

Screening pulmonary arteriovenous malformations in a large cohort of Spanish patients with hemorrhagic hereditary telangiectasia.

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Key words

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Transthoracic contrast echocardiography

Computed tomography

Ultrasound contrast agents

ABSTRACT

Background and objectives

Because of serious nature of potential complications, screening for pulmonary arteriovenous malformations is required in patients with hereditary hemorrhagic telangiectasia. The aim of this study was to evaluate the utility of contrast echocardiography and compare the performance of two contrast agents: agitated saline and Gelofusine.

Material and Methods

Two hundred and five patients screened for PAVMs using TTCE and computed tomography (CT) performed with an interval of less than 180 days. Contrast echocardiography studies were graded on a 4-point semiquantitative scale based on the amount of microbubbles seen in left heart chambers.

Results

Positive TTCE findings were seen in 137 (66.8%) patients, whereas CT confirmed PAVMs in 59 (43.1%). Two of 67 grade 1 patients; 18 of 42 grade 2; 17 of 22 grade 3 and all grade 4 had PAVMs on CT. Embolotherapy was feasible in 38.9% patients in grade 2 and 82.3% and 95.2% in grade 3-4. No patients in grade 1 were embolized. The mean cardiac cycle in which bubbles were first seen in the left heart in patients without and with PAVMs on CT was 6.1 and 3.9 ($P<0.0001$). Compared to saline, Gelofusine produced an overall increase in grade.

Conclusions

No grade 1 patients had treatable PAVMs. There is a need for improvement in the selection of patients for CT in grade 2, where less than half have PAVMs on CT. The cardiac cycle may help to differentiate between patients with and without PAVMs. Gelofusine was not better than saline for PAVM screening.

Introduction

Hereditary hemorrhagic telangiectasia (HHT) is a rare (1 in 5000 to 8000 people) disorder, transmitted in an autosomal dominant trait; with more than 90% of the cases caused by mutations in Endoglin (*ENG*; HHT1), activin A receptor type II-like 1 (*ACVRL1/ALK1*; HHT2), or SMAD4 (Hereditary hemorrhagic telangiectasia and Juvenile polyposis; HTJP) genes (1, 2). These mutations, together with other environmental factors promote the development of small (telangiectasia) or large arteriovenous malformations (AVMs) in almost all organs of the economy (1, 2); including the lung, where pulmonary arteriovenous malformations (PAVMs) are a common finding. This is especially true among HHT1 patients in whom pulmonary shunts are seen in up to 85% of these patients (3-7). PAVMs create a right-to-left shunt between the pulmonary arterial and venous circulation that can lead to hypoxemia, paradoxical embolism, or rupture and, as a consequence, not infrequently a brain abscess, stroke, or hemoptysis are seen in individuals with a previously undiagnosed PAVM (2-4). Recent data also, highlight that reduced oxygenation of blood due to pulmonary AVMs results in a graded erythrocytotic response to maintain arterial oxygen content, and higher stroke volumes and/or heart rates to maintain oxygen delivery (8). This latter situation can also be aggravated in HHT patients by the anemia resulting from iron losses due to bleeding from nasal and gastrointestinal telangiectasies. (8). Anatomic resolution of PAVMs through embolization, has been shown to reduce strokes, migraines, and hematologic, cardiac and ventilatory demands (2).

Because of the serious nature of these potential complications and benefits for embolization screening for PAVMs is recommended in HHT patients (9).

Transthoracic contrast echocardiography (TTCE) followed by CT in patients testing positive with this technique are the recommended imaging methods in the last 2010 clinical guideline (9). Nonetheless, positive TTCE findings are relatively common in patients with HHT (60% to 85% of patients tested) (3, 10, 11), whereas a much smaller percentage of between 21% and 52.3% actually show PAVMs on CT (12). Therefore, adherence to clinical guideline recommendations would result in a large number of unwarranted CT studies, implying unnecessary radiation exposure and healthcare expenditure (2,13-15). The results of a previous study by our group (13) and those of other authors (10, 14, 16) have indicated that use of a system to grade the passage of contrast material on TTCE to estimate the size of pulmonary shunts is of value for selecting patients who should subsequently undergo CT evaluation. Two TTCE grading systems have been proposed: a 4-grade scale (10, 13, 17) and a 3-grade scale (7, 14, 16). The main difference between the two options is that grades 3 and 4 in the 4-grade scale correspond to grade 3 in the 3-grade scale. At present, the indication for CT seems clear in patients showing moderate or extensive contrast passage using these scales (grades 2, 3, and 4). In patients with a pulmonary shunt grade 1, according to the work of Velthuis et al (18), a conservative management strategy (without CT and antibiotic prophylaxis) is probably justified considering the absence of treatable PAVMs and the negligible risk of cerebral paradoxical embolism in this subset of patients. An interesting question in TTCE grade 1 is to know if these positive results are a consequence of very small PAVMs or the results of normally present intrapulmonary arteriovenous anastomosis (19). An increase in cardiac output has been shown to produce an increase of blood flow through to this normally present intrapulmonary arteriovenous anastomosis in healthy patients (20).

Most echocardiography studies carried out in HHT patients use an agitated saline solution as the contrast agent. However, other contrast options are available, such as the colloid solution, Gelofusine (Braun Medical, Melsungen, Germany). Gelofusine is reported to provide better opacification of the heart chambers than agitated saline solution (21), as the bubbles formed are smaller (22, 23) and more stable (24). Use of Gelofusine may result in a smaller number of suboptimal studies relative to saline contrast(21, 24).

Based on this background, the aim of this study was to assess the use of graded contrast echocardiography for selecting Spanish HHT patients to undergo CT, and evaluate the performance of agitated saline solution versus Gelofusine as contrast media. To the best of our knowledge, this is the first study comparing these two contrast agents in the screening of PAVMs of HHT patients.

Materials and Methods

From January 2003 to December 2014, 664 patients with suspected HHT were seen in our HHT unit through one of the following channels: ambulatory visit, hospital admittance, or genetic testing alone. Patients seen through either of the first 2 channels underwent a hospital protocol that included a clinical history-taking, physical examination, laboratory testing, genetic testing, TTCE, chest or abdominal CT, abdominal ultrasound, and brain or lumbar MRI, when clinically justified. Only patients who underwent TTCE and CT within an interval of ≤ 180 days between the two techniques were included in the study. Patients in whom a persistent foramen ovale was detected were excluded. The hospital ethics committee for clinical research of the Autonomous Community of Cantabria

approved the study protocol. All patients provided written consent for participation. The presence or absence of anemia was considered in women and men when hemoglobin (Hb) levels were lower than 12 g/dL, and 13,5 g/dL, respectively

The TTCE technique, previously used by our team and described by others (13, 14), was performed by 3 experienced echocardiographers, following intravenous line placement in an antecubital vein. The two contrast agents, agitated saline (9 ml saline solution mixed with 0.5 ml room air and 0.5 ml of blood from the patient) and Gelofusine (10 ml), were administered with the patient in left lateral decubitus. A four-chamber view was used, and TTCE was considered positive for pulmonary shunting when bubbles were observed in the left atrium without application of the Valsalva maneuver. The number of cardiac cycles that occurred before bubbles appeared in the left atrium was recorded and a delay of >3 cycles was established to differentiate PAVMs from intracardiac shunt (25). Shunts visualized through a pulmonary vein were classified as pulmonary shunts regardless of the cardiac cycle (12). A second study with Valsalva manoeuvre was performed when contrast was present in the left atrium in less than four cardiac cycles. The study was considered positive for PAVM if intracardiac shunts were not visualized by color Doppler and the pattern of appearance of bubbles was not modified by Valsalva. Based on the amount of bubbles visible in the left ventricle, TTCE findings were rated according to the grading system of Barzilai et al (17), modified by Zukotynski et al. (10) and used in our previous study (13) into x4 grades: grade 1, minimal left ventricular opacity (up to 20 bubbles); Grade 2,

moderate opacity; grade 3, extensive opacity without outlining the endocardium; and grade 4, extensive opacity with endocardial definition.

TTCE with agitated saline contrast was performed in all patients. Between 2008 and 2014, patients additionally underwent TTCE using Gelofusine to determine whether there were differences in screening outcomes versus saline. The same protocol described above was used, and the Gelofusine study was performed a few minutes after the saline study in the same session **after making sure that there were no bubbles from the previous study.**

Chest CT was carried out on 3 different multidetector systems over the study period: a 2-row Somatom AR.T scanner (Siemens, Erlangen, Germany), a 32-row LightSpeed scanner (General Electric, New York, USA), and an 80-row Aquilion Prime TSX-303A scanner (Toshiba Medical Systems Corporation, Japan), using ≤ 3 mm slice thickness (9). Nonionic contrast agent (300 mg/mL iodine at a rate of 3 mL/sec and maximum dose of 2 mL/kg) was routinely administered **because in most of these patients the liver was included in the study in order to rule out hepatic arteriovenous malformations (HAVMs). These studies were performed in a caudo-cranial direction and always included an image acquisition in an early arterial phase (5, 26).** The images obtained were evaluated by 3 radiologists with experience in HHT. Visualization of a nodule with an afferent artery and efferent vein was considered diagnostic for PAVM (5). **The presence of telangiectases, perfusion disorders, vascular confluent masses or arteriovenous or veno-venous shunts were considered diagnostic for HAVMs (27).** In the assessments of TTCE and CT features, readers were blinded to the results of the other imaging study.

Revascularization of a previously treated PAVM was defined based on CT findings of a persistent aneurysmal sac with prominent afferent arteries and drainage veins

(28, 29). Patients with an afferent artery measuring $\geq 3\text{mm}$, a PAVM with less size but suitable for embolization and those with CT criteria indicating revascularization (30) were sent to the angiography suite to plan embolotherapy.

Statistical analysis

Baseline characteristics of the patients were compared using a Mann-Whitney U test or Kruskal-Wallis test for continuous variables. For categorical variables the chi-square test or Fisher exact test when expected values in any of the cells of a contingency table were below 5 were calculated (Fisher exact Test were estimated in case of $m \times n$ tables by Monte Carlo method). Kappa statistics with 95% confidence intervals (95% CI) were used to assess agreement between TTCE studies performed using agitated saline or Gelofusine solution. Sensitivity, specificity, and positive and negative predictive values were calculated, using the CT results as the reference test; 95% CI were calculated as exact binomial confidence intervals. The diagnostic accuracy of cardiac cycle was evaluated by constructing a receiver-operating-characteristic (ROC) curve. *P*-values < 0.05 (two-sided) were considered statistically significant. Statistical analyses were performed using SPSS v 19.0 (IBM Corp, Armonk, NY), and Stata statistical software (Release 10.0, Stata Corporation, College Station, TX).

Results

Of the 664 patients evaluated in the HHT Unit of Hospital Sierrallana (Cantabria, Spain), 449 (65.2%) were diagnosed with HHT based on clinical criteria or genetic testing. Among this total, 312 (69.5%) patients had TTCE to rule out PAVMs, and 205 (45.7%) underwent both TTCE and chest CT at a mean interval between

assessments of 14 days (SD 34.8, range 0-180 days). There were no significant differences in demographic and genetic characteristics between the overall population and those undergoing the two imaging assessments (Table 1). Ninety-five of these 205 patients had been included in a previous study (13).

The demographic characteristics of the 205 patients who underwent TTCE and CT stratified by TTCE grade are shown in Table 2. Patients in higher grades were significantly younger than those TTCE negative or in lower grades ($p=0.04$). Female predominate in grades 2 and 3 and men in grade 4 ($p=0.003$). HHT type 2 patients were more frequent in grades 0 and 1, and type 1 patients predominated in the remaining grades; particularly grade 4 where 90.5% of the patients were HHT1 ($p<0.0001$). Hb values were found in case files of 191 patients (93.2%) were determined. Seventy-seven patients (40.3%) were found to be anemic. No statistically significant differences in Hb values ($p=0.749$) were observed when comparing grades, nor were there differences found in the number of patients with anemia ($p=0.91$) or in the Hb values of these patients ($p=0.154$). CT studies of the liver were performed for 188 patients (91.7%). In 62.2% of these, the tests revealed HAVMs. In addition to the Hb values, we do not find significant differences in the number of HAVMs when comparing grades ($p=0.87$).

Fifty-nine (38.8%) of 152 TTCE-positive patients had a PAVM on CT (Table 3): 2 (3%) grade 1, 18 (42.9%) grade 2, 17 (77.3%) grade 3, and 21 (100%) grade 4. One patient categorized as TTCE-negative (no passage of contrast visualized) had a previously embolized PAVM showing no signs of revascularization on CT. Twenty-four patients had a single PAVM and 35 (59.3%) multiple PAVMs, with no significant differences in the incidence of single or multiple PAVMs between

grades. However a trend was detected in which the higher the grade, the greater the probability of having multiple PAVMs (test for trend: $P=0.10$).

Among 152 TTCE positive patients, the cardiac cycle at which microbubbles first appeared in the left heart chambers after contrast administration was recorded in 137 patients. The mean cardiac cycle at visualization of bubbles in the left ventricle was 6.6 in grade 1, 4.7 in grade 2, 4 in grade 3, and 3.2 in grade 4 (Table 4). In patients classified into higher grades, contrast bubbles appeared earlier ($p<0.0001$), such that in grade 4, 61.9% of patients showed bubbles in the left atrium during the third cardiac cycle.

In patients without PAVM on CT, bubbles were first seen in the left heart chambers at a mean of 6.1 cardiac cycles (SD 1.7) and 3.9 (SD 0.1) in those with PAVM **on CT** ($p<0.0001$) (Table 5). A cut-off of ≤ 4 cycles had a sensitivity of 81.5% (95% CI, 76.1%-86.8%) and specificity of 77.1% (95% CI, 71.3%-82.8%) to detect PAVMs and correctly classified 78.8% of patients. The area under the curve for cardiac cycle in the diagnosis of PAVM was 0.86 (95% CI: 0.79-0.91) (Fig. 1). Nonetheless, we were unable to establish a clear differentiation between patients with and without CT-proven malformations in each grade based on the cardiac cycle. In grade 2, the area under the curve for cardiac cycle in the diagnosis of PAVM was 0.63 (95% CI: 0.46-0.78), with 3 cycles being the cut-off that best classified patients (60.5%), with a specificity of 100% (95% CI: 83.9%-100%), but much lower sensitivity (11.8%; 95% CI: 1.5-36.4%). A count of 4 cardiac cycles had a sensitivity of 64.7% (95% CI 38.3-85.8%) and specificity of 47.6% (95% CI 25.7%-70.2%) to detect PAVM, and correctly classified 55.3% of patients in this grade.

Sixteen (27.1%) of the 59 patients with PAVM had at least 1 embolized PAVM (mean 1.9; range 1-5) when they were first seen in our hospital. Over the period studied, 29/59 (49.2%) underwent one or various embolizations (mean 3.2; range 1-12), 9 of which were for PAVM revascularization. In 12 patients (2 to treat revascularization) (20.3%), embolotherapy was recommended based on CT findings (afferent artery ≥ 3 mm) (Table 6). When TTCE findings were used as the reference, only 7/42 (16.7%) patients in grade 2 (2 revascularizations) required or were recommended embolotherapy. In Grade 3, treatment was performed or recommended in 14 (63.6%) patients (1 revascularization) and in Grade 4, in 20 (95%) patients (8 revascularizations). When CT findings were used as the reference, 7/18 (38.9%) patients with grade 2 PAVM, 14/17 (82.3%) with grade 3, and 20/21 (95.2%) with grade 4 underwent or were recommended treatment. The single grade 4 patient who was not treated had multiple PAVMs on CT, but none were amenable to embolotherapy.

In the comparative study, 114 patients underwent TTCE using the 2 contrast agents (Table 7). As compared to saline TTCE, Gelofusine TTCE resulted in upgrading of a large percentage of patients in each grade: 64.3% of TTCE-negative; 54% in grade 1, 60% in grade 2, and 64.3% in grade 3 (videos 1 and 2). A correlation between the two contrast agents was only seen in grade 4. Because of this considerable overestimation of grade, there was a poor correlation between results for the two contrast agents: 0.32 quantified by kappa statistics (95% CI 0.25-0.38). In total, 41/114 (36.0%) undergoing both contrast studies had a PAVM on CT. To detect the same number of PAVMs according to clinical guideline recommendations (positive TTCE, followed by CT), 104 CT studies would be performed based on Gelofusine results and 86 on agitated saline results. No

significant contrast-related adverse events occurred with either agent. Only one patient, an 11-year-old boy with a huge PAVM in the right lung (TTCE grade 4), experienced dizziness after Gelofusine administration.

Discussion

Several findings from the present study are consistent with those of our previous research (13) and the results of other authors (7, 10, 14, 16). The data further support the idea that although small PAVMs can be detected on contrast TTCE (grade 1), the afferent artery is not large enough to undergo embolotherapy, and therefore, CT would not be needed (7, 13, 14). Analysis of the situation regarding TTCE grade 2 requires special consideration. In patients classified as grade 2, some treatable PAVMs are detected and CT would be indicated in these cases. Nonetheless, the percentage of PAVMs ultimately identified by CT is low: only 25% to 45.3% of these patients (42.9% in this series) have a PAVM on CT (10, 13, 14). Furthermore, the percentage amenable to embolotherapy is even lower: 16.7% in this series and 25.3% in the study of Velthuis et al (14). Thus, more than half the patients rated as TTCE grade 2 using the current criteria would actually not need CT evaluation. Hence, it would be of value to further refine the criteria to differentiate between TTCE grades 1 and 2 to reduce the number of unnecessary CTs in these patients.

Without a doubt, CT is needed to characterize the malformation in patients in TTCE grades 3 and 4 (13, 14). More than 75% of these patients (77.3% in our series, 77.4% in the series of Velthuis et al. (14) have PAVM on CT and more than 80% are accessible to embolotherapy (82.2% in grade 3 and 95% in grade 4 in our study and 83.7% in the study of Velthuis et al) (14)

Previous reports found a relationship between TTCE grade and HHT genotype (7, 14) and our results concur with these findings: HHT2 predominated in TTCE-negative patients and grade 1, whereas HHT1 predominated in the remaining grades. Velthuis et al (14) reported no differences in age or sex between patients categorized into the different grades, but in our series, **patients with higher grades were significantly younger than those in the lower grades.** These findings support the notion that lung involvement is more frequent and more severe in patients with the HHT1 subtype, as described (7, 31). **Female predominate in the study group although there was a slight predominance of men in grade 4.** Further studies in larger samples are needed to determine whether these are generalizable differences or result from a collateral effect of the sample. The number of patients with multiple PAVMs (60%) was similar to previously reported values (32).

Reduced oxygenation of blood due to pulmonary PAVMS, anemia and/or the presence of HAVMs could in HHT patients increased cardiac output as reported (8, 32). An increase in cardiac output has been shown to lead to an increase of blood flow through normally present intrapulmonary arteriovenous anastomosis and therefore be the cause of false positive TTCE results in HHT patients (20). However, in our study, patients testing negative for TTCE had similar levels of Hb and HAVMs to those with positive TTCE results; suggesting that these parameters do not significantly affect the TTCE findings.

With regard to the cardiac cycle time point, our current results confirm those of our previous study (13): contrast passage occurred earlier in higher grades. Furthermore, and in contrast to the results of Zukotynski et al (10), we found a statistically significant difference in the cardiac cycle at which bubbles were first seen in the left chambers on TTCE between patients with and without PAVMs on

CT ($p < 0.0001$). The fourth cycle seemed to be the best cut-off for this purpose, yielding a sensitivity of 81.5% and specificity of 71.5%, and correctly diagnosing 79% of patients. However, we were unable to establish a useful cardiac cycle to differentiate between patients with and without CT-confirmed PAVMs within each TTCE grade. Studies in larger patient samples may clarify this aspect.

The treatment of choice for PAVMs is currently embolotherapy, a technique that has minimal functional repercussions and relatively low invasiveness (34, 35). Sixteen patients had previously undergone embolotherapy of one or several PAVMs when they were first seen in our unit. Most (15 of 16 patients) tested positive on TTCE; a normal finding according to Lee et al. (36) who reported a high incidence of persistently positive TTCE findings following embolotherapy (90%). Forty-one patients showed PAVMs on CT with afferent arteries large enough to be treated at follow-up (35); most were in grades 3 and 4 and embolotherapy was feasible in more than 82%. These results concur with those of Velthuis et al (14): 83.7% of their grade 3 patients had embolotherapy.

To our knowledge, this is the first study comparing contrast TTCE using agitated saline versus Gelofusine for PAVM screening in an HHT population. In all TTCE grades, there was a clear trend to categorize patients into a higher grade with use of Gelofusine than with saline. We attribute this to the smaller size of the Gelofusine bubble, although other characteristics of this contrast medium, such as more stable bubbles and longer bubble retention (21, 24), may contribute to this difference. Of particular note, a number of patients classified as TTCE-negative using saline were passed to grade 1 or even 2 with Gelofusine. We cannot say whether this is attributable to a greater capacity of Gelofusine to detect small PAVMs or simply false-positive detection. Small pulmonary shunts on TTCE have

with the use of either contrast agent in our study.

One limitation of this study is that 2-detector CT was used in some examinations. More sensitive systems would likely detect a larger number of small PAVMs, and this may justify the low incidence of PAVMs in our grade 1 group relative to other studies (7, 14). However, we believe that this limitation does not significantly influence the interpretation of the results, as detection of significant PAVMs would not be affected. Second, semiquantitative measurement was used to differentiate between grades 2 and 3, and 3 and 4, although the results were comparable to those found in a study using a quantitative cut-off (14). Third, patients who had undergone previous PAVM embolization were not excluded because in clinical practice, TTCE findings of grade 2 to 4 PAVM in these patients indicate the need for CT assessment of possible revascularization.

In summary, the results of this study further support our previous interpretation that CT is not needed in patients with grade-1 shunt on TTCE. We believe that the definition of grade 2 should be refined to better differentiate between grades 1 and 2 and further reduce unnecessary CT studies. The cardiac cycle at which bubbles first appear can help to identify patients with and without PAVMs. Use of the fourth cycle correctly classified 79% of patients. Gelofusine use did not improve

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Supplemental material Video 1 A, B

Video 1. A, B. Transthoracic contrast echocardiography, performed in the same patient. Using an agitated saline solution (A) and Gelofusine (B). In this case there is an upgrade from grade 2 with saline to grade 4 with Gelofusine.

Supplemental material

Video 2.A, B. Transthoracic contrast echocardiography performed in the same patient: with a saline solution (A) and Gelofusine (B). In this case there is an upgrade from grade 3 with saline to grade 4 with Gelofusine

Figure 1

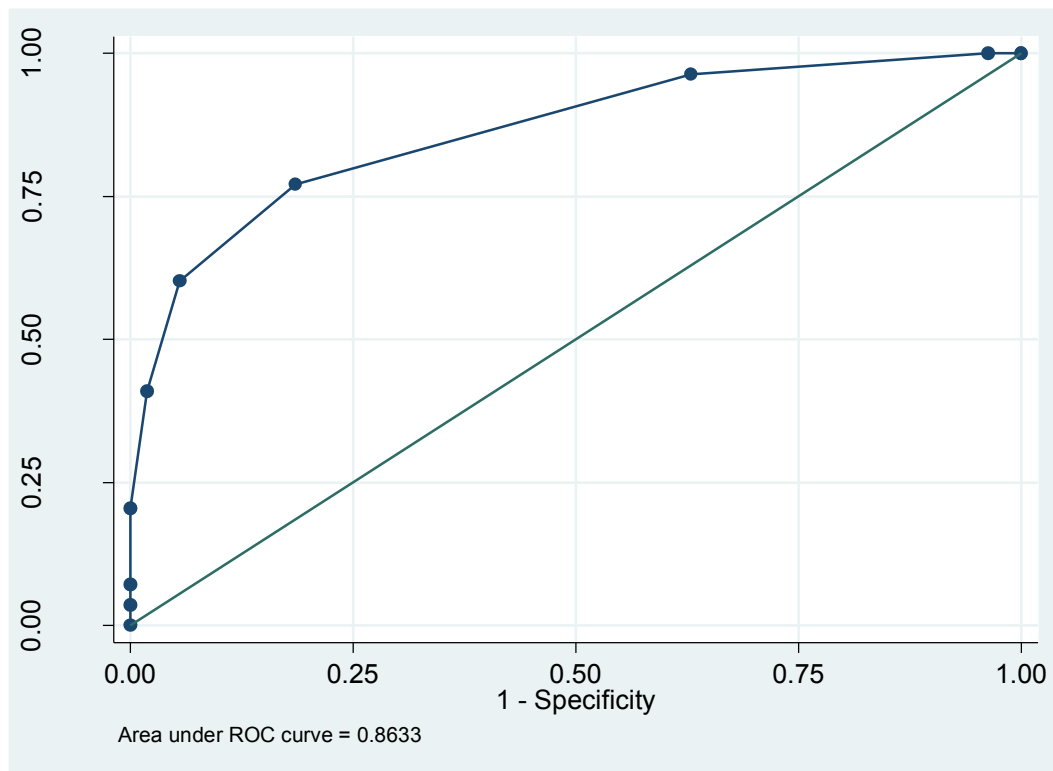


Fig. 1: ROC curve. Cardiac cycle in the diagnosis of pulmonary arteriovenous malformation.

Table 1. Demographics and genetic characteristics.

	HHT patients n=449	TTCE+CT n=205	P value*
Age, years, median; range	43; 0-85	50; 6-85	<0.001
Sex			0.74
Male (%)	204 (45.4)	96 (46.8)	
Female (%)	245 (54.6)	109 (53.2)	
Genetic testing (%)	424 (94.4)	197 (96.1)	
HHT1 (%) / median; range	192 (42.8) / 40; 1-85	87 (42.4) / 47; 6-85	<0.001
<i>Male</i>	97 (50.5)	44 (50.6)	0.993
<i>Female</i>	95 (49.5)	43 (49.4)	
HHT2 (%) / median; range	229 (51.00) / 46; 0-81	109 (53.2) / 50; 11-78	0.03
<i>Male</i>	97 (42.4)	51 (46.8)	0.443
<i>Female</i>	132 (57.6)	58 (53.2)	
SMAD4 (%) / median; range	3 (0.7) / 45; 42-48	1 (0.5) 42	--
<i>Male</i>	0 (0)	0 (0)	--
<i>Female</i>	3 (100)	1 (100)	
Other** (%) / median; range	25 (5.5) / 50; 8-74	8 (3.9) / 53; 35-72	0.93
<i>Male</i>	10 (40)	1 (12.5)	0.22
<i>Female</i>	15 (60)	7 (87.5)	
N TTCE performed	312	205	
TTCE positive (%)	222 (71.1)	152 (74.1)	0.49

*The two-tailed chi-square test/Fisher exact test for categorical variables or the Mann-Whitney U test for continuous variables. **Other: Diagnosis established based on 3 or 4 Curaçao criteria.

Table 2. Demographics, genetics, hemoglobin values and hepatic arteriovenous malformations, according to TTCE grade

TTCE results	Patients, n	Age median; range	Male (%) / Female (%)	HHT1 (%)	HHT2 (%)	SMAD4 (%)	Other* (%)	HB (g/dL); median; range	Anemia (%) / HB (g/dL), median; range	HAVMs, n (%)
Negative	53	56; 19-85	28(52.8)/25 (47.2)	13 (24.5)	38 (71.7)	0 (0)	2 (3.8)	12.95; 6-17.3	22 (28.6%) / 11.2 (6-13.1)	32 (27.4)
Grade 1	67	49; 18-78	34(50.7)/33 (49.3)	17 (25.4)	46 (68.7)	0 (0)	4 (6)	13.05; 6.1-17.2	24 (31.2) / 10 (6.1-13.4)	37 (31.6)
Grade 2	42	46; 12-73	17(40.5) / 25 (59.5)	27 (64.3)	14 (33.3)	0 (0)	1 (2.4)	13.0; 5.4-17.4	17 (22.1) / 10.7 (5.4-13.4)	25 (21.4)
Grade 3	22	43.5; 26-70	4 (18.2) / 18 (81.8)	11 (50)	9 (40.9)	1 (4.5)	1 (4.5)	12.3; 8.9-18.0	8 (10.4) / 9.5 (8.9-11.8)	13 (11.1)
Grade 4	21	40; 6-73	13 (61.9) / 8 (38.1)	19 (90.5)	2 (9.5)	0 (0)	0 (0)	14.0; 8.3-19.0	6 (7.8) / 9 (8.3-10)	10 (8.5)
p-value**		0.004	0.003	<0.0001				0.749	0.91/0.154	0.87

TTCE: Transthoracic contrast echocardiography; HHT1: deficit of *Endoglin*; HHT2: deficit of *ACLVI/ALK1*; Smad4: association of Hereditary hemorrhagic telangiectasia and Juvenile polyposis. Negative, no signs of contrast passage on TTCE. * Diagnosis established based on 3 or 4 Curaçao criteria; HB: Hemoglobin; HAVMs: hepatic arteriovenous malformations. ** The two-tailed chi-square test/Fisher exact test for categorical variables or the Kruskal-Wallis test for continuous variables.

Table 3: Correlation between TTCE and chest CT in the 205 patients undergoing both studies

TTCE results	Patients, n (%)	Patients with PAVMs on CT n (%)	Patients with only one PAVM seen on CT (%)	Patients with multiple PAVMs seen on CT (%)	PAVM, mean (SD)
Negative	53 (25.9)	1* (1.9)	1 (1.9)	0 (0.0)	0
Grade 1	67 (32.7)	2 (3)	2 (100)	0 (0.0)	1 (0)
Grade 2	42 (20.5)	18 (42.9)	7 (38.9)	11 (61.1)	3.2 (2.9)
Grade 3	22 (10.7)	17 (77.3)	8 (47.1)	9 (52.9)	2.6 (2.0)
Grade 4	21 (10.2)	21 (100)	6 (28.6)	15 (71.4)	4.1 (3.6)
All	205	59 (28.8)	24 (40.7)	35 (59.3)	3.2 (3.0)

CT, computed tomography; PAVM, pulmonary arteriovenous malformation; TTCE, transthoracic contrast echocardiography. Negative, no signs of contrast passage on TTCE; *previously embolized and no signs of revascularization seen on CT

Table 4. Correlation between TTCE grade and cardiac cycle.

TTCE grade	Patients n (%)	Cardiac cycle: Mean cardiac cycle, SD; range
Grade 1	60 (43.8)	6.6, 1.7; 3-10
Grade 2	38 (27.7)	4.7, 1.1; 3-7
Grade 3	18 (13.1)	4, 0.7; 3-5
Grade 4	21 (15.4)	3.2, 0.6; 2-4
All	137 (100)	5.2, 1.8; 2-10

TTCE, transthoracic contrast echocardiography

Table 5. Sensitivity and specificity of different cardiac cycle cut-offs in the diagnosis of PAVM

Cut-off point*	Sensitivity, % (95% CI)	Specificity, % (95% CI)	Correctly Classified, %	LR+	LR-
1	0.0 (0-6.6)	100.0 (95.7-100)	60.6	1.0	
2	3.7 (0.5-12.7)	100.0 (95.7-100)	62.0	1.0	0.0
3	37.0 (24.3-51.3)	96.4 (89.8-99.2)	73.0	1.5	0.1
4	81.5 (68.6-90.7)	77.1 (66.6-85.6)	78.8	4.1	0.3
5	94.4 (84.6-98.8)	60.2 (48.9-70.8)	73.7	10.8	0.4
6	98.2 (90.1-99.9)	41.0 (30.3-52.3)	63.5	22.1	0.6
7	100.0 (93.4-100)	20.5 (12.4-30.8)	51.8		0.8
8	100.0 (93.4-100)	7.2 (2.7-15.1)	43.8		0.9
9	100.0 (93.4-100)	3.6 (0.8-10.2)	41.6		1.0
10	100.0 (93.4-100)	0.0 (0.0-4.4)	39.4		1.0

*Cardiac cycle at which bubbles were seen in left heart chambers. The cut-off point includes all values less than or equal to this value.

LR, likelihood ratio.

Table 6: Correlation between TTCE grade, CT findings, and embolotherapy treatment

TTCE results	Patients, n	Patients with PAVM on CT, n (%)	Patients with PAVMs embolized, n (%) ¶	Embolotherapy, n (%) §	PAVMs treated per patient, mean	Embolotherapy recommended n (%) ♦
Negative	53	1 (1.8)	1 (1.8)	0 (0.0)	0	0 (0.0)
Grade 1	67	2 (3)	1 (1.5)	0 (0.0)		0 (0.0)
Grade 2	42	18 (42.9)	5 (11.9)	6 (2*) (14.3)	2.1	1 (2.4)
Grade 3	22	17 (77.3)	2 (9.1)	9 (41.0)	1.8	5 (1*) (22.7)
Grade 4	21	21 (100)	7 (33.3)	14 (7*) (66.7)	3.3	6 (1*) (28.6)

¶ Patients with a PAVM embolized the first time they come to the HHT Unit. § Patients treated with embolotherapy during the study period. ♦ Patients with embolotherapy recommended because of the size of the feeding artery. * Patients with a PAVM and CT signs of revascularization seen by CT. TTCE, transthoracic contrast echocardiography. Negative, no signs of contrast passage on TTCE

Table 7. Comparison of TTCE Grading Using Agitated Saline Contrast Vs. Gelofusine: **first and second injections.**

TTCE		Gelofusine						Patients	PAVM on CT
		Negative	Grade 1	Grade 2	Grade 3	Grade 4			
Agitated saline	Negative	10	17	1			28	1 (*)	
	Grade 1		14	15	2		31	2	
	Grade 2			12	14	4	30	16	
	Grade 3				5	9	14	11	
	Grade 4					11	11	11	
	Patients	10	31	28	21	24	114		
	PAVM on CT	0	1 (*)	7	11	22		41	

(*) One previously embolized PAVM with no CT evidence of revascularization.

TTCE, transthoracic contrast echocardiography; negative, no signs of contrast passage on TTCE

Video 1 A

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