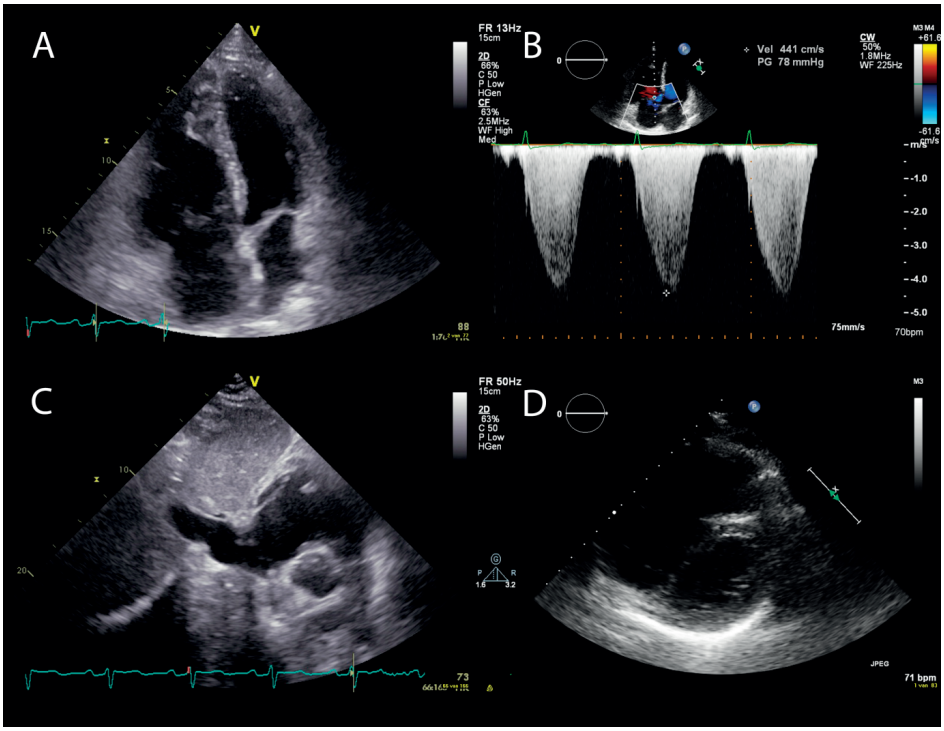

Figure 1. Characteristic echocardiogram of a patient with pulmonary hypertension

a. Apical 4-chamber view showing dilatation of the right ventricle. b. Apical 4-chamber view with Doppler signal (continuous wave) showing an increased right ventricular- right atrial pressure gradient (4.4 m/s). c. Subcostal view showing dilatation of the inferior caval vein corresponding with an increased pressure in the right atrium. d. Parasternal short axis view showing flattening of the interventricular septum (D-sign) and dilatation of the right ventricle.

Since the treatment strategies differ between post-capillary high-output PH and pre-capillary PAH, it is important to differentiate between these two different entities. RHC is the gold standard for making the diagnosis of both high-output PH and PAH [13,17,25]. In PAH, the mPAP is usually higher with an increase in PVR and transpulmonary gradient due to arteriopathy. Most often a normal or decreased CO and PAWP is seen. In high-output PH on the other hand, there is only a moderate increase in mPAP, with a normal PVR, elevated PAWP and most importantly, an increased CO (table 2) [13,17].

HIGH OUTPUT PULMONARY HYPERTENSION

High-output heart failure is the most common initial presentation of HAVMs in HHT. Liver involvement is present in 32-78% of the HHT patients and is predominantly seen in HHT type 2 [1,26-28]. The presence of symptoms is directly associated with significant morbidity and mortality and therefore, screening for liver AVMs with Doppler ultrasound is warranted

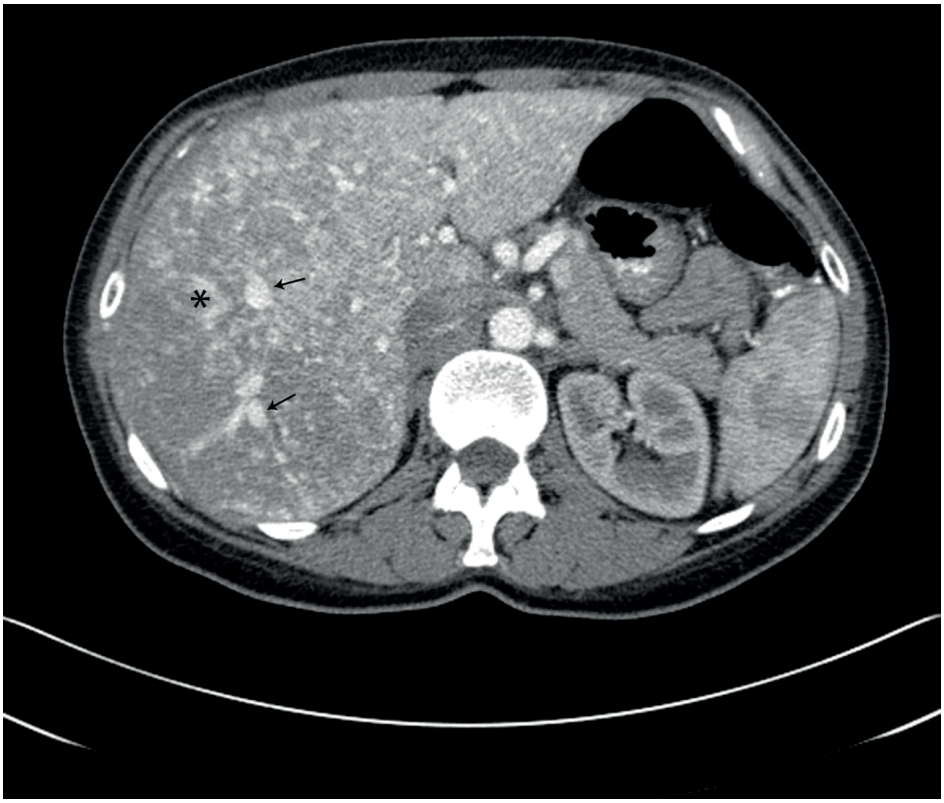

Figure 2. Hepatic arteriovenous malformations
Computed tomography with contrast in arterial fase showing extensive filling of the hepatic veins (arrows), and diffuse hepatic arteriovenous malformations (asterix).

Table 2. Haemodynamics in pulmonary hypertension associated with hereditary haemorrhagic telangiectasia

	High output PH	PAH
mPAP (mmHg)	+	++
PAWP (mmHg)	=/+	=
PVR (Wood units)	=	++
CO (L/min)	++	-

PH, pulmonary hypertension; PAH, pulmonary arterial hypertension; mPAP, mean pulmonary artery pressure; PAWP, pulmonary artery wedge pressure; PVR, pulmonary vascular resistance; CO, cardiac output. +, increase; =, normal; -, decrease. Adapted from Faughnan et al.[17], with permission of the publisher.

in all patients who are symptomatic or have abnormal liver enzymes [1,25]. In the majority of cases, only small telangiectasia are seen, which do not lead to symptoms. However, large HAVMs exist in typically three different and often concurrent types of intrahepatic shunting; from the hepatic arteries to hepatic veins, from the hepatic arteries to portal veins, and from the portal veins to hepatic veins [17,25]. These hepatic shunts can lead to high-output cardiac failure, portal hypertension, biliary ischaemia or encephalopathy with a wide range of symptoms [25]. Overall, symptoms due to HAVMs occur in 8% of HHT patients and predominantly in females [29]. Symptoms of high-output cardiac failure usually develop in females between 50 and 70 years of age and are characterised by dyspnoea on exertion, fatigue, orthopnoea, ascites and/or oedema [17,29].

Pathophysiology of high output PH

Exercise testing in healthy persons revealed that an increase in CO leads to an elevation in pulmonary artery pressure (increase in mPAP up to 0.5 to 3.0 mmHg/L per minute) [30]. In patients with HAVMs, shunting of blood from the hepatic arteries and/or portal veins to the hepatic veins results in a hyperdynamic state, in which the CO can be elevated two-to-three fold [31]. Besides this cause of high cardiac output, severe epistaxis or gastro-intestinal bleeding in patients with HHT may lead to anaemia with a compensatory increase in CO as well.

In HHT, a multifactorial cascade will eventually lead to high-output cardiac failure. At first, the increase in CO will be compensated by dilatation of the pulmonary arteries and thereby pulmonary pressure will still be maintained. An increase in left atrial (LA) pressure will predispose patients for atrial fibrillation (due to enlargement of the LA) and diastolic dysfunction of the left ventricle. Increased LA pressure and impaired pulmonary vasodilatation will eventually result in PH. The combination of volume and pressure overload leads to right ventricular (RV) dilatation, decreased systolic function of the RV and subsequent right heart failure. Severe bleeding (e.g epistaxis or gastro-intestinal bleeding) may trigger the cascade because of the subsequent increase in CO [17,29,31].

Treatment of high output PH

The first-line treatment of PH associated with a high-output state consists of intensive medical treatment including salt restriction and diuretics, correction of anaemia, antihypertensive and antiarrhythmic agents and digoxin if necessary [9]. In patients refractory to medical-therapy, liver transplantation is the best option, with a 5-year survival of 83% in a series of 40 patients [29]. However, a high post-operative morbidity is seen [25,32]. Recently, Dupuis-Girod *et al*. [33] treated 25 patients with severe HAVMs and a high CO (median cardiac index (CI) 5.1 L/min per square meters (range 4.1-6.2 L/min per square meters)) with bevacizumab, a vascular endothelial growth factor inhibitor. This treatment resulted in a significant decrease in CO (median CI at 6 months 4.1 L/min per square

meters (range 3.0-5.1 L/min per square meters), normalisation of the pulmonary pressure in 5 out of 8 patients with PH at baseline and clinical improvement of dyspnoea [33].Other invasive treatments such as surgical hepatic artery ligation or transcatheter therapeutic embolisation of the hepatic artery are associated with a high morbidity and mortality and therefore not recommended [1,17].

PULMONARY ARTERIAL HYPERTENSION

PAH is a clinical condition characterised by the presence of pre-capillary PH due to arteriopathy with media hypertrophy and intima proliferation. It is increasingly recognised as a severe complication of HHT. There have been a few case series that describe the association between PAH and HHT, however these series all included patients with PH in which HHT symptoms were also present [18-22].

Pathophysiology and genetics of PAH

In 2001, it was demonstrated for the first time that different mutations in *ACVRL1* predispose patients for the development of PAH [18]. This was confirmed in a few case series describing the presence of PAH in patients with an *ACVRL1* mutation and clinical features of HHT [19,21,22]. Trembath *et al*. [18] described that mutations in *ACVRL1* may lead to both occlusion of the pulmonary arteries together with vascular dilatation, manifested as AVMs in HHT. Although less frequently, *ENG* mutations have also been identified in patients with both HHT and PAH, suggesting a less potent association between endoglin and PAH [18,19]. Mutations in the *bone morphogenic protein receptor type (BMPR2)* gene, which is another gene encoding the endothelial surface protein components of the TGF-β receptor that is detected in approximately 70% of the patients with hereditable PAH, were not found in HHT associated PAH [34].

Prognosis

The clinical outcomes of patients with PAH caused by an *ACVRL1* mutation have been analysed in 32 patients and compared to other PAH patients without this mutation. PAH caused by an *ACVRL1* mutation was found in significantly younger patients (mean age 21.8 ± 16.7 years) and had a significantly shorter survival, despite similar therapy [34]. No data exist about the prognosis of patients with PAH and *ENG* mutations. The overall prognosis of PAH in general ranges from 6 months to several years based on the underlying disease [13]. It is noteworthy that *ACVRL1* mutation carriers may develop severe PAH without any clinical evidence of HHT because of the early development of PAH in these patients and the late-onset penetrance of *ACVRL1* mutation for HHT manifestations [34].

Treatment of PAH in HHT

No systematic evidence exists for treatment of HHT patients with PAH. It seems rational to treat patients according to the guidelines for PAH, with PAH-specific medication and supporting therapy (diuretics, oxygen, and digoxin) [13]. Today there are three different groups of PAH specific medication; endothelin receptor antagonists (ERA), phosphodiesterase inhibitors (PD5I) and prostacyclins. There are two case-reports that describe successful treatment of PAH in HHT patients with the ERA bosentan. After treatment, improvement of symptoms, exercise capacity and laboratory findings and a decrease in mPAP were found [35,36]. There are no reports describing the treatment with other PAH specific medication in patients with PAH and HHT. Since there was no response to acute vasodilator challenge in 32 patients with HHT and PAH, there is probably no role for the use of calcium channel blockers in this population [17,34]. And due to an increase in bleeding complications regular treatment with oral anticoagulation is not advised [1]. However, based on recent literature, treatment with anticoagulation could be considered on a case by case basis [37].

Pulmonary arteriovenous malformations in PH

The coexistence of PH and pulmonary arteriovenous malformations (PAVMs) has specific clinical and therapeutic implications. PAVMs are low-resistance, high-flow abnormal vascular structures that bypass the normal capillary filter and thereby result in permanent pulmonary right-to-left shunting (figure 3) [38-40]. Paradoxical embolisation through these PAVMs can lead to severe neurological complications, such as a stroke or brain abscess [1,40]. Contrast echocardiography is the screening test of choice (sensitivity up to 98.6%), with a direct relationship between shunt grade and prevalence of cerebral manifestations in patients screened for HHT [40-42]. To avoid neurologic and bleeding complications, PAVMs can be treated with transcatheter embolisation with coils or plugs (figure 3d) [1,43]. It may be expected that closing this low resistance system will result in a rise in mPAP. Measuring the pulmonary pressure before and after embolisation of PAVMs in 43 patients, Shovlin *et al*. [44] found no significant found no significant increase in mPAP after emboliation, even in patients with pre-existing mild to moderate PH. A possible explanation is a decrease in CO after embolisation which has a greater effect on the PVR than occlusion of the PAVMs. This fall in CO immediately after PAVM closure was recently described in 29 HHT patients by Vorselaars *et al*.[45] Furthermore, PAVM-related hypoxemia can induce vasoconstriction with a concomitant increase in PVR. Both studies described an increase in saturation after embolisation which may result in a decrease in pulmonary vasoconstriction and thereby PVR [44,45]. One case report described a fatal rupture of a PAVM in a patients with severe PAH. Although patients with severe PH were excluded from the above studies, it would be prudent to consider that the higher the mPAP and PVR at baseline and the larger the PAVM, the greater likelihood of worsening PH after embolisation [44].

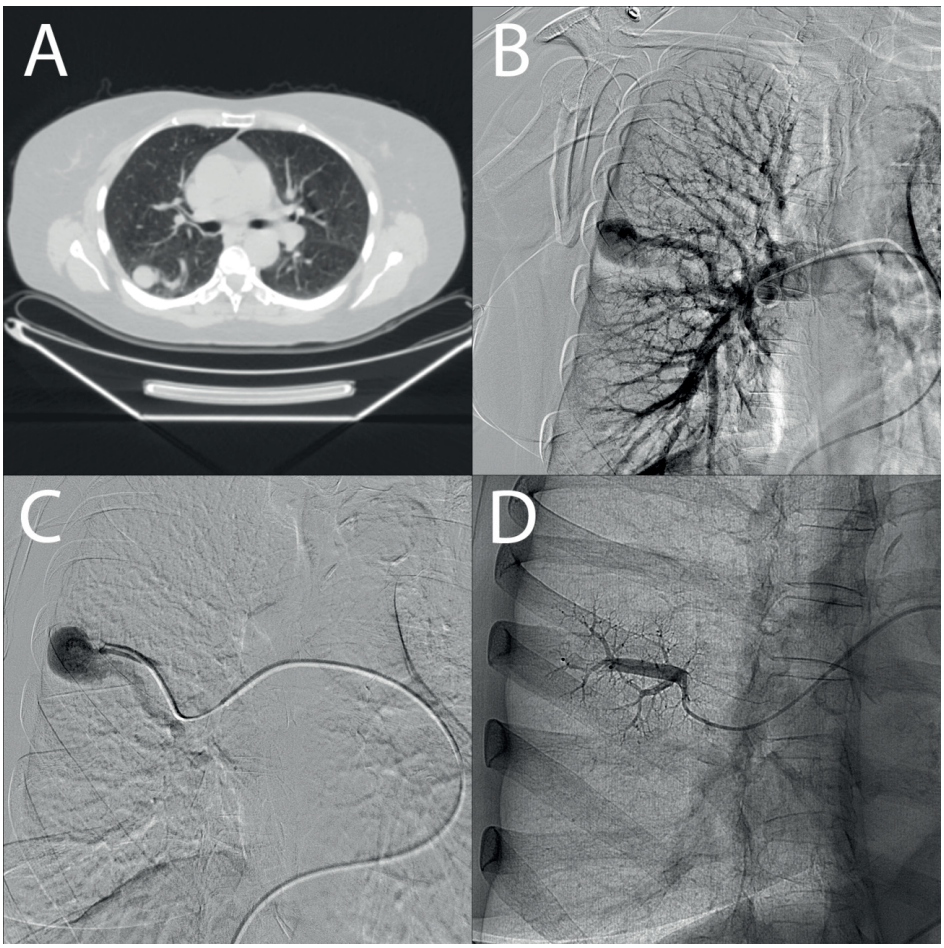

Figure 3. Pulmonary arteriovenous malformations
a. Computed tomography of the chest with large pulmonary arteriovenous malformation (PAVM) in the lower lobe of the right lung. b. Pulmonary angiogram of the PAVM in the same patient. c. Selective pulmonary angiogram of the PAVM in the apex of the lower lobe of the right lung. d. Repeat angiogram after transcatheter embolisation of the PAVM with a vascular plug.

FURTHER RESEARCH AND RECCOMDATIONS

Although a number of studies described patients with PH and HHT, no data are available about the exact prevalence of PH in the overall HHT population. Most studies used a small sample size of highly selected patients and data from RHC are lacking. Therefore we recommend to perform a systematic screening to reveal the true prevalence of both forms of PH with their different aetiologies in a HHT population. Because of the non-specific symptoms and potentially fatal prognosis, all HHT patients should be referred to an HHT centre of excellence.

CONCLUSION

PH is increasingly recognised as a severe complication of HHT, but the true prevalence of PH in HHT is still unknown. PH in HHT is mostly post-capillary in origin and results from high cardiac output due to HAVMs and anaemia. Rarely *ACRVL1* or *ENG* mutations results in pre-capillary PAH. Differentiation between both forms of PH in HHT by RHC is essential, since both entities are associated with severe morbidity and mortality with different specific treatment options. Therefore all HHT patients should be referred to an HHT centre.

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CHAPTER 8.3

Pulmonary arterial hypertension and hereditary haemorrhagic telangiectasia

Submitted

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ABSTRACT

Hereditary haemorrhagic telangiectasia (HHT) is an autosomal dominant inherited disease characterised by multi-systemic vascular dysplasia. Heritable pulmonary arterial hypertension (HPAH) is a rare but severe complication of HHT. Both diseases can be the result of genetic mutations in *ACVLR1* and *ENG* encoding for proteins involved in the transforming growth factor-beta (TGF-β) superfamily, a signalling pathway which is essential for angiogenesis. Changes within this pathway can lead to both the proliferative vasculopathy of HPAH and arteriovenous malformations seen in HHT. Clinical signs of the disease combination may not be specific but early diagnosis is important for appropriate treatment. This review describes the molecular mechanism and management of HPAH and HHT.

PULMONARY ARTERIAL HYPERTENSION

Pulmonary arterial hypertension (PAH) is a rare (25/million cases), but severe vascular disorder with increased mean pulmonary arterial pressures (mPAP) as a result of vascular remodelling [1]. Proliferation of endothelial cells (EC) and vascular smooth muscle cells reduce the intraluminal space of the pulmonary arterioles thereby increasing the arterial pressure, eventually leading to right ventricular failure.

PAH is defined by an increased mPAP (of ≥ 25 mmHg at rest), pulmonary capillary wedge pressure ≤15 mmHg and pulmonary vascular resistance of > 3 Wood units, for all of which the gold standard of measurement is a right heart catheterisation (RHC), although right ventricular pressure measurement on an echocardiogram can also give an estimation of the severity of the disease [1]. Clinical features are the result of decrease in cardiac output due to right heart failure and include progressive dyspnoea, decreased exercise tolerance and fatigue.

PAH is associated with several conditions and factors including specific drugs (anorexigens), congenital left-to-right shunt, connective tissue disease, human immunodeficiency virus, several genetic mutations which are known as heritable PAH (HPAH). Idiopathic PAH is a diagnosis per exclusionem when no underlying cause is found. Mutations in the bone morphogenetic protein receptor (*BMPR2*) are most frequently described in HPAH, however other associated genetic mutations include *ACVLR1, ENG* and *BMP9* which are also associated with hereditary haemorrhagic telangiectasia (HHT) [2,3]. These genes encode for proteins that play a role in the transforming growth factor-beta (TGF-β) superfamily signalling pathway.

HEREDITARY HAEMORRHAGIC TELANGIECTASIA

HHT, also known as Rendu-Osler-Weber disease (ROW), is an autosomal dominant inherited disease with multi-systemic vascular dysplasia characterised by mucocutaneous telangiectasia, arteriovenous malformations (AVMs) and recurrent spontaneous epistaxis [4]. The estimated worldwide prevalence is at least 1 in 5000 individuals. The majority of cases is caused by mutations in the *ENG* or *ACVRL1* gene, causing a haploinsufficiency with reduced levels of functional proteins of Endoglin and Activin receptor like kinase 1 (ALK1), respectively. These mutations can be found in up to 95.7% of HHT patients [5]. *ENG* mutations cause HHT type 1 which is characterised by a higher prevalence of pulmonary and cerebral AVMs, mucocutaneous telangiectasia and epistaxis compared to *ACVRL1* mutations, or HHT type 2. The second has a higher prevalence of hepatic AVMs.

Patients with HHT, especially women with an *ENG* mutation, who have not been screened and treated pre-emptively have a slightly lower life expectancy than family members without HHT and severe epistaxis can result in a decreased quality of life [6]. Complications from pulmonary AVMs mainly include hypoxemia and paradoxal (sterile or septic)

emboli, although many patients remain asymptomatic. Hepatic shunting can lead to portal hypertension, biliary necrosis and high output cardiac failure due at least two-to three fold elevation of cardiac output [7,8]. Complications of cerebral AVMs are rare (approximately 0.5% per year) but its consequence can be devastating [9]. Clinical signs are not only variable in subtype but also variable in severity between family members with identical mutations [10]. Etiological factors and genetic modifiers are thought to explain this clinical variability [11,12].

MOLECULAR MECHANISM

The TGF-β superfamily signalling pathway has been recognised to play an important role in different cellular processes including proliferation, migration and apoptosis [13]. The TGF-β is a complex pathway which plays a pivotal role in the process of angiogenesis using two distinct signalling pathways; the activin receptor-like kinase 5 (ALK5)–Smad2/3 pathway and the ALK1–Smad1/5/8 pathway [14,15] (figure 1). Although much research has been done on the effects of ALK1, its role in angiogenesis has been shown inconsistent [16-18]. When vessels are formed EC migrate and proliferate. Once the capillary wall is formed, pericytes help stabilize the vessel and inhibit EC proliferation and migration. This leads to vascular maturation, a process in which ALK5 plays an important role. Endoglin is upregulated by ALK1 and is an accessory receptor in the TGF-β signalling pathway which is particularly expressed on proliferating EC. [19] It has been found that endoglin counterbalances the stabilizing role of ALK5 [20]. Mutations in *ENG* and *ACVRL1* genes disrupt TGF-β signaling, altering EC tubulogenesis and pericyte recruitment causing abnormal capillary formation and maturation leading to venous enlargement, vascular hyperbranching and arteriovenous malformations explaining the abnormal morphogenesis of vasculature in HHT [14,21].

These EC also regulate vascular function by controlling the production of vasoconstrictors, vasodilators and the activation and inhibition of smooth muscle cells (SMC). Disruption of the SMAD1/5/8 pathway and BMP signalling, as a consequence of a *BMPR2* or *ACVRL1* mutation, results in inhibition of apoptosis of SMC leading to SMC proliferation and vascular remodelling, ultimately causing PAH [22-24]. Interestingly, both these diseases originate in defects in the BMP9/ALK1/Endoglin pathway (figure 1). BMPR2 forms a signaling complex with ALK1 which responds to BMP9 by binding with high affinity to ALK1 and Endoglin [2,25]. A case report has shown that a mutation in BMP9 can lead to a syndrome with phenotypic similarities with HHT [26]. Recently, BMP9 has been used in animal studies to treat PAH by stimulating BMPR2 signalling. So hypothetically it might be possible that BMP9 treatment has a therapeutic effect on HHT [27,28].

PAH AND HHT

Heritable PAH is a rare but severe complication of HHT. *ACVRL1* mutations have been recognised to lead to this combined syndrome for several years. Thirty-nine patients with PAH and *ACVRL1* mutations have been described in literature [29-39]. Many different

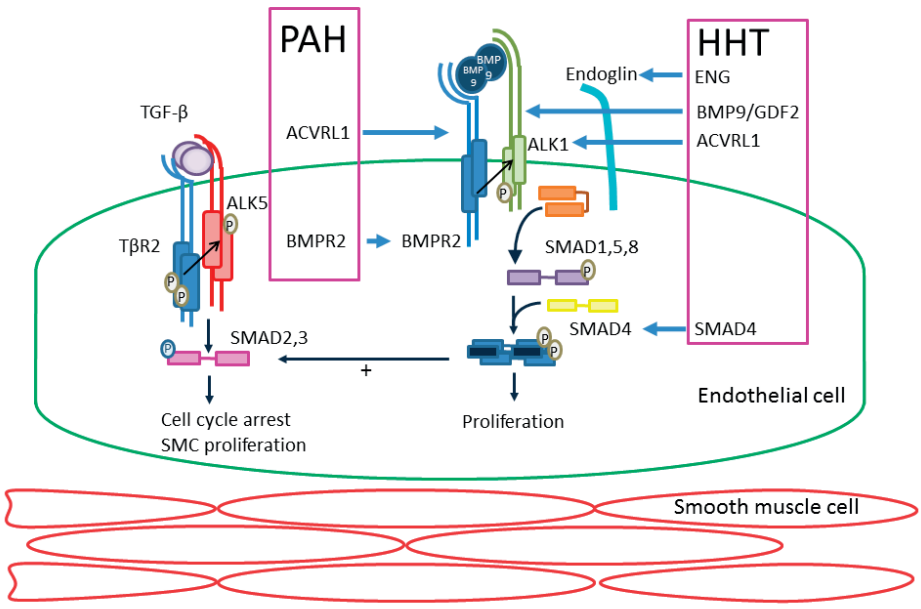

Figure 1. Schematic diagram illustrating the TGF-B pathway and the genes and proteins involved in PAH and HHT. *Illustrated are two pathways of ALK5/SMAD2-3 and ALK1/SMAD1-5.*

ACVRL1 mutations have been described in HPAH patients, but there seems to be a predominance of mutations in exon 10 (http://www/hhtmutation.org) [34]. Knowledge of PAH in the field of HHT is especially important since this combination usually leads to a worse outcome than PAH alone [34]. Twenty-two of the patients described in these case reports were diagnosed under the age of 18 (56%). Compared to *BMPR2* mutation carriers and non-carriers (idiopathic PAH), *ACVRL1* mutation carriers are diagnosed at a younger age and have a worse prognosis despite similar therapy and better haemodynamics at time of diagnosis [34]. This suggest that the disease progresses more rapidly with severe consequences. Even though it is rare for HHT to be complicated by PAH, physicians should be aware of the combination and perform an echocardiogram when clinical signs indicate so, especially in patients with *ACVRL1* mutations. Conversely, clinical signs of HHT in patients with HPAH based on *ACVRL1* mutations might not always be apparent initially.

Ten patients with HPAH and HHT resulting of *ENG* mutations have been described in literature [32,39-42]. However there are a few more cases with reported *ENG* variants and HPAH but no clinical signs of HHT. Interestingly in some of these cases, family members with the same DNA variant show no signs of PAH or HHT [43,44]. No data exist about the prognosis of patients with PAH and *ENG* mutations. Although more patients with HHT and pulmonary hypertension (PH) have been described in various reports, this often involves PH due to left sided heart disease or high output PH due to a left to right shunt in the presence of AVMs in the liver [35,45,46]. Furthermore, both PAH and HHT show an impaired inflammatory response and inflammation often leads to disease progression [47,48]. This information may be interesting for future therapy, although this is yet to be explored.
Furthermore, in both diseases a difference is seen in men and women. Epidemiologic data shows a female predominance in many types of PAH and life-expectancy of females with HHT caused by an *ENG* mutation seems to be impacted greatly [6,49]. Although it is thought female hormones play an important role in both diseases, the exact mechanisms are not yet fully understood [50-52].

MANAGEMENT OF HPAH IN HHT

Although literature is limited, treatment with the typical therapies used for HPAH is recommended. This includes a combination of different PAH specific medication (endothelin receptor antagonists (ERA), phosphodiesterase inhibitors (PD5I), prostacyclins and soluble guanylate cyclase stimulators) and supporting therapy (e.g. diuretics and oxygen) [1]. Two case reports describe successful treatment with the ERA bosentan in PAH and HHT, which improves exercise capacity, laboratory findings and hemodynamic parameters [53,54]. Recently the first case of a patient successfully treated with sildenafil (PD5I) was documented [55]. Although vasoreactivity testing is recommended in all patients with HPAH, there was no reaction on pulmonary vasodilators in a study with 23 *ACVRL1* patients. Treatment with calcium channel blockers seems therefore not indicated [1,34].
It is important to realize that embolisation of pulmonary AVMs could potentially increase the pulmonary arterial pressure, although to which extent this might contribute to the progression of PAH is not yet known [56-59]. Furthermore, the risk of sudden rupture of pulmonary AVMs may be increased in PAH patients [60].

PULMONARY HYPERTENSION AS COMPLICATIONS OF HHT

This review discusses the role of PAH and HHT particularly, but it is important to note that other types of PH, associated with HHT, can occur by several different mechanisms. Most often, post capillary PH develops as a final result of the hyperkinetic state associated with liver AVMs. Especially in HHT, anaemia due to epistaxis and gastro-intestinal bleeding may

trigger this cascade due to increased cardiac output. Pre-capillary PH may be the result of chronic thromboembolic PH (CTEPH) since HHT patients may encounter an increase thrombotic risk [61].

CONCLUSION

The combination of PAH and HHT is rare but may have severe consequences. Both diseases can be the result of genetic affecting the TGF-β signalling pathway, essential for angiogenesis. Clinical signs may not specific but early diagnosis is important for appropriate treatment. Therefore awareness of this disease combination is important for all clinicians working with HHT or PAH patients.

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CHAPTER 9

Percutaneous left atrial appendage closure—An alternative strategy for anticoagulation in atrial fibrillation and hereditary haemorrhagic telangiectasia?

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ABSTRACT

Many patients with hereditary haemorrhagic telangiectasia (HHT) are unable to sustain oral anticoagulation (OAC) because of severe epistaxis, gastrointestinal (GI) bleeding and the risk of life threatening bleeding from cerebral arteriovenous malformations (CAVMs) or pulmonary arteriovenous malformations (PAVMs). In patients with atrial fibrillation (AF), most thromboembolic complications arise from the left atrial appendage (LAA) and percutaneous transcatheter LAA closure proved to be non-inferior to OAC at mid-term follow-up. We report our experience with LAA closure in HHT with a follow-up of 12 months. Percutaneous LAA closure was performed in five patients with both HHT and high thromboembolic risk AF (CHA_2DS_2-VASc score ≥2) without peri-procedural complications. At 3 months no thromboembolic event occurred. After 12 months one patient had a transient ischaemic attack while another patient had recurrence of stroke, this latter patient had a significant stenosis of the carotid artery and an incomplete closure of the LAA without any signs of thrombus on echocardiogram. Both patients had a non-treatable pulmonary right-to-left shunt (RLS). Percutaneous closure of the LAA may provide an alternative strategy to long-term OAC therapy in HHT patients with AF induced high stroke risk and intolerance for OAC.

INTRODUCTION

Atrial fibrillation (AF) is the most common cardiac arrhythmia worldwide and is a major risk factor for cerebral embolic stroke [1-3]. Oral anticoagulation (OAC) is highly effective in stroke prevention [1]. However, a substantial number of patients are unable to sustain chronic OAC. Among these are patients with hereditary haemorrhagic telangiectasia (HHT). HHT is an autosomal dominant inherited disease characterised by vascular malformations ranging from small telangiectasia in skin and mucosal membranes to large arteriovenous malformations in brain, liver and lungs [4].

These patients frequently encounter severe epistaxis and gastrointestinal (GI) bleedings leading to anaemia and a substantial decrease in quality of life (QOL). Furthermore, since cerebral arteriovenous malformations (CAVMs) and pulmonary arteriovenous malformations (PAVMs) increase the risk of life threatening bleeding, a relative or absolute contraindication for OAC exists [5]. Most thromboembolic complications in patients with AF arise from the left atrial appendage (LAA) [1,6]. This is the first case series describing the feasibility of percutaneous LAA closure in HHT patients with AF and a high thromboembolic risk [7].

CASE REPORTS

Between 2010 and 2012, five consecutive patients (patient number 1-5, 3 males, mean age 71.4±5.0 years) with HHT and high thromboembolic risk AF (median CHA_2DS_2-VASc score of 4, range 2-5) received a LAA closure device. The baseline characteristics are described in Table 1.

Before LAA closure, patients 1 to 4 used OAC (CHA_2DS_2-VASc score 2, 5, 2, 4 respectively). All patients had progressive bleeding problems during this therapy and patient 1 and 2 needed several blood transfusions. Patient 5 (CHA_2DS_2-VASc score 5) only used aspirin because of a history of severe epistaxis and both an ischaemic and haemorrhagic stroke.

LAA closure

Before closure of the LAA, a three dimensional transoesophageal echocardiogram (3DTEE) was performed to evaluate the anatomy of the LAA and to exclude pre-existent thrombus formation (Figures 1,2). All procedures were performed as written before.[3] In all cases the implantation of the LAA closure device (Watchman Left Atrial Appendage Occlusion Device®, Atritech Inc., Plymouth, Minnesota, USA) was acutely successful and there were no peri-procedural complications. Directly after implantation, patient 1, 3 and 4 continued OAC, patient 5 continued aspirin and patient 2 combined aspirin and clopidogrel.

Follow-up

At three-month follow-up, TEE showed residual flow from LA to the LAA in patient 5 as a sign of incomplete LAA closure. No thromboembolic complications occurred. All patients

discontinued OAC because of progressive bleeding; four patients switched to aspirin and patient 5 stopped the aspirin.

Table 1. Baseline characteristics

Number, N	5
Age (years)	71.4 ± 5.0
Sex, N	
Male	3 (60%)
Female	2 (40%)
CHA_2DS_2-VASc-score	4 (2-5)
HHT type	
Type 1	1 (20%)
Type 2	4 (80%)
PAVMs	3 (60%)
HAVMs	0 (0%)
Bleeding tendency	
Epistaxis	5 (100%)
GI bleeding	2 (40%)
Hb (mmol/L)	6.7 ± 0.3

Values are in number with percentages (%), mean ± standard deviation (SD) or median with range. HHT, hereditary haemorrhagic telangiectasia; PAVMs, pulmonary arteriovenous malformations; HAVMs, hepatic arteriovenous malformations; GI, gastrointestinal; Hb, haemoglobin.

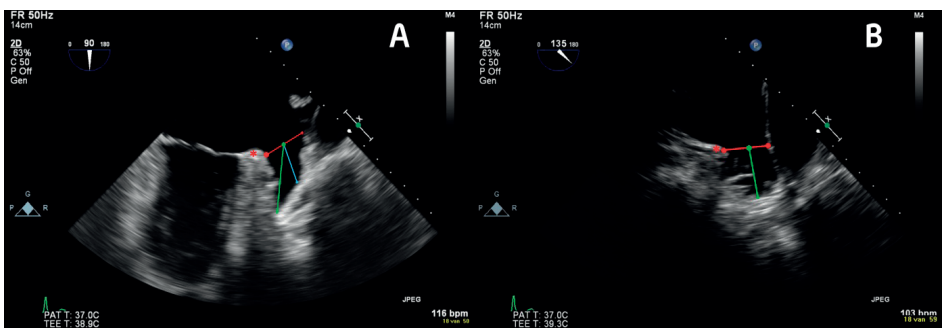

Figure 1 (A) 2D TEE before LAA closure; two-chamber view at 90°; (B) 2D TEE before LAA closure; long-axis view at 135°.
Red line: landing zone; green line: LAA total depth; blue line: LAA depth perpendicular to landing zone; red astrix: circumflex coronary artery. TEE, transoesophageal echocardiogram; LAA, left atrial appendage.

At 12-month follow-up (Table 2), one symptomatic episode was documented as a transient ischaemic attack (TIA) in patient 1. The MRI showed no signs of ischaemia. The TEE 2 months before and after the event showed a small residual flow but the criteria

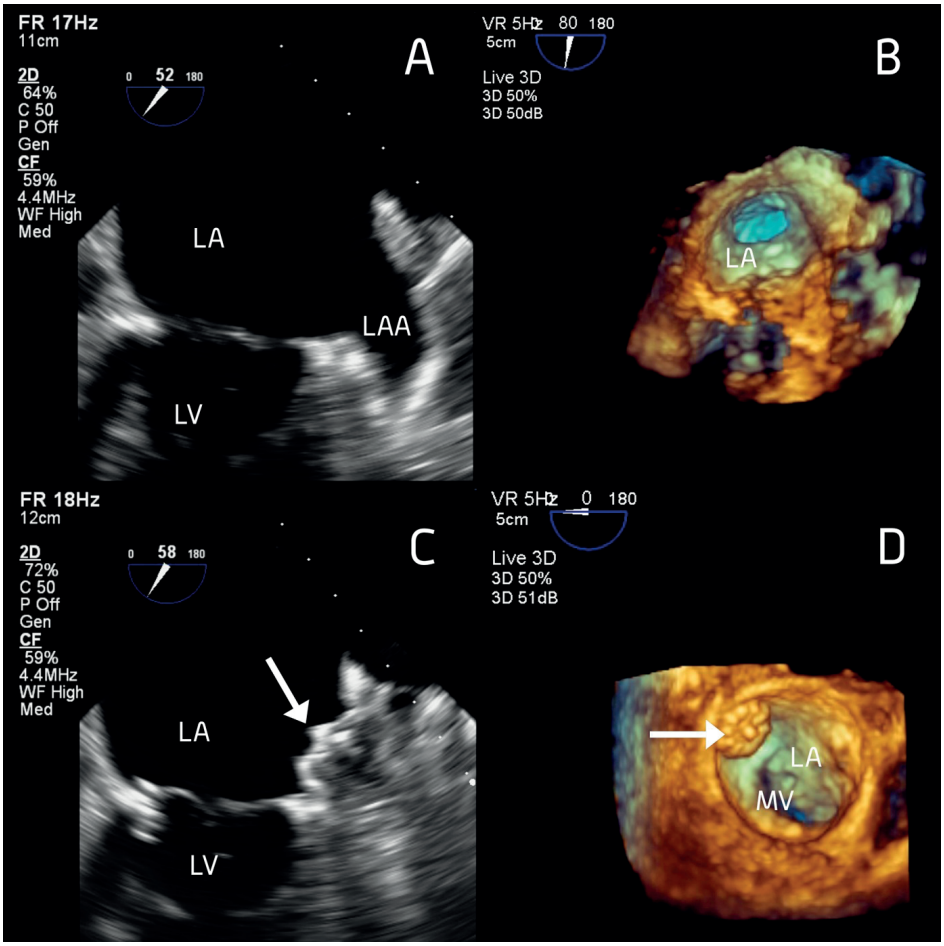

Figure 2 (A) 2D TEE of LAA, LA and LV before LAA closure; (B) 3D TEE of LAA and LA before LAA closure; (C) 2D TEE of LA, LV with the Watchman® closure device in the LAA; (D) 3D TEE of MV and LA with the Watchman® closure device in the LAA.

3D TEE, three dimensional transoesophageal echocardiogram; LAA, left atrial appendage; LA, left atrium; LV, left ventricle; MV, mitral valve.

for complete closure were still fulfilled and no thrombus was seen. This patient had a history of smoking, had non-treatable PAVMs and was treated with thalidomide for refractory epistaxis. At the time of the event, the patient used no OAC or antiplatelet therapy. Although the exact cause of the TIA remained unknown, this patient restarted OAC which resulted in severe GI bleeding. Due to recurrent symptomatic AF rhythm surgery with LAA resection was performed. A minor stroke was reported in patient 5. This patient had a significant stenosis of the carotid artery and embolized PAVMs with a persistent right-to-left shunt (RLS) on contrast echocardiogram. At follow-up, an incomplete LAA closure

but no thrombus formation was seen. In the remaining three patients, OAC was withheld without any complications.

DISCUSSION

The treatment of patients with HHT and high thromboembolic risk AF is an increasing problem. Current guidance on the use of antiplatelet and anticoagulant agents in HHT is based on anecdotal evidence and expert opinion [5,8]. This leads to insufficient treatment in many patients with a high stroke risk. In the United Kingdom, over 50% of the HHT patients were advised not to use OAC or antiplatelet therapy [8]. To decrease the risk of

Table 2. Results at 12 month follow-up

Complete closure	4 (80%)
Complication	
TIA	1 (20%)
CVA	1 (20%)
Systemic embolus	0 (0%)
Therapy	
Acenocoumarol	1 (20%)*
Aspirin	1 (20%)
No therapy	3 (60%)

*Values are in number [percentages (%)]. * acenocoumarol was restarted in one patient after a TIA. TIA, transient ischemic attack; CVA, cerebrovascular accident.*

thromboembolic complications originating from the LAA, a percutaneous LAA closure may be performed safely. At mid-term follow-up, LAA closure proved to be non-inferior with regard to the prevention of stroke, systemic embolism and cardiovascular death in a large study with 707 patients [6].

In this current study, there was a CHA_2DS_2-VASc score of 4 estimating a yearly stroke risk of 4.0% [9]. The thromboembolic complications that occurred during follow-up could be caused by either paradoxical embolisation trough PAVMs, carotid artery disease, incomplete closure of the LAA or the use of thalidomide. A PAVM causes a permanent RLS that bypasses the pulmonary capillary filter, which carries the risk of cerebral paradoxical embolization [7,10]. An incomplete closure of the LAA may provoke thrombus formation and might allow thrombotic embolisation of LAA thrombus through the remaining defect, although current evidence seems contradictory [6,11,12]. A small peri-device flow (jet width ≤5 mm) is seen after LAA closure in >30% and the PROTECT-AF trial revealed that this is not associated with an increased thromboembolic risk [12]. In this case series, thalidomide could also have contributed to thrombus formation. Thalidomide is frequently

used for the treatment of refractory incapacitating epistaxis in HHT and the thrombotic complications are well known in cancer patients treated with thalidomide [13].Currently, no guidelines regarding the treatment of patients with an incomplete LAA closure and an absolute contraindication for OAC exist. One report describes the safety of percutaneous LAA closure with another LAA closure device [the Amplatzer cardiac plug (ACP)] in 60 patients (no HHT patients) with a contraindication to OAC [14]. After LAA closure, antiplatelet therapy was started without any thromboembolic complications (device related thrombus occurred in 3.5%).[14] Although there is less evidence, the ACP device may be an option for patients in which the LAA anatomy is not suitable for implantation with a Watchmann device [15].

There is no literature on the use of LAA closure without OAC or antiplatelet therapy. However, this therapy seems most important in the first months after implantation when endothelialisation of the device is not complete.

Recently, it has been suggested that HHT patients tolerate antiplatelet therapy better than OAC [8]. Besides this bridging therapy with OAC might not be necessary after LAA closure, based on the recent ASAP trial [16]. Therefore, LAA closure seems especially valuable in HHT patients with intolerance for OAC who otherwise would be treated with antiplatelet therapy alone.

The treatment strategy in patients with both HHT and AF induced high stroke risk remains challenging and no sufficient answer for this specific population has been found. Treatment with OAC may lead to progressive and severe bleeding with a decrease in QOL. However, guidance for the treatment of patients with an incomplete LAA closure in this specific subgroup is lacking. Secondly, other thromboembolic risk factors may exist, especially in HHT.

Based on our current experience, a tailor made approach is necessary in which the choice for OAC or LAA closure should be based on the thromboembolic risk, the presence of visceral arteriovenous malformations and the bleeding tendency of the patient. We recommend to select patients with HHT for percutaneous LAA closure when OAC is not tolerated and after an observational period with and without antiplatelet therapy prior to LAA closure.

In conclusion, percutaneous closure of the LAA may provide an alternative strategy to OAC therapy in HHT patients with AF induced high stroke risk and intolerance for OAC. Future larger studies are needed to reveal the risks and benefits of this therapy in patients with HHT.

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CHAPTER 10

Summary and general discussion

SUMMARY

This thesis focuses on the different clinical aspects of hereditary haemorrhagic telangiectasia (HHT). Both new insights on the diagnostic use of transthoracic contrast echocardiography (TTCE) and disease associations are described.

The clinical diagnosis of HHT is established according to the Curaçao criteria, including the presence of recurrent and spontaneous epistaxis, telangiectasia at characteristic locations, visceral arteriovenous malformations (AVMs) and a first-degree family member with HHT. **Chapter 2** demonstrates that the addition of a pulmonary right-to-left shunt (RLS) ≥ 2 on TTCE to these clinical criteria increases its sensitivity (from 88% to 90%) without a subsequent decrease in specificity (74% to 74%). Small pulmonary RLS (RLS grade 1) should not be accepted as clinical criterion.

In **chapter 3** the diagnostic accuracy of pulmonary shunt fraction measurement with the 100% oxygen method is compared to TTCE. This method, based on alveolar-arterial oxygen differences after breathing 100% oxygen for 30 minutes, has a very low sensitivity (51%) for detection of any pulmonary RLS in HHT patients. However, there is a correlation between pathologic shunt fraction and an increased pulmonary RLS grade on TTCE. Since many moderate and large RLS remain undetected (80% and 30% for pulmonary RLS grade 2 and 3 respectively), the 100% oxygen method is not useful to exclude the presence of a pulmonary RLS in HHT.

Chapter 4 focuses on the reproducibility of pulmonary RLS quantification using TTCE. Our study confirms the previous described excellent inter-observer agreement (κ coefficient 0.97) and is the first to report the extremely high inter-injection agreement (κ coefficient 0.95) that was measured by performing two different contrast injections in a single patient. A good acoustic window and sufficient contrast opacification of the right ventricle are indispensable for reliable results.

Chapter 5 addresses the important subject of follow-up of HHT patients and shows that TTCE is the cornerstone in the diagnostics of pulmonary RLS. Follow-up with TTCE was performed in 200 non-treated HHT patients five years after the baseline TTCE. Increase in pulmonary RLS grade occurred in 18% of patients, although never more than one grade, and both in patients with and without pulmonary RLS at baseline. This implies that development of pulmonary arteriovenous malformations (PAVMs) can occur in all HHT patients. Embolisation was possible in 12% of the patients with non-treatable pulmonary RLS at baseline. In the subgroup of patients without pulmonary RLS at baseline, no treatable PAVMs were found at five years follow-up. Based on the results of this study, we recommend follow-up of patients with a pulmonary RLS with TTCE at least every five years. In

patients with no pulmonary RLS at initial screening a conservative management strategy with an interval of more than five years might be safe.

Treatment of PAVMs consists of percutaneous transcatheter embolotherapy with vascular coils or plugs. **Chapter 6** analyses the haemodynamic effects of PAVM embolisation by measurement of cardiovascular haemodynamics using non-invasive finger pressure measurement (Finometer®) and calculation of pressure registrations with Modelflow® methodology. During embolisation in 29 patients, blood pressure, heart rate, stroke volume, cardiac output (CO), total peripheral resistance and delta pressure/delta time were continuously monitored. Although haemodynamic changes were not consistent in all patients, we most notably measured a decrease in stroke volume and CO after embolisation.

In **chapter 7** our scientific efforts were combined with the HHT centre in Toronto (Canada) to create a cohort of *SMAD4* mutation carriers. This retrospective cohort study aimed to describe the prevalence of aortic dilation in HHT. *SMAD4* positive patients were compared to both *ENG* and *ACVRL1* mutation carriers and definite HHT negatives. Chest computed tomography (CT) scans of 178 consecutive subjects were reviewed. The presence of aortopathy was significantly increased in *SMAD4* mutation carriers compared to the other groups (p=0.007). Aortic root dilatation was found in 31% of *SMAD4*, 2% of *ENG*, 6% of *ACVRL1* mutation carriers and in 4% of non-HHT controls (p<0.001). *SMAD4* was an independent predictor for increased aortic root diameter (β-coefficient 3.5, p<0.001). Although the *SMAD4* gene mutation is independently associated with a higher risk of aortic root dilation as compared to other HHT patients and non-HHT controls, no complications occurred.

Pulmonary hypertension (PH) is a previous described complication of HHT. In **chapter 8.1** data are presented on the prevalence of PH assessed with echocardiography in patients with HHT as compared to HHT negative controls. To our knowledge, this is the largest echocardiographic study describing the presence of PH in patients with genotyped confirmed HHT as compared to a genotyped confirmed HHT negative control group. The PH probability was defined according to the tricuspid regurgitation velocity. In total 127 HHT type 1, 150 HHT type 2 and 106 non-HHT subjects were analysed showing an increased prevalence of PH in HHT compared to HHT negative controls (HHT type 1 9%, HHT type 2 18%, controls 4%; p=0.005). This increase is most prominent in HHT type 2 (18%) and mainly results from the high CO state associated with HAVMs. Hereditary pulmonary arterial hypertension (HPAH) is rare in our cohort of HHT patients as it was diagnosed in two patients (<1%; both HHT type 2).

Chapter 8.2 gives a complete overview of the literature on PH in HHT and focuses on the most important subgroups namely: (I) high-output PH due to shunting of blood from the hepatic arteries and/or portal veins to the hepatic veins, and (II) HPAH with arteriopathy due to the HHT-related gene mutations in *ENG* or in particular *ACVRL1*. Recommendations for management are provided since differentiation between pre- and post-capillary is uttermost important for treatment and prognosis.

Chapter 8.3 focusses on the relationship between HPAH and HHT. Both diseases can be the result of genetic mutations affecting the TGF-β signalling pathway, which is essential for angiogenesis. Alteration of TGF-β signalling in endothelial cells causes abnormal capillary formation and maturation leading to venous enlargement, vascular hyperbranching and arteriovenous malformations explaining the abnormal morphogenesis of vasculature in HHT. Disruption of the pathway as a consequence of (mostly) *ACVRL1* mutation, results in inhibition of apoptosis of smooth muscle cells leading to proliferation and vascular remodelling, ultimately causing HPAH. Patients with the combined HHT-HPAH syndrome seem to have more progressive disease with a worse prognosis despite similar therapy compared to other PAH patients. Therefore, although HPAH is a rare complication of HHT, physicians should be aware of this disease combination to diminish the poor prognosis and start early treatment.

Chapter 9 reports our experience with five HHT patients with high thromboembolic risk (CHA_2DS_2-VASc score ≥2) atrial fibrillation (AF) and severe bleeding due to HHT. Left atrial appendage (LAA) closure is suggested as an alternative strategy compared to oral anticoagulation. Percutaneous LAA closure with a Watchman® device was performed without peri-procedural complications. At three months no thromboembolic event occurred. After 12 months one patient had a transient ischemic attack while another patient had recurrence of stroke. The thromboembolic cause remains unknown since both patients had pulmonary RLS, and one patient had a significant stenosis of the carotid artery and an incomplete closure of the LAA without any signs of thrombus on echocardiogram. Percutaneous closure of the LAA may provide an alternative strategy to oral anticoagulation in HHT patients with AF induced high stroke risk.

DISCUSSION AND FUTURE PERSPECTIVES

Pulmonary shunting

Although the risks of a pulmonary right-to-left shunt (RLS) have been extensively described before, no high-level recommendations for the diagnostic approach exist. After previous publications on the prognostic and diagnostic value and safety of transthoracic contrast echocardiography (TTCE), this thesis confirms that TTCE is the cornerstone diagnostic mo-

dality for pulmonary RLS detection in patients with hereditary haemorrhagic telangiectasia (HHT).

The three key findings on the use of TTCE in HHT are as following: (I) a grade 1 pulmonary RLS should not be accepted as clinical criteria for HHT, (II) TTCE is safe and reproducible when performed in experienced hands, and (III) growth of pulmonary RLS can occur in all HHT patients, although development of large pulmonary arteriovenous malformations (PAVMs) does not occur in the first five years in patients without a pulmonary RLS at baseline. This implies that only grade 2 and 3 (moderate and large) RLS have clinical implications. These key findings will be further discussed below.

The current clinical Curaçao criteria have a very good diagnostic performance compared to genetic testing [1]. However, in these criteria the presence of PAVMs are diagnosed with chest CT. Since current guidelines advise the use of TTCE for screening of PAVMs and chest CT is not made in all HHT patients, we evaluated the role of pulmonary shunting on TTCE as new clinical Curaçao criterion. Small RLS, both pulmonary and cardiac, are present in up to 28% of healthy persons. We have shown that small pulmonary RLS (grade 1) should not be accepted as a clinical criterion for HHT since it will lead to more false-positive clinical HHT diagnoses [2-5].

Two previous concerns about TTCE were safety and reproducibility. The most feared complication of TTCE is systemic air embolism, although this is extremely rare based on the literature and our own experience (described in none of our patients). We found an almost perfect inter-injection and inter-observer agreement, however learning curve and experience can influence the outcome. Moreover, a good acoustic window and sufficient contrast opacification of the right ventricle are indispensable. Besides, not all RLS are of clinical interest and other RLS origins are prevalent (e.g. patent foramen ovale or hepatopulmonary syndrome) [6]. Therefore, medical decisions based on TTCE need to be in the hands of experienced cardiologists or pulmonologists. Especially since false negative TTCE could have tremendous outcome. Due to the rareness of the disease, centralisation of care in specialised HHT centres and education of clinicians working with HHT is necessary to maintain reliable results. Because reproducibility was only evaluated in a single and experienced centre, future research should prove the safety and reproducibility in other centres.

When TTCE is not available due to logistic reasons or lack of experience, chest computed tomography (CT) can be used to screen for PAVMs [7]. However, use of ionized radiation is linked to increased presence of solid tumours and leukaemia. The risk of repeated CTs, especially in children and young adults, should not be underestimated [8,9]. Therefore, the European Society of Cardiology advises the use of non-ionizing tests, such as TTCE, when

appropriate [10]. Screening with TTCE can especially diminish the use of radiation in HHT patients with small RLS or screened healthy family members (which is approximately 1/3 of screened population in the Dutch HHT centre).

Both the pathophysiology of growth and occurrence of new vascular defects such as PAVMs, is not exactly clear in HHT. HHT mouse models have surprisingly shown that there is a relatively normal vasculature with no major defects during developmental angiogenesis. This suggests that additional triggers are probably needed for AVM development [11]. Direct predictors for PAVM growth are unclear, but may include: (I) genetic factors (e.g. more growth in HHT type 1), (II) genetic modifiers (e.g. *GJA5* haploinsufficiency in HHT type 2 resulting in impaired angiogenesis [11]), (III) potentially other modifiers (e.g. pregnancy or other causes of increased cardiac output (CO), hypoxemia or inflammation), and (IV) intrinsic growth due to the high flow low resistance pathway of PAVMs. Moreover, different patterns of growth are seen and therefore increase in pulmonary RLS can be due to both increases in number of (diffuse) microscopic PAVMs, growth of macroscopic PAVMs or genesis of new PAVMs. Future scientific research should focus on potential modifiers of PAVM growth to differentiate between high and low risk patients.

The algorithm for follow-up is complex and no clear recommendations for timing of follow-up are stated by the current guidelines. This results in inconsistency in practice regarding PAVM screening, management, and follow-up as demonstrated in a recent global survey [12]. We have given important answers on the question how to follow HHT patients. Firstly, we demonstrated the necessity of follow-up for all HHT patients. Increase in pulmonary RLS occurred in patients with and without RLS at first screening, implying that new RLS may appear during follow-up, besides growth of untreated small pulmonary RLS resulted in larger treatable PAVMs. Secondly, we showed that follow-up after five years was safe in 200 HHT patients with no or non-treated pulmonary RLS at baseline.

Given the expected low risk of TTCE, potentially benefits of minimizing radiation and the recent advances in understanding the clinical implications of pulmonary RLS diagnosed with TTCE, we suggest a new algorithm for follow-up (figure 1). Frequency and diagnostic modality depends on the presence of (macroscopic) PAVMs. In HHT patients with no pulmonary RLS or a pulmonary RLS grade 1, follow-up is advised every five years using TTCE. Chest CT is only performed in case of an increase to a pulmonary RLS grade 2. Potentially, in patients without pulmonary RLS a more conservative follow-up strategy may suffice, however clinical data are lacking yet. Although symptoms of HHT may increase with age, future research may provide evidence for an age limit where after follow-up is not needed for patients without RLS.

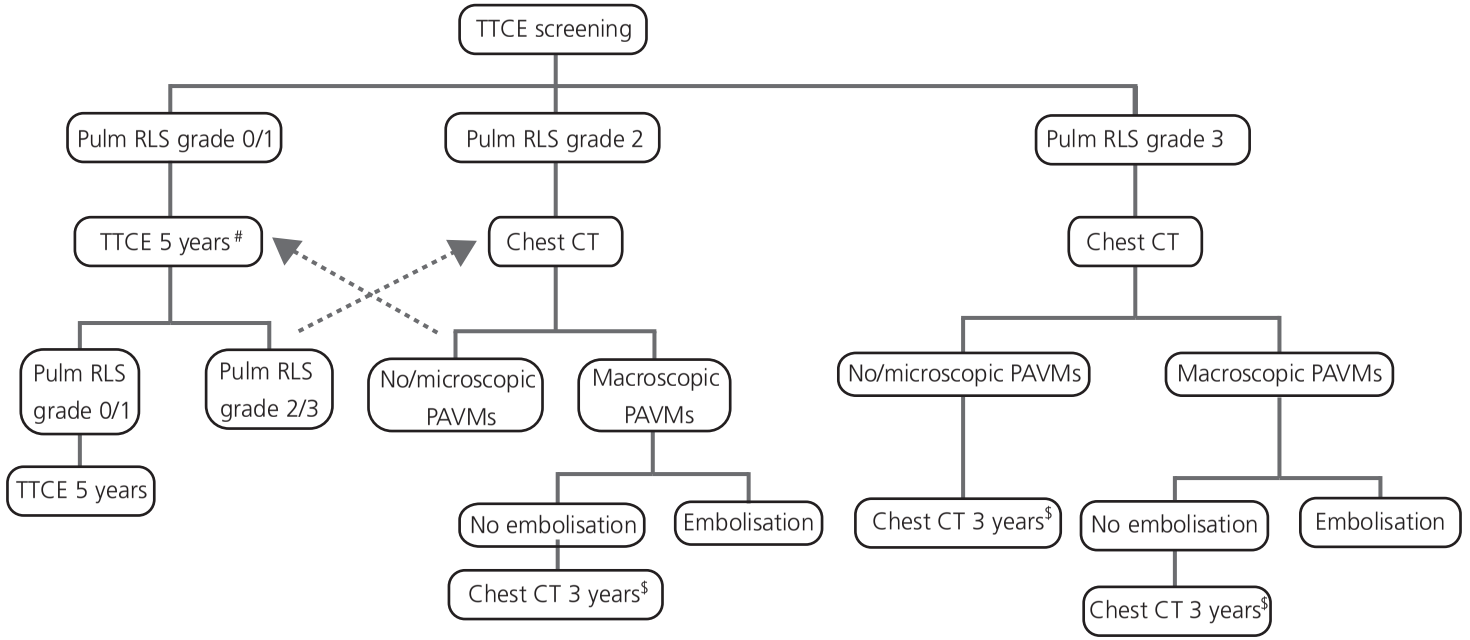

Figure 1. Algorithm for follow-up of pulmonary RLS in HHT patients.
TTCE, transthoracic contrast echocardiogram; RLS, right-left-shunt; HHT, hereditary haemorrhagic telangiectasia. #In patients without pulmonary RLS follow-up with TTCE after more than 5 years might be safe. $No data on exact time interval known.

Follow-up of patients with a pulmonary RLS grade 2 of 3 is more complex and depends on the presence of macroscopic PAVMs. Follow-up of patients with a pulmonary RLS grade 2 and no or microscopic PAVMs, based on chest CT, should be performed afters five years with TTCE. For patients with a pulmonary RLS grade 2 and macroscopic PAVMS it is advised to perform a CT after three years, although there are no data supporting this time-interval yet. Follow-up of patients with a pulmonary RLS grade 3 should be performed with chest CT only. Follow-up after embolisation is not described in this thesis but is usually performed with chest CT since older studies showed a low sensitivity of TTCE. However, with the increased imaging quality of TTCE and experience with TTCE these days, the sensitivity may increase. Although the diagnostic value of TTCE may still improve in time, one must bear in mind that it will not replace chest CT as pre- and post-embolisation diagnostic modality because it is a functional measurement and localisation, size and complexity of PAVMs are not visualised. Future research should focus on (I) personalised algorithms depending on HHT type, age and presence of (small) PAVMs (III) the diagnostic value of other, non-ionizing, tests such as magnetic resonance imaging for PAVM detection and the use of TTCE after embolisation of PAVMs (especially in case of a solitary PAVM).

Global collaboration, research and education of HHT experts may improve reliable use of TTCE and improve patient care by the development of a personalised diagnostic strategy. HHT is therefore included in the VASCERN (European Reference Network on Rare Multi-systemic Vascular Diseases), aiming to enhance access to care and improving quality and quantity of life of this population.

Clinical characteristics of hht

HHT is frequently described as a disease characterised by telangiectasia and vascular malformations in the lungs, brain and liver. Different symptomatology exists per genotype, mutation and between family members with the same mutation. These different phenotypes may influence prognosis, quality of life and potentially interact with each other. We described the following important cardiovascular disease associated with HHT and possible new treatment options: (I) aortopathy in *SMAD4*-related HHT, (II) prevalence of overall pulmonary hypertension (PH) and hereditary pulmonary arterial hypertension (HPAH) in HHT patients**,** (III) percutaneous left atrial appendage (LAA) closure in HHT patients suffering atrial fibrillation (AF). These different associations will be broadly discussed below.

Although *SMAD4* is one of the three most common mutations in HHT, it may affect less than 2% of the total HHT population. In these patients all the classic clinical characteristics of HHT are present in combination with symptoms of the juvenile polyposis syndrome [13]. Furthermore, misbalance in TGF-β due to a mutation in the *SMAD4* mimics other TGF-β associated connective tissue disease resulting in enlargement of the thoracic aorta (mainly

the aortic root). Aortic dilation is probably caused by loss of contractile smoot muscle cells [14]. Importantly, we found no association between the other HHT mutations and aortopathy. The pathogenic differences between *SMAD4* and *ENG/ACVRL1* resulting in aortic dilation are not completely understood. At this moment, the exact clinical consequences for the *SMAD4* mutation carriers are not clear since no complications were observed and no serial measurements were performed. Potentially, *SMAD4* mimics other characteristics of connective tissue disease as well. Therefore, longitudinal research should focus on actual growth of the aorta, occurrence of complications and other symptoms of connective tissue disease. Due to the rarity of this combination, collecting prospective data on the outcome will be extremely time consuming and may not be expected within the upcoming years.

PH is one of the most feared complications of HHT and chapter 7 of this thesis indeed shows an increased prevalence of PH compared to HHT negative controls. However, this mostly includes PH related to HAVMs and we described a relatively low prevalence of HPAH in our cohort of genotyped HHT patients. The effect of TGF-β dysfunctioning is context depended and may lead to smooth muscle cell dysfunction and proliferation of endothelial cells resulting in HPAH [15]. Shunting of systemic AVMs and anaemia will increase CO resulting in a hyperdynamic state with increase of pulmonary pressures. Previous data on the prevalence of PH in HHT were based on relative small single centre studies which were lacking power, invasive pressure measurement or genetic testing and were containing conflicting data. Recent publicized data confirm the diminished prognosis of HHT related PH (hazard ratio 3.8; $p< 0.0001$ adjusted for age, not defined by PH subclass) [16]. Therefore, although prevalence may be low, clinicians should be aware of this dangerous disease combination. Further increase of pulmonary pressure could be expected after embolisation of low resistance PAVMS, however decrease of CO seems to prevent this. Further research should focus on results of treatment of both high output PH and HPAH.

Life expectancy of HHT patients will increase due to screening and pre-emptive treatment. With aging more HHT patients with other (e.g. cardiovascular) disease will seek medical care. Arrhythmias are already relatively common in HHT patients (11% in a cohort of 1025 HHT patients) and the prevalence of arrhythmias such as AF will rise further due this increased aging [17]. Compared to other patients, both adrenergic stimuli to maintain high CO and increase of left atrial dimensions due to high pulmonary pressure (irrespectively of origin) will further increase the risk of AF [17]. Prevention of thromboembolic complications in this specific population may be challenging since different determinants, including classic thrombotic risk factors, increased coagulation factor VIII, and increased bleeding risk may be present. Many HHT patients are withheld from treatment with anticoagulation for partly unknown reasons. Literature states that there is a wide variation of haemorrhagic complications associated with the use of anticoagulation in HHT patients [18],

although most feared complications such as pulmonary or cerebral haemorrhage are not frequently described [18,19]. However, clinical practice shows that use of anticoagulation frequently leads to an increase of epistaxis and anaemia resulting in a decreased quality of life [18,20]. Given the reasonable tolerance and low prevalence of severe complications, HHT should not be considered an absolute contraindication for anticoagulation. Risks and benefits of all treatment options should be carefully considered on a case-by-case basis including extensive pre-screening for AVMs and close follow-up of complications. When classic anticoagulation (e.g. vitamin K antagonists) is not tolerated, closure of the LAA or a conservative strategy can be considered. Because there is no randomized controlled evidence for percutaneous LAA closure without short-term antiplatelet therapy, individual antiplatelet treatment test (using an epistaxis diary) is necessary to safely implant the LAA closure device. There is no literature on the use of non-vitamin K oral anticoagulation (NOAC) in HHT patients.

In conclusion, all the aspects mentioned above show the complexity of HHT, the inhomogeneity of the population and the need for multidisciplinary and specialised care including pulmonologists, cardiologists, interventional radiologists, gastroenterologists, neurologists and otorhinolaryngologists. Clinicians should be aware of the possibility of different and potentially severe complications depending on the HHT type. Echocardiography should be used to screen for PAVMs, is important for the follow-up of small pulmonary RLS and can be used to screen for other complications such as PH and aortic dilation. Hopefully, further investigations and global registration of data will lift HHT diagnostics and treatment from partly experienced and eminence based medicine towards evidence based and personalised medicine.

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APPENDIX

NEDERLANDSE SAMENVATTING/ DUTCH SUMMARY

Introductie (hoofdstuk 1)

Hereditaire hemorragische teleangiëctasieën (HHT), ook wel bekend als de ziekte van Rendu-Osler-Weber, is een zeldzame vaataandoening. De ziekte wordt gekenmerkt door verwijding van kleine bloedvaatjes (teleangiëctasieën) en arterioveneuze malformaties (AVMs), waarbij als gevolg van vaatmisvorming een abnormale directe verbinding is ontstaan tussen de arteriële (slagaderlijke) en veneuze (aderlijke) circulatie. De AVMs zijn voornamelijk gelokaliseerd in de longen (pulmonale AVMs (PAVMs)), lever (hepatogene AVMs (HAVMs)) en/of hersenen (cerebrale AVMs (CAVMs)). HHT heeft een autosomaal dominant overervingspatroon en wordt meestal veroorzaakt door een mutatie in de genen *ENG* of *ACVRL1,* overeenkomend met HHT type 1 en HHT type 2. In zeldzame gevallen wordt het veroorzaakt door een mutatie in het *SMAD4* gen. Ondanks dat er grote interfamiliaire en interindividuele verschillen bestaan, is er een evidente genotype-fenotype relatie waarbij PAVMs en CAVMs vaker voorkomen in HHT type 1 en HAVMs in HHT type 2. HHT kan worden gediagnostiseerd met behulp van genetisch onderzoek of met gebruik van de klinische Curaçao criteria, welke bestaan uit: (I) aanwezigheid van spontane en recidiverende epistaxis (bloedneuzen), (II) teleangiëctasieën op voorkeurslocaties, (III) viscerale AVMs en (IV) een eerstegraads familielid met bewezen HHT. Bij aanwezigheid van 3-4 criteria is er sprake van een definitieve diagnose, bij 2 criteria is de diagnose mogelijk en bij 0 of 1 criterium is de diagnose onwaarschijnlijk.

Dit proefschrift richt zich op verschillende klinische aspecten van HHT.

Deel I richt zich met name op PAVMs waarbij diagnostiek, follow-up en gevolgen van behandeling worden besproken. PAVMs omzeilen het capillaire filter (de haarvaten) in de longen, resulterend in een pulmonale rechts-links shunt (RLS). Sommige patiënten hebben hierdoor een lagere zuurstofwaarde in het bloed. Echter het belangrijkste probleem is het risico op paradoxale embolisatie van septische of trombotische origine, waarbij bacteriën of trombi door de RLS in de arteriële circulatie terecht komen en leiden tot een hersenabces of beroerte. Een pulmonale RLS is aanwezig in 91% van de patiënten met HHT type 1 en 53% van de patiënten met HHT type 2.
Behandeling kan veilig worden verricht middels transcatheter embolisatie waarbij via een bloedvat vanuit de lies het aanvoerende vat van de AVM wordt afgesloten met vasculaire plugs of coils. Gezien door deze behandeling de bovengenoemde complicaties kunnen worden voorkomen, is diagnostiek naar PAVMs van groot belang. Transthoracale contrast echocardiografie (TTCE) is de hoeksteen in de screening naar PAVMs. Bij TTCE wordt gebruik gemaakt microbubbels, bestaande uit natriumchloride oplossing, bloed en lucht. Na contrastinjectie wordt het aantal microbubbels in het linker ventrikel geteld in een stilsta-

and echo frame. Hierbij wordt onderscheid gemaakt in een pulmonale RLS graad 1, 2 en 3 voor respectievelijk 1-29 microbubbels, 30-99 microbubbels en 100 of meer microbubbels. Eerdere onderzoeken, gepubliceerd vanuit het St. Antonius Ziekenhuis, beschrijven een duidelijke associatie tussen de pulmonale RLS graad op TTCE en de aanwezigheid van behandelbare PAVMs op CT-thorax. Tevens blijkt de pulmonale RLS graad een prognostische voorspeller voor het optreden van neurologische complicaties.

In **hoofdstuk 2** wordt de diagnostische waarde van de Curaçao criteria beschreven. Hierbij wordt aangetoond dat een pulmonale RLS graad ≥ 2 op TTCE als toevoeging aan de criteria, de sensitiviteit verhoogt (88% naar 90%) zonder verlaging van de specificiteit (persisterend 74%). Het toevoegen van een kleine pulmonale RLS (RLS graad 1) aan de criteria resulteerde niet in een verbetering van de diagnostische waarde.

In **hoofdstuk 3** is de diagnostische nauwkeurigheid van de 100% zuurstof methode in het detecteren van pulmonale RLS vergeleken met TTCE. De 100% zuurstof methode is gebaseerd op het alveolaire (longblaasjes)-arteriële (slagaderlijke) zuurstof verschil na inademing van 100% zuurstof. Ondanks dat er wel een correlatie werd gevonden tussen de shunt fractie en een grotere pulmonale RLS graad, heeft deze methode een lage sensitiviteit (51%) voor de detectie van een pulmonale RLS in HHT patiënten. Gezien een groot deel van de pulmonale RLS graad 2 en 3 (respectievelijk 80% en 30%) niet gedetecteerd worden middels de 100% zuurstof methode, is deze methode niet bruikbaar als diagnosticum voor pulmonale RLS in HHT.

Hoofdstuk 4 focust op de reproduceerbaarheid van pulmonale RLS kwantificatie middels TTCE. Deze studie bevestigt de eerder beschreven uitstekende 'interobserver' overeenkomst (κ coëfficiënt 0.97). Hiernaast is het de eerste studie die de bijna perfecte 'interinjectie' overeenkomst (κ coëfficiënt 0.95) beschrijft. Deze 'interinjectie' overeenkomst werd gemeten door twee verschillende contrast injecties te verrichten in één patiënt. Een goed echovenster en aanbod van voldoende contrast in de rechter ventrikel zijn onmisbaar voor een betrouwbaar resultaat.

Hoofdstuk 5 beschrijft het belangrijke onderwerp van de follow-up van HHT patiënten. Follow-up met TTCE is verricht in 200 onbehandelde HHT patiënten vijf jaar na de initiële TTCE op baseline. Vergroting van de pulmonale RLS werd gezien in 18% van de patiënten, echter deze groei was nooit meer dan één shunt graad. Vergroting werd beschreven in zowel patiënten met als zonder pulmonale RLS op baseline. Dit impliceert dat ontwikkeling van PAVMs kan voorkomen bij alle HHT patiënten. Embolisatie werd uitgevoerd in 12% van de patiënten met niet-behandelbare pulmonale RLS op baseline. In de subgroep van patiënten zonder pulmonale RLS op baseline, werden geen behandelbare PAVMs gevonden

na vijf jaar follow-up. Gebaseerd op de resultaten van deze studie, wordt geadviseerd om follow-up minstens eens per vijf jaar te verrichten in alle patiënten met een pulmonale RLS. In patiënten zonder pulmonale RLS bij initiële screening is een meer conservatieve strategie met een follow-up interval van meer dan vijf jaar mogelijk veilig, echter data ontbreken nog op dit moment om dit te bevestigen.

Hoofdstuk 6 analyseert de hemodynamische effecten tijdens PAVM embolisatie door het meten van de cardiovasculaire hemodynamica tijdens embolisatie. Hiervoor werd gebruik gemaakt van non-invasieve vingerdrukmeting (met behulp van de Finometer®). De calculatie van de druk registraties werd verricht volgens de Modelflow® methodologie. Tijdens embolisatie in 29 patiënten werden de bloeddruk, hartfrequentie, slagvolume, cardiac output, perifere vaatweerstand en de delta pressure/delta time continu gemonitord. De hemodynamische veranderingen waren niet consistent in alle patiënten. De voornaamste veranderingen na embolisatie waren daling van het slagvolume en de cardiac output.

Deel II van dit proefschrift bespreekt bekende en nieuwere klinische karakteristieken van HHT. Naast de hierboven beschreven veel voorkomende manifestaties van HHT leiden ook andere complicaties tot morbiditeit bij HHT patiënten. Het is bekend dat er verschillen zijn in de symptomatologie per genotype, mutatie en tussen familieleden met dezelfde mutatie. Deel II van dit proefschrift probeert bewustwording te vergroten van het brede scala aan problemen, die zich kunnen voordoen bij verschillende HHT patiënten.

In **hoofdstuk 7** bundelen we onze wetenschappelijke krachten met het HHT centrum in Toronto, Canada, om een grote populatie HHT patiënten met een *SMAD4* mutatie te kunnen analyseren. Het doel van deze retrospectieve cohort studie was het beschrijven van de prevalentie van aortadilatatie in HHT patiënten. *SMAD4* mutatie dragers werden vergeleken met *ENG* en *ACVRL1* mutatie dragers en een HHT negatieve controle groep. Een CT-thorax werd beoordeeld van 178 opeenvolgende personen. De aanwezigheid van aortadilatatie was significant verhoogd in *SMAD4* patiënten in vergelijking met de andere groepen: aortawortel dilatatie werd gevonden in 31% van de *SMAD4* patiënten, ten opzichte van 2%, 6% en 4% in de *ENG, ACVRL1* en controle groep. De *SMAD4* mutatie was daarbij een onafhankelijke voorspeller voor een hogere diameter van de aortawortel. Er werden geen complicaties gezien.

Pulmonale hypertensie (PH) is een veel beschreven complicatie van HHT. In **hoofdstuk 8.1** wordt de aanwezigheid van PH in patiënten met HHT vergeleken met patiënten zonder HHT. Binnen deze studie was de waarschijnlijkheid van PH gebaseerd op de maximale snelheid van de tricuspidalisklepinsufficiëntie bij echocardiografie. Voor zover bekend, is dit de grootste echocardiografische studie, die de aanwezigheid van PH beschrijft in een

cohort van patiënten met genetisch bewezen HHT ten opzichte van personen, waarbij genetisch onderzoek de diagnose HHT heeft uitgesloten. In totaal werden 127 HHT type 1, 150 HHT type 2 en 106 HHT-negatieve controles geïncludeerd. De resultaten toonden een verhoogde aanwezigheid van PH in de HHT subgroep ten opzichte van de HHT-negatieve subgroep (HHT type 1 9%, HHT type 2 18%, controlegroep 4%). PH werd met name gevonden in HHT type 2 en werd voornamelijk veroorzaakt door de hoge cardiac output ten gevolge van HAVMs. Hereditaire pulmonale arteriële hypertensie (HPAH) was zeldzaam in dit cohort en werd enkel gediagnosticeerd in 2 patiënten (<1%, beiden HHT type 2).

Hoofdstuk 8.2 geeft een compleet overzicht van de literatuur over PH in HHT waarbij de focus ligt op de twee belangrijke subgroepen: (I) "high-output PH" door shunting vanuit de arteria hepatica of portale vaten naar de vena hepatica en (II) HPAH met de daarbij horende arteriopathie door de HHT genmutaties in *ENG* en met name *ACVRL1*. Differentiatie tussen deze verschillende vormen is van uiterst belang, gezien beide vormen andere behandeling en prognose kennen.

Hoofdstuk 8.3 beschrijft de relatie tussen HPAH en HHT, welke beide kunnen worden veroorzaakt door een genetische mutatie, coderend met een van de eiwitten uit de transforming growthfactor-β (TGF-β) pathway. De TGF-β pathway is belangrijk bij de angiogenese (vorming van bloedvaten). Derhalve kunnen veranderingen in de TGF-β signalering op endotheelcellen leiden tot de vorming van abnormale capillairen en AVMs. Hiernaast kunnen deze veranderingen leiden tot inhibitie (remming) en apoptose (celdood) van gladde spiercellen en tot vasculaire proliferatie en remodelling met HPAH tot gevolg. HPAH wordt met name veroorzaakt door de *ACVRL1* mutatie, al blijkt het ook in deze populatie zeldzaam. Patiënten met het gecombineerde HHT-HPAH ziektebeeld hebben een slechtere prognose dan andere patiënten met HPAH, ondanks vergelijkbare behandeling. Derhalve is het herkennen van HPAH binnen HHT van groot belang om zo de prognose van deze patiënten te verbeteren door vroege behandeling.

Hoofdstuk 9 bespreekt onze ervaring van vijf HHT patiënten met atriumfibrilleren (AF) met een hoog trombo-embolisch risico (gedefinieerd als (CHA_2DS_2-VASc score ≥2)) en ernstige bloedingscomplicaties ten gevolge van HHT. Het merendeel van de patiënten met hoog trombo-embolisch risico en AF worden behandeld met orale antistolling in de vorm van vitamine K antagonisten of NOACs (non vitamin K oral anticoagulation). Linker hartoor (LAA) afsluiting is eerder beschreven als een mogelijke alternatieve behandeling voor patiënten met een zeer hoog bloedingsrisico. In deze studie werd percutane LAA sluiting met een Watchman® device verricht zonder peri-procedurele complicaties en werden gedurende een follow-up van 3 maanden geen trombo-embolische complicaties gezien. Follow-up na 12 maanden toonde een 'transient ischemic attack' (TIA) in één patiënt en

een ‘cerebro-vascular accident’ (CVA) in één patiënt met een eerdere CVA in de voorgeschiedenis. Gezien beide patiënten een pulmonale RLS hadden en één van de patiënten een significante stenose van de arteria carotis had en tevens een incomplete sluiting van het LAA zonder aanwijzingen voor trombus op het echocardiogram, is de daadwerkelijke trombotische origine niet vast te stellen. Concluderend is percutane LAA afsluiting een mogelijke alternatieve behandeling ten opzichte van orale antistolling in HHT patiënten met een hoog trombotisch risico ten gevolge van AF.

Dit proefschrift adviseert het gebruik van TTCE als diagnosticum voor pulmonale RLS. Echter ervaring met zowel HHT als met TTCE is van groot belang voor een betrouwbaar resultaat. Ondanks dat groei van PAVMs wordt beschreven zijn de exacte risicofactoren hiervoor niet bekend. In de discussie wordt een nieuwe flowchart beschreven, waarbij frequentie en geadviseerd diagnosticum bij follow-up afhankelijk is van de aanwezigheid van een pulmonale RLS. Naast het gebruik van echocardiografie in de diagnostiek van PAVMs, kan het ook worden gebruikt om te screenen naar complicaties zoals PH en aortadilatatie.

Tot besluit: HHT is een zeer divers en complex ziektebeeld, waarbij door de verschillen binnen de patiëntenpopulatie behoefte is aan uitgebreide multidisciplinaire en gespecialiseerde zorg. Hierbij is samenwerking noodzakelijk tussen longartsen, cardiologen, interventieradiologen, maag-darm-lever artsen, neurologen en keel-neus-oor artsen. De wereldwijde inzet van betrokken onderzoekers en registratie van data heeft geleid tot steeds meer ‘evidence based medicine’, waarbij hopelijk in de toekomst nog meer kennis voor handen is om plaats te maken voor steeds verder gepersonaliseerde HHT zorg.

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The prevalence of pulmonary hypertension in hereditary haemorrhagic telangiectasia.
ERS congress Amsterdam 2015. (poster discussion session)

Reproducibility of right-to-left shunt quantification using transthoracic contrast echocardiography using echocardiography in hereditary haemorrhagic telangiectasia.
International HHT congress Dubrovnik 2017.

Age penetrance of pulmonary right-to-left shunt in patients with hereditary haemorrhagic telangiectasia.
International HHT congress Dubrovnik 2017.

Awards

Young travel award HHT congress 2015: *Prevalence of pulmonary hypertension in a large cohort of patients with hereditary haemorrhagic telangiectasia & Follow-up of the pulmonary right-to-left shunt with transthoracic contrast echocardiography in hereditary haemorrhagic telangiectasia.*

1st price poster presentation NVVC voorjaarscongres 2015: *Aortic Dilation in Patients with hereditary haemorrhagic telangiectasia and a SMAD4 Gene Mutation.*

2nd price oral presentation international HHT congress 2015: *Follow-up of the pulmonary right-to-left shunt with transthoracic contrast echocardiography in hereditary haemorrhagic telangiectasia.*

3rd price poster presentation international HHT congress 2015: *Aortic Dilation in Patients with Hereditary Haemorrhagic Telangiectasia and a SMAD4 Gene Mutation.*

1st price poster presentation St. Antonius wetenschapsavond 2014: *Prevalence of pulmonary hypertension in a large cohort of patients with hereditary haemorrhagic telangiectasia.*

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CURRICULUM VITAE

Veerle (Veronique Marguerite Marie) Vorselaars, was born on April 11th 1987 in Tilburg. After graduating Gymnasium 'Cum Laude' at Theresia Lyceum Tilburg, she started Medicine at Utrecht University in 2005. In 2009 she interrupted her study for one year to join the Lustrumboard of C.S. Veritas to expand her management qualities. During her studies she did an internship social medicine at Curaçao, she volunteered at Young Africa Skills centre, Mozambique and performed a research internship at the pulmonary department of University Hospital Leuven, Belgium. During her study she did an internship at the cardiology department of St. Antonius Hospital, where her interest in cardiology was generated. In 2012 she joined the hereditary haemorrhagic telangiectasia (HHT) research group as research student which was the foundation for this thesis. From 2013 she worked as research fellow at the HHT centre of excellence under supervision of dr. M. Post, dr. J. Mager en drs. R. Snijder. She was awarded with an Young Travel Award at the international HHT Conference 2015 Captiva Island, United States. During this period she was board member of the hospitals PhD group 'De promovendiclub'. In January 2016 she started working as resident cardiology. In January 2017 she started her specialist training in cardiology at St. Antonius Hospital, Nieuwegein (head dr. J. ten Berg), which commenced with two years training in internal medicine (head dr. P. de Jong) were she is currently working.