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ABSTRACTS

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ORAL COMMUNICATIONS

Session 1 Mechanisms of HHT

Arterial-venous malformation form through canonical SMAD4 signaling

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Hereditary Hemorrhagic Telangiectasia (HHT) is an inherited vascular disorder caused by mutations in the TGFB/BMP signaling components including *Growth Differentiation Factor 2* (*GDF2)* encoding the Bone Morphogenic Protein (BMP9), the surface receptors Endoglin (*ENG*, mutated in HHT1) and *ACVRL1* encodingActivin-receptor-like kinase 1 (*ALK1*, mutated in HHT2), and the signaling pathway effector *SMAD4* (*SMAD4*, mutated in a combined syndrome of HHT and juvenile polyposis (HHT-JP)). HHT patients develop multiple focal vascular malformations including small caliber cutaneous capillary telangiectasias and large caliber visceral arterial-venous malformations (AVMs). These lesions in which blood flow is shunted from an artery directly into a vein are fragile and prone to rupture causing hemorrhages. We recently showed that BMP9/10

ligand blockade or homozygous *Alk1* deletion in the endothelium both lead to increased endothelial PI3K pathway activation that may be a novel target for the treatment of vascular lesions in HHT patients. To test if increased PI3K signaling is due to canonical Smad signaling, we created inducible, endothelial-specific homozygous *Smad4* mice. Our preliminary results show that Smad4 deletion mimics BMP9/10 blockade and endothelial *Alk1* inactivation, suggesting that lack of canonical SMAD signaling downstream of BMP9/10-Alk1 contributes to AVM pathogenesis. Current studies aim to establish the exact mechanisms leading to vascular malformations.

Identification of TGF-Beta downstream effectors: a SMAD4 directed approach reveals a link between angiopoietin signaling and HHT

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Objectives: To identify the downstream effectors of the transforming growth factor-beta (TGF-Beta) pathway that are responsible for the vascular defects associated with HHT, including the development of arteriovenous malformations (AVMs). Our experiments focus on the role of Smad4 in HHT as TGF-Beta signaling converges at Smad4 to transcriptionally regulate target genes of this pathway.
Methods: Utilizing the Cre-LoxP system and an inducible, endothelial cell specific Cre mouse line (Cdh5-Cre^{ERT2}), we developed a Smad4 animal model of HHT (termed Smad4-iECKO). RNA-sequencing and ChIP-sequencing experiments were conducted on Smad4-iECKO mice and primary human endothelial cells to uncover downstream target genes of the TGF-Beta pathway. In vivo experiments, including conditional knockout mouse models, will be used to evaluate the causal role (s) of these effectors in the formation of AVMs.
Results: Depletion of Smad4 specifically in blood vessels leads to AVM formation in the mouse retina, similar to Alk1 and Endoglin mouse models of HHT. Combined RNA- and ChIP-sequencing data revealed 184 potential Smad4 target genes, including the vascular receptor Tek and its antagonistic ligand, Angiopoietin 2 (Ang2). We find that Tek and Ang2 are significantly mis-regulated in retinal blood vessels of Smad4-iECKO mice, and have begun examining their regulatory effects on the formation and resolution of AVMs.
Conclusion: Integration of next generation sequencing methods and our Smad4-iECKO model have uncovered prospective downstream effectors of TGF-Beta signaling. Our preliminary data suggests that among these effectors, the Tek/Angiopoietin pathway may have a critical and previously unexplored role in the pathogenesis of AVMs and HHT.

Cardiosphere derived cells require endoglin to promote paracrine-mediated angiogenesis

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Aim/Methods: Cardiosphere-derived cells (CDCs) are a heterogeneous heart-derived cell population that are currently being evaluated in the clinical setting for their ability to improve outcomes following myocardial infarction (MI). Their beneficial effects occur primarily via paracrine mechanisms that include promoting neo-angiogenesis that is critical for revascularisation of ischemic tissues. CDCs express endoglin (also known as CD105), which is a defining marker of mesenchymal stem cells (MSCs) and a TGFβ family co-receptor. The role of endoglin is best understood in endothelial cells, where it binds to specific ligands of the TGFβ family, including BMP9, to promote activation of the SMAD1/5/8 pathway and stimulation of angiogenesis. However, whether it has a similar pro-angiogenic role in CDCs has not yet been determined. Our objective was therefore to investigate whether endoglin promotes the paracrine pro-angiogenic properties of CDCs. This was achieved using conditioned media prepared from wild-type and endoglin-depleted murine CDCs. Their pro-angiogenic properties were then evaluated in a range of in vitro and in vivo angiogenesis assays.
Results: In all cases, the CDC secretome promoted a pro-angiogenic response that was endoglin-dependent. Furthermore, endoglin expression in CDCs was also required for these cells to mediate pro-angiogenic effects in a mouse model of MI. There was reduced activation of the SMAD1/5/8 pathway in CDCs in the absence of endoglin, which could be rescued by BMP9. Importantly, BMP9 pre-treatment of endoglin-depleted CDCs led to restoration of their pro-angiogenic paracrine properties.
Conclusion: Our findings show the critical importance of endoglin and BMP9 signaling in promoting the pro-angiogenic properties of CDCs.

Session 2 Treatment of HHT

Effect of systemic bevacizumab on invasive cardiac hemodynamics among patients with hereditary hemorrhagic telangiectasia and high output heart failure

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Objectives: High output heart failure is a serious complication observed in hereditary hemorrhagic telangiectasia (HHT). Previous work has suggested a decrease in cardiac output among individuals treated with intravenous bevacizumab as measured by echocardiography. Cardiac catheterization is the gold standard measure of cardiac output. We describe the impact of bevacizumab on cardiac output by invasive hemodynamic assessment.
Methods: We retrospectively reviewed our database of HHT patients between May 2010 and October 2016. We identified patients treated with intravenous bevacizumab (5 mg/kg for at least 6 doses) and recorded cardiac hemodynamic parameters pre and post-treatment. Cardiac output (CO) and cardiac index (CI) were assessed invasively by catheterization. Statistical assessment comparing pre and post-intervention was performed via 2-tailed Wilcoxon matched-pairs signed rank test.
Results: We identified 14 patients treated with intravenous bevacizumab; 8 of those patients underwent both baseline and post-intervention catheterization. Mean CO pre and post-intervention were 8.2 L/min and 7.1 L/min respectively ($p = 0.1953$). Mean CI pre and post-intervention were 4.3 L/min/m^2 and 3.7 L/min/m^2 respectively ($p = 0.1230$) a 13% reduction in mean CI from baseline. In a subset of 5 of these patients with both invasive and echocardiographic data at baseline and post-intervention, echocardiography identified a 34% reduction in CI, while catheterization identified only a 19% reduction.
Conclusion: Cardiac index tended to decrease among patients with high output heart failure treated with intravenous bevacizumab, but we lacked statistical power to prove significance. Our series suggests that improvement is not as substantial by gold-standard catheterization as suggested by echocardiography. Further studies with more patients are warranted.

Intravenous bevacizumab for the management of transfusion dependent blood-loss anemia in hereditary hemorrhagic telangiectasia

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Background: RBC transfusion dependence from severe HHT related bleeding due to epistaxis and/or gastrointestinal bleeding is a major clinical problem with few durable treatment options. We present a multi-year clinical experience with intravenous (IV) Bevacizumab in the management of RBC transfusion dependent HHT patients with refractory anemia.

Methods: All RBC transfusion dependent HHT patients with refractory anemia treated with IV Bevacizumab from June 2013 to Jan 2017 were included in this study. Patients were categorized as RBC transfusion dependent if they had been receiving regular or scheduled RBC transfusions in the 6 months preceding initiation of Bevacizumab. Patients were treated with a standardized protocol for IV Bevacizumab (Fig. 1). After completion of the initial cycle of Bevacizumab treatments, patients were re-treated with Bevacizumab as per clinical need (recurrent epistaxis/GI bleeding; worsening anemia). Number and frequency of PRBC transfusions, hemoglobin levels along with iron studies, ESS and quality of life were collected serially in all patients. Quality of Life was assessed at each follow up appointment using a 7 point self-reported Likert scale with the following question: *How would you rate your overall quality of life since the last appointment?* (1 = very poor QOL; 4 = fair/average QOL and 7 = excellent QOL). We also assessed the impact of epistaxis on quality of life (E-QOL) life using a similar 7 point Likert scale question: "How often has nose bleeding affected or interfered in your day-to-day life since the last appointment" (1 = very seldom; 4 = occasionally and 7 = very often).

Results: IV Bevacizumab was administered to 34 patients overall. Of them, 16 patients (47.1%) were RBC transfusion dependent with a median duration of RBC transfusion dependence of 6 years (range 1–15). Median lifetime RBC transfusions before start of Bevacizumab was 75 (range 4–650) compared to a median of 1.5 (range 0–10) in non-transfusion dependent patients (n = 18). Median transfusion frequency in the RBC transfusion dependent cohort (n = 16) was 2 blood transfusions/month (range 0.3–16).

The median number of transfusions at baseline and at 3, 6, 9, 12, 24 and 36 months after the initiation of IV Bevacizumab therapy are shown in Table 1. After initiation of IV Bevacizumab, there was complete cessation of blood transfusion needs in all but 3 patients; 2 of whom required continuous Bevacizumab top up infusions to maintain their hemoglobin. Patients were followed for a median of 21.4 months (range 3–42.5 months) from the start of Bevacizumab treatment. Median number Bevacizumab doses administered were 13.5 (range 1–48 doses) at a frequency of 11.7 Bevacizumab doses/patient/year of follow up. In the 4 patients requiring continuous top-up Bevacizumab dosing; the median number of doses administered was 41 (range 33–48 doses) at a frequency of 15.4 Bevacizumab doses/patient/year of follow up. In the other 12 patients (requiring only intermittent re-dosing of Bevacizumab); the median number of doses administered was11 (range 1–24 doses) at a frequency of 7.3 Bevacizumab doses/patient/year of follow up. Table 2 illustrates the median hemoglobin, iron, ferritin, epistaxis severity scores (ESS), quality of life (QOL) and epistaxis impact on quality of life (E-QOL) measured serially in these patients. Whereas Table 3 illustrates those same variables from the time of Bevacizumab initiation until patients required re-treatment with Bevacizumab for worsening bleeding and/or anemia. This depicts the natural history of these patients after a single initial treatment cycle of IV Bevacizumab.

Conclusion: Intravenous Bevacizumab is a very effective treatment option for HHT patients with RBC transfusion dependence secondary to severe bleeding from epistaxis and/or GI bleeding. Bevacizumab resulted in complete freedom from RBC transfusions in more than 80% of patients with maintenance of adequate hemoglobin, iron levels and quality of life. Further studies are needed to establish a dose–response relationship as well as clinical, genetic and biomarker predictors of response.

A standardized treatment protocol of Intravenous Bevacizumab for refractory HHT related epistaxis and gastrointestinal bleeding

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Background: Refractory anemia due to severe HHT related bleeding (from epistaxis and/or gastrointestinal (GI) bleeding) is often an intractable problem with few durable treatment options and transfusion dependence. We present a multi-year clinical experience with intravenous (IV) Bevacizumab for the management of these patients.

Methods: All patients treated with IV Bevacizumab for severe HHT related bleeding at the Mayo Clinic in Rochester, Minnesota from June 2013 to Jan 2017 were included in this report. Epistaxis severity scores (ESS) and hemoglobin levels were obtained serially in all patients. Data presented here covers each patient from the date of first Bevacizumab infusion to the date they required re-treatment with Bevacizumab (for either worsening anemia and/or epistaxis).

Results: A total of 34 HHT patients with refractory anemia were treated with IV Bevacizumab using a uniform pre-specified treatment protocol typically consisting of 4 infusions 2 weeks apart followed by 4 infusions 1 month apart (Fig. 1). The refractory anemia was primarily related to severe epistaxis (n = 15, median ESS: 8.19, range 4.7–10); GI bleeding (n = 4, median ESS: 0.72: range 0–0.9) or both (n = 15, median ESS: 5.25, range 0.7–7.3). One or more RBC transfusions had been administered to 27 patients (79%) and 16 patients (47%) were RBC transfusion dependent in the 6–12 months leading up to Bevacizumab treatment. Median time from first Bevacizumab dose to the time of re-treatment (or last date of follow-up if not re-treated) was 8.1 months (range 3–27.3 months).

Three patients were still receiving Bevacizumab doses from the initial cycle. Of the remaining 31 patients who had completed the initial dosing cycle; those not requiring re-treatment with Bevacizumab at 1, 3, 6, 9, 12, 15, 18, 21 months post initial cycle completion were 29, 21, 14, 10, 5, 3, 1, and 1 respectively.

Serial ESS scores in the 30 patients with significant epistaxis (excluding the 4 patients with isolated GI bleeding) are shown in Table 1 and Fig. 2. The median ESS declined from **6.52** (n = 30) at baseline to **4.06** (n = 15); **3.89** (n = 14); **2.28** (n = 10); **1.92** (n = 13); **3.15** (n = 14); **2.12** (n = 8); 3.65 (n = 4); **4.24** (n = 4); **6.56** (n = 1) at 1, 3,

6, 9, 12, 15, 18, 21 and 24 months after initiation of Bevacizumab without any top-up infusions (re-treatment).
Serial hemoglobin values in the entire cohort (n = 34) are shown in Table 2 and Fig. 3. The Median Hemoglobin levels increased from **9.1** g/dl (n = 33) at baseline to **11.7** (n = 16); **12.4** (n = 21); **12.3** (n = 22); **11.7** (n = 11); **11.5** (n = 11); **12.2** (n = 8); **13.2** (n = 5); **10** (n = 3) at 1, 3, 6, 9, 12, 15, 18 and 21 months after initiating Bevacizumab without any top-up infusions (re-treatment).
Serial Iron, Ferritin and RBC transfusion data are shown in Tables 3 and 4. The total number of lifetime blood transfusions in all 34 patients prior to Bevacizumab was 2965 units (median 6, IQR 1–62.5, range 0–650). Total number of blood transfusions after starting Bevacizumab until re-treatment or last follow up (in cases not re-treated) in the 34 patients was 79 units of blood (median 0, IQR 0–0.5, range 0–36). Thus, a substantial reduction in RBC transfusions was noted after initiation of Bevacizumab treatment and this effect appeared to be sustained through follow up.
Conclusion: Intravenous Bevacizumab is a very effective treatment option for HHT patients with refractory anemia secondary to epistaxis and/or GI bleeding. There appears to be a durable response after a standardized treatment protocol typically consisting of 8 infusions. RBC transfusion needs were dramatically reduced or even eliminated along with significant improvements in hemoglobin, iron and ferritin levels. The ESS was also dramatically improved. Over time, most patients required top-up infusions (re-treatment) with Bevacizumab due to worsening epistaxis and/or anemia. Further studies are needed to establish a dose–response relationship as well as clinical, genetic and biomarker predictors of response.

Dietary assessments suggest many patients with hereditary hemorrhagic telangiectasia do not ingest recommended dietary allowance (RDA) for iron, and may spontaneously modify their diet to avoid nosebleed precipitants

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Objectives: Nosebleeds (epistaxis) occur very frequently in patients with hereditary hemorrhagic telangiectasia (HHT), leading to iron deficiency, and detrimental impact on quality of life. There is much evidence demonstrating that sensible dietary choices optimize health and well-being in the general population. Our goal was to examine if people with HHT actively manipulate their diet in order to meet recommended dietary iron intake requirements and/or improve nosebleeds.
Methods: With ethical approval, unselected groups of 50 and 25 HHT patients measured their food intake using a food frequency questionnaire (FFQ) and 7-day weighed food diary (7dWFD) respectively. Nosebleeds were quantified using the epistaxis severity score. All data were collected before perceived effects of dietary items on HHT nosebleeds were captured in unbiased international surveys of independent HHT cohorts. Ingested 7dWFD items were blindly assigned to 18 food groups where nosebleed associations were reported by ≥ 238 patients.
Results: By FFQ, none of the 5 pre-menopausal females met their recommended dietary allowance (RDA) of 18 mg iron/day. Only 4/50 (8%) met this RDA that did not take account of HHT haemorrhagic iron losses. The food items in the least ingested tertile foods by 7dWFD were reported to precipitate nosebleeds in 108/1542 cases (7.0%) compared to 49/1501 (3.2%) for the mid tertile and 52/1575 (3.3%) for the most ingested foods ($p < 0.001$ by Chi squared test.)
Conclusion: Dietary iron intake is often below even the general population recommendations. People with HHT may choose to avoid specific food items that they perceive to affect their nose bleed severity.

Long term follow-up of hereditary haemorrhagic telangiectasia patients who underwent liver transplantation for severe liver involvement

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Introduction: Liver transplantation (LT) has been reported as the only definitive curative treatment in hereditary haemorrhagic telangiectasia (HHT) with severe liver involvement leading to high-output cardiac failure and/or biliary ischemia and/or portal hypertension. The aim of this study was to evaluate HHT patients' long-term follow-up after liver transplantation 10–24 years after transplant.
Methods: Patients who underwent liver transplant for HHT in the Lyon liver transplant Unit from 1993 to 2009 were followed prospectively at this centre and in the French reference centre for HHT.
Results: Fifteen patients were included in this study (14 women and 1 man). Mean age at transplant was 52 years (33–66). Main indication for LT was cardiac failure. Twelve patients are still alive (80%) 16 years (8–24) after LT. One patient died from cardiac failure 65 days after LT and two late deaths unrelated to the disease occurred. In patients with cardiac failure, mean cardiac index failed from 5 to 3 L/min respectively ($p = 0.001$). Eleven patients out of 15 also experienced a dramatic improvement in epistaxis and quality of life. Among the 14 long survivors, recurrent liver telangiectasia was found on CTscan in 8 patients (57.1%) and on histology in 2 patients (14.2%) after a mean delay after LT of 11.6 years (6–15).
Conclusion: LT is a successful option for the treatment of severe hepatic HHT with low mortality in this cohort. Although, long term follow-up also suggests that hepatic recurrence of the disease seems to happen in an increasing number of patients.

Metafore study: 8 years later. Follow-up of HHT patients treated with bevacizumab for severe hepatic vascular malformations and high cardiac output

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Context: Twenty-five patients with severe hepatic arterio-vascular malformations (AVMs) have been included between March 2009 and November 2010 in a single-center phase-II trial (the "Metafore" study). Bevacizumab was given at a dose of 5 mg/kg every 14 days with a total of 6 injections.
Objective: To study long-term efficacy and safety of 25 HHT patients treated in this study.
Results: Twenty-two patients are still followed and 3 patients died 28, 29 and 45 months after the beginning of the treatment from unrelated complications.
All 3 patients classified as "non-responders" are alive. One of them underwent a liver transplantation 6 years after the treatment. The 2 others have clinical symptoms related to high cardiac output. Liver transplantation was refused by one and was ruled out for the other one because of severe hypoxemia related to diffuse pulmonary AVMs. All 5 patients classified as "complete-responders" patients, are also alive. Of them, 3 have been retreated with bevacizumab, without toxicity and with good response on cardiac index in 2 cases. Of the 17 patients classified as "partial-responders" patients, 14 are alive. Two of them underwent a liver transplant 5 and 6 years after the treatment and 2 are waiting for liver transplantation. Eight patients received a second or third course of bevacizumab or maintenance therapy with a good tolerance except for one who stopped the treatment after 2 injections because of articular pain. No arterial or venous thrombosis was observed in this cohort.
Conclusion: A second or a third cycle of treatment as well as maintenance therapy is possible and can be effective.

Session 3 Diagnosis of HHT

Clinical, radiographic, and genetic findings of a cohort of 39 patients with hereditary hemorrhagic telangiectasia and brain vascular malformations

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Aim/Method: We present a retrospective review of a prospectively maintained database on a cohort of all HHT patients with single or multiple BVMs evaluated at the University of Utah's HHT Center with known BVMs since 1995. Medical records were reviewed for each patient, and data, when available, were collected with a focus on presenting signs and symptoms, BVM imaging characteristics, and genomic findings.
Results: Thirty-nine patients with HHT and at least one BVM were diagnosed either clinically (38%, 15/39) or clinically and genetically (62%, 24/39). Males (49%, 19/39) and females (51%, 21/39) were equally represented, and mean age of diagnosis of HHT was 26 years (median 20 years, range 0–57 years; data available for 34/39 patients). Three patients have died since their diagnosis. Most patients presented with at least one of the following Curacao criteria: epistaxis (100%, 33/33; data available for 33/39), mucocutaneous telangiectasia (100%, 26/26), at least one visceral AVM (81%, 25/31; 92% pulmonary, 24% hepatic, 4% pancreatic, 4% gastrointestinal), or known family history (84%, 26/31). The majority of patients (21/35) sought evaluation, in part, due to recurrent epistaxis and/or family history. Two patients presented with solitary brain abscess and concurrent pulmonary or hepatic AVM, prior to receiving a diagnosis of HHT. Five patients complained of headache as a significant impetus for their evaluation. The BVM ruptured in 9 patients, with 2 patients suffering a second re-bleed years after stereotactic radiosurgery (SRS) treatment of the original lesion. In 8 of the 9 patients with BVM rupture, the rupture was the presenting symptom heralding the diagnosis of HHT. A total of 78 BVMs were identified in the 39 patients. Twenty-six patients had a solitary BVM whereas a third of patients (13/39) had at least two lesions (range 2–16). Supratentorial cerebral hemisphere BVMs comprised over three-quarters of all lesions (78%), and laterality of these lesions was approximately equivalent (Right: 46%, 28/61 vs. Left: 54%, 33/61). Infratentorial lesions accounted for the remainder. More specific location distribution, in order of frequency, was frontal (37%), parietal (17%), cerebellar (15%), occipital (13%), temporal (13%), and brainstem (3.9%), or in deep structures (Vein of Galen; 1%). Of the 30 BVMs with accurate measurements, size was generally small, with mean of 14 mm (median 10 mm, range 4–30 mm). Spetzler-Martin grading was performed for 55 of the 78 BVMs. The majority of patients were Grade 1 (73%, 40/55), whereas 24 and 4% were Grades 2 (13/55) and 3 (2/55), respectively. BVM management was clearly documented in 42 patients as involving surgical management alone (63%, 22/35), embolization followed by surgery (9%, 3/35), embolization alone (14%, 5/35), SRS (14%, 5/35), or observation (20%, 7/35). Twenty-four patients (62%, 24/39) had a pathogenic mutation found in either the ENG (63%, 15/24) or ACVR1 (38%, 9/24) gene. In the remaining cases, genetic testing was not performed.
Conclusion: Here we report on our cohort of 39-patients with HHT, diagnosed either clinically or genetically, having single or multiple BVMs. We found the majority of BVMs were small, singular lesions within the supratentorial anatomical compartment. Nearly all were Spetzler-Martin Grade 1 or 2, and most were amenable to surgical treatment, although SRS, embolization, and observation can hold promise in definitive treatment of these patients. Increasing the use of molecular genetic diagnostics for characterization of larger, more robust patient cohorts will continue to improve our understanding of the varied natural history of these lesions so that we may improve patient prognostication, safety, and outcomes.

Comparison of MRI and digital subtraction angiography for detection of cerebral arteriovenous malformations in hereditary hemorrhagic telangiectasia

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Objective: To evaluate the relative utility of screening MRI and diagnostic digital subtraction angiography (DSA) in detecting cerebral arteriovenous malformations (AVMs) within the Hereditary Hemorrhagic Telangiectasia (HHT) population.
Methods: Of 343 consecutive patients evaluated at the UCSF HHT Center of Excellence, 64 met study inclusion criteria. Specifically, known or suspected HHT defined by meeting at least two Curacao

criteria or positive genetic testing, and at least one brain MRI and DSA with available images. MRIs were retrospectively reviewed and the number of AVMs identified was compared with those identified on DSA as determined by neurointerventionalist evaluation.
Results: Of the 64 patients, 46 (72%) had AVMs with a total of 96 AVMs identified on either modality. 27 AVMs (28%) were seen only on DSA, 6 (6%) only on MRI, and 63 (66%) on both. Of those seen on MRI, 45 (65%) were seen on the 3D T1 post-gadolinium sequence, 44 (63%) on the 2D T1 post-gadolinium sequence, 42 (61%) on the SWI sequence, 36 (52%) on T2 sequence, and 30 (43%) on the MRA sequence. The sensitivity and specificity of MRI in detecting if a patient had any AVMs then confirmed on DSA were 83.7 and 84.7% respectively and the PPV and NPV were 92.3 and 72.0% respectively.
Conclusion: This study reinforces the use of MRI with gadolinium as a primary screening tool for cerebral AVMs in HHT patients and suggests that 3D T1 post-gadolinium, 2D T1 post-gadolinium, and SWI are the highest yield sequences for brain AVM detection in HHT.

Vitamin D levels are associated with decreased epistaxis in patients with hereditary hemorrhagic telangiectasia (HHT)

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Objectives: Heredity Hemorrhagic Telangiectasia (HHT) is a hereditary vascular malformation disorder with considerable clinical variability. The identification of clinically measurable biomarker (s) for disease severity may help patient management. In other vascular malformation syndromes Vitamin D has been shown to act as a marker for disease severity, improve endothelial cell stability and modulate lesion development. Vitamin D also modulates the TGF-beta pathway.
Methods: A retrospective chart review identified 198 patients seen at the University of Utah's HHT Center for whom vitamin D levels, Epistaxis Severity Score (ESS) and its components were available. Patients were divided into three groups based on the severity of their epistaxis: mild, moderate and severe based on pre-determined criteria. Vitamin D levels were compared between mild vs. severe epistaxis. Patients were also divided into four groups based on their 25-OH vitamin D levels ($\leq$ 10 ng/mL, 11–20 ng/mL, 21–30 ng/mL, $\geq$ 31 ng/mL) and ESS and its components were compared for each.
Results: Those with milder epistaxis had statistically higher levels of vitamin D ($M = 34.6$, $SD = 6.43$); than those who had severe epistaxis ($M = 17.8$ ng/mL, $SD = 9.72$, $t(61) = -8.23$, $p < 0.001$). A significant difference was also found between the four groups and their associated ESS ($p = 0.013$). Of the six components that comprise the ESS, vitamin D levels were significantly different for the duration of epistaxis ($p = 0.016$).
Conclusion: Sixty-two percent of patients in our study were vitamin D deficient. Vitamin D deficiency was associated with a higher ESS and longer bleeding duration. Higher vitamin D levels were found in patients with historically mild epistaxis.

Screening children for pulmonary arteriovenous malformations: evaluation of 18 years of experience

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Background: Hereditary Hemorrhagic Telangiectasia (HHT) is an autosomal dominant disease with multi-systemic vascular dysplasia. Early diagnosis through screening is important to prevent serious complications. How best to screen children of affected parents for pulmonary arteriovenous malformations (PAVMs) is often subject to debate. Transthoracic contrast echocardiogram (TTCE) is considered optimal in screening for PAVMs in adults. Guidelines for the screening of children are not specific, reflecting the lack of scientific evidence on the best method to use.
Objective: Aims of this study are (i) to evaluate our current screening method, consisting of history, physical examination, pulse oximetry and chest radiography and (ii) to assess whether postponing more invasive screening for PAVMs until adulthood is safe.
Methods: This is a prospective observational cohort study using a patient database.
Results: Over a period of 18 years (mean follow-up 9.21 years, SD 4.72 years), 436 children from HHT families were screened consecutively. 175/436 (40%) children had a diagnosis of HHT. PAVMs were detected in 39/175 (22%) children, 33/39 requiring treatment by embolotherapy. None of the screened children suffered any PAVM-associated complications with this screening method.
Conclusion: This study shows that a conservative screening method during childhood is sufficient to detect large PAVMs and protect children with HHT for PAVM-related complications. Postponing TTCE and subsequent chest CT scanning until adulthood to detect any smaller PAVMs does not appear to be associated with major risk.

Efficacy and safety of propranolol for epistaxis in hereditary hemorrhagic telangiectasia; retrospective, then prospective study, in a total of 21 patients

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Objectives: Hereditary Hemorrhagic Telangiectasia (HHT) is a genetic disorder of angiogenesis associated with disabling epistaxis. More effective treatments are required for the management of epistaxis. Propranolol, a beta-blocker, is a potentially useful therapeutic considering its anti-angiogenic properties. The aim of this study is to explore the efficacy and safety of propranolol for epistaxis in HHT patients.
Methods: We retrospectively recorded the epistaxis severity score (ESS) of 10 HHT patients receiving propranolol for cardiologic or neurologic indications both, prior to and while undergoing treatment. We then prospectively studied the efficacy of propranolol administered to 11 patients with disabling epistaxis for a 3-month period. We recorded the cumulative duration of epistaxis and the number of epistaxis episodes per month assessed with specific grids completed by participants.
Results: Nine of ten patients retrospectively analyzed significantly improved their ESS. In the prospective study, after 3 months of propranolol treatment, the median duration of epistaxis per month significantly decreased ($p = 0.007$), as did the number of epistaxis episodes per month ($p = 0.015$). Tolerance of propranolol was quite satisfactory with only one hypotension among the overall 21 HHT patients.
Conclusion: Propranolol should be considered as a therapeutic option for epistaxis in HHT patients.

Session 5 Treatment of HHT

Effect of bevacizumab nasal spray on epistaxis duration in hereditary hemorrhagic telangiectasia: a randomized clinical trial

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Epistaxis is the most frequent and disabling manifestation in hereditary hemorrhagic telangiectasia (HHT). Bevacizumab is effective on nosebleeds intraveinously, however, the efficacy of an intranasal treatment has yet to be evaluated.

Objective: To evaluate the efficacy on the duration of nosebleeds of 3 different doses of bevacizumab administered as a nasal spray in a repeated manner in patients with HHT complicated by nosebleeds.

Methodology: This study was a randomized multicenter placebo-controlled, combined phase II/III clinical trial, with dose selection at an intermediate analysis. Patients were recruited from 5 French HHT centers from April 2014 to January 2015 with a 6-month follow-up after the end of the treatment. Participants were aged 18 years and older and had been diagnosed with HHT and nosebleeds. The primary efficacy end point was mean monthly epistaxis duration for 3 consecutive months immediately after the end of the treatment.

Results: Eighty consecutive HHT patients were randomized and treated in the phase II study, with four parallel groups in a 1:1:1:1 ratio. One group received a placebo (n = 21) and the others received three doses of bevacizumab administered as a nasal spray (25 mg (n = 20), 50 mg (n = 20), or 75 mg (n = 19) per treatment). Mean monthly epistaxis duration measured at 3 months was not significantly different in the 60 patients receiving bevacizumab in comparison with the placebo group ($p = 0.57$) or between groups. Toxicity was low and no severe adverse events were reported. This study was terminated prior to phase III for treatment futility after interim analysis.

Conclusion: In patients with HHT, a bevacizumab nasal spray treatment of 3 administrations at 14-day intervals with doses of 25 mg, 50 mg or 75 mg per spray, as compared to a placebo, did not reduce monthly epistaxis duration.

Embolotherapy of pulmonary arteriovenous malformations with the MVP micro vascular plug: technical success and intermediate-term follow up

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Objective: To evaluate technical success and intermediate-term results for embolotherapy of pulmonary arteriovenous malformations (PAVMs) with the Micro Vascular Plug (MVP).

Methods: Patients with HHT and PAVMs treated by MVPs between October 2014 and January 2017 were retrospectively reviewed. Data collected included number and type of PAVM, feeding artery size, device migration, aneurysmal sac size and perfusion. Technical success was defined as intraprocedural angiographic occlusion of the feeding artery. Successful treatment was defined as no PAVM persistence (no perfusion of the sac on follow-up contrast-enhanced CT). MVP-3, 5, 7 and 9 were chosen to embolize feeding arteries measuring $\leq$ 3, 3–5, 5–7, and 7–9 mm respectively.

Results: 29 patients were identified (18 female, 11 male) with an average age of 41. 91 MVP-3, 28 MVP-5, 2 MVP-7, and 1 MVP-9 plugs were deployed to 82 simple and 24 complex PAVMs. Average feeding artery size was 2.6 mm (range 1.1–7.1). Technical success was achieved in 115 of 122 (94%) deployments. CT follow-up (average 176 days, range 71–466) revealed successful treatment, as well as $>$ 30% reduction of the sac in 62 of 67 (92.5%) PAVMs. No recanalizations were seen when follow-up pulmonary angiography was available. One migration occurred when a MVP-5 placed in a 5 mm feeding artery moved to the draining vein on follow-up CT.

Conclusion: Embolization of PAVM feeding arteries with the Micro Vascular Plug results in a high rate of immediate angiographic occlusion, successful treatment of the aneurysmal sac, and a low-rate of persistence on intermediate-term follow-up CT.

Bevacizumab (Avastin) treatment for severe pulmonary hypertension in patients with hereditary hemorrhagic telangiectasia

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Introduction: Pulmonary arterial hypertension (PAH) in Hereditary Hemorrhagic Telangiectasia (HHT) can result from high output cardiac failure due to hepatic Arteriovenous malformations (AVMs) or secondary to arteriopathy with media hypertrophy and intima proliferation. Treating hepatic AVMs can decrease pulmonary pressures in the first group. We describe the treatment outcome of 4 patients with HHT and PAH treated with Bevacizumab.

Methods: Retrospective case series.

Results: Four patients with HHT were diagnosed with moderate-severe PAH. All patients had hepatic AVMs. The patients had right heart catheterization prior to treatment. Patients' baseline characteristics:

	Age/gender	Mutation	PA pressure	PCWP	CO	PVR
1	64/F	ACVRL1	108/45 (70)	31	7.5	5.2
2	69/F	ACVRL1	60/30 (38)	30	9.7	0.8
3	68/F	Not done	85/33 (55)	30	9	2.7
4	42/F	ACVRL1	120/55 (71)	11	6.8	9.4

Patients were treated with Bevacizumab 5 mg/kg every 2 weeks × 6 courses and then every 4–8 weeks. Marked clinical improvement was demonstrated in 3 of them with a significant improvement in WHO functional class ($p = 0.05$). A significant decrease in pulmonary pressures and in cardiac output was demonstrated by catheterization or by echocardiography. Patient number 4 who had clinical signs of arteriopathy had only mild clinical improvement with Bevacizumab.

Conclusion: Bevacizumab is an effective treatment for PAH secondary to Hepatic AVMs and high cardiac output. The etiology of PAH in HHT has significant implications on treatment decisions.

Pazopanib reduces bleeding in hereditary hemorrhagic telangiectasia?

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Background: Pazopanib (Votrient) is an orally administered tyrosine kinase inhibitor that blocks VEGF receptors and is of interest as anti-angiogenic treatment for hereditary hemorrhagic telangiectasia (HHT).

Methods: Prospective, multi-center, open-label, dose-escalating study [50 mg, 100 mg, 200 mg and 400 mg], designed as a proof of concept study to demonstrate efficacy of pazopanib on HHT-related bleeding, and to measure safety. Patients, recruited at 5 HHT Centers, required ≥ 2 Curacao criteria AND {anemia OR severe epistaxis with iron deficiency}. Primary outcomes were hemoglobin and epistaxis severity, measured at end-treatment and compared to baseline, with multiple secondary outcome measures, as well as safety monitoring every 1.5 weeks.

Results: Seven patients were recruited and treated with 50 mg pazopanib daily. Three/7 patients had ≥ 50% decrease in epistaxis duration during weeks 11 and 12 and a total of 5/7 had ≥ 50% decrease in epistaxis duration during treatment. Two/7 patients had ≥ 50% decrease in epistaxis frequency during weeks 11 and 12. Six/7 patients showed a decrease in ESS of > 0.71 (the MID). One/7 had ≥ 2 g/dL increase in hemoglobin, averaged over last 3 treatment measures (total three/7 patients had ≥ 2 g/dL increase in hemoglobin during treatment). SF-36 was improved (exceeding MID) in 7/8 domains at week 6 and/or week 12. There were 19 adverse events (AE) including one severe AE (elevated liver functions tests); there were no serious AE.

Conclusion: We observed improvements in hemoglobin and epistaxis in this small series of HHT patients, at a low dose, with no serious AE. Further studies of pazopanib efficacy are warranted.

Session 6: Diagnosis of HHT

Can graded contrast echocardiography be used for post-treatment surveillance of pulmonary arteriovenous malformations?

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Objectives: Chest CT is the gold standard for post-embolotherapy PAVM surveillance. Graded transthoracic contrast echocardiography (TTCE) can predict need for embolotherapy based on feeding artery diameter when used in screening. This prospective study is designed to determine whether graded TTCE can be used as an alternative to CT in determining response to treatment and in long-term surveillance.

Methods: To date, twenty patients (5 M:15 F, mean age 52.4 ± 12.4 years, range 35–75 years) with PAVMs, prior PAVM embolotherapy, and at least one follow-up chest CT have been enrolled in this IRB-approved study (Clinicaltrials.gov identifier: NCT02936349). Subjects underwent graded TTCE. TTCE grade and chest CT findings were compared.

Results: Twenty-five embolization procedures were performed in the 20 subjects with a total of 48 treated PAVMs. Median time from treatment to most recent post-treatment chest CT was 5.8 ± 3.8 years. Fifty percent (10/20) of patients had no PAVMs and 45% (9/20) had PAVMs with feeding arteries < 2 mm on chest CT. One patient was thought to have a PAVM with a 2 mm feeding artery that was found to be 3 mm on further review of imaging prompted by TTCE results. Median time from most recent chest CT to TTCE was 328 days. TTCE revealed 5% (1/20) of patients had grade 0 shunts, 50% (10/20) had grade 1, 30% (6/20) had grade 2, and 15% (3/20) had grade 3. TTCE grade and chest CT findings are correlated in Table 1. The patient in which TTCE prompted review of imaging had a grade 3 TTCE and underwent repeat embolotherapy.

Conclusion: Graded TTCE may be effective in determining whether a patient needs CT follow-up after PAVM embolization, but likely cannot replace CT in all patients.

Table 1 Comparison of TTCE and chest CT findings

Maximum PAVM feeding artery size on CT (mm)	Number of patients	Grade 0 TTCE	Grade 1 TTCE	Grade 2 TTCE	Grade 3 TTCE
0 (no PAVMs)	10	1/10 (10%)	8/10 (80%)	1/10 (10%)	–
1–2	9	–	2/9 (22.2%)	5/9 (55.6%)	2/9 (22.2%)
> 2	1	–	–	–	1/1 (100%)

Diagnostic yield of rescreening adults for pulmonary arteriovenous malformations

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Objective: To determine the yield of rescreening adults with initial negative screening for pulmonary arteriovenous malformations (PAVMs).
Methods: Patients with a definite diagnosis of HHT were identified in the University of Toronto, University of Montreal and Mayo Clinic HHT Databases. Inclusion criteria were: (1) definite diagnosis of HHT, (2) initial negative bubble echocardiography and/or chest CT and (3) minimum of 2 years of imaging follow-up. A positive screen required confirmed PAVMs on CT. Frequency of PAVMs was calculated at four time-points 3 ± 1 years, 5 ± 1 years, 7–9 years and at ≥ 10 years with the primary endpoint being rate of de-novo PAVM at 5 ± 1 years.
Results: 170 patients with a total of 1320 patient-years of follow-up were included. 100 patients (58.8%) were female. Mean age at initial screening was 48.3 ± 17.0 years. Nine patients (5.3%) had de novo PAVM following initial negative screen. Among patients who had screening performed at the 3, 5, 7 and ≥ 10 year time points, the rate of new PAVMs were 1.8% (3/164), 3.6% (5/140), 6.6% (6/91) and 13.8% (9/65) respectively. Median feeding artery size was 1.3 mm. One patient had a feeding artery > 3 mm discovered at 6 years and was treated with embolization. Overall rate of de-novo PAVM formation was 0.7%/patient-year. Two patients had positive bubble echocardiograms but no follow-up CT so their status was unknown.
Conclusion: No new clinically significant PAVMs were identified in patients rescreened up to 5 years after initial negative screening. These findings suggest that longer time intervals beyond 5 years may be considered between screenings.

Optimizing shunt quantification in hereditary hemorrhagic telangiectasia screening for pulmonary arteriovenous malformations

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Use of agitated saline contrast in transthoracic cardiac echocardiography (TTCE) is a mainstay in screening for pulmonary arteriovenous malformations (PAVMs) in hereditary hemorrhagic telangiectasia (HHT). Increased shunting during TTCE is associated with PAVMs on computerized tomography (CT). Two semi-quantitative grading systems have been proposed to define shunt grade: a 3-grade and a 4-grade scale.
Objectives: We compare these semi-quantitative grading systems to a new frame-by-frame quantification scale and a 5-grade qualitative scale used at our center.
Methods: We retrospectively studied patients seen in the Utah HHT Center from March 2002 and July 2015 with suspected or definite HHT, quality echocardiograms compatible for frame-by-frame quantification, who underwent TTCE and CT. The McNemar test without Yates correction was used to compare grading systems.
Results: The frame-by-frame quantification scale had a sensitivity of 95% and specificity of 76.1%. The 3-grade scale had a sensitivity of 95% and specificity of 70.9%. The 4-grade scale had a sensitivity of 95% and specificity of 61.5%. The 5-grade scale had a sensitivity of 97.5% and specificity of 55.5%. The frame-by-frame quantification scale had the highest specificity, and statistically outperformed the 4-grade and 5-grade scales. By McNemar's test, all scales were equivalent in sensitivity (not missing treatable PAVM), with the frame-by-frame and 3 point scales achieving this goal with the fewest excess CT studies, but requiring the most observer time.
Conclusion: Future automation of the frame-by-frame quantification scale will result in a truly quantitative shunt evaluation tool useful for research applications and clinical convenience.

Gastrointestinal affection in HHT patients: cross sectional study

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Objective: To describe the gastrointestinal affection in patients with HHT-related digestive bleeding.
Methods: Cross sectional study of the Institutional HHT registry (*Clinical Trials.gov NCT01761981*), of a tertiary referral centre, between 2010 and 2017.
Results: We reviewed 466 patients. One hundred eighty-five underwent endoscopy, half of them ($n = 92$) had telangiectases in the gastrointestinal tract. Sixty-one (61/92) patients studied in our center were included, 67% were females and the median age was 64 (IQR 57–70). The median of endoscopies per patient was 1 (IQR 1–2). Thirty-nine patients (64%) had anaemia, 15 (29%) presented severe epistaxis, 23 (38%) melena, 8 (13%) hematemesis, 10 (16%) haematochezia and 11 (18%) shock. Almost half of the patients had multiple (2–10) telangiectases, 31% diffuse (> 10) telangiectases and isolated (< 2) were seen in 29%. Telangiectases were observed in oesophagus 16%, stomach 82%, duodenum 53%, jejunum 18%, ileum 13% and colon 35%. Around 70% of the patients had medium (2–10 mm) and large (> 10 mm) telangiectases. Thirty-eight patients (62%) received intravenous iron therapy, the median of infusions was 4.5 (IQR 2–9.5). Twenty-two (36%) patients required blood transfusion, the median was 8 (IQR 2.5–18.5 Fifty-two patients (85%) received medical therapy. Twelve patients (20%) were anticoagulated, antiaggregated or presented other coagulation disorders. Fifty-two (85%) patients underwent at least one endoscopic treatment. Argon plasma coagulation was the treatment applied in almost all the patients. Endoscopic complication was observed in two patients.
Conclusion: A high proportion of patients suffer from severe gastrointestinal involvement. Postmenopausal women and predominant proximal affection seemed to be characteristic.

Predominance of RASA1 mutations in a cohort of children with central nervous system arteriovenous fistulas

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Cerebral or spinal cord arteriovenous fistulas (AVFs) are rare vascular malformations found particularly in children and are mainly due to two types of hereditary vascular diseases: Hereditary Haemorrhagic Telangiectasia (HHT) and capillary malformations-arterio venous malformation (CM-AVM). Here, we sought to assess the spectrum of genetic anomalies in a cohort of children presenting at least one cerebral or medullary pial fistula, and to describe their baseline clinical characteristics at time of diagnosis.

Sequencing analysis and search for large rearrangements of *ACVRL1*, *ENG* and *RASA1* genes were performed for 39 patients with cerebrospinal arteriovenous malformations presenting at paediatric age. A germline mutation was identified in 20 out of 39 probands (51%): 6 in *ENG* (15.3%), 1 in *ACVRL1* (2.5%) leading to HHT molecular diagnosis and 13 in *RASA1* (33.3% leading to CM-AVM molecular diagnosis.

At onset, HHT mutation carriers were characterized by a high rate of haemorrhage whereas skin capillary haemangioma are predominant in the RASA1 mutation carrier group. The location of the AVF was equally distributed between cerebral and spinal cord in the HHT mutation carrier group whereas it was mostly cerebral in RASA1 mutation carrier and non-carrier group. None of HHT mutation carriers had cardiac failure at onset whereas almost 50% of patients present with this symptom in the RASA1 mutation carrier group and in non-carrier patients.

In conclusion, our results highlight the importance of genetic testing in children presenting with cerebral or spinal cord arteriovenous fistulas setting in view of the high frequency of gene mutations in paediatric cerebrospinal AVFs, and the predominance of RASA1 over HHT mutations.

Applicability of the Curacao criteria in the diagnosis of hereditary hemorrhagic telangiectasia in children and young adults

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Objective: To evaluate the utility of the Curacao criteria for the diagnosis of HHT in pediatric and young adult populations.

Methods: We conducted a multi-center, retrospective study of patients seen at the HHT centers at UNC and Yale from 2002 to 2016. Data collected included age at evaluation, gender, Curacao criteria present, and genotype positive or negative. Patients were divided into five age groups; 0–5, 6–10, 11–15, 16–20, and 21–25 years. Sensitivity and specificity of the Curacao criteria was calculated for each group.

Results: A total of 196 patients were included, with 46 patients aged 0–5, 45 patients 6–10, 59 patients 11–15, 23 patients 16–20, and 23 patients aged 21–25. Sensitivity was 3% for the 0–5 year age range, 15% for 6–10, 36% for 11–15, 44% for 16–20, and 70.5% for 21–25 years. Specificity was 100% for all age groups except for the 11–15 year olds (96%).

Conclusion: This is the first study to evaluate the utility of the Curacao criteria for diagnosis of HHT in the pediatric setting. We found the Curacao criteria had a high specificity in all age groups. Sensitivity was low in the very young subjects and improved significantly with increasing age. This is likely because of decreased frequency of disease related symptoms in childhood. Our findings suggest that the Curacao criteria are useful and can be applied in the pediatric setting. However, genetic testing would be preferred in asymptomatic subjects, particularly in the presence of a family history of HHT.

Paediatric presentations of hereditary haemorrhagic telangiectasia in Tayside: Is brain arteriovenous malformation screening justified?

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Objective: In the international consensus guidelines for HHT management (2009), the recommendation with the least agreement (64%) was that neonates and children with HHT should have MRI screening for a brain arteriovenous malformation (BAVM). To quantify the risk of a clinical BAVM presentation in childhood, we reviewed HHT-related serious clinical events occurring before the age of 18 in all HHT patients in Tayside.

Methods: Retrospective cohort study of all individuals with a known mutation in the *Endoglin* or *ACVRL1* gene in Tayside, Scotland. Outcomes were identified from review of all available clinical data sources.

Results: 49 patients had a confirmed diagnosis of HHT with a known mutation. 5 patients had post-diagnostic MRI brain scanning (2 asymptomatic; 3 due to abnormal neurology, seizure or migraine); none had a BAVM. 2 unrelated children, both with *Endoglin* mutations presented with neurological symptoms, prior to diagnosis of HHT. Patient 1 had a cerebral haemorrhage at age 6. A BAVM was identified and excised, and the patient made a good neurological recovery. Patient 2 died at age 3 years during a seizure. A de-novo *Endoglin* mutation was found. Previous MRI for investigation of seizures and post-mortem, including neuropathology, did not identify a BAVM.

Conclusion: Whilst HHT can present with serious childhood complications, we have not identified an individual in our population who would have benefited from paediatric BAVM screening. Given the risks of BAVM screening and treatment, such screening in children requires further evaluation before it is recommended as routine clinical practice in the UK.

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

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Objectives: 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, $p = 0.04$).
Conclusion: *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. Therefore, screening for aortic dilation in HHT patients with a *SMAD4* gene mutation is recommended.

Session 8: Translational aspects of HHT

Evaluation of stop codon read through as a therapy for HHT

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Aim/Method: A common mechanism by which pathogenic DNA mutations cause inherited disease is by generating premature termination codons (PTCs), either through a nonsense substitution, or insertions/deletions that alter the reading frame. PTC-containing transcripts frequently undergo nonsense mediated decay (NMD) resulting in null alleles. Drugs such as ataluren, recently approved in the UK for Duchenne Muscular Dystrophy, allow ribosomes to read through nonsense mutations without affecting natural translational stop codons. Our goal is to develop this strategy to treat HHT, where approximately 20% of published mutations in *ACVRL1, ENG* and *SMAD4* are nonsense substitutions. Our approach combines in vitro reporter assays, analysis of NMD in fresh peripheral blood mononuclear cells from patients with known mutations, and isolation of blood outgrowth endothelial cells (BOEC) to analyze correction of endogenous cell signaling abnormalities.
Results: a dual-reporter plasmid system has been established to measure the efficiency of nonsense readthrough in constructs with a premature stop codon, modeling the same mutations that are present in the Imperial HHT cohort. Initial validation using the aminoglycoside antibiotic G418 demonstrates efficient induction of full length protein. Comparison with ataluren and other novel readthrough agents is underway. Previous analysis of BOEC from patients with pulmonary hypertension shows robust correction of endogenous signaling defects using G418 and ataluren. Similar studies with HHT cells are now ongoing.
Conclusion: our preliminary results suggest that nonsense readthrough is effective for HHT stop mutations to restore full-length protein. Studies are ongoing to assess correction of signaling abnormalities and tolerance of possible missense mutations.

Mice lacking endoglin in macrophages show an impaired immune response

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Endoglin is an auxiliary receptor for members of the TGF-β superfamily and plays an important role in the homeostasis of the vessel wall. Mutations in endoglin gene (ENG) or in the closely related TGF-β receptor type I ACVRL1/ALK1 are responsible for a rare dominant vascular dysplasia, the Hereditary Hemorrhagic Telangiectasia (HHT) or Rendu-Osler-Weber syndrome. Endoglin is also expressed in human macrophages, but its role in macrophage function remains unknown. In this work, we show that endoglin expression is triggered during the monocyte- macrophage differentiation process, both in vitro and during the in vivo differentiation of blood monocytes recruited to foci of inflammation in wild-type C57BL/6 mice. To analyze the role of endoglin in macrophages in vivo, an endoglin myeloid lineage specific knock-out mouse line (Engfl/flLysMCre) was generated. These mice show a predisposition to develop spontaneous infections by opportunistic bacteria. Engfl/flLysMCre mice also display increased survival following LPS-induced peritonitis, suggesting a delayed immune response. Phagocytic activity is impaired in peritoneal macrophages, altering one of the main functions of macrophages which contributes to the initiation of the immune response. We also observed altered expression of TGF-β1 target genes in endoglin deficient peritoneal macrophages. Overall, the altered immune activity of endoglin deficient macrophages could help to explain the higher rate of infectious diseases seen in HHT1 patients.

Mosaicism involving non-blood tissue: implications for genetic testing in HHT families

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Mosaicism in HHT has been reported in a few cases in which it was detected in blood.
Objective: Here, we report the first case of mosaicism not detected in blood from a family proband, and discuss implications for genetic testing algorithms in HHT families.
Methods: A proband with classic HHT (epistaxis, oral, cutaneous and GI telangiectases, PAVM and HAVM) had no pathogenic variant identified by sanger sequencing and large deletion/duplication analysis of *ENG, ACVRL1* and *SMAD4.* Exome sequencing was then performed on the proband as well as her affected child (epistaxis, oral, cutaneous and GI telangiectases and PAVMs).

Results: A pathogenic *ENG* c.1131 + 1G > A splice-site variant was detected in the proband's affected child, but not in DNA extracted from the peripheral blood of the affected parent/proband. Additional tissue samples (saliva and hair bulbs) were obtained from the proband to test for mosaicism. Analysis for the presence of this variant was also negative for saliva, but positive for the hair bulb sample (at 33%).
Conclusion: This is the first report of an HHT patient with mosaicism in whom the disease-causing mutation was not detected in blood. This suggests that mosaicism, which does not involve blood cells, should be considered in individuals/families with classic HHT who have "negative" genetic test results in a proband. This mechanism may explain the small percentage of patients with classic HHT in whom a pathogenic variant has not been identified in one of the known HHT genes from a blood sample.

Endoglin antibody therapy produces the small vessel manifestations of HHT-1 and is associated with clinical activity when dosed with inhibitors of the VEGF pathway

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Methods: TRC105 is an endoglin antibody being studied in three randomized clinical trials of advanced cancer patients: Phase 3 in angiosarcoma (AS), Phase 2 in renal cell carcinoma (RCC), and Phase 2 in glioblastoma (GBM), each with a primary endpoint of progression free survival (PFS), as well as single arm Phase 2 trials in hepatocellular carcinoma (HCC) and gestational trophoblastic neoplasia (GTN), each with a primary endpoint of overall response rate (ORR). TRC105 has been administered to more than 400 cancer patients as a single agent or in combination with VEGF inhibitors (e.g., bevacizmab, axitinib, pazopanib and sorafenib). Combination treatment with VEGF inhibitors has been the focus of clinical development, as VEGF up-regulation was observed following TRC105 single agent treatment and endoglin up-regulation is observed following VEGF inhibitor treatment.
Results: TRC105 has a side effect profile that is consistent with the small vessel manifestations of HHT type 1 (HHT-1), a genetic syndrome caused by endoglin heterozygosity that results in endoglin haploinsufficency. Patients treated with TRC105 develop mucocutaneous telangiectasia that causes one or more signs and symptoms of HHT-1, including epistaxis, gingival bleeding and cutaneous telangiectasia, within the initial month of dosing. The development of mucocutaneous telangiectasia in unaffected by whether TRC105 is dosed as a single agent or dosed with a VEGF inhibitor. Separate trials of TRC105 and VEGF inhibitors have demonstrated signs of activity beyond that expected with the companion VEGF inhibitor: PFS in a single arm trial of TRC105 + axitinib was 11.3 months compared to PFS of 4.8 months in a separate trial of axitinib in RCC patients; ORR in a trial of HCC patients treated with TRC105 + sorafenib was 23% compared to ORR of 2% in a separate trial of sorefenib in HCC patients; ongoing durable complete responses have observed in trials of TRC105 + pazopanib in AS and of TRC105 + bevacizumab in GTN.
Conclusion: TRC105 is an endoglin antibody that has on-target effects in cancer patients that mimic the small vessel manifestations of HHT-1. Importantly, HHT is associated with superior stage stratified cancer survival (Duarte et al., Cancer Epidemiology, Biomarkers and Prevention, 2013), and clinical trials indicate that targeting endoglin with TRC105 combined with VEGF inhibitor therapy is efficacious. Randomized clinical trials are ongoing in three oncology indications. Endoglin antibodies are also being studied in age related macular degeneration and fibrotic diseases.

Session 9: New perspectives in HHT

VASCERN HHT priority evaluations 2016–2017

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Objectives: In 2016, the European Commission established networks across Europe, in order to improve the care of European citizens with Rare Diseases. A specific HHT Working Group was established within VASCERN, the European Reference Network for Rare Multisystemic Vascular Diseases. The objective of this study was to bring together the views of HHT professionals and HHT patients/representatives to establish priorities for action.
Methods: Between 11/2016 and 01/2017, a survey asked respondents for their HHT role, country of origin, and in free text, what they considered to be the 3 most important problems for HHT. A second question asked them to indicate using a grid of options, what proportion of HHT patients they thought would have particular HHT manifestations. Data were analysed using STATA IC v13.
Results: 61 respondents from 8 European countries completed the survey. 32 (53%) were healthcare professionals, 17 (28%) were members of HHT families and/or HHT patient advocates, and 10 (17%) were scientists. The top 7 priorities across all 61 individuals were anemia, arteriovenous malformations, bleeding, childhood/young people, the hereditary nature of the disease; lack of effective medications and problems finding informed care. There was broad agreement between groups regarding the prevalence of particular arteriovenous malformations; anemia; nosebleeds; pregnancy complications and impaired quality of life. There were differences in the perceived prevalence of gastrointestinal bleeding; dyspnea and pulmonary hypertension.
Conclusion: Across respondent groups, there was good concordance of views on HHT. The data provide a solid platform to continue the joint and informed focus on priority areas.

Narratives describing the impact of living with HHT: a qualitative study

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Objectives: Hereditary Haemorrhagic Telangiectasia (HHT) is a condition that affects individuals throughout their lifetimes and its management requires input from a multidisciplinary team. The medical literature focuses on the complications and management of HHT, however there is little documented about the psychosocial impact of this condition.
This qualitative study therefore aimed to understand the impact of living with HHT.
Methods: Twenty semi-structured interviews with Australian participants were undertaken and a narrative analysis was performed. Participant ages ranged from 22 to 65 years and gender as equally divided between men and women.
Results: Preliminary analyses suggest that the relationship between the health care professional and the individual living with HHT is crucial. Most participants were frustrated by their health care professionals' lack of knowledge and at the same time often felt dismissed. As a result, participants felt forced to self-advocate and struggled to find professionals in whom they could develop trust and confidence. Most participants voiced the desire for a specialist centre to coordinate their care. The visibility of symptoms also affected multiple aspects of individuals' lives including in their relationships, socialising and work life. Stigma and embarrassment were commonly experienced. Individuals also demonstrated coping strategies relating to living with HHT with many participants minimising the severity of their own condition compared to others. Many tried to find control over the unpredictability of their symptoms by searching for triggers and altering their lifestyle.
Conclusion: These themes highlight the large impact of living with HHT and may help to inform both genetic counselling and interactions with other health care professionals.

Assessment of an age-related threshold of nasal, oral and cutaneous telangiectasia number for diagnosis of hereditary hemorrhagic telangiectasia: HHT Paris Center experience in 832 genotyped HHT patients

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Objectives: To determine and validate according to age the lower threshold of telangiectasia number which should predict HHT.
Methods: In a first study, 156 family members of genotyped HHT patients were examined by the same dermatologist and otorhinolaryngologists not aware of the diagnosis between 2004 and 2008. The lower thresholds of telangiectasia number were calculated according to age by using Receiver Operator Characteristic (ROC) analysis.
In the second study, the same specialists used the thresholds between 2009 and 2016 in 676 distinct family members. Sensitivity and specificity were calculated for each threshold. The number of telangiectasia was counted within nasal cavity and in the face, oral cavity, hands, fingers and feet.

Results:

Age	0–16 year	16–30 year	30–50 year	> 50 year
First study (N = 156)				
Nasal thresholds	3	3	3	3
Sensitivity	67 [45–84]	84 [60–97)	90 [70–99]	92 [73–99]
Specificity	89 [52–100]	85 [55–98]	83 [52–98]	86 [42–100]
Oral and skin thresholds	2	10	20	20
Sensitivity	47 [29–65]	59 [36–79]	86 [75–97]	100 [80–100]
Specificity	100 [55–100]	100 [74–100]	86 [57–98]	100 [52–100]
Second study (N = 676)				
Nasal thresholds	3	3	3	3
Sensitivity	59 [48–69]	86 [77–93]	95 [91–98]	99 [95–100]
Specificity	93 [68–100]	100 [76–100]	93 [76–99]	88 [47–100]
Oral and skin thresholds	2	10	20	20
Sensitivity	46 [36–46]	64 [54–74]	73 [66–80]	91 [85–95]
Specificity	88 [64–99]	100 [75–100]	96 [78–100]	100 [55–100]

Conclusion: Specificity of the lower thresholds of telangiectasia number was higher in the second study than in the first one both for nasal and oral/skin telangiectasia, except in youngest patients for oral/skin telangiectasia. This result confirms the pertinence of our lower thresholds. Interestingly, the lower threshold of telangiectasia number was 3 in nasal cavity whatever the age. For oral cavity and skin, the lower threshold of telangiectasia number increased with age and should predict HHT.
Acknowledgment: To Bénédicte Chesneau for her excellent technical assistance.

Improved life expectancy when screened for pulmonary arteriovenous malformations preemptively

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Introduction: In 2004 the screening protocol in the St Antonius Hospital was changed to include TTCE as a standard screening tool for PAVMs. Untreated PAVMs may cause serious complications including death. The goal of this study is to evaluate the life expectancy of HHT patients, when screened according to the current screening protocol using TTCE and/or chest CT scans.
Methods: All patients with a HHT diagnosis based on the internationally accepted Çuracao criteria (i.e. three or more criteria) and/or genetic conformation of the family mutation were assigned to the HHT cohort (n = 1425). All individuals considered not to have HHT based on the Çuracao criteria (one or no criterion) or genetic tests confirming the individual had not inherited the family mutation were assigned to the control cohort (n = 1357). Social security numbers were cross checked with the ministry of healthcare to confirm status alive or deceased, and date of death. The HHT group was divided in two groups screened

before 2004 and screened in or after the year 2004. After exclusion the HHT group contained 482 and 709 individuals (screened < 2004 and ≥ 2004, respectively), and the control group 901 individuals. To validate the control group, life expectancy data of the control group was compared to data of the general Dutch population.
Results: In total 1191 HHT patients and 901 controls were included. The control group could be validated using data from the general Dutch population; life expectancy figures were identical. The HHT patients screened before 2004 were slightly older with mean ages of 53.5 years (range 5–92, SD 19.5 years) versus 46.8 years (range 5–89, SD 19.5) and 47.5 years (range 3–100, SD 17.0 years), for patients screened after 2004 and controls respectively. When all three groups were compared a significant difference was found, at the detriment of the HHT group screened without TTCE (mean survival 76.4 years, 95% CI 75.3–78.6). Difference between the mean survival of the control group (88.8 years, 95% CI 84.8–92.7) and HHT group screened using TTCE or chest CT (82.0 years, 95% CI 80.3–83.4) was not statistically significant (mantel-cox test $p = 0.42$).
Conclusion: Screening for PAVMs, using TTCE and chest CT scans, has improved life expectancy. The HHT group screened preemptively for PAVMs using TTCE shows similar life expectancy to the validated control group.

Hereditary haemorrhagic telangiectasia and the 100,000 genomes project

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Objectives: Genomic diagnostics is being mainstreamed into UK medical practice via the 100,000 Genomes Project which is recruiting patients with specified rare diseases without a known molecular diagnosis, for whole genome sequencing (WGS) [1].
Methods: Genomics England protocols were followed for the nomination of hereditary haemorrhagic telangiectasia (HHT); generation of a data model based on human phenotype ontology (HPO) terms [2]; development and review of a PanelApp gene panel [3]; and recruitment of HHT families from NHS Genomic Medicine Centres (GMCs). Separately, a Respiratory GeCIP (Clinical Interpretation Partnership) subdomain was established specifically to analyse HHT WGS data.
Results: HHT recruitment opened in October 2015, for patients where gene tests had not identified a causative pathogenic gene variant ("mutation"), and affected individuals had at least three inclusion criteria: nosebleeds from nasal telangiectasia; mucocutaneous telangiectasia; visceral AVMs (pulmonary, cerebral, hepatic) or gastrointestinal telangiectasia; and a first degree relative with HHT. To date, from the 13 GMCs, 75 participants in 49 families have been recruited. In the majority of cases, phenotypic data have been entered into an electronic data capture tool, and genomic DNA has been sequenced by Illumina Inc. at the Genomics England Sequencing Centre in Hinxton. For data analyses, the GeCIP subdomain has recruited an unprecedented 20-strong international faculty from 9 countries with expertise spanning HHT gene identification; clinical genetics; bioinformatics; and downstream modelling and therapeutics. First results are expected in 2017.
Conclusion: A critical, collaborative resource for the advancement of HHT genomics has been established from HHT families reviewed within NHS England.

Increased infection rates in HHT patients: results of an online survey

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Aim/Method: Several publications have reported an increased risk of bacterial infections for HHT patients. We developed an internet based questionnaire on various topics of HHT. The dissemination was supported by CureHHT and the German self-help group. Several questions addressed the rate of infections of HHT patients compared to their partners.
Results: A total of 701 questionnaires were filled in by patients in January 2017. Patients reported a significantly ($p < 0.05$, McNemar's test) higher risk to have a sinusitis (n = 352), brain abscess (n = 348), abscess at another location of the body (n = 344), urinary tract infection (n = 353), and pneumonia (n = 349) in the past than their partners. No significantly higher risk was reported for colds (n = 355), gastritis (n = 352), sepsis (n = 342), wound infections (n = 345), skin and soft tissue infections (n = 345), bone and joint infections (n = 343).
Conclusion: To the best of our knowledge this is the first report on increased rates of sinusitis, pneumonia, and urinary tract infections in HHT patients. An increased risk of abscesses is known from the literature in association with pulmonary arteriovenous malformations. However, an increased susceptibility to other infections has been postulated as a result of an impaired immune system in HHT. The results of our survey support our hypothesis that some infectious diseases are more common in HHT patients. It is interesting that an increased risk could only be documented for some types of infections. This might serve as a hint for the underlying mechanism of immune modulation.

Hepatic involvement and atrial fibrillation in geriatric patients

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Aim: Complications secondary to hepatic involvement in HHT tend to become more relevant with increasing age, possibly due to interactions with concomitant morbidities and/or polypharmacy. We aimed to carry out an overview of HHT phenotype in geriatric age, to investigate correlations between cardiac arrhythmias and hyperdynamic state secondary to hepatic arterio-venous malformations (HAVMs), Moreover, we aimed to identify HAVM-correlated predictors of AF risk in geriatric HHT patients.
Methods: The study was designed as a retrospective cohort study of geriatric patients with confirmed HHT. Inclusion criteria: (i) full clinical-instrumental evaluation, (ii) age ≥ 65 years at initial screening or subsequent monitoring. Hepatic screening: Echo-Color Doppler examination, Multi-Slice Computed Tomography, biohumoral functional analysis. Heart rate abnormalities were investigated by basic electrocardiography.
Results: A total of 76 patients were included in the study (mean age 70.04 ± 4.22 years). Chronic anoemia was present in 52/76 patients

(68.4%). Geriatric patients had a higher prevalence of hepatic AVMs and a reduced prevalence of pulmonary and brain AVMs if compared to the global prevalence of our cohort. Signs of chronic atrial fibrillation were detected in 11/76 (14.4%) patients. Hepatic examination disclosed more severe haemodynamic alterations, as well as significantly higher γ-GT and Alkaline Phosphatase levels, and significantly more serious anoemia, in AF vs. non-AF patients. Multiple logistic regression analysis disclosed γ-GT levels as the only significant variable contributing to AF onset.
Conclusion: Geriatric age in HHT entails a significant association between HAVM-related subclinical haemodynamic alterations and AF risk, with γ-GT representing a potential predictor of AF onset.

To dive or not to dive: can we revise our recommended avoidance of scuba diving on patients with microscopic or treated PAVMS?

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Objective: The 2011 International Guidelines for Diagnosis and Management of HHT advise patients with PAVMs against SCUBA diving regardless of size, even once the PAVM is treated. Since this publication, there has been greater understanding of the relationship of decompression sickness (DCS) and shunt size in the intracardiac shunt literature. This review offers an updated discussion on the risk of DCS in patients with PAVMs.
Methods: A meta-analysis was conducted using the Medline database from 1997 to January 2017 using key words PAVMs or intracardiac shunt and DCS.
Results: Doppler evidence of venous gas emboli (VGE) is common even after shallow ascents (1–3 m) in asymptomatic healthy subjects. Arterialization of VGE in right to left shunts is thought to increase DCS risk. Studies have shown in intracardiac shunts, however, that DCS risk is related to shunt size rather than just presence of shunt. Furthermore, majority of divers are able to return to unrestricted diving following intracardiac shunt closure.
Conclusion: There is discordance between the recommendations in the PAVM and intracardiac shunt literature and the risk of SCUBA diving. Shunt size has been investigated as a determinant of DCS in intracardiac shunts but not in PAVMs. Evidence from intracardiac shunt data suggests that size of the shunt is proportional to the risk of DCS. Extrapolation of the intracardiac shunt literature suggests that for HHT patients with microscopic or successfully treated PAVMs, the risk of paradoxical embolus is exceptionally low. Moreover, shallow dives and free diving may be acceptable.

Session 10 Mechanisms of HHT

Decreased expression of VEGFR1 contributes to the pathogenesis of Hereditary Hemorrhagic Telangiectasia type 2

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Aim/Method: Mutations in *ACVRL1* gene cause HHT2, a disease characterized by excessive angiogenesis with AVMs. How haploinsufficiency in *ACVRL1* gene leads to HHT2 remains unresolved. Here, we took advantage of $Acvrl1^{+/-}$ embryonic stem cell (ESC) models and of $Acvrl1^{+/-}$ mice to demonstrate the key role of VEGFR1 in HHT2 pathogenesis.
Results: We first focused on the neonatal retina. $Acvrl1^{+/-}$ retinas showed excessive angiogenesis at P7 compared to control littermates. The newly formed capillaries showed numerous endothelial Tip cells at the vascular front. Migration of the vessel sprouts was also reduced with defective local guidance. At the molecular level, we found that VEGFR1 expression was reduced in $Acvrl1^{+/-}$ retinas. VEGFR1 is expressed in Stalk cells to regulate endothelial Tip cell specification and vessel guidance, limiting angiogenesis. To gain insights into the role of VEGFR1 in HHT2, we generated $Acvrl1^{+/-}$ ESC lines and found that this model system was able to copy the in vivo HHT2 retinal phenotype. Introduction of a transgene allowing the expression of VEGFR1 in endothelial cells was sufficient to restore the $Acvrl1^{+/-}$ phenotype. The tracheal vasculature of adult $Acvrl1^{+/-}$ mice infected by *Mycoplasma pulmonis* also showed excessive angiogenesis with the formation of multiple AVMs when compared to control littermates. Blocking antibodies targeting VEGFR2 were able to prevent pathological angiogenesis in infected $Acvrl1^{+/-}$ mice.
Conclusion: We provide new insights into the mechanisms underlying HHT2 elicited by reduced expression of VEGFR1. We are currently evaluating the expression levels of soluble VEGFR1 in HHT2 patients to determine whether they may predict disease severity.

Nitric oxide synthase inhibition attenuates Notch-mediated brain arteriovenous malformation

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Aim/Method: The core pathology of HHT is arteriovenous malformation (AVM). Mechanisms underlying pathogenesis remain poorly understood, hindering therapeutic development. In in vitro systems, HHT genes affect endothelial nitric oxide synthase (eNOS). We report here that Notch shares this molecular link with HHT and further demonstrate that eNOS mediates AVM formation in vivo.
Results: Endothelial expression of constitutively active Notch4 (Notch4*) initiates brain AVMs in mice through enlargement of microvessels without an increase in endothelial cell number or proliferation. Instead, initial enlargement of AV shunts correlates with area expansion of individual endothelial cells, raising the possibility that enhanced vasodilation may play a role in the early stages of AV shunting. We hypothesized that Notch4* disrupts eNOS signaling, thereby permitting vessel enlargement and AV shunting. We found that pharmacological inhibition of NOS by administering the NOS inhibitor NG-nitro-L-arginine or genetic deletion of eNOS both attenuated brain AVM formation in mice expressing Notch4*, as indicated by decreased AV shunt diameter. Furthermore, NOS inhibition or eNOS deletion improved survival of mice expressing Notch4* and reduced severity of brain AVM-associated pathologies.
Conclusion: Our results show that inhibiting NOS/eNOS signaling attenuates Notch4*-mediated brain AVM formation and suggest that NOS/eNOS pathway is a critical mediator of AVM formation and potential therapeutic target.

Identification of new genes and genetic modifiers in HHT that alter clinical severity

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Hereditary hemorrhagic telangiectasia (HHT) is the most common inherited vascular dysplasia with extensive clinical heterogeneity. Yet, approximately 15% of individuals identified as having HHT currently have no known genetic cause. Mutations in *ENG*, *ACVRL1*, and *SMAD4* have been identified in affected individuals. However, the genetic heterogeneity of HHT does not explain the variable clinical manifestations routinely seen within families. Recent studies have suggested that variation in several vascular genes may impact both the severity and location of arteriovenous malformation (AVM) formation.
Objective: Our goal was to identify additional HHT genes and genetic modifiers that may govern AVM formation and HHT severity.
Methods: Transcriptomes from 38 HHT cases and 8 healthy controls were prepared using RNA extracted from peripheral blood and the globin-zero removal TruSeq stranded RNA-seq kit (Illumina). Indexed samples were pooled and sequenced using 2 × 100 paired end reads on a HiSeq2500 instrument. Reads were aligned to the human genome using Bowtie-2. Data were analyzed and compared using DESeq2 in R to identify differences in gene expression.
Results: Over 100 genes were differentially expressed in HHT patients versus the controls. Many are involved in hypoxia response and vascular injury. Phenotypic analyses were performed to identify genetic modifiers and novel transcripts in HHT patients who have pulmonary arterial hypertension (PAH), pulmonary AVMs, and/or severe epistaxis. This analysis revealed differentially expressed genes that may be involved in modifying nosebleed severity.
Conclusion: The identification of new genes and genetic modifiers in HHT may lead to better diagnostics and the development of new therapeutics.

Session 11A Genetics and HHT

Variants in *endoglin* and non-HHT phenotypes

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Aim/Method: A phenome-wide association study (PheWAS) of a large population cohort who have not been diagnosed with HHT.
Results: Moderately rare coding variants in ENG (Pro131Leu–rs1800956 and Asp366His–rs139398993) are associated with HHT-like phenotypes including vascular anomalies. In addition, ENG variant rs139398993 is associated with increased risk for polycystic kidney disease.
Conclusion: Non-HHT causing coding variants in ENG may be associated with vascular anomalies and other pleiotropic effects.

Next generation sequencing in HHT genetic diagnosis

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Objective: Three genes have been implicated in hereditary hemorrhagic telangiectasia (HHT). They are responsible of typical HHT (*ENG*, *ACVRL1*) and HHT with juvenile polyposis (*MADH4*). Mutations of two other genes cause overlapping but distinct phenotypes: epistaxis and telangiectases of particular localization (*GDF2*) and telangiectases with capillary malformations (*RASA1*). We developed a next-generation sequencing (NGS) gene panel for the diagnosis of HHT and related disorders.
Methods: We designed targeted sequences of all exonic regions and intronic flanking regions of these five genes with the software SureDesign (Agilent technologies). We included targeted sequenced for promoter regions of *ENG* and *ACVRL1*. We used SureSelet^{QXT} targed enrichment system for Illumina Multiplexed sequencing (NextSeq 500). We first validated the methods with DNA from 10 patients with a previously identified disease-causing variant. Then, we sequenced DNA from 122 novel patients addressed for molecular diagnosis of HHT. Large deletions were screened by mlPA.
Results: All the 10 previously identified mutations were found by NGS. Among the 122 novel patients, we found a potentially pathogenic variant in 73: 40 in *ACVRL1*, 32 in *ENG*, and one in *MADH4*; all variants were confirmed by Sanger sequencing. Among the 49 patients without identified mutation, only 2 had a definite diagnosis of HHT. No large deletion was found.
Conclusion: Our study assesses NGS is an efficient and cost-effective method for HHT genetic diagnosis. A retrospective study will be performed for patients with a confirmed clinical diagnosis but without mutation identified by Sanger sequencing and mlPA.

Two interesting cases of molecular diagnosis for HHT: low-level mosaicism and abnormal splicing of ACVRL1

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Objectives: Here we describe two cases demonstrating pathogenic changes in ACVRL1 that can be difficult to identify using routine genetic testing procedures. For the first case, when subsequent clinical description of the proband indicated the potential for mosaicism, sequencing data was reassessed to investigate for low-level mutations. For the second case, a familial variant of uncertain significance (VUS) had been identified in intron 5 of the ACVRL1 gene. Using RNA analysis, we investigated the functional impact of this previously unreported variant. METHODS: To confirm low-level mosaicism, alternate primers were employed to bi-directionally re-sequence a suspicious alteration detected in the original ACVRL1 sequencing trace. The sample was also tested by an allele-specific PCR assay. To determine splicing aberrations in the second case, the ACVRL1 cDNA coding sequence was analyzed using Sanger sequencing to characterize splicing at the exon 5/6 junction.
Results: We identified an ACVRL1 mosaic c.200G > A mutation in the first patient with suspected HHT, estimated to be present on one allele in 22% of the patient's blood leukocytes. The previously observed familial VUS (c.625 + 56G > A) predicted to cause a cryptic preferred splice site in the second patient was shown by ACVRL1 cDNA sequencing to cause a proportion of an affected patient's transcripts to have abnormal retention of nucleotides c.625 + 1 to c.625 + 57.
Conclusion: Communication of clinical symptoms to clinical laboratories greatly facilitates their investigations of pathogenetic changes. RNA analysis findings of aberrant splicing associated with a VUS can support molecular and clinical diagnosis of HHT.

Session 11B HHT around the world

Clinical Analysis of 200 Japanese HHT patients

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Objectives: To analyze clinical characteristics of Japanese HHT patients and false negative factors of Curaçao criteria (1–2 points) among them.
Methods: 200 Japanese HHT patients who were diagnosis by genetic test and/or Curaçau criteria (≥ 3) were included. Thus, all patients were definite HHT. There were 102 men and 98 women aged 0–82 years (mean 40.1). 170 patients underwent genetic tests. Essentially all patients underwent MR for bAVM and CT for pAVF. Additionally, dynamic CT for hAVM, enhanced CT or MR for sAVM, and endoscope for GI tract AVM were performed in selected patients. Genetically confirmed HHT patients with Curaçao 1–2 points were further analyzed.
Results: There were 97 HHT1, 57 HHT2, and 16 mutation-undetected patients. Mean ages were 33.2, 46.6, and 50.6 years, respectively. pAVFs ware found in 63 HHT1 and 10 HHT2 patients. bAVM were found in 28 HHT1 and 2 HHT2 patients. hAVMs were found in 24 HHT1 and 43 HHT2 patients. Among 154 genetically confirmed patients, 18 patients (12 children and 6 adults) were either probable or unlikely. Six adult patients (mean age 31.2) lacked either nosebleed or telangiectasia as well as visceral AVMs. Only 1 of nine screened children had bAVM and pAVF. Only one of 6 adult patients had pAVF.
Conclusion: Younger age of HHT1, higher prevalence of pAVF and bAVM among HHT1 and hAVM among HHT2 were confirmed in Japanese HHT patients. Curaçao diagnostic criteria are generally reliable except for children, but false negative must be taken into account in adult patients.

Hereditary hemorrhagic telangiectasia (HHT): a retrospective study of ten patients

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Objectives: The aim of this retrospective study was to present our clinical experience with HHT patients treated at our Department.
Methods: From 2008 to 2016, patients diagnosed and treated for HHT were identified by the hospital database review. The following parameters were evaluated: signs and symptoms at presentation, family history, and survival.
Results: Ten patients (6 males, 4 females) were identified with a median age of 70 years at presentation (range 46–80 years). All patients were referred to a hematologist or gastroenterologist for severe iron deficiency anemia. Median hemoglobin level at presentation was 7.3 g/dL (range 5.4–10.0 g/dL). Eight patients required emergency blood transfusions. Only two patients met all of Curacao criteria, six met 3 of 4 criteria, and two patients met 2 criteria. Four patients had family history of HHT. The most common presentation was recurrent epistaxis (in all patients). Nine patients had mucocutaneous telangiectasia, seven had gastrointestinal angiodysplasia (gastrointestinal bleeding was present in 6 patients), two had pulmonary and one had hepatic arteriovenous malformations. Four patients were treated with Argon plasma coagulation and one patient with repeated nasal packing. After a median follow up of 60 months, six patients are alive and four patients died. Two deaths were caused by severe gastrointestinal bleeding.
Conclusion: In this cohort of patients, the most common presentation of HHT was iron deficiency anemia caused by recurrent epistaxis and gastrointestinal bleeding which significantly contributed to mortality. Since most patients were diagnosed at an older age, efforts should be made to identify HHT patients earlier.

The relevance of HHT Europe (Federation of Patient Organizations) in improving patients centered activities and patient involvement in outcomes for HHT

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Objective: For people with hereditary hemorrhagic telangiectasia (HHT), our objective is to improve Patient Organization (PO) expertise, share best practices, create patient centered policies, promote patient involvement and improve cooperation with the HHT clinical and research community. Further objectives included fostering the creation of HHT organizations in under-represented European countries.
Methods: To achieve this HHT Europe has encouraged participation of PO members in training on orphan drugs and clinical trials while also conducting annual training sessions on non profit management, communication, fundraising and patient involvement. Patient participation in the new European Reference Network for Rare Multisystemic Vascular Diseases (VASCERN) was promoted. **Results:** The outcomes result in 5 Eurordis Summer School trainees, 1 Eupati patient expert, HHT Europe representatives in the European Medicine Agency (EMA), and 4 European Patient Advisory Group (Epag) representatives in the VASCERN. There are 3 new organizations and 3 more in the making. The Federation has coordinated European HHT awareness campaigns and directly supported new patient organizations on the continent. A survey on Federation

benefits revealed a completely new perception of PO role as a stakeholder in the decisional processes on disease related decisions (90% of answers) and of the benefits that the clinical and research community can reap from an improved cooperation with the patient community. (80% of replies).
Conclusion: The overall outcomes of the Federation's activities are perceived as successful and patient involvement improves all PO practices. The survey supports the critical need for essential exchange and cooperation between the PO and clinical community.

HHT: a South African perspective on diagnosis, management and follow up

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Background: Hereditary Haemorrhagic Telangiectasia has a worldwide prevalence of 1:5000. This figure, however is not represented by data in South Africa as there is no database tracking the diagnosis and follow up of HHT patients. HHT is very likely underdiagnosed in South Africa, partly due to lack of awareness and resources. There are no clear South African guidelines on its management or on when to refer the patient for further specialist care. Our aim is to try to establish a system to improve the care of patients suffering from HHT in an area where no specialised HHT centres are available. Furthermore, we would like to create a multidisciplinary unit within our academic hospital (Tygerberg Hospital, Cape Town, South Africa) to manage those patients.
Methods: An overview of the following will be presented in poster format: 1. A pamphlet providing information and guidance aimed at the health professionals—specially the ENT surgeons; 2. an information pamphlet aimed at patients suffering from HHT; 3. an 'insert' for the patients' files in order to track their progress during their visits to hospital (this will contain a severity scoring system, there investigations and their management to date); 4. a database that will be used to capture each patient's details and record their management.
Conclusion: Once the system in place, we would strive to expand our services to a greater patient population in South Africa and Africa within the scope of our resources. Ultimately creating a HHT platform for much needed diagnosis, management, and research.

Session 12 Mechanisms of HHT

Isogenic induced pluripotent stem cells (IPSCS) derived from mosaic hereditary hemorrhagic telangiectasia type 1 (HHT1) patient

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Objectives: We recently identified a mosaic HHT1 patient with an ENG mutation present in the peripheral blood, but not other somatic cells, such as skin fibroblasts. We reasoned that human induced pluripotent stem cells (hiPSCs) generated from a mosaic HHT1 patient could be an isogenic source of heathy and diseased endothelial cells (ECs) from the same patient.
Methods: Reprogramming of erythroblasts expanded from peripheral blood and skin- derived fibroblasts was done in parallel in order to generate hiPSC lines with and without the mutation. hiPSC lines were generated using non-integrating reprogramming method with episomal vectors. Three independent hiPSC clones were generated by reprogramming erythoblasts and fibroblasts. The presence of the mutation was confirmed in erythroblast-derived hiPSCs, but not detected in fibroblast-derived hiPSCs, as expected. We further confirmed the genetic identity of the lines as being from the same patient by DNA fingerprinting.
Results: Erythroblast and fibroblast-derived HHT1 hiPSCs were differentiated to ECs. Importantly we confirmed reduced ENG cDNA and protein expression in the ECs derived from mutant hiPSC compared to wt hiPSC isogenic controls. We next examined functionally of HHT1-iPSC-ECs in vitro in assays that included TGFb-mediated signal transduction, proliferation, migration, barrier function and sprouting in a 2D EC-pericyte co-culture system.
Conclusion: Our data indicate that HHT1 hiPSC ECs derived from isogenic hiPSC lines possess normal functionality in vitro and reduced ENG expression levels had no effect on TGFb-mediated signal transduction, proliferation, migration, barrier function and EC sprouting in 2D co-culture system. Additional studies are ongoing to elucidate triggers of a disease phenotype in HHT1-hiPSC-derived ECs in vitro.

Telangiectasia-on-a-chip: an in vitro model of hereditary hemorrhagic telangiectasia incorporating perfused vessels

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Objectives: The genetic cause of HHT has been known for more than 20 years, but still little is understood about how these mutations cause vascular malformations. Current models include genetically-modified mice and 2D in vitro platforms. A better model is needed that incorporates the complexity of a 3D environment, including functioning blood vessels, while allowing for precise manipulation and evaluation. We aimed to develop a model of telangiectasias to meet these needs, based on our in vitro vascularized micro-organ (VMO) platform incorporating perfused, living microvessels.
Methods: The VMO platform is a microfluidic device in which endothelial cells (ECs), suspended within an extracellular matrix, are stimulated by interstitial flow and co-suspended stromal cells to self-assemble into microvascular networks. Expression of endoglin and Alk1, mutations in which cause HHT Type 1 and 2, were knocked down and microvascular network formation was observed.
Results: Alk1 and endoglin-deficient ECs formed aberrant microvascular networks—resembling telangiectasias, with increased total vessel length and branching, and irregular diameters.
Conclusion: With this system, processes thought to be important in the development of telangiectasias can be studied, including recruitment of mural cells. Vascular permeability can also be assessed, allowing us to address vessel fragility. We are currently testing modifications to the platform that allow for testing the role of shear in the generation of AVMs. Finally, we hope to use the platform to identify drugs that can reverse the mutant phenotypes.

Somatic *ALK1* gene mutations mediated by an adenoviral vector expressing CRISPR/CAS9 induces arteriovenous malformation in the brain angiogenic region of adult mice

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Objectives: Current large animal models for brain arteriovenous malformation (bAVM) lack true AVM nidus in the brain. We have established several bAVM mouse models by genetically deleting hereditary hemorrhagic telangiectasia causative genes, *Alk1* or *Eng*. However, it is difficult to monitor bAVM progression and hemodynamics using non-invasive imaging in mice. CRISPR/Cas9 genetic editing can induce somatic gene mutations that bypass the potential mortality caused by germline mutations. We tested the feasibility of using CRISPR/Cas9 to mediate somatic *Alk1* mutations for bAVM model generation.
Methods: Two sequence-specific guide RNAs (sgRNA) targeting mouse exons 4 and 5 of *Alk1* gene were designed and cloned into an adenoviral vector to generate pAd-Cas9-Alk1e4 + e5sgRNA. After verifying gene mutations in the bEND.3 endothelial cells, the vector was packaged into an adenovirus, which was co-injected with an adeno-associated viral vector expressing vascular endothelial growth factor (AAV-VEGF, to induce brain angiogenesis required for bAVM formation in adult mice) into the mouse brain. Gene deletion efficiency in the virus injection sites was analyzed.
Results: pAd-Cas9-Alk1e4 + e5sgRNA knocked down ALK1 protein expression to 80% compared with untransfected bEND.3 cells. Co-injection of Ad-Cas9-Alk1e4 + e5sgRNA and AAV-VEGF into wild-type C57bl/6 adult mouse brain induced bAVM 8 weeks later. Injection of Ad-Cas9-Alk1e4 + e5sgRNA mutated 14% of *Alk1* gene in the brain around injected sites. Latex perfusion showed dilated tangled vessels that resembled the bAVM phenotype.
Conclusion: We successfully induced the bAVM phenotype in the adult brain angiogenic region using Ad-Cas9-Alk1e4 + e5sgRNA-induced somatic *Alk1* gene mutation. Future studies will use this method to create bAVMs in larger animals.

A novel SMAD4 mouse model of HHT

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Objectives: TGF-Beta signaling through Alk1 and Eng converge at Smad4 to regulate gene expression. Although 2% of HHT patients exhibit mutations in the Smad4 gene, no animal model exists to study this central mediator of the pathway. Our objective is to develop and characterize a mouse model of Smad4-mediated HHT that can be further used to identify and study the TGF-Beta downstream effectors.
Methods: Utilizing the Cre-LoxP system, we developed a Smad4 inducible, endothelial specific knockout (Smad4-iECKO) mouse model whereby Smad4 is deleted specifically in blood vessels. We used immunofluorescent staining, confocal microscopy and real-time quantitative PCR methods to provide a thorough characterization of the developing retinal vascular network in our Smad4 HHT model.
Results: Loss of Smad4 leads to AVM formation in the mouse retina, similar to Alk1 and Eng mouse models of HHT. In Smad4 mutant retinas, we observe an increase in both artery and vein diameter, an overall decrease in vascular outgrowth and a distortion in artery-vein identities. By screening multiple arterial and venous markers, we found that several vascular pathways are affected by the absence of Smad4, including TGF-β, Notch, Eph, and VEGF. Furthermore, we find that Smad4 mutants exhibit increases in vascular remodeling in areas containing AVMs.
Conclusion: Using Smad4, the central mediator of the TGF-Beta pathway, we have established and characterized a universal model of HHT. Using this model, we will be able to gain insight into the direct mechanisms of HHT located downstream of Alk1 and ENG.

Session 13 Treatment of HHT

Long-term outcomes for radiosurgical management of brain arteriovenous malformations in patients with hereditary hemorrhagic telangiectasia

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Background: Cerebral arteriovenous malformations (AVM) are common in patients with hereditary hemorrhagic telangiectasia (HHT). However, due to rarity of HHT, and little published evidence of outcomes from management of brain AVMs in this disease. Due to their multiplicity and relatively small size, radiosurgery is an appealing treatment option for these lesions. However, the outcomes for radiosurgical treatment of these lesions have not been reported to date.
Objective: We report long-term outcomes of the radiosurgical treatment of brain AVMs in HHT patients.
Methods: From the database of Brain Vascular Malformation Consortium HHT project, 23 patients with 27 brain AVMs who underwent radiosurgery were studied. Long-term outcomes are reported.
Results: The patients were followed for an average of 19.3 years after treatment. Average lesion size was 8 mm before treatment. Median modified Rankin scale (mRS) score of the patients before treatment was 1. At the time of last follow-up, median mRS score was 1.5.
Conclusion: Our long-term outcome data along with the delayed post-operative imaging show that radiosurgical treatment is an effective option for HHT patients with brain AVM malformation and should be continued as a treatment option in these cases.

Surgical treatment versus non-surgical treatment for brain arteriovenous malformations in patients with hereditary hemorrhagic telangiectasia: a retrospective multicenter consortium study

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Background: Cerebral arteriovenous malformations (AVM) are common in patients with hereditary hemorrhagic telangiectasia (HHT). However, due to rarity of HHT, and little published evidence of outcomes from management of brain AVMs in this disease, current International HHT Guidelines recommend an individualized approach. Specifically, the outcomes for surgical versus non-surgical management of these lesions have not been reported to date.
Objective: We report long-term outcomes of surgical resection of brain AVMs in HHT patients compared to outcomes in non-surgically treated patients.
Methods: From the database of Brain Vascular Malformation Consortium HHT project, 19 patients with 20 resected AVMs (group 1), and 22 patients with 33 AVMs who received non-surgical treatment (group 2) were studied. The groups were retrospectively reviewed for changes in functional status (modified Rankin Scale score) during the follow-up period.
Results: During the follow-up period, 9% of patients in group 1 suffered from worsening of functional status whereas this figure was 16% for group 2 ($P > 0.05$). Functional outcomes were not statistically different between the two groups at the latest the follow-up ($P > 0.05$).
Conclusion: HHT patients treated surgically for brain AVMs appear to have long-term functional outcomes comparable to non-surgical (including observational) therapy with fewer unfavorable outcomes. It is therefore reasonable to consider surgical resection as a management option, in the multidisciplinary team's individualized treatment strategy for HHT patients with brain AVMs.

Systemic to pulmonary arteriovenous malformations (SPAVM): a distinct entity from HHT PAVMS clinically, on imaging, and for embolization, with whole genome analysis

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Objectives:

1. Elucidate clinical presentation, imaging, and embolization techniques for rare SPAVM that is mistaken for HHT PAVM.
2. Determine if a distinct gene for SPAVM.

Methods: Retrospective evaluation 6 patients for Curacao criteria, right-left shunt, CT scans, pulmonary and systemic angiography, right heart pressures; embolization results in 4/6. Whole exome sequencing. PhenoDB variant analysis tool for heterozygous and homozygous rare functional variants (missense, nonsense, splicing, indels). ClinVar and Human Gene Mutation Database classification of variants and associated online Mendelian Inheritance in Man phenotypes. Then merge to identify genes mutated in ≥ 3 probands. Final candidate gene list submitted: GeneMatcher and Matchmaker Exchange databases to identify other patients.
Results: 6 patients, 4 M, 2F, 26–70 years (44 years). Curacao criteria: epistaxis = 0, telangiectasiae = 0, family history = 1/6 (17%), cerebral AVM = 1/6, pulmonary AVM = 6/6, hepatic AVM = 0, GI bleeding = 0. CT scans 6/6 read as HHT PAVMs, unilateral 5/6 (83%), bilateral 1/6 (17%). Aneurysms of ascending aorta 2/6 (33%), family 3/5 (60%).
Angiography 6/6: systemic artery supply to pulmonary sacs; drainage into segmental pulmonary artery. Pulmonary pressures normal 5/6 (83%), slightly elevated 1/6 (17%). No right-left shunt: agitated saline echography 3/6 (50%), angiogram 3/6 (50%). Peripheral blood genetic analysis 6/6 (100%), also systemic and pulmonary artery blood 4/6 (67%). Five family members, 10–58 years, 2 M, 3F, having genetic analysis. Embolization of SPAVMs 4/6 (67%); aneurysmal sac occlusion for successful treatment, systemic and/or pulmonary approach.
Conclusion: SPAVMs: sacs fill from systemic feeders, drain via pulmonary artery. Do not shunt right-left. Risks leaving untreated: hemoptysis, pulmonary hypertension. Occlusion of aneurysmal sac required. 2/6 (33%) ascending aortic aneurysmal dilatation. Whole genome analysis on 11 patients.

Progression of pulmonary arteriovenous malformations in children with hereditary hemorrhagic telangiectasia

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Objectives: Pulmonary arteriovenous malformations (PAVMs) may occur in children with hereditary hemorrhagic telangiectasia (HHT). A longitudinal study of PAVMs in children with HHT was undertaken to assess for progression of these malformations.
Methods: Retrospective, single center study from May 2002 to December 2016 of 130 children with HHT diagnosed using Curacao criteria or by genetic confirmation. PAVMs were diagnosed with contrast echocardiography or chest CT. Embolization of PAVMs were performed according to HHT consensus guidelines.
Results: Of 130 children with HHT, 67 (52%) were positive on initial screening and 76 (58%) had PAVM during their course. Of 63 children without PAVM on initial screening, 31 were followed for > 1 year. Nine of 31 (29%) developed PAVM after initial negative study (mean

time to detection, 5.6 years). Fifty percent (38/76) of the children with PAVM underwent embolization; 76% of those (29/38) were treated after initial screening. Nine children (12%) who were initially found to have PAVM too small to treat had progression of PAVM size allowing embolization at a later stage (mean time from PAVM diagnosis to embolization, 3.4 years). After embolization, 21% (8/38) underwent repeat interventions (mean time between embolizations, 5.5 years). Genetic diagnosis, age, and gender were not associated with risk of having PAVM nor with need for repeat interventions.
Conclusion: Nearly one-third (29%) of children initially negative for PAVMs subsequently developed PAVMs within 5 years of follow-up. An additional 12% with small PAVMs demonstrated growth to a size that met criteria for embolization. After embolization, 1/5 (21%) will undergo subsequent interventions.

Human endoglin as a potential new partner involved in platelet-endothelium interactions

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Objectives: Hereditary hemorrhagic telangiectasia type 1 (HHT1) is characterized by a bleeding tendency that is postulated to be a consequence of telangiectasia fragility rather than a platelet defect. HHT1 patients present heterozygous mutations in the endoglin gene resulting in a loss of expression of membrane endoglin (Eng) on endothelial cells (EC). We reported that endothelial Eng is involved in inflammation via its RGD motif, through Integrin mediated leukocyte adhesion and transmigration. These data prompted us to hypothesize that Eng may act as an adhesion molecule involved in the interaction between EC and platelets through integrin recognition.
Methods: Blood samples were obtained from healthy donors, HHT1 and Glanzmann´s thrombasthenia patients. Bleeding time in Eng haplodeficient (Eng ±) and wildtype (Eng +/+) mice was studied. Generation of stable cell transfectants in L6E9 rat myoblasts, expressing human Eng, and in Chinese hamster ovary (CHO) cells, expressing human αIIbβ3, were carry out. Microfluidic devices were used to evaluate shear resistant platelet adhesion.
Results: We find that the extracellular domain of human Eng promotes platelet adhesion under static conditions and confers resistance of adherent platelets to detachment upon exposure to flow. Also, platelets adhere to confluent EC in an Eng mediated process. CHO cells ectopically expressing the human αIIbβ3 integrin acquire the capacity to adhere to myoblast transfectants expressing human Eng, whereas platelets from Glanzmann´s thrombasthenia patients lacking the αIIbβ3 integrin are defective for Eng dependent adhesion to EC. Furthermore, the bleeding time, but not the prothrombin time, is significantly prolonged in Eng +/mice compared to Eng +/+ mice.
Conclusion: These results suggest a new and critical role for Eng in αIIbβ3 integrin mediated adhesion of platelets to the endothelium and may provide a better understanding on the basic cellular mechanisms in thrombo-inflammatory events.

Genetic background-dependent vascular alterations of BMP9-KO mice

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Objectives/Methods: Our team previously identified bone morphogenetic proteins BMP9 and BMP10 as physiological high affinity ligands of the endothelial receptors ALK1 and Endoglin, whose genes are mutated in 85% of HHT patients. Bmp9 mutations have also recently been described in HHT.
In order to understand the role of BMP9 in HHT development, we generated *Bmp9* knock-out (*Bmp9*-KO) mice in the C57/Bl6 genetic background. These mice were viable but presented alterations of their lymphatic vasculature, whereas their blood vasculature was normal (unless BMP10 was simultaneously neutralized with antibodies). Because *Eng* ± mice have been shown to present a more pronounced phenotype in the 129/Ola background, we wanted to study the phenotype of B*mp9*-KO mice in this background.
Results: Follow-up of 129/Ola *Bmp9*-KO mice viability revealed an abnormal gender-dependent mortality. *Bmp9*-KO male mice in the 129/Ola background had a mean survival age of 28 weeks whereas 77% of females were still alive at this age. In males, deaths were preceded by a rapid weight loss (− 20% within 1 week). Analysis of their vasculature suggested enlarged blood and lymphatic vessels. Interestingly, *Bmp9*-KO mice in 129/Ola background often presented macroscopic alterations of their kidneys (53% of males and 25% of females) and liver (23% of males and 75% of females).
Conclusion: Taken together, these observations suggest that the 129/Ola genetic background is prone to major physiological alterations when the B*mp9* gene is deleted whereas this is compensated in other genetic contexts. This observation paves the way for the search of susceptibility genes that can protect or worsen the clinical alterations observed in HHT patients.

POSTER PRESENTATIONS

Antiangiogenic Therapy

Phenotype assessment and pilot observational study of intranasal bevacizumab in hereditary haemorrhagic telangiectasia (HHT)

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Objectives: A significant number of patients with HHT attend The Royal Melbourne Hospital for specialist multidisciplinary clinical care. Nearly all suffer recurrent bleeding, especially epistaxis. The cohort have been assessed clinically but 2 major clinical questions remained unanswered:

A. Does intranasal Bevacizumab offer symptomatic relief; and
B. Are there renal manifestations of HHT?

This study addressed these two questions in the existing cohort.
Methods: Up to 20 participants with a confirmed diagnosis of HHT were recruited via the Australian HHT registry (www.hht.org.au). We documented frequency, severity and clinical impact of nose bleeding in our HHT patients. This was captured via structured diary entry and monitored monthly by a study team member for 3 months. Participants completed questionnaires including an Epistaxis Severity Score and had blood count and iron levels checked at the completion of this period. We then assessed the symptomatic (clinical) response to the treatment of intranasal Bevacizumab spray. This 3-month pilot study used a short course of low dose intranasal Bevacizumab, and assessed outcomes via comparing scores pre and post Bevacizumab use. The study evaluated potential kidney involvement by testing the urine and by renal ultrasound and MRI. Measures included RBC count, spot urine alumin/creatinine and protein/creatinine ratios. Colour and spectral Doppler of renal arteries and renal veins looked for evidence of obvious intrarenal shunting.
Results and Conclusion: Renal findings in the cohort will be presented with comparison of MRI and ultrasound for interrelator reliability and genotype–phenotype correlation.

How we do intranasal bevacizumab injection in Norway

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Objectives: To describe our experience in treating HHT associated epistaxis with intranasal injections of bevacizumab.
Patients: Fifty HHT patients have been treated by intranasal bevacizumab injection between June 2011 and January 2015. Total number of treatments is 200.
The main indication was the lack of clinical benefit, in spite of increasing the frequency of the laser therapy, and additional medical therapy.
Method: Eighty percent of the treatments (40/50) were done in local anesthesia with sedation. Four or five sites were injected in each nasal cavity based on the vascular anatomy of the nasal cavity. Four ml of 25 mg/ml bevacizumab was injected in each nasal cavity. Total dose was 200 mg. No laser photocoagulation was done during or after the procedure.
Results: Improvement in the epistaxis severity was reported in the majority of the patients. The duration of effect of the treatment varies among patients (mean = 6 months, range 3–15 months). Some of the patients did not show any beneficial effect of repeated intranasal bevacizumab injections. The effectiveness of the treatment was gradually decreased in some patients who showed initial good effect (detailed data will be presented at the conference).
Conclusion: Intranasal bevacizumab injection is effective and quite safe. Restricting the injection to 4–5 sites in each nasal cavity makes it possible to do the treatment in local anesthesia with sedation. This treatment modality has now found its place in the treatment algorithm of HHT associated epistaxis.

VEGF and other angiogenic factores in HHT associated epistaxis

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Objective: To find possible correlation of VEGF and other angiogenic factors in HHT patients with the grade of epistaxis.
Material and method: 110 blood samples from 66 HHT patients were collected to measure the VEGF and other angiogenic factors in addition to hemoglobin, serum iron and ferritin. The grade of epistaxis was evaluated using the ESS and IFT epistaxis scoring systems.
Results: The preliminary results showed no strong correlation of the level of VEGF and the grade of epistaxis.
Conclusion: VEGF level cannot be used as a predictive factor for choosing the patients suitable for bevacizumab injections.

Bevacizumab: a case study in management of a diffuse liver AVM causing presinusoidal portal hypertension and ascites

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Objective: We present the case of a favorable therapeutic effect of intravenous bevacizumab in a patient with hereditary hemorrhagic telangiectasia (HHT) who suffered from symptomatic high-output heart failure in the setting of diffuse liver AVMs, pre-sinusoidal portal hypertension, chronic ascites, and chronic anemia.
Methods: A 51-year old woman with definite HHT presented with chronic GI bleeding and a diffuse hepatic artery to portal vein liver AVM resulting in high-output heart failure, cirrhosis, pre-sinusoidal portal hypertension, and chronic ascites. The patient received bevacizumab 5 mg/kg IV every 2 weeks for 6 infusions followed by 5 mg/kg every 2 weeks for an additional 4 infusions.
Results: Prior to treatment, the patient underwent weekly paracentesis yielding 6–7 L. Following 6 infusions, the patient's ascites drainage reduced to 4 L every 2–3 weeks and continued to improve prior to and during additional bevacizumab infusions. Her ascites ultimately resolved and she has not required paracentesis since 10/2016. Pre-treatment echocardiogram demonstrated high cardiac output and post-treatment echocardiogram demonstrated normal cardiac output. Quantitative flow measurements using phase-contrast MRI demonstrated celiac artery flow of 4577 mL/min pre-treatment and 1885 mL/min post-treatment.
Conclusion: In our experience, diffuse AVMs respond better to bevacizumab than large vascular communications. Hepatic artery to portal vein liver AVMs are uncommon. Resolution of ascites can be used as a clinical endpoint when evaluating bevacizumab treatment response in patients with pre-sinusoidal portal hypertension. Flow measurements with phase contrast MRI can be used to evaluate treatment response to IV bevacizumab in patients with liver AVMs.

Bevacizumab therapy for brain arteriovenous malformations

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Objectives: The objective of this study is to provide feasibility and preliminary safety and efficacy data for use of IV bevacizumab in the treatment of unruptured brain arteriovenous malformations (bAVM).
Methods: The trial is a 10 patient one-armed, open-label study that will administer bevacizumab (5 mg/kg every 2 weeks) by IV infusion over the course of 12 weeks. Patients with unruptured bAVM deemed unsuitable for invasive treatment or patients that have elected to defer invasive treatment or failed conventional therapy will be included in this study. The primary endpoint will be bAVM volume following

completion of 12 weeks of bevacizumab treatment. Our primary research hypothesis is that bAVM nidal volumes will decrease over the study course as evidenced by comparing baseline MRI and 26-week MRI. A 52-week interval MR study will aid in our assessment of the durability of drug effect on bAVM in addition to providing more safety data. We will compare pre- and post-treatment volumes using a paired *t* test. Secondary endpoints include MRA-based computational fluid dynamic parameters in primary bAVM feeding arteries and the incidence of symptomatic intracranial hemorrhage. Safety will be evaluated at each infusion visit by physical examination, routine lab work, and assessment for adverse events.
Results: This study is actively enrolling patients and preliminary data will be presented.
Conclusion: This study is the first medical intervention trial for bAVM. The study will meaningfully impact the clinical and research communities managing AVMs, regardless of their anatomic location, and in turn those patients who suffer from them.

BABH study: a double-blind, randomized national phase 3 study to evaluate efficacy and safety of bevacizumab for the treatment of severe bleedings in hemorrhagic hereditary telangiectasia (HHT)

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Aim/Method: Antiangiogenic drugs, such as bevacizumab, a humanised monoclonal antibody targeting vascular endothelial growth factor (VEGF), are a new treatment strategy in Hereditary Hemorrhagic Telangectasia (HHT). Patients with severe anemia related to nose bleeds and/or digestive bleedings need multiple blood transfusions. Many case reports and a non-comparative study have shown dramatic improvement of HHT bleedings after bevacizumab treatment. However, bevacizumab efficacy and safety in HHT need to be confirmed by a randomized phase II.
Objective: To evaluate, 6 months after the beginning of the treatment, the efficacy of bevacizumab on blood transfusions in patients with HHT complicated by bleedings responsible for severe anemia.
Methodology: Randomized, double blind phase III study (24 patients). Bevacizumab will be administered intravenously at a dose of 5 mg/kg/injection every 14 days for 2.5 months. Infusions of 0.9% NaCl will be used as Placebo.
Inclusion criteria: Patients with definite HHT diagnosis (based on Curaçao criteria), age ≥ 18 years old with severe anemia defined by need of blood transfusion despite appropriate care and iron supplementation.
Endpoint and analysis: Comparison of the number of transfusions during the last 3 months before treatment and between the 3rd and the 6th months after the beginning of treatment. Efficacy will be considered if the number of red blood cell transfusions is decreased by at least 50% between these 2 periods.
Results: Inclusions will start in 2017 as soon as regulatory approvals are received. The study has been granted by the French Ministry of Health (PHRC 2016).
Conclusion: On going.

Effect of a standardized protocol of intravenous bevacizumab on quality of life (QOL) in severe HHT related bleeding and refractory anemia

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Background: Severe HHT related bleeding (from epistaxis and/or gastrointestinal (GI) bleeding) have a severe impact on quality of life (QOL) and affect the day-to-day activities of patients. We report on QOL changes during a multi-year clinical experience with intravenous (IV) Bevacizumab for the management of these patients.
Methods: All patients treated with IV Bevacizumab for severe HHT related bleeding from June 2013 to Jan 2017 were included in this study. Patients were administered IV Bevacizumab using a standardized treatment protocol consisting typically of 8 initial infusions (Fig. 1). Subsequent re-dosing of Bevacizumab was based on worsening bleeding and/or anemia. Quality of Life was assessed at each follow up appointment using a 7 point self-reported Likert scale with the following question: *How would you rate your overall quality of life since the last appointment?* (1 = very poor QOL; 4 = fair/average QOL and 7 = excellent QOL). We also assessed the impact of

epistaxis on quality of life (E-QOL) life using a similar 7 point Likert scale question: "How often has nose bleeding affected or interfered in your day-to-day life since the last appointment". (1 = very seldom; 4 = occasionally and 7 = very often). Overall QOL and E-QOL were collected on the following days: (1) day of the first Bevacizumab infusion, (2) all follow up appointments during the initial treatment cycle (8 doses) and (3) subsequent follow up appointments until they required re-dosing with Bevacizumab or were lost to follow up.

Results: IV Bevacizumab was administered to 34 patients using a uniform treatment protocol (Fig. 1). The results on hemoglobin, ESS and transfusion needs are presented elsewhere. Median time to re-treatment with Bevacizumab was 8.1 months (IQR 5.7–14.1 months) (range 3–27.3 months) from the start of the treatment cycle. The number of patients not requiring re-treatment at 1, 3, 6, 9, 12, 15, 18, 21 and 24 months post initiation of Bevacizumab were 34, 33, 26, 14, 13, 8, 5, 2, and 1 respectively.

QOL data is presented in Tables 1, 2 and Figs. 1, 2. The median QOL score was **3.5** (n = 26) at baseline and improved to **5** (n = 15), **5** (n = 14), **5** (n = 10), **5** (n = 9), **5** (n = 14), **6** (n = 6), **5** (n = 3), **6** (n = 3), **5** (n = 1) at 1, 3, 6, 9,12, 15, 18, 21 and 24 months respectively.

The median E-QOL score declined from **4** (n = 26) at baseline to **3** (n = 15), **3.5** (n = 14), **1.5** (n = 10), **2** (n = 9), **1** (n = 14), **1.5** (n = 6), **4** (n = 3), **5** (n = 3), **4** (n = 1) at 1, 3, 6, 9,12, 15, 18, 21 and 24 months respectively after starting Bevacizumab therapy without further top-up doses.

Conclusion: Our study showed significantly improved patient reported QOL after a standardized treatment protocol utilizing intravenous Bevacizumab. We also found a significant reduction in the degree to which epistaxis interfered with day-to-day life. The adverse impact of epistaxis on QOL in HHT patients has been reported in a number of studies and treatment with Bevacizumab is an effective option in these patients.

Maintenance dosing of intravenous Bevacizumab in the treatment of severe HHT related bleeding and refractory anemia

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Background: Intravenous Bevacizumab has become an important agent in the management of severe HHT related bleeding (epistaxis and/or gastrointestinal (GI) bleeding). There is very little published information regarding need for repeat Bevacizumab infusions after initial treatment is completed. We present our clinical experience with re-dosing or 'top-up' Bevacizumab infusions after initial treatment using a standardized treatment protocol.

Methods: All patients treated with IV Bevacizumab for severe HHT related bleeding from June 2013 to Jan 2017 were included in this study. Patients were administered IV Bevacizumab using a standardized treatment protocol consisting of around 8 initial infusions. Data regarding re-dosing of Bevacizumab (top-up treatments) along with epistaxis severity scores (ESS), hemoglobin, iron studies and quality of life data were collected serially in all patients.

Results: 34 patients received the IV Bevacizumab treatment protocol and were followed for a median of 13.6 months (range 0.1–39.3 months) after the end of the initial treatment cycle. Of these; 18 patients (53%) required subsequent re-dosing of IV Bevacizumab due to worsening bleeding and anemia at an average of 5.1 re-treatments/patient/year of follow up. Overall time to re-dosing was 4.25 months (IQR 1.9–8.2 months; range 1.3–13.2 months) amongst these 18 patients.

A total of 4 out of 18 patients (22%) required continuous scheduled re-dosing of Bevacizumab at an average of 13.9 re-treatments/patient/year of follow up. The median time period to re-dosing of Bevacizumab in these 4 patients was 2.7 months (IQR 1.4–4 months, range 1.3–4.1 months). The remaining 14 patients (78%) required intermittent re-dosing at an average of 2.5 re-treatments/patient/year of follow up. The median time to re-dosing in these 14 patients was considerably longer at 5.7 months (IQR 2.2–8.8 months; range 1.4–13.3 months). The serial hemoglobin and ESS values for these patients around each top-up cycle are presented in Tables 1, 2, 3 and Figs. 1, 2. Top-up doses were administered at 7.5 mg/kg (2 patients; 36 doses); 5 mg/kg (16 patients; 92 doses); 2.5–3 mg/kg (7 patients; 56 doses) and 1 mg/kg (2 patients; 21 doses).

Conclusion: More than 50% of patients in this study required re-dosing after receiving a standardized initial dosing protocol of IV Bevacizumab with continued benefit both in terms of hemoglobin values and the ESS. The majority of patients (78%) could be managed successfully with an intermittent re-dosing strategy. Bevacizumab maintenance dosing is a very effective treatment option for patients with severe HHT related bleeding and refractory anemia.

Bevacizumab (Avastin) treatment for severe hepatic encephalopathy in a patient with hereditary hemorrhagic telangiectasia

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Introduction: Hepatic encephalopathy is a rare manifestation of hepatic AVMs in patients with HHT. We describe a case of a patient with hepatic encephalopathy that resolved after Bevacizumab treatment.

Case Report: a 69 year old female with HHT2, large hepatic AVMs, high cardiac output and severe pulmonary hypertension was admitted because of general deterioration over several weeks prior to hospitalization. On admission she was lethargic, with confusion and irritability. On physical examination she was somnolent, with flapping tremor and palmar erythema. Her blood ammonia level was elevated to 236 mcg/dl. She was diagnosed with hepatic encephalopathy. Treatment with diuretics and Rifaximin did not result in significant improvement. Treatment with Bevacizumab, 5 mg/kg was initiated and repeated after 2 weeks. Marked improvement was observed after the first dose. The lethargy resolved and she became alert regaining her previous daily activities. Ammonia level was down to 89 mcg/dl after the second dose of Bevacizumab. Six doses were given every 2 weeks and then every month. She remained well on Bevacizumab treatment only.

Conclusion: Bevacizumab treatment improves symptoms related to hepatic AVMs. This case report suggests that Bevacizumab might have a role in treating hepatic AVMs-related hepatic encephalopathy.

Bevacizumab for the severe liver involvement in HHT patients. A case series

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Table 1 Median hemoglobin values before and after each IV Bevacizumab top-up dose in the 14 patients receiving intermittent re-dosing

	Post initial cycle (34 patients)	Day of top-up 1	Post top-up 1	Day of top-up 2	Post top-up 2	Day of top-up 3	Post top-up 3	Day of top-up 4	Post top-up 4	Day of top-up 5	Post top-up 5	Day of top-up 6	post top-up 6	Day of top-up 7	Post top-up 7
#re-dosed		14	14	9	9	8	8	7	7	3	3	2	2	2	2
HgB-17.1	median	12.35	11	12.1	11.25	12.9	9.9	10.2	11	10.75	9.3	11.65	11.3	13	12.75
IQR	10.52–13.92	9.87–12.45	10.5–12.9	8.77–13.27	8.8–14.1	8.57–10.65	8.8–14.85	9.35–11.7	8.4–11.82	9.3	12.4–16.9	11.3	9.2–16.8	8.4–17.1	17.1
Range	8.6–15.8	8.8–13.2	10.2–15.8	8.4–13.5	7.9–15.8	7.9–11.1	8.3–16.1	8.3–12	7.8–12	9.3	12.4–16.9	11.3	9.2–16.8	8.4–17.1	17.1

Table 2 Median ESS before and after each IV Bevacizumab top-up dose in the 14 patients receiving intermittent re-dosing

	Post initial cycle (34 patients)	Day of top-up 1	Post top-up 1	Day of top-up 2	Post top-up 2	Day of top-up 3	Post top-up 3	Day of top-up 4	Post top-up 4	Day of top-up 5	Post top-up 5	Day of top-up 6	post top-up 6	Day of top-up 7	Post top-up 7
Number re-dosed		14	14	9	9	8	8	7	7	3	3	2	2	2	2
ESS (median)	2.14	3.69	2.69	2.75	1.92	3.49	2.59	3.66	3.29	1.41	1.95	4.96	0.9	0	0
IQR	1.37–3.75	1.3–4.55	1.71–4.62	1.47–4.54	0–4.68	0.38–5.71	0–5.62	2.64–5.04	1.41–5.18	0–2.83	1.95	4.96	0–1.81	0	0
Range		0–5.14	1.63–5.76	0.51–5.76	0–5.87	0–10	0–5.87	2.64–5.04	1.41–5.18	0–2.83	1.95	4.96	0–1.81	0	0

Table 3 Bevacizumab top-up characteristics in all 18 patients who needed re-treatment

	End of initial cycle	0–1 Month post initial cycle	1–3 months	3–6 months	6–9 months	9–12 months	12–15 months	15–18 months	18–21 months	21–24 months
Number of patients at risk	34	34	29	21	14	10	5	3	1	1
Number of patients retreated	0	0	6	5	4	2	1	0	0	0
% re-treated	0	0	20.7%	23.8%	28.6%	20%	20%	0	0	0

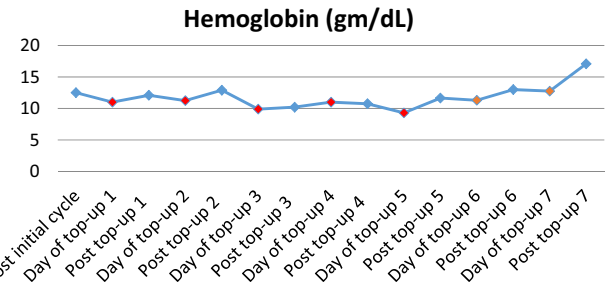

Fig. 1 Hemoglobin values on follow up

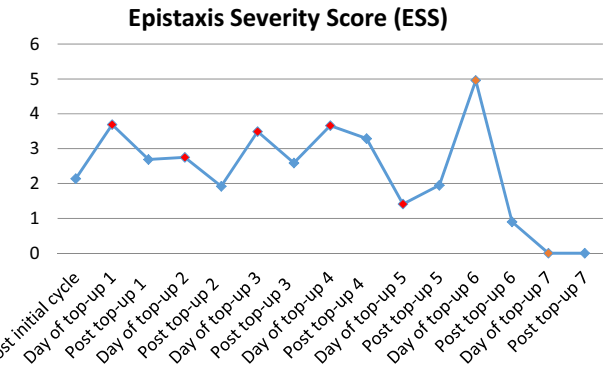

Fig. 2 ESS values on follow up

Objective: To report preliminary results of bevacizumab treatment in four patients with severe hepatic involvement.
Results: Three patients were females whose age range was 36–58. All patients presented high output cardiac failure (HOCF) with pulmonary hypertension, one also presented ischemic biliary necrosis. All four patients suffered from anemia mainly due to gastrointestinal bleeding. Two patients presented PAVMs and one needed pulmonary embolization. Three patients suffered self-limited supraventricular arrhythmia episodes. Two had endocarditis, one in the aortic valve as a young person who needed prosthetic valve replacement and anticoagulation, the other one in the tricuspid valve developing severe valve regurgitation, progressive right ventricle and biatrial enlargement. All patients exhibited hepatomegaly of which two were severe with ascites and edema. The cardiac index per patient was 6.3, 4.5, 5.1, 6.8 L/min/m^2. All reported low quality of life (QoL). The patients were treated with progressive doses of diuretics, digoxin or propranolol, and iron supplements. Also, received tranexamic acid presenting a mild response.
The four patients received bevacizumab 5 mg/kg every 15 days/six infusions after the intensive medical therapy failed. A patient received two extra bevacizumab cycles to control the anemia. All patient improved the QoL, clinical, hemodynamic and hematologic parameters after bevacizumab treatment. The follow-up range was 2–36 months. All patients were assessed for liver transplantation. The patient with severe tricuspid regurgitation died the following year due to progressive HOCF. Two patients developed a slow-healing venous ulcer as an adverse event.
Conclusion: Bevacizumab might represent a useful tool in the management of patient with HHT severe hepatic disease.

Osteonecrosis of the knees after intranasal injection with bevacizumab in treating HHT associated epistaxis: a case report

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Objective: Description of a patient who developed osteonecrosis in both knees while undergoing regular intranasal submucosal injections with bevacuzimab.
Methods: Case report.
Materials: A 66 year old man with HHT related epistaxis. This patient had been treated by laser and argon-plasma cautery over the course of several years. Due to unsatisfactory response, intranasal submucosal injections with bevacizumab was started in 2012 and repeated every 6 months. Starting in 2014 he developed gradually increasing severe bilateral pain in the knees to such an extent that he almost was unable to walk and required a wheelchair. CT and MRIs confirmed avascular osteonecrosis affecting the knees. This was judged as an adverse effect of bevacuzimab as the patient presented no other possible risk factors for avascular osteonecrosis. The bevacuzimab treatment was discontinued.
Conclusion: Otolaryngologists treating HHT patients with bevacuzimab injections should be aware of this potential side effect. Patients undergoing this particular treatment with new onset of skeletal pain should be referred to imaging.

Long-term experience with intranasal bevacizumab therapy for HHT associated epistaxis

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Objective: Long term follow up of intranasal bevacizumab therapy in HHT.
Methods: Patients who were treated for HHT-associated epistaxis by repeated intranasal submucosal injection of bevacizumab between June 2011 and August 2013 were included. The effectiveness of the treatment was evaluated by two grading scores; (1) epistaxis severity score (ESS) and (2) epistaxis intensity, frequency and the need of blood transfusion (IFT) score. Scoring was done before and 6–8 weeks after each treatment. In addition hemoglobin levels were evaluated before the treatment and regularly after the treatments. End point of follow up was January 2015.
Results: Thirty-three patients were included. The mean follow up period was 20.5 months (range 1–45 months). The mean number of treatments per patient was 8 treatments (range 1–20). The mean ESS score before the first treatment and at the end point of follow up were 6.20 and 3.40 respectively ($p < 0.000$). The mean IFT score before the first treatment and at the end point of follow up were 12.64 and 6.66 respectively ($p < 0.000$). The mean hemoglobin levels before the first treatment and at the end point of follow up were 11.23 and 12.64 respectively ($p = 0.006$).
The mean interval between treatments was 6 months (median = 6, range = 1–13).

No other epistaxis specific treatment modality has been used during this period.

Five patients (15%) showed no improvement after the treatment.
Conclusion: Repeated intranasal bevacizumab injection is an effective and quite safe treatment for most of the moderate and severe grades of HHT-associated epistaxis. Six months is the usual interval between treatments.

BRAIN MANIFESTATIONS OF HHT

High detectability of non-contrast-enhanced MR angiography using a silent scan for screening of cerebral arteriovenous malformations in HHT patients

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Objectives: Cerebral vascular malformations (CAVMs) in HHT patients contain nidus-type arteriovenous malformations (AVMs), cavernous malformations, developmental venous anomalies, capillary telangiectasias, and pial arteriovenous fistulas (PAVFs). The main purpose of screening for CAVMs in HHT patients is to detect AVMs and PAVFs bearing rupture risks with less invasiveness. The usefulness of Non-Contrast-Enhanced MR Angiography Using a Silent Scan (silent-MRA) for detection of CAVM was assessed.
Methods: We performed cerebral screening in 10 HHT patients between 2015 and 2017. Evaluation methods are as followed, (1) non-contrast-enhanced MRI screening including TOF-MRA and silent-MRA, (2) 4D-CT angiography when CAVMs are suspected in MRI, and (3) catheter-based cerebral angiography (DSA) if any interventions are required. Silent MRA uses a Silenz pulse sequence (GE Healthcare) containing an ultra-short time TE combined with arterial spin-labeling (ASL).
Results: 3 AVMs and 1 PAVF in 3 HHT patients were confirmed by DSA. 2 AVMs were below 1 cm in size with early venous drainage and one of them had bled. All of 4 lesions were detectable in silent MRA, meanwhile 2 lesions were hard to detect in TOF-MRA.
Conclusion: This non-contrast method using ASL detects most of high-flow arterio-venous shunt lesions with rupture risks, not venous or capillary malformations. High detection rate of small CAVMs in HHT patients in silent-MRA suggests its usefulness as cerebral screening method in HHT patients.

Hypermagnesemia in HHT patients with brain T1 MRI signal abnormalities

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Objectives: Brain MRI T1 abnormalities may be common in HHT. This signal may be due to manganese deposition. It is unknown if manganese levels are abnormal in patients with HHT and if this correlates with brain MRI imaging abnormalities. Brain manganese has neuropsychological consequences in other diseases. We hypothesized HHT patients with abnormal brain MRI T1 signal would have elevated blood manganese levels, and that this may be related to iron deficiency.
Methods: We reviewed brain MRI reports of 50 definite (by Curçao criteria) HHT patients seen at University of Colorado Anschutz Medical Campus from December 2015 to January 2017. Eight patients had T1 hyperintensity. Of those eight, four had serum manganese levels drawn. One patient with iron deficiency had a repeat serum manganese level drawn after intravenous iron treatment.
Results: All four HHT patients with abnormal brain T1 MRIs had elevated serum manganese levels (31.9, 36.3, 19.3 and 33.8 μg/L; normal = 4.2–16.5 μg/L). One subject treated with two 750 mg doses

of Injectafer had a decreased manganese level following this treatment (pre-level = 36.3; post level = 24.7).
Conclusion: Based on this small series, there may be a correlation between T1 brain abnormalities and high serum manganese levels in HHT. Further study is necessary to determine if manganese levels are elevated in HHT patients without T1 signal abnormalities, or if elevated serum levels and brain deposition are due to iron deficiency and aberrant electrolyte absorption.

Cerebrovascular manifestations of hereditary hemorrhagic telangiectasia: A look beyond AVMs

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Objectives: Hereditary hemorrhagic telangiectasia (HHT) patients have an increased risk for cerebral arterio-venous malformations (AVMs). However, it is currently unknown what the association of HHT is with other cerebrovascular abnormalities, particularly with imaging manifestations of cerebral small vessel diseases (CSVD), such as microbleeds and white matter hyperintensities (WMH). Furthermore, it has been shown that many manifestations of CSVD are preceded by abnormalities in cerebrovascular reserve, as measured by the so-called cerebrovascular reactivity (CVR). The objectives of this study were: (1) to evaluate the occurrence and distribution of CSVD in HHT patients as compared to healthy age matched controls and (2) to evaluate the cerebrovascular reserve in HHT patients using CVR measurements with MRI.
Methods: For this study 17 adult patients (mean age 47, range 19–68) with proven endoglin gene mutation (HHT1 patients) and 13 healthy age matched subjects were included. After providing written informed consent, the subjects were scanned on a 3 Tesla MRI scanner using a protocol for the assessment of cerebrovascular reserve and manifestations of CSVD. The results were evaluated using appropriate statistical tests.
Results: The prevalence of CSVD manifestations was very low in our study population. WMH was most prevalent and was higher in the HHT1 group compared to the healthy controls, 8 subjects in the HHT 1 group vs. 3 subjects in the healthy control group, respectively; however, this was not statistically significant. The number of patients with (silent) infarcts was low in both groups, 3 in the patient group vs. 1 in the control group. There were no microbleeds present in either group.
The preliminary analysis indicated differences in whole brain CVR reactivity in HHT1 patients compared to healthy controls. Furthermore, some subjects who did not have macroscopic cerebral AVMs on the MR angiography, showed focal increases in cerebral blood flow (CBF) on MR perfusion, possibly in agreement with recent reports of capillary vascular malformations in HHT patients.
Conclusion: Although we found no increased association between HHT type 1 and cerebral small vessel disease markers, patients with HHT1 showed impaired cerebrovascular reactivity, possibly pointing to a poorer regulating capacity of the cerebral vasculature. In future work, we will study these differences in detail and will also look on the association of these changes with pulmonary AVMs.

Cerebrospinal AVFS treated by glue in HHT patients

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Aim: Cerebrospinal AVMs in HHT with 4 patients are analyzed respectively. All of them diagnosed by Crasao criteria or genetic analysis as HHT.
Methods: 456 pial brain AVMs, 126 spinal AVFs, 317 dural AVFs, 86 choroidal AVFs are analyzed retrospectively in our clinical database. 4 patients diagnosed as HHT. Case1: 3 Y.O. boy presented huge perimedullary Type3 spinal SAVF, multiple cerebral micro-AVM and capillary vascular malformation, and pulmonary AVFs. Endoglin type1. Case2: neonate boy, multiple high flow cortical fistula presented. HHT suspected by Crasao Criteria. Case3: neonate boy, multiple choroidal fistulas draining into the dilated median vein of prosencephalon. Endoglin HHT type1. Case4: neonatal girl. Mesencephalic AVM presented hemorrhage at neonate. ALK1. HHT type2.
Results: 2 patients classified as HHT type1 (Endoglin), and 1 patient as type2 (ALK1). 1 patient diagnosed as HHT with suspected crasao criteria.4 different distinct phenotypes of angioarchtecture were described independently: (1) high-flow "single-hole" pial fistulas in 1 infant, (2) Nidus type brain AVMs in 1 infant and 1 neonate. (3) "micro-AVM" "capillary vascular malformations," defined as small lesions without clear evidence of a shunt in 1 children. (4) Multiple choroidal shunts droning to the dilated median vein of prosencephalon in 1 neonate.
Conclusion: All of our cerebrospinal AVFs with HHT could be treated by NBCA glue embolization. Although natural history of capillary malformation is still unknown, management of these lesion should be careful, and demanded meticulous endovascular technique. There are no dural AVFs associated with HHT.

Clinical presentation and treatment paradigms in patients with hereditary hemorrhagic telangiectasia and spinal vascular malformations

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Background: Hereditary hemorrhagic telangiectasia (HHT) is a rare autosomal dominant disorder resulting in angiodysplasia including mucocutaneous telangiectasias (responsible for epistaxis) and arteriovenous malformations (AVMs) of organs. Although central nervous system vascular malformation can occur in the entire neuroaxis, spinal vascular malformations are rare. In those harboring spinal vascular malformations, the most common presentation is myelopathy due to mass effect or hemorrhage. Herein we present our center's experience with management of spinal vascular malformations in patients with HHT.
Methods: We retrospectively reviewed our prospectively collected and maintained database of patients with HHT treated at the University of Utah HHT center and identified those patients who harbor a spinal vascular malformation. The clinical, radiographic and genetic information of this cohort was reviewed and supplemented with review of all reported cases of spinal vascular malformation in patients with HHT form the literature.
Results: The majority of patients with spinal vascular malformations presented with radiculopathy (3/4; 75%). One patient presented after subarachnoid hemorrhage and was noted to have a spinal vascular malformation. The mean age of presentation was 21.5 years (range 1 month–77 years). Two patients harbored cervical lesions, while one had a thoracic and another patient a lumbar lesion. Three of patients underwent treatment (surgery in 2 cases; surgery and embolization in 1 case) while a fourth patient was treated conservatively. Our review

of the literature demonstrated 29 total perimedullary spinal AVFs in 28 HHT patients (71% male; 29% female). The lesions were located in the cervical spine in 5/29 (17.2%), thoracic in 12/29 (41.4%), lumbar 4/29 (13.7%), thoraco-lumbar in 7/29 (24.1%) and conus medullaris (1/29; 3.4%). Three (10%) lesions were not treated, 17/29 (58.6%) underwent embolization, 6/29 (20.6%) underwent surgical resection, and 3/29 (10%) were treated with a combination of embolization and surgery. In 14 cases, the patient presented with hemorrhage of the AVF; 15 AVFs presented without hemorrhage. Overall, 76% of patients achieved complete or near-complete occlusion, with most recovering neurological function.
Conclusion: Spinal AVMs in patients with HHT are commonly perimedullary AVFs. Discovery of spinal lesions often occurs secondary to neurological deficits. Most patients benefit from intervention, including endovascular embolization followed by surgery. These patients generally achieve a favorable outcome. We favor screening of the neuroaxis in patients with diagnosis of HHT to identify lesions prior to catastrophic hemorrhage or progressive myelopathy.

Preliminary results of systematic screening of brain and spinal cord vascular malformations in HHT infants

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Objectives: To screen brain and spinal cord arteriovenous malformations in HHT neonates, in order to prevent further hemorragic event.
Methods: This prospective study was conducted since 2007. All HHT adult patients visiting the multidisciplinary outpatient clinic were informed of the program of screening brain and spinal cord arteriovenous malformations in their child after birth. During pregnancy, a clinical genetic consultation for the couple was provided. After birth, blood sample from the cord was tested for endogline and Alk1 in 50 neonates. In case of positive results (n = 25), MRI (Brain: axial SET2, SET1, T2*, diffusion, coronal SET2, and TOF MRA if needed; Spine: sag SET1 and SET2) was performed (n = 16), as well as neuropediatrical examination. If necessary (n = 1) digital substracted angiography (DSA) was performed.
Results: Four patients presented haemorrhagic and/or thrombotic abnormalities: One patient had a cerebral arteriovenous malformation (CAVM) defined on DSA as a small capillary telangiectasia. It was depicted on MRI with combined focal T2* hyposignal as a previous thrombohemorragic event, very mild vascular ectasia on morphological sequences (SET2 and SET1), and arteriovenous shunt present on TOF sequence. Three other patients had T2* punctate hyposignal intensities, without vascular ectasia nor TOF hypersignal. Two of these five patients presented cortical migration abnormalities.
All of the patients with T2* hyposignal or cortical abnormalities had an endogline genotype. No spinal cord arteriovenous malformation was depicted on this cohort. No neurological symptoms related to the arteriovenous shunt could be depicted, but later on, language acquisition delay was observed in the one presenting cortical abnormalities and CAVM.
Conclusion: This small cohort of brain and spinal MRI performed in HHT children could depict one arteriovenous shunt assessed on DSA with previous thrombohemorragic event on T2*. Other T2* abnormalities and cortical migration abnormalities were depicted, all in endogline phenotype.

Evaluation of cerebral arteriovenous malformation in HHT: magnetic resonance imaging vs. digital subtraction cerebral angiography

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Objectives: Brain vascular malformations (BVM) are prevalent in individuals with HHT. Recently BVMs were classified into two distinct phenotypes: AVMs, when arteriovenous shunting is present on digital subtraction angiography (DSA), and capillary malformations (CM), when absent. This distinction is important since CM is considered low risk for intracranial hemorrhage. The purpose of this study was to compare MRI to DSA in identification and characterization of BVMs.
Methods: Brain MRIs and DSAs obtained in 2008–2015 in 25 patients with HHT were retrospectively reviewed by diagnostic and interventional neuroradiologists, respectively. The number of BVMs was recorded and classified as AVM or CM. Concordance between the two imaging methods was assessed using Cohen's kappa test.
Results: MRI identified 41 BVM lesions in 17 patients. DSA confirmed 27/41 lesions (66%) and identified 2 additional lesions not observed on MRI. Arteriovenous shunting could be identified on DSA in 27/29 (93%) confirmed BVMs, mostly microAVMs. Of the 27 lesions observed on both modalities, classification as AVM or CM showed 74% concordance between MRI and DSA (Kappa = 0.266, 95% CI − 0.139, 0.671). 14 MRI-detected lesions were occult on DSA.
Conclusion: MRI identified more BVM lesions than DSA, including angiographically occult lesions that likely represent telangiectasias. The vast majority of BVM lesions in HHT represented microAVMs rather than CMs in this cohort, as arteriovenous shunting could be identified on DSA. In classifying CMs vs. AVMs, MRI and DSA had only modest strength of agreement. Therefore, both imaging modalities are essential in the characterization of BVM lesions to allow accurate prediction of hemorrhagic risk.

Identification of subclinical pathology by cerebral MRI

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Objectives: Where patients with pulmonary arteriovenous malformations (PAVMs) have experienced a clinical ischemic stroke, conventional management, as in the general population, includes antiplatelet agents that may aggravate HHT blood losses. As a result, although recognised to be at risk of silent ischemia (Moussatas et al. 2000), there has not been a blanket recommendation to treat all PAVM patients with such agents. The goal of this study was to evaluate evidence for ischemia in reports of cerebral MRI scans performed in patients with known or suspected hereditary haemorrhagic telangiectasia (HHT) for the purpose of cerebral MRI screening, and catagorise by the presence or absence of a PAVM diagnosis.
Methods: MRI scan reports were downloaded into an Excel chart and abnormalities catagorised, blinded to patient demographics such as age, gender, presence of HHT and presence of PAVMs. Data were analysed using STATA IC v13 (Statacorp, Texas).
Results: Between 10/07/2001 and 02/12/2016, 45 individuals (21 males; 24 females) with known or suspected HHT underwent a cerebral MRI. Ages ranged from 17 to 74 (median 42.9) years. Twenty-five (56%) were known to have PAVMs, and 37 had definite HHT. 25 (56%) of scans were reported as normal. Only three patients

(6.7%) had findings compatible with cerebral telangiectasia, one had a cerebral aneurysm, and no cerebral AVMs were identified. However 13 patients (28.9%) had one or more cortical infarcts, and a further five (11.1%) had periventricular and/or deep white matter small vessel ischemic changes. Ischemic changes were reported in 13/24 (54.2%) of patients with PAVMs, and 4/21 (16.7%) of patients without PAVMs (Chi squared $p = 0.015$). For patients with PAVMs, the age and gender-adjusting odds ratio for any ischemic change was 80.1 (95% confidence intervals 2.0, 3145, $p = 0.019$), and for large vessel infarcts, 8.1 (95% CI 1.2, 54.5, $p = 0.032$).
Conclusion: The findings identify a high rate of silent cerebral ischemic changes in patients with PAVMs, and raise the question whether all patients with persistent PAVMs after treatment should have pharmacological stroke prevention therapy, in the absence of a clinical stroke.

Capillary vascular malformations with previous hemorrhage in hereditary hemorrhagic telangiectasia: case report

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Objective: To report capillary vascular malformations (CVMs) with previous hemorrhage in a hereditary hemorrhagic telangiectasia (HHT) type 1 patient.
Case presentation: A 43-year-old woman had recurrent epistaxis since her elementary school days. She was a member of a HHT type 1 family. She presented with syncope at the age of 19 years old, which led the diagnosis of asymptomatic multiple cerebral vascular malformations and multiple pulmonary arteriovenous fistulas. Pulmonary lesions were partially treated with embolization and she was followed-up until the age of 29 elsewhere. She came to us 14 years later at the age of 43 without any neurological or respiratory symptoms. Contrast-enhanced brain MR imaging revealed 9 cerebral vascular malformations in total. All these lesions had contrast enhancement without dilated vessels. T2*-weighted imaging disclosed 2 of them being with an evidence of previous hemorrhage. Since it was difficult to diagnose them either small arteriovenous malformations (AVMs) with nidi or CVMs solely by MR imaging, catheter cerebral angiography was performed. It showed 3 small AVMs and 3 typical CVMs. The remaining 3 lesions were not demonstrated even by high-resolution flat-panel angiography due to their smallness. Two lesions with previous hemorrhage on T2*-weighted imaging were confirmed to be CVMs.
Conclusion: We conclude that CVMs may have a potential to bleed although most of them are asymptomatic.

CLINICAL MANIFESTATIONS OF HHT

Hereditary haemorrhagic telangiectasia (HHT) is more than a bleeding nose, results from the Danish HHT database

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Background: The Danish HHT database was established in 1995 including all HHT patients in the County of Fyn. Odense University Hospital has been acknowledged as HHT treating center in Denmark. Patients from all parts of Denmark are referred for evaluation.
Purpose: Presenting 20 years research results from the Danish HHT database.
Materials and methods: The Danish HHT-centre was established at Odense University Hospital on the basis of several years with ongoing epidemiological studies in the County of Fyn. Since 1.1.1995 all patients seen at the Danish HHT center have been clinically evaluated concerning manifestations of HHT. All participants have been offered genetic testing. All patients are offered screening for Pulmonary AVM. Screening for CAVM and HAVM are only offered on demand.
Results: The most common mutations are found in Endoglin and ACVRL1, causing alterations in the TGF-β pathway. Identification of a disease causing mutation is possible in 85% of cases. Around 90% of patients experience epistaxis, very variable in severity but increasing with age. We will present results regarding PAVM and mutation diagnostics and epistaxis. We will focus on how referral bias may cause altered prevalence of severity amongst patients.
Conclusion: HHT includes different clinical manifestations all due to development of arteriovenous malformations. Patients with HHT should be provided with information on how to handle their systemic disease in different phases of life. Screening for silent clinical manifestations as PAVM, may prevent serious morbidity. Luckily many HHT patients live near normal lives the majority of their lifespan.

Venous thromboembolism in patients with hemorrhagic hereditary telangiectasia

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Aim: We used the RIETE registry to assess the clinical characteristics and outcomes of patients with venous thromboembolism (VTE) and Hemorrhagic Hereditary Telangiectasia (HHT).
Methods: RIETE is an ongoing, observational registry of consecutive patients with symptomatic, objectively confirmed, acute deep vein thrombosis (DVT) or pulmonary embolism (PE).
Results: As of January 2017, 20 patients with HHT were included (55% male), mean age 62 ± 18 years. Eight patients presented as DVT and 12 as PE. Mean hemoglobin levels were 11.9 ± 2.8 g/dL, platelet count 280 ± 103.2 × 1000/mm^3. Two patients developed VTE after surgery, 5 had recent immobility, one recent travel and 2 had history of VTE. Sixteen patients were initially treated with low-molecular-weight heparin (LMWH), 3 with unfractionated heparin (UFH) and one with rivaroxaban. Of 16 patients on LMWH, 8 received lower than recommended doses. Then, 10 patients received long-term LMWH (8 at low doses), 8 vitamin K antagonists (VKA)

and one, rivaroxaban. Three patients underwent a vena cava filter. Mean duration of therapy was 8.2 ± 7.5 months. After a mean follow-up of 12 ± 13.4 months, one patient died of PE after receiving UFH for 4 days, two developed VTE recurrences (1 during VKA treatment and 1 after long-term LMWH withdrawal), 8 bled (2 were on low-dose LMWH, 3 on therapeutic LMWH). In all, 2 bleeds were major, 4 were epistaxis and 3 rebled (2 epistaxis). Six bleeds occured during the first 2 months of anticoagulation.

Conclusion: During anticoagulation for VTE, both bleeding and recurrences are common. Accurate identification of at-risk patients is urgently needed.

Ischemic stroke or brain abscess in patients with hemorrhagic hereditary telangiectasia

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Aim: To assess the clinical characteristics and outcomes of patients with Hemorrhagic Hereditary Telangiectasia (HHT) and ischaemic stroke or brain abscess.

Method: We included all HHT patients with history of ischaemic stroke or brain abscess attended at a HHT unit of a university hospital, from September 2011 to January 2017. All patients have been diagnosed by Curaçao criteria or positive genetic study. Right-left shunt (RLS) at transthoracic contrast echocardiography (TTCE), graded in four grades according to the system proposed by Barzilai, was used as screening method for pulmonary arteriovenous malformations (PAVM).

Results: Of 230 patients followed in our HHT Unit, 14 (6%) had ischaemic stroke (9 patients) or cerebral abscess (5 patients). Mean age was 55 (28–78) years. HHT was considered "Definite" according to Curaçao Criteria in 13 patients. Mean Epistaxis Severity Score was 3.45 and mean haemoglobin levels were 11.7 g/dL. All patients but one (80%) with brain abscess and 50% with ischaemic stroke, were females. All neurological events occurred prior to our HHT Unit referral, 8 patients with undiagnosed HHT. Oxygen saturation levels were ≤ 95% in 5 patients. RLS occurred in 11 patients, 9 with grades ≥ 2 at TTCE. All patients with a positive TTCE, received antibiotic prophylactic education before high-risk surgery or dental manipulations. PAVM were diagnosed in 10 patients, 9 of them underwent PAVM percutaneous embolization. After a follow-up period of 22.5 (2–51) months no patient presented new neurological event and the modified Rankin Scale was grade 2 and 3 in two patients and 0 in the remaining patients.

Conclusion: Ischemic strokes and brain abscesses occur commonly in undiagnosed HHT patients with PAVMs. Risk reduction could be improved referring these patients to a HHT Unit for early diagnosis and treatment of PAVMs in order to prevent neurological complications. Antibiotic prophylaxis for patients with positive TTCE is mandatory.

Liver involvement associated to hereditary haemmorrhagic telangiectasia in pediatric age

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Aim: Liver involvement in Hereditary Haemorrhagic Telangiectasia (HHT) is usually detected in adult patients. The present study aimed to systematically estimate the HHT-associated hepatic angiodynamic profile in pediatric age.

Methods: The study was designed as a cross-sectional survey. Inclusion criteria: age 4–18 years, carrier of an HHT-causing familial mutation, enrolled in the full clinical-instrumental protocol. Patients were subclassified as HHT1 and HHT2 according to the mutated gene. Hepatic screening was performed by Echo-Color Doppler examination in all patients, and by a second methodology (Multi-Slice Computed Tomography, MSCT, or Magnetic Resonance Angiography, MRA) in > 12-years-old patients. Both quantitative and qualitative ultrasonographic parameters were employed.

Results: In > 12-years-old patients, MSCT/MRA examination disclosed silent hepatic involvement in 35.0% of cases, and nodular regenerative hyperplasia in two cases. Diameter of common hepatic artery was significantly larger in HHT2 (0.45 ± 0.15 cm) compared to HHT1 (0.33 ± 0.09, $p < 0.01$) and control children (0.32 ± 0.08, $p < 0.05$), whereas no statistical difference was found for other extrahepatic parameters. None of the patients had clinical manifestations of liver involvement. Moreover, comparison of angiodynamic profiles evidenced a clear worsening of ultrasound parameters in adults vs pediatric HHT patients, likely associated to an ongoing subclinical hyperkinetic state.

Conclusion: Liver involvement are detected in pediatric HHT patients with a lower frequency compared to adults, thus suggesting that hepatic arterio-venous shunts may arise with increasing age. HHT2 children show a higher HAVM frequency and a trend to hepatic artery dilation when compared to HHT1 children, whereby anticipating the gene-associated difference, already observed in adult HHT patients.

Hepatic involvement and stiffness evaluation by elastography

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Aim: Hepatic involvement in HHT can be associated to hyperplastic micronodular regeneration and deposition of fibrous tissue, often not associated to clinically significant portal hypertension or cirrhosis. Elastonography has recently been proposed as a non-invasive effective tool to estimate liver stiffness, as a parameter for liver fibrosis. The present study aims to investigate subclinical hepatic stiffness alterations in HHT patients with liver involvement.

Methods: The study was designed as a prospective cohort study of patients with either genetically or clinically confirmed HHT diagnosis. Patients were consecutively recruited, among patients who were

subjected to full clinical-instrumental evaluation, independent of clinical symptoms. Hepatic screening was performed by Echo-Color Doppler examination and Multi-Slice Computed Tomography. Stiffness evaluation was carried out by wave elastography (echosens Fibroscan), with semiquantitative scale grading currently employed for HCV-related hepatitis.
Results: A total of 40 patients were included in the study (mean age 53.4 ± 6.39 years). Hepatic arterio-venous malformations were detected by MSCT in 31/40 (77.5%) patients. Elastography-based stiffness alterations were detected in 19/40 patients (47.5%), seven of which showed HHT-related or –unrelated concomitant hepatic disease. For the remaining 12 patients with elevated stiffness, no overt liver disease or other fibrosis signs were evident. We found a statistically significant stiffness increase in liver-disease vs. non-liver-disease patients (10.03 ± 5.94 and 5.30 ± 2.03, respectively, $p < 0.006$). Stiffness was positively correlated with hepatic artery diameter and maximum velocity.
Conclusion: Liver stiffness in HHT may be altered in certain patients, due to diffuse hypervascularization and/or subclinical fibrotic deposition.

Severe pulmonary involvment of SMAD4-mutated patients with juvenile polyposis/hereditary hemorrhagic telangiectasia combined syndrome

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Aim: To describe clinical pulmonary features of patients affected by Juvenile polyposis/hereditary hemorrhagic telangiectasia (JP/HHT) syndrome and confirmed mutations in *SMAD4* gene and to compare lung arterio-venous malformation (AVM) radiological features with HHT1 and HHT2 patients.
Methods: The study was designed as a cross-sectional prospective survey. Inclusion criteria: carrier of a *SMAD4* mutation, completed clinical-instrumental screening. Phenotypic data of enrolled patients were then compared with HHT1 and HHT2 patients' data. Clinical-instrumental protocol included screening for pulmonary, hepatic and cerebral AVMs for all patients, independent from clinical symptoms, and upper and lower endoscopy for JP/HHT patients only.
Results: A total of 5 SMAD4-mutated patients were recruited in the study. All of the 5 patients had pulmonary AVMs (PAVMs) and GI polyps. Silent hepatic involvement was disclosed in 4/5 patients and brain AVMs in 0/5 patients. Clinical overt manifestations secondary to PAVMs was reported by 4/5 patients, including hypoxaemia, digital clubbing, brain abscess/stroke. Anatomical characteristics of HHT/JP, HHT1 and HHT2 PAVM-positive patients showed a trend towards more frequent bilateral involvement (16/22, 72%, 7/17 (41%), and 4/5 (80.0%), respectively, $p = 0.09$). HHT/JP patients had significantly higher prevalence of complex PAVMs, when compared to HHT1 and HHT2 patients (3/5, 60.0%, 2/22, 9%, and 0/17, 0%, respectively, $p < 0.01$), and of large (feeding artery > 3 mm) PAVMs (4/5, 80%, 12/22, 54.5%, 3/17, 17.6%, respectively, $p < 0.02$). Aggressive GI polyp phenotype was observed as soon as in adolescence.
Conclusion: HHT/JP patients show a severe phenotype, mainly involving pulmonary AVMs and GI polyps, which require an appropriate instrumental survey.

Hereditary haemorrhagic telangiectasia and oral anticoagulation: efficacy of percutaneous left atrial appendage occlusion in patients with non-valvular atrial fibrillation

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Objective: Severity of epistaxis in Hereditary Hemorrhagic Telangiectasia (HHT) varies widely, from mild, self-limited nosebleeds to severe, life-threatening nasal hemorrhage. In Patients with severe grade of epistaxis, gastrointestinal bleeding and life-threatening bleeding from visceral arteriovenous malformations, oral anticoagulation (OAC) therapy may often result unsustainable. However, some of these patients are affected by atrial fibrillation (AF). In this particular high stroke risk setting, percutaneous closure of left atrial appendage (LAA) may represent a viable and efficacious alternative strategy to long-term (OAC).
Methods: Between 2009 and 2016, five consecutive patients with non-valvular AF-related high thromboembolic risk, CHA2DS2-VASc ≥ 2, and severe epistaxis, underwent percutaneous closure of LAA with Amplatzer cardiac plug device. The procedure was performed percutaneously using a trans-septal approach under transesophageal echocardiography and fluoroscopy guidance. Procedure- and device-related major adverse events (MAEs) were defined as the composite of death, stroke, major or life-threatening bleeding, serious pericardial effusion, device embolization, major access-site vascular complication, or need for cardiovascular surgery within 30 days following the intervention.
Results: Early procedural success was complete and no MAEs occurred in all patients. At 12 months follow-up no thromboembolic event occurred in HHT treated patients. Follow-up transesophageal echocardiography showed complete LAA sealing in all patients with no residual leaks and no device embolization.
Conclusion: Percutaneous closure of LAA may represent a safe and efficacious alternative strategy to long-term OAC therapy in HHT patients with AF-induced high stroke risk and poor tolerance for OAC.

Physiological hormonal changes and severity of Osler-Weber-Rendu syndrome-results from an online questionnaire

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Objectives: A better understanding of the role of hormonal changes in women on the severity of HHT may help in improving its treatment. The aim of this study is to assess the influence of changing hormonal status on disease severity in women across age groups compared to men.
Methods: An online questionnaire was distributed through the Osler self-help group. We extracted questions relating to epistaxis severity score (ESS), changes in telangiectases and epistaxis severity during

periods of physiological hormonal changes. Statistical analyses were done using Stata 14.0.
Results: 389 respondents answered the questionnaire. Out of the 173 women respondents, 38% described an increase and 42% a decrease in the severity of epistaxis after puberty. During the postpartum period and while breastfeeding, 31% experienced an increase and 40% a decrease in the epistaxis severity. 58% of the women reported a worsening and 13% an improvement of epistaxis after menopause. 68% of the women reported an increase in the telangiectases during menstruation. Women reported a lower mean ESS than men (women = 6.6; men = 7.5; p value = 0.02). Two-sided linear regression analyses showed a statistically signifcant increase in mean ESS with increasing age in women (b = 0.04, CI 0.003, 0.078, p value = 0.036). Men did not show a significant increase in ESS (b = 0.02, CI − 0.02, 0.054, p value = 0.370).
Conclusion: Women had a lower ESS than men but showed a significant progression in the epistaxis severity with increasing age in our study group. This may be explained by the protective effect of estrogen in younger women.

Nailfold capillaroscopy in hereditary hemorrhagic telangiectasia

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Objectives: To describe nailfold capillaroscopy results in a cohort of Spanish patients with hereditary hemorrhagic telangiectasia (HHT)/ Rendu Osler Weber disease and to evaluate potential associations with organ involvement.
Methods: Between 1 January 2002 and 31 December 2013, 195 HHT patients diagnosed either clinical or genetically, underwent a nailfold capillaroscopy performed with a digital microscope with100x magnification connected with a computer image processor.
Results: 195 patients were evaluated and pathological findings were observed in 53.85% of cases. Patients with HHT type 1 and those over 50 years old showed a significant higher incidence of anomalies in capillaroscopy while no significant differences were observed considering gender. The association between internal organ affectation and a pathological capillaroscopy result was only significant in pulmonary arteriovenous malformations (MAVp). This was true both with computed tomography (CT) as well as contrast echocardiography. The sensitivity of the capillaroscopy in detecting MAVp was 85.41%.
Conclusion: The findings of a pathological capillaroscopy (especially in the form of mega-capillaries) is common in patients with HHT (especially HHT1) and in patients with an advanced age. The association between pulmonary arteriovenous malformations, which are seen by both CT and contrast echocardiography, and a pathological capillaroscopy result is significant. This observation can help to guide the prioritization of lung screening.

EPISTAXIS AND GASTROINTESTINAL BLEEDING

Treatment algorithm for HHT associated epistaxis at OSLO University Hospital in Norway

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Objective: To describe the algorithm for treating HHT associated epistaxis at Oslo University hospital in Norway.
Material and method: The ENT department at Oslo University hospital in Norway is the main treating center for HHT associated epistaxis in Norway. Almost all patients with HHT associated are epistaxis referred to this center. In average 125 diode laser, 40 pulsed dye laser, 5 septodermoplasy operations and 44 intranasal injection of bevacizumab are performed each year.
Results: The treatment algorithm, we depend, based on the start with laser therapy. Argon-plasma cautery will be the next step if the patient does not respond to the laser therapy. The following step is septodermoplasy. Intranasal bevacizumab injection is performed only if the patient refused septodermoplasy, there is a contraindication for septodermoplasy, or there was no good effect of the septodermoplasy. There was so far no need for nasal closure (Modified Young's procedure) yet.

TACRO: Efficiency and safety of a 0.1% tacrolimus nasal ointment as a treatment for epistaxis in hereditary hemorrhagic telangiectasia (HHT). A double blind, randomized, placebo controlled, multicenter trial

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Background: Improvement in epistaxis has been shown in HHT patients after a liver transplantation. It was hypothesized that the immunosuppressive treatment (FK506) used to prevent rejection may have an anti-angiogenic effect. Furthermore, Albiñana et al. found that the immunosuppressor FK506 increases the protein and mRNA expression of ENG and ALK1 in cultured endothelial cells, and enhances the TGF- β1/ALK1 signaling pathway and endothelial cell functions such as tubulogenesis and migration. These results suggest that FK506 may be an interesting drug for use in patients with HHT.
Objectives: The main objective of this trial is to evaluate efficacy of a tacrolimus (FK506) nasal ointment treatment, administered for 10 weeks twice daily, on the duration of nosebleeds. Secondary objectives are to evaluate the tolerance and the efficacy of the treatment on progress in the clinical and biological parameters.
Method: It will be a phase II multicenter, randomized study carried out in double blind. 48 patients will be included with an active/placebo ratio = 1:1. Patients will be monitored for 140 days: 10 weeks' treatment and 10 weeks' follow-up. The treatment tested is Protopic® (tacrolimus at 0.1%). The placebo is the same formulation without tacrolimus.
Results and conclusion: Inclusions of patients will start in 2017 as soon as regulatory approvals are received. The study will last 2 years and we could conclude on efficacy of tacrolimus nasal ointment on epistaxis at the end of 2019. The study has been granted by the Hospices Civils de Lyon.

Necrosis of the upper lip, lateral nasal cartilage and forehead skin after embolization of a HHT patient

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Aim/Method: Case report for clinical poster. 70 years old female with a recurrent epistaxis caused by HHT refereed to Uppsala University hospital for embolization Maj 2016. The patient was treated twice by embolization, first time 2004 and the second time 2007 with a good result. The patient embolized for 3rd time in June 2016.
Results: After only 24 h she develops a discolouration of the skin of the forehead and the cheek on the left side with paraesthesia and a reduction in the sensation and palpitation with dyspnoea. Acute CT of the brain and the chest shows no sign of CVL or intracerebral haemorrhage and no signs of pulmonary embolism. The patient received Aspirin 75 mg once a day and cortison100 mg once iv. But because of signs of heart failure and arterial fibrillation the patient moved to cardiology department and then discharged to local hospital. One week after embolization the patient showed first sign of skin necrosis in the left forehead, left side of the lateral nasal cartilage and the left side of the upper lip.
Conclusion: After consultation of plastic surgeon how decided for reconstruction of the necrotic skin, a series of plastic surgeries end with a reasonable cosmetic and functional results. In this case report can we show a series of pictures taken from day one after embolization until today.

Causes and severity of anemia in hereditary hemorrhagic telangiectasia

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Objective: To determine anemia severity at initial evaluation for HHT, and to identify predictors of anemia source and severity.
Methods: We conducted a multi-center, retrospective study of patients seen for an initial visit at the HHT centers at UNC and UAMS. Data collected included documented anemia on CBC, gender, genotype, and presence of visceral organ arteriovenous malformations (AVMs). Anemia severity was defined as mild if hemoglobin ≥ 10, moderate if 8–10, and severe if ≤ 8 g/dl.
Results: In total, 116 patients had documented anemia, with the majority being female (58.6%), and possessing an ALK1 mutation (42.4%). 57.8% of patients had mild anemia, 33.6% moderate, and 8.6% severe. Epistaxis was the most common source in patients with mild and moderate anemia. In patients with severe anemia, both GI bleeding and epistaxis was the most common source (50%), with an additional 20% having GI bleeding alone as an anemia source. Overall genotype ($p = 0.0003$) and presence of visceral organ AVM ($p = 0.0007$) was linked to anemia source. Neither gender, genotype or presence of AVM was significantly linked to anemia severity.
Conclusion: Iron deficiency anemia can be associated with significant morbidity. We found that in patients with severe anemia, GI bleeding was a more frequent source than those with mild or moderate anemia. Genotype and the presence of visceral organ AVMs was linked to anemia source. Further analysis is needed to understand disease related predictors of anemia, thereby identifying patients at highest risk in order to facilitate aggressive screening within this population.

Education intervention regarding nasal hygiene and epistaxis management: patient perspective

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Rationale: Epistaxis is common in Hereditary Hemorrhagic Telangiectasia (HHT) and affects quality of life. There is consensus regarding need for nasal hygiene, including lubrication for management. Also, HHT patients often consult for management of epistaxis. Yet, there are no reported protocols for educating HHT patients about nasal care and epistaxis management. We identified this as a clinical care gap.
Methods: We designed an epistaxis education intervention for HHT patients, to address the gap. Education was provided by an HHT nurse, including verbal and written information about a nasal hygiene routine (saline spray and lubrication) and education about epistaxis self-management (nasal pressure, avoidance of self-packing with tissue, etc.). Fourteen patients received education, including 3 who were educated on self- packing with dissolvable SURGIFOAM^{TM}. Patients were assessed with Epistaxis severity score (ESS) pre and post-education and a structured interview post-education.
Results: Pre-education, ESS category was mild in 3/14 (21%), moderate in 11/14 (79%). Nine/14 (64%) reported self-packing with tissue. Thirteen (97%) were contacted post-education (3–8 months post); ESS category improved in 3/13 (23%) and unchanged in 10/13 (77%). Three/13 (23%) continued recommended hygiene, 9/13 (69%) an adapted version and 2 did not. Eight/13 (62%) reported no tissue self- packing, 5/13 (38%) continued this for "convenience". Ten/13 (77%) reported improved perception of self-management, feeling less "stressed/panicked" during epistaxis; 3 (23%) reported no change.
Conclusion: Epistaxis education in HHT patients leads to changes in patient behavior and improvements in patient perception of disease management. Education protocols should be further tested and studied in this population.

Hereditary haemorrhagic telangiectasia: the otolaryngologist's role. Case presentation of a novel technique for nostril closure surgery

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Objectives: Nine out of ten patients with HHT present to the otolaryngologist with varying degrees of epistaxis. The otolaryngologist plays a pivotal role in initial diagnosis of HHT, the management of epistaxis, identifying associated malformation and referral to appropriate specialities. This is particularly the case in resource- limited settings where there are no HHT centres.
Methods: Surgical treatment options are considered for persistent or severe cases. A stepwise approach is advocated and typically entails several attempts of cautery, followed by septodermoplasty. Closure of nostrils, known as Young's procedure, is reserved for cases with severe epistaxis with symptomatic anaemia refractory to surgical and medical treatment options.
Results: We present a patient with HHT, who presented with severe iron deficiency anaemia after multiple nasal cautery procedures and septodermoplasty procedure. A modified Young's was performed. He developed a slight wound dehiscence, a common and often accepted complication, with persistent severe epistaxis. A revision procedure failed to close the dehiscence. A novel technique using an underlay cartilage graft was used to close the dehiscence.

Conclusion: This case highlighted the important role of the otolaryngologist in managing a patient with HHT (especially in a limited resource setting) and describes a novel technique to obtain full nostril closure.

Role of the combined approach capsule endoscopy and device assisted enteroscopy for diagnosis and treatment of small bowel lesions in patients with hereditary hemorrhagic telangiectasia: results of single-centre prospective study

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Objectives: Hereditary hemorrhagic telangiectasia (HHT) is an autosomal-dominant vascular disorder leading to telangiectases and arteriovenous malformations of the skin, mucosa, and viscera. Telangiectases in the upper gastrointestinal tract are known, but data regarding possible small-bowel involvement are poor due to the technical difficulty of exploring the entire gastrointestinal tract. The aim of the present study was to use capsule endoscopy (CE) to determine the prevalence of small-bowel telangiectases in HHT patients and device assisted enteroscopy (DAE) to treat.
Methods: From December 2004 to September 2015, 24 consecutive adult HHT patients (13 men, 11 women; mean age 62.5, range 23–80) who were undergoing regular follow-up at the outpatient clinic underwent gastrointestinal exploration to verify possible locations of HHT. Esophagogastroduodenoscopy (EGD), Colonoscopy and CE were carried out regardless of the presence of clinical or biochemical signs of active bleeding or to identify a possible source of bleeding.
Results: Upper EGD revealed telangiectases in 10/24 patients (41.6%). Pancolonoscopy revealed telangiectases in 3/24 patients (12.5%). CE revealed small-bowel telangiectases in all patients. All patients underwent operative DAE to treat vascular lesions.
Conclusion: CE is indicated to confirm a potential source of hemorrhage in the small bowel of patients with HHT. CE is important to direct the treatment by DAE.

GENETICS

Mutation affecting the proximal promoter of *Endoglin* as the origin of hereditary hemorrhagic telangiectasia type 1

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Aim: Pathogenic mutations have been described in exons, splice junctions and, in a few cases with ENG mutations, in the proximal promoter, which creates a new ATG start site. However, no mutations affecting transcription regulation have been described to date in HHT, and this type of mutation is rarely identified in the literature on rare diseases.
Methods: Sequencing data from a family with HHT lead to single nucleotide change, c.-58G > A. The functionality and pathogenicity of this change was analyzed by in vitro mutagenesis, quantitative PCR and Gel shift assay. Student t test was used for statistical significance.
Results: A single nucleotide change, c.-58G > A, in the proximal ENG promoter co-segregated with HHT clinical features in an HHT family. This mutation was present in the proband and in 2 other symptomatic members, whereas 2 asymptomatic relatives did not harbor the mutation. Analysis of RNA from activated monocytes from the probands and the healthy brother revealed reduced ENG mRNA expression in the HHT patient ($p = 0.005$). Site-directed mutagenesis of the ENG promoter resulted in a three-fold decrease in luciferase activity of the mutant c.-26 58A allele compared to wild type ($p = 0.005$). Finally, gel shift assay identified a DNA–protein specific complex.
Conclusion: The novel ENG c.-58G > A substitution in the ENG promoter co-segregates with HHT symptoms in a family and appears to affect the transcriptional regulation of the gene, resulting in reduced ENG expression. ENG c.-58G > A may therefore be a pathogenic HHT mutation leading to haploinsufficiency of Endoglin and HHT symptoms. To the best of our knowledge, this is the first report of a pathogenic mutation in HHT involving the binding site for a transcription factor in the promoter of ENG.

Chromosomal translocation as a cause of JP/HHT

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Introduction: Common molecular genetic screening technics in HHT patients disclose mutations in app. 90% of the analyzed patients. In the remaining families despite of adherence to the Curaçao criteria's the genetic event will often remain unknown. Patients with germline mutations in SMAD4 can present symptoms of both juvenile polyposis syndrome (JPS) and hereditary hemorrhagic telangiectasia (HHT): the JP-HHT syndrome.
Methods: A patient fulfilling the Curacao criteria's was submitted to molecular genetic analysis in ENG, ACVRL1 and SMAD4 using standard technics. In brief, DNA was extracted and sequenced with targeted NGS using the Agilent targeted sequence capture method followed by sequencing on the Illumina HiSeq1500 NGS platform. Cytogenetic analysis was performed using standard procedures. In brief, leucocytes were cultured and cell divisions stopped by colcemid. Chromosomes were stained using Leishman´s color. A minimum of 15 metaphases was analyzed.
Results: No pathogenic variation was observed. The daughter of the index patient was submitted for chromosomal analyses due to recurrent miscarriages. The translocation t(1;18)(p36.1;q21.1) was observed. The same translocation was found in the patient. The family presents with both colorectal cancer and HHT. The translocation segregates with the SMAD4 related phenotypes.
Conclusion: A translocation involving a breakpoint in the SMAD4 locus co-segregates with JP/HHT in an extended family. This observation warrants analysis for chromosomal rearrangements in HHT patients with no other variants identified.

Mosaicism in a patient with HHT

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Introduction: HHT is an autosomal dominant genetic disorder caused by any of the three genes ENG, ACVRL1 or SMAD4. Hence,

usually patient's presents with pathogen genetic variation in a heterozygous state with an equal representation of the two alleles Occasionally an individual will be found negative for mutation in either of the three genes. Here we provide evidence of the presence of a mosaicism that would not be observed in the clinical routine if using Sanger sequencing or a NGS read coverage below app. 100.
Methods and materials: The proband, who fulfilled the Curacao criteria was submitted to the HHT Center in Odense. DNA was extracted from peripheral blood leukocytes. The coding region, exon–intron boundaries, and the flanking sequences of the genes were sequenced with targeted NGS using the Agilent targeted sequence capture method followed by sequencing on the Illumina HiSeq1500 NGS platform.
Results: A pathogenic genetic variant c.704dupC; p.Val236Glyfs*98 was found in the *ENG* gene. Her mother with clinical HHT was found negative when analyzing for the familial mutation using Sanger sequencing. Analyzing the mothers DNA extracted from either peripheral blood leukocytes, cheek swaps or urine using a NGS HHT panel the familial pathogenic genetic mutation was found at different levels dependent on tissue providing evidence of the presence of mosaicism in the mother.
Conclusion: Evidence of mosaicism for a pathogenic genetic variation in an individual presenting with symptoms of HHT is provided. This finding illustrates the importance of evaluating for mosaicism in index cases fulfilling the Curacao criteria's but found negative for pathogenic genetic mutation in either of the candidate genes.

Utility of HHT patient specific gene mutations to link families in databases

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Aim/Method: The HHT Outcomes Registry will contain de-identified information from consented Hereditary Hemorrhagic Telangiectasia (HHT) patients from North American HHT Centers. Information collected will include clinical, genetic, treatment and radiographic data, with long-term clinical follow-up. Given issues of confidentiality, information regarding family relationships will not always be collected, for example, in cases of families recruited across multiple centres. Family relation data may be required for certain research questions. To assess this data issue, we proposed to determine family relatedness using identified HHT mutations, within our HHT Centre database.
Results: 92 sequential HHT patients with known mutations were selected. Exact mutations were matched and family relations were determined by pedigree. Of 92, 66 (72%) patients could be assigned to a unique family, while 26 (28%) could not, with 10 mutations shared among unrelated individuals.
Conclusion: These data suggest that the use of genetic mutations to assign family relation is promising for database analyses, given that $> 70\%$ of individuals could be assigned to a unique family. This may be an underestimate as we may have not been able to identify relatedness in some large families. The reliability of this technique could be improved by removing known hot-spot mutations.

Genotype–phenotype correlation in children with HHT: data from the Brain Vascular Malformation Consortium

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Objective: To investigate genotype–phenotype correlations in a pediatric cohort of patients with Hereditary Hemorrhagic Telangiectasia (HHT).
Methods: Clinical manifestations of HHT were examined in the first 125 children enrolled with and without brain arteriovenous malformation (AVM) in the multicenter Brain Vascular Malformation Consortium (BVMC) HHT Project. HHT gene mutation results were assessed for correlation with clinical phenotypes.
Results: Of 125 children, 71 (57%) were male, with a mean age of 11.7 years (SD = 4.9, range 1–18 years). Epistaxis was reported in 111/125 (89%) children. and telangiectasia in 62/125 (50%). Fifty/125 (40%) had Pulmonary AVMs (PAVMs), 45/125 (36%) had brain AVM, 6/125 (5%) had a history of HHT-related GI bleeding and 4/125 (3%) had been diagnosed with liver VMs. Genetic mutations were known in 87/125 (70%) of the children of which 42/87 (48%) were endoglin, 38/87 (44%) ALK1 and 7/87 (8%) were SMAD4. All the children with SMAD4 mutation were found to have epistaxis and none had PAVM, BAVM or liver VMs, although 3/7 (43%) had GI bleeding. Of the children with endoglin mutation; 37/42 (88%) had epistaxis, 22/42 (52%) had PAVM, 20/42 (48%) had BAVM and 1/42 (2%) were found to have GI bleeding and similarly liver VMs. Of the children with ALK1 mutation, 30/38 (79%) had epistaxis, 11/38 (29%) had PAVM, 7/38 (18%) had BAVM and none had GI bleeding and 1/38 (3%) had liver VMs. Children with endoglin mutations were more likely to have either Endoglin mutation was significantly associated with PAVM ($p < 0.05$) and BAVM ($p < 0.05$) compared to ALK1 mutation.
Conclusion: Endoglin mutation was associated with PAVM and BAVM in children, as observed previously in adults. Though rare, GI bleeding and liver VMs, were reported in this pediatric series.

12q13.12q13.13 Microdeletion: a new contiguous gene syndrome encompassing *ACVR1* and *SCN8A* genes. A case report

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Aim/Method: Case report.
Results: We herein report the case of a 50 year-old woman who was referred to the French National Hereditary Hemorrhagic Telangiectasia (HHT) Centre, without familial history, for a definite HHT diagnosis using the Curaçao criteria, associating epistaxis, cutaneous-mucous telangiectases, symptomatic pulmonary arteriovenous

malformations (AVMs), and asymptomatic liver AVMs. PAVMs were treated using transcatheter vaso-occlusion. As the patient also has a mild intellectual disability, the HHT genetic testing (*ACVRL1, ENG* and *SMAD4*) was completed by an oligonucleotide array comparative genomic hybridization (array CGH) study. This revealed that the patient carried a de novo 1.58 Mb deletion of chromosome 12q13.12q13.13, including the *ACVRL1* and *SCN8A* gene, thus making it possible to define a new contiguous gene syndrome. The *ACVRL1* gene, in which autosomal dominant mutations or large rearrangements for one or more exon have been reported in patients with HHT type 2, was responsible for HHT and the deletion of *SCN8A* gene is likely to be the cause of the cognitive impairment. Some missense de novo or inherited variant of the neuronal sodium channel gene *SCN8A,* which encodes the sodium voltage-gated channel alpha subunit 8, have been reported in patients with a wide range of epileptic disorders, extending from early-onset epileptic encephalopathy to benign infantile epilepsy with dyskinesia. Heterozygous loss of function mutations, however, has been reported in a few patients with mild intellectual deficiency with or without ataxia. Interestingly, in this particular case of a large *SCN8A* gene deletion, epilepsy was absent and clinical examination identified moderate neurological symptoms.

Conclusion: In conclusion, we reported a new contiguous gene syndrome encompassing *ACVRL1* and *SCN8A* genes. This case highlighted the interest of the search for large rearrangement in case of unusual symptoms in HHT.

RASA1 mutation (capillary malformation-arteriovenous malformations syndrome) may have phenotypic overlap with hereditary hemorrhagic telangiectasia. A report of two cases

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Objectives: To report two cases of capillary malformation-arteriovenous malformations (CM-AVM) syndrome associated with RASA1 mutation with a clinical presentation mimicking hereditary hemorrhagic telangiectasia (HHT).

Methods: Two patients with clinical presentation of HHT (epistaxis or muco-cutaneous telangiectasia and visceral involvement) were screened in our pluridisciplinary HHT Center, using clinical examination, including dermatology, ENT, thoraco-abdominal CT-scan and genetic tests.

Results: A 28-year-old man, already treated for cerebral AVM had epistaxis, typical nasal involvement of HHT with fifty telangiectasia and 14 non-typical cutaneous telangiectasia. CT-scan revealed a large porto-caval shunt between the right branch of the portal vein and the inferior vena cava. A 9-year-old girl presented cyanosis due to a huge and complex pulmonary AVM of the right lower lobe and multiple muco-cutaneous telangiectasia typical of HHT. Nasal endoscopy was normal. CT-scan also confirmed the PAVM and revealed a hepatic AVM within the left lobe. The PAVM was embolized and surgically resected because of the development of a large systemic lung supply to the AVM 3 years after embolization. Both had no family history of HHT but HHT was considered possible. The genetic tests did not find classical mutations (ENG, ALK1, SMAD4) but identified RASA 1 mutation observed in the CM-AVM syndrome that classically induces cutaneous or subcutaneous capillary malformations, cerebro-medullar or musculo-skeletal AVM. PAVM and porto-caval shunt have not yet been described.

Conclusion: Clinical and radiological features of HHT may be encountered in RASA1 mutation and probably conversely. Thoraco-abdominal visceral AVMS described here have not been reported in the CM-AVM syndrome.

Landscape of HHT mutations from large gene panel routine sequencing

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We used next-generation sequencing—based method for molecular diagnosis of hereditary vascular disorders including HHT. We developed a targeted capture panel including twelve genes (ACVRL1, ENG, GDF2, TEK, RASA1, SMAD4, BMPR2, CAV1, KCNK3, EIF2AK4, SMAD9, TBX4) using SeqCap EZ (Roche Nimblegen) coupled with multiplexing and high-throughput sequencing using the Miseq system (Illumina). We analyzed 120 patients with a diagnosis of HHT clinically suspected on the basis of the Curaçao criteria. Mutations identified are both point mutations and large rearrangements. Any potential pathogenic variant was reassessed by Sanger sequencing or mlPA analysis.

A mutation was identified in 47.5% of suspected HHT cases. Most of HHT mutations were identified in ACVRL1 (53%) and ENG (44%). One SMAD4 mutation (2%) was identified in a patient without any sign of juvenile polyposis. Interestingly, a RASA1 mutation was identified in a family suggestive of HHT. Additionally nine variants of unknown significance (VUS) were detected in *ENG*, *SMAD4* and *GDF2* genes. The pathogenic effect of four mutations initially classified as VUS was confirmed by RNA analysis and in vitro functional analysis is in progress for several *ENG* and *ACVRL1* VUS.

Gene panel NGS sequencing is an efficient tool to better define the genetic architecture of HHT offering the molecular analysis of all known genes in one time. Furthermore, identification of mutations in genes responsible for clinically related diseases is helpful for correcting the diagnosis. The main challenge in the future will be the interpretation of growing number of VUS identified by this approach requiring development of functional and splicing defect analysis in routine lab.

Functional studies of the *ACVRL1* c. 314-35A > G polymorphism in HHT1

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Objectives: A common single nucleotide polymorphism (SNP), *ACVRL1* c.314-35A > G, is associated with an increased risk of both sporadic BAVMs and of any AVM in HHT1 patients. This SNP is located near a branch point for mRNA splicing, suggesting altered splicing as the functional basis of the genetic association. We investigated the possible effect(s) of *ACVRL1* c.314-35A > G on splicing and expression of *ACVRL1*.
Methods: We used reverse transcription PCR (RT-PCR) on RNA isolated from 15 lymphoblastoid cell lines (LCLs) from HHT1 patients homozygous for the A or G allele at *ACVRL1* c.314-35 to amplify the different mRNA splice variants present in each sample. We compared the intensity of the variants between samples of the two genotypes. We also cloned each allele from *ACVRL1* exon 3–8 into the pSPL3 splicing vector and these were transfected into cells to evaluate the effect of *ACVRL1* c.314-35A > G in its genomic context on mRNA splicing. Real-time quantitative PCR (qPCR) experiments to examine total *ACVRL1* mRNA expression levels in LCLs are ongoing.
Results: Several different splice isoforms were present in most cells. However, there was no statistical difference in splicing patterns between cell lines with the AA or the GG genotype at *ACVRL1* c.314-35. The in vitro transfection experiments also showed no differences in splicing between the A and G alleles.
Conclusion: Although *ACVRL1* c.314-35 does not appear to alter splicing, it is possible that it exerts a functional effect via other mechanisms, e.g. mRNA expression, or is in linkage disequilibrium with other functional polymorphism(s).

Whole-exome sequencing in HHT patients

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Objective: Despite the analysis of the genes *ENG*, *ACVRL1*, *MADH4* and *GDF2* we did not find mutation in about 10% of patients fulfilling the clinical criteria of definite hereditary hemorrhagic telangiectasia (HHT). Recently mutations of *RASA1* were implicated in some HHT patients but involvement of this gene accounts for a minority of patients. In order to identify new genes causing HHT we performed whole-exome sequencing (WES).
Methods: We selected 12 patients with definite HHT diagnosis (3, 4 or 5 Curaçao criteria). All DNA were previously analyzed by Sanger sequencing and mlPA: 7 non related patients (6 with familial history of HHT), four patients belonging to 2 independent families and one putative new case with his healthy parents. WES was performed by IntegraGen society. Data extracted from vcf files were analyzed with the ERIS on-line interface.
Results: Around 85% of the exome was covered with a minimum depth of 25X. We did not find any deleterious mutations in genes coding for proteins implicated in the TGF-beta pathway or variants in genes common to all the affected patients. We found a de novo missense mutation in *MIIP*, and a missense in *TGFBR3* in two related patients, both reported in dbSNP but not validated. Other variants identified in this series are in the way of characterization.
Conclusion: According to the number of variants obtained by exome sequencing, this approach should be practiced preferentially on well-characterized HHT pedigrees. We will complete this study looking for mosaicism and variants in regulatory regions.

Characterization of pulmonary arteriovenous malformations in *ENG* versus *ACVRL1* mutation carriers in hereditary hemorrhagic telangiectasia

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Objectives: Symptomatic pulmonary arteriovenous malformations (pAVMs) are major contributors to morbidity and mortality in hereditary hemorrhagic telangiectasia (HHT). Mutations in *ENG* (HHT type 1) and *ACVRL1* (HHT type 2) account for approximately 96% of clinically diagnosed cases, and earlier studies indicated differences in pAVM frequency in the two genotypes. The aim of this study was to compare the features of pAVMs and severity of disease between these two genotype groups.
Methods: Sixty-six patients with HHT and affected family members were characterized genetically in our HHT Center between 2008 and 2016, and 62 of them with either *ENG* (28) or *ACVRL1* (34) mutations were included in this retrospective analysis. Morphologic features of pAVMs were analyzed using computed tomography angiography. HHT symptoms, pAVM imaging characteristics, frequency of procedural intervention, and overall HHT severity scores were compared between the genotype groups using Fisher's exact or Mann–Whitney test.
Results: *ENG* mutation carriers were more likely than *ACVRL1* mutation carriers to have pAVMs (72 vs. 18%, $p < 0.001$), multiple pAVMs ($p = 0.03$), and undergo procedural intervention ($p = 0.02$). Additionally, pAVMs in *ENG* carriers were more likely to exhibit bilateral lung involvement and growth over time, although not statistically significant. Finally, HHT severity score was significantly higher in *ENG* than *ACVRL1* ($p = 0.02$).
Conclusion: Significant differences of pAVM incidence and characteristics are found based on genotype. These pAVM features may contribute to the higher disease severity in HHT type 1. Future exploration of this data may lead to more personalized screening protocols, earlier interventions, and better utilization of imaging resources.

Hereditary haemorrhagic telangiectasia: a record linkage study

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Objectives: To use record linkage to estimate the prevalence of HHT and the spectrum of complications found in a population based cohort of patients.
Methods: With Caldicott Guardian Approval, all known cases of HHT in Tayside, Scotland were identified from clinical information systems, pedigree analysis and DNA laboratory records. Data was collected on mutation status, clinical presentation, screening and management.
Results: 47 cases in 20 families (27 female; 20 male) had confirmed molecular or pedigree diagnoses. 33 (70%) had *ACVRL1* mutations and 14 (30%) *Endoglin* mutations. A de novo *Endoglin* mutation was found after a child died, likely from complications of HHT. Minimum point prevalence in Tayside is 1:8831 (47 in 415,040), but is expected to be higher due to methodological limitations. 44 (94%) of cases had recurrent spontaneous epistaxis; 39 (83%) had mucocutaneous telangiectases. Of those without epistaxis or telangiectasia, 100 and 89% were < 25 years old respectively. 38 (81%) of cases had pulmonary arteriovenous malformation (PAVM) screening. 4 (44%) and 3 (13%) of screened *Endoglin* and *ACVRL1* cases had PAVMs respectively. One case with an *Endoglin* mutation presented symptomatically with a brain AVM. 7 (15%) had asymptomatic hepatic vascular abnormalities (all *ACVRL1* cases). No other AVMs were documented. 7 (15%) of the total cohort had acute epistaxis-related hospital admissions; 6 (13%) required blood transfusion because of epistaxis.
Conclusion: record linkage can identify cases across an entire region. Estimated prevalence and genotype–phenotype correlation corroborates previous data demonstrating that PAVM appear to be more common in *Endoglin* cases than *ACVRL1* cases, and hepatic abnormalities are largely restricted to *ACVRL1* cases.

Genetic characteristics of the HHT Italian population: the experience of the three Italian Reference Centres

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Objectives: To describe the disease-causing mutations distribution of *ENG* and *ACVRL1* in the HHT Italian population.
Methods: Patients were recruited for the clinical screening and the diagnosis in the three main HHT Italian reference centres of Pavia, Crema and Bari. Mutation analyses were performed by DHPLC and/or Sanger sequencing of *ENG* and *ACVRL1* coding exons. A dedicated mlPA kit (Salsa mlPA Probe mix P093-C2, HHT/PPH1; MRC-Holland) was used to detect large deletions and duplications.
Results: From 2000 up to October 2016, we collected about 2000 samples from more than 500 families. Until now, we have studied 450 families; a disease-causing mutation was identified in 390 families and about 68% of them have HHT type 2. We found 240 different mutations spread among the whole coding sequence and flanking introns of both genes with the exception of *ACVRL1* exon 2 and *ENG* exons 11 and 15, where no mutation was detected. Noteworthy, about 47% of Patients have a mutation in exons 3, 7 or 8 of *ACVRL1*. If we consider the geographical origin of the families, a founder effect is suggested for at least 5 mutations.
Conclusion: We confirm the peculiar genetic characteristic of the HHT Italian population with a higher incidence of HHT2. In particular, an unexplained 22% of cases carry a mutation in *ACVRL1* exon 3. In addition, a founder effect is to be supposed in some geographic regions.

Functional analysis of a novel ENG variant in a patient with hereditary hemorrhagic telangiectasia (HHT) and pulmonary arterial hypertension (PAH) identifies a new SP1 binding site

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Objectives: To investigate the role of a novel intronic *ENG* variant found in a Patient with a PAH diagnosis followed by the identification of typical HHT clinical signs. The pathogenic role of this variant was demonstrated.
Methods: We analysed all coding exons of *ENG*, *ACVRL1* and *BMPR2* by Sanger sequencing and mlPA. We expressed the *ENG* variant in vitro and evaluated protein levels by western blotting. Then, we confirmed the results by qRT-PCR on an RNA sample of the Patient. We used in silico tools to evaluate the presence of putative transcription factor binding-site, changed in the variant. EMSA analyses were performed to validate the involvement of the transcription factor Sp1.
Results: We identified the *ENG* novel variant c.1852 + 42 C > T in a Patient with both PAH and HHT. No other disease-causing mutation was found. We proved by western blotting and qRT-PCR that the variant significantly reduced ENG expression. *In silico* analyses predicted that the variant changes a putative binding-site for the transcription factor Sp1, already demonstrated as involved in *ENG* expression. By EMSA, we observed that nuclear extract proteins of human fibroblasts bind with different affinity wild-type and mutated oligonucleotides.
Conclusion: We identified a novel Sp1 binding-site in *ENG* intron-14. We demonstrated the pathogenic role of *ENG* c.1852 + 42 C > T mutation, which impairs this Sp1 binding-site reducing the transcription level of the gene. These results stress the importance of joining genetic findings to functional studies, in order to understand the role of novel variants of uncertain significance in the disease pathogenesis.

Characterization of a mutation in 5′UTR of endoglin causing hereditary hemorrhagic telangiectasia

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Objectives: Most HHT patients have a mutation in the coding region of the activin A receptor type II-like 1 (*ACVRL1*) or Endoglin (*ENG*) gene. However, in approximately 4% of cases, sequencing analysis and deletion/duplication testing fail to identify mutations in the coding regions of these genes. Previous work from our group has emphasized the importance of including the study of mutations in the *ENG* gene 5'untranslated region (UTR) in the molecular diagnosis of HHT (Damjanovich et al. Orphanet J. Rare Dis. 2011 Dec 22;6:85; Fontalba et al., BMC Med. Genet. 2013 Nov 25;14:121).
Methods: Sequencing of the ENG 5′UTR region in a 48-year-old Caucasian male with pulmonary arteriovenous malformations and 1–2 episodes/week of epistaxis revealed the presence of a variant (c.-142A > T). This variant generates a putative new initiation codon leading to a new open reading frame with a different translation initiation site. Family segregation studies confirmed this gene variant in two affected second degree relatives. Site directed mutagenesis was performed to generate the mutant using an endoglin expression vector as template. The monkey kidney COS-7 cell line was transfected with expression vectors and cell lysates were subjected to SDS-PAGE, followed by immunoblotting.
Results: In vitro expression studies show that cell transfections with an expression vector encoding the c.-142A > T endoglin mutant results in a marked decrease of protein levels compared with a wild type endoglin sequence, suggesting that the mutation interferes endoglin expression.
Conclusion: Our results reinforce the need for further studies of the ENG 5′UTR region in HHT molecular diagnostic tests.

Directing HHT patients to the 100,000 genomes project

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Objectives: Genomic diagnostics is being mainstreamed into UK medical practice via the 100,000 Genomes Project [1] which is recruiting patients with hereditary haemorrhagic telangiectasia (HHT). Our goals were to identify which HHT families were suitable for recruitment, and evaluate what proportion of patients in different categories had pathogenic variants in the recognised HHT genes.
Methods: From September 2015, patients attending our clinical service with known or suspected HHT, but with no known molecular diagnosis, were offered an NHS HHT gene test through the UKGTN. [2] DNA findings were characterised by variant subtype/classification on the HHT mutation database [3], and represented graphically using GraphPad Prism.
Results: 52 DNAs were sequenced—44 from probands with ≥ 3 Curaçao Criteria, and 8 where HHT was strongly suspected, due to the presence of pulmonary AVMs and at least one other HHT feature. Of the 27 pathogenic variants (Fig. 1), 13 (48.1%) were not previously reported in the literature. Pathogenic sequence variants were identified in 23/44 (53%) with ≥ 3C Curaçao Criteria and 4/8 (50%) with < 3. 20/52 (38%) of probands had no identified variants in *ENG*, *ACVRL1*, *SMAD4*, or *GDF2*. Of the 20 probands with no identified DNA sequence variants, 16 (80%) had 3 Curaçao Criteria and 12 (60%), a strong family history.
Conclusion: Although there was an element of selection bias, a surprisingly high proportion of patients with 3 or more Curacao criteria were suitable for recruitment to the 100,000 Genomes Project. We are exploring if this reflects local or UK referral biases.

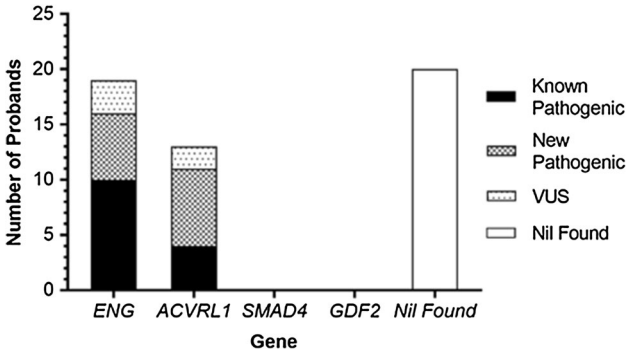

Fig. 1 Characterisation of molecular findings from NHS HHT gene test. Findings are characterised by the gene affected, and classification of the identified variant (known pathogenic, new pathogenic, or variant of uncertain significance (VUS)). Probands with no variants found are also indicated

Unraveling the mystery: analysis of the human genome with refus highlights the genetic complexity of HHT

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Approximately 15% of individuals identified as having hereditary hemorrhagic telangiectasia (HHT) currently have no known genetic cause. Mutations in *ENG*, *ACVRL1*, and *SMAD4* have been identified in affected individuals. In 2013, we identified that mutations in *BMP9* cause a phenotype similar to HHT using exome sequencing. Yet, the genetic cause remains unknown for some families even after exome analysis. Unlike exome sequencing, genome sequencing enables the detection of coding and noncoding intronic and promoter variation and large rearrangements.
Objective: Use genome sequencing to identify new genes and genetic modifiers in families with HHT who do not have an identifiable mutation.
Methods: Genomes from 35 individuals with HHT and 2 healthy controls from 13 families were prepared and sequenced (Illumina). Data was analyzed using RUFUS, a reference-independent variant discovery tool that directly compares raw sequence data from two or more samples to identify novel and rare mutations.
Results: Three families had a causative noncoding variant in *ENG* or *ACVRL1* that was missed by exome sequencing. Two families had a variant in *ACVRL1* intron 9 that disrupts splicing, including one family with an *ACVRL1* intron 9: chromosome 3 translocation.

Analysis is currently underway to identify additional HHT causative genes and genetic modifiers in the other families. Several hits are being pursued and will be presented.
Conclusion: Genome sequencing suggests that noncoding variation in the known genes plays a more significant role in HHT than previously thought. This is the first report to show the role of chromosomal translocation as a mechanism of HHT.

A novel SMAD 4 mutation in a child with probable emerging phenotypes of JP-HHT syndrome

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Patients with SMAD4 mutations can present with symptoms of both juvenile polyposis syndrome (JPS) and hereditary hemorrhagic telangiectasia (HHT): the JP-HHT syndrome. This germline mutations explains only 1–2% of the HHT cases and are associated with JPS in approximately 20–30% of cases in many cohorts.

We describe clinical characteristics of a 6 year old girl who first presented with a history of iron deficiency anemia and rectal bleeding for over a year. More than 100 juvenile polyps were detected and partially removed by colonoscopy and polypectomies. A novel heterozygous mutation of SMAD4 was noted at Exon 9, c.1052A > T (p.Asp351Val). This sequence change replaces aspartic acid with valine at codon 351 of the SMAD4 protein (p.Asp351Val). The aspartic acid residue is highly conserved. Since there is a large physicochemical difference between aspartic acid and valine, we think this variant can manifest with SMAD4-related disease.

The patient subsequently developed fever, poor weight gain, lower extremity joint swelling and upper extremity swelling where she cannot walk, and chest pain. Due to swelling and erythema of great toes, ankle, knees, wrists, and IP joints bilaterally, juvenile idiopathic arthritis (JIA) was diagnosed. She remains steroid dependent for her JIA and also receives tocilizumab every 2 weeks. Asthma was diagnosed for a symptom of chest pain and she had further evaluations for chest pain and digital clubbing.

Chest CT scan showed multiple nodules in the lungs and the liver suggesting pulmonary and hepatic arteriovenous malformations (AVMs). Pulmonary angiography was performed, demonstrating multiple smaller lesions on the left, and larger lesions on the right with some clearly shunting. Pulmonary AVMs in the posterior lateral right upper lobe, with 3 feeding arteries measuring approximately 2.29 mm were embolized using 4 MVP vascular plugs (1–3 mm size). MPA pressure was 30/16 (23) with systemic 94/49 (66). Cardiac output 4.7 L/m.

Since the patient has a known history of JIA, other differential diagnosis included vasculitis, due to the thickened and less well defined malformation walls. Her brain imaging does not demonstrate evidence of prior stroke, abscess, or AVMs.

There are several challenges in diagnosing this young child with HHT. First, young children may not manifest common symptoms like most HHT adults. The most common presenting symptom is epistaxis, occurring eventually in more than 90% of patients and beginning on average at the age of 12 years. Furthermore, in reported cohorts of JP-HHT syndrome, spontaneous and recurrent epistaxis and telangiectasia are less affected compared with the majority of HHT patients in general. We give the consideration of the JP-HHT syndrome to obtain an extensive evaluation and an appropriate care of the child and her family. The phenotypic picture of this syndrome may just emerge over time. We also reported hepatic AVMs in this child. Although most cases with hepatic AVMs present in the adulthood, the onset of development of AVMs in the liver is unknown and deserve discussion for screening. Finally, our case stresses that surveillance for the disease requires increased awareness of the JP-HHT syndrome. We emphasize that a multidisciplinary approach is essential and more effective.

MECHANISMS OF DISEASE

MicroRNA dysregulation in peripheral blood mononuclear cells and early endothelial progenitor cells from HHT patients

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Aim/Method: The goal of this study was to profile and characterize microRNAs (miRs) in early endothelial progenitor cells (eEPCs) and peripheral blood mononuclear cells (PBMCs), from hereditary hemorrhagic telangiectasia (HHT) patients.28 HHT patients with confirmed mutations, and 25 controls were recruited. 40 ml of peripheral blood was collected for eEPC culture and PBMC isolation. Total RNA was isolated from PBMCs and eEPCs. PBMC and eEPC expression profiling was conducted with a human miR array analysis. Dysregulated miRs identified from the analyses were then validated with RT-qPCR. Significant differences were determined using a two-tailed *t* test.
Results: Of the 800 miRs screened, 167 and 121 dysregulated miRs were identified in PBMCs and eEPCs, respectively. Selected PBMC miRs (MiRs-28-5p, -30b-5p, -361-3p and -374a-5p) and eEPC miRs (miRs-132-3p, -221-3p and -424-5p) were validated with RT-qPCR. PBMC miR-361-3p ($p = 0.025$) known to target IGF1, and eEPC miR-132-3p ($p = 0.047$) known to target SMAD2, were found to be significantly decreased compared to controls. Subsequently, IGF1 messenger RNA (mRNA) levels were significantly increased ($p = 0.005$) in PBMCs of HHT patients compared to controls.
Conclusion: Our results show dysregulation of select miRs in PBMCs and eEPCs from HHT patients. The finding of increased IGF1 mRNA and its possible relation to decreased miR-361-3p levels is both novel and exciting. This may represent a putative pathogenic mechanism involved in HHT, and may provide a unique miR therapeutic target for the treatment of clinical manifestations. Future work will include the characterization of the other dysregulated miRs to gain further insight into their role in HHT pathogenesis.

Next-generation sequencing reveals downstream targets of the TGF-BETA pathway in HHT

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Objectives: To identify downstream targets of the TGF-Beta pathway that contribute to AVM formation in HHT patients. We aim to provide a direct link between the TGF-Beta pathway and its downstream effectors.
Methods: We performed RNA-sequencing experiments on our novel Smad4 inducible, endothelial cell specific knockout (Smad4-iECKO) mouse model of HHT and identified genes that were up- or down-regulated in mutant retinas. In addition, ChIP-sequencing was used to identify Smad4 binding sites in human umbilical vein endothelial cells (HUVECs). We then integrated these RNA- and ChIP-sequencing datasets to provide a list of potential therapeutic targets for HHT pathogenesis.

Results: Our RNA sequencing experiment revealed 328 differentially expressed genes (178 downregulated, 150 upregulated, FDR = 0.05) including genes associated with blood vessel formation and genes previously not associated with HHT. For example, we found TEK and Rho to be downregulated while Scai and Col20a1 were upregulated. ChIP-sequencing methods identified over 25,000 Smad4 binding sites in HUVECs. Integration of these two experiments revealed 184 genes in common that represent potential direct targets of Smad4 that regulate AVM formation.
Conclusion: Using next generation sequencing, we have uncovered many novel TGF-Beta effectors which will provide the HHT-field with new potential therapeutic targets. Additionally, this is a first step in establishing a mechanism downstream of Alk1, ENG and Smad4.

Role of type III Transforming Growth Factor-β receptor in regulating ALK1 signaling and endothelial biology

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Aim/Method: The Transforming Growth Factor-β (TGF-β) superfamily of ligands and receptors play critical roles in angiogenesis, evidenced by their upregulation in both physiologic and pathologic angiogenesis, including metastatic cancer. In most human cancers, loss of expression of the type III TGF-β receptor (TβRIII), a TGF-β superfamily co-receptor, correlates with increased angiogenesis, tumor growth and metastatic potential. However, in development, global genetic deletion of TβRIII causes embryonic lethality in mice, characterized by severe defects in cardiac and hepatic vasculogenesis. Despite these observations, how TβRIII controls angiogenesis remains unknown.
Results: Our lab has recently discovered that vascular endothelial cells express relatively high levels of TβRIII, alongside the canonical endothelium-restricted co-receptor endoglin. Moreover, we have established that TβRIII forms stable complexes with Activin receptor-Like Kinase 1 (ALK1), an endothelium-specific TGF-β superfamily receptor kinase, leading to increased phosphorylation of Smad transcription factors that regulate angiogenic gene expression.
Conclusion: Through genetic knockout approaches using CRISPR technology, we have demonstrated that loss of TβRIII impairs endothelial function and angiogenic potential, including tubulogenesis. Our studies have uncovered a novel TβRIII/ALK1 interaction, the role of TβRIII in ALK1-mediated signaling and TβRIII's role in functional endothelial biology. Understanding TβRIII's function in the normal and tumor endothelium may lead to the development of innovative pharmacological treatments targeting angiogenesis and also improve our ability to anticipate the potential adverse effects of therapies targeting TGF-β superfamily receptors.

Is soluble endoglin inducer of inflammation in endothelium?

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Objectives: A soluble form of tissue endoglin (sEng) circulating in plasma has been proposed to be at least partially responsible for the induction of endothelial dysfunction (but not in atherosclerosis prone blood vessels) and related to hypercholesterolemia. We provided couple of experiments in order to reveal whether high levels of soluble endoglin might upregulate endothelial dysfunction markers and induce endothelial dysfunction and inflammation both in vitro and in vivo.
Methods: Transgenic mice overexpressing human sEng (high *Sol-Eng*$^+$) and their and age-matched transgenic littermates that do not develop high levels of human sEng (low *Sol-Eng*$^+$) were fed high fat diet for 3 months. HUVEC and HEK were exposed to recombinant human endoglin at concentration 40, 500 ng/mL at different times (16–48 h).
Results: High sEng levels and the presence of mild hypercholesterolemia resulted in induction of inflammation (increased expression of P-selectin, ICAM-1, pNFκB and COX-2) in aorta of high *Sol-Eng*$^+$ mice. sEng treatment in endothelial cells resulted in activation of NF-κB/IL-6 expression, increased expression of membrane endoglin and reduced expression of Id-1.
Conclusion: Current results show that soluble endoglin induce signs of inflammation in vascular endothelium both in vivo and in vitro. On the other hand, not all typical markers of endothelial dysfunction in endothelial cells or functional properties in aorta were changed in the presence of high levels of soluble endoglin. In other words, ongoing studies in our lab will try to answer the question whether soluble endoglin can induce endothelial dysfunction, inflammation and promote atherogenesis.
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Continued overexpression of endoglin impedes the correct course of angiogenesis

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Objectives: The main cause of the clinical symptoms of HHT is a deficient angiogenesis due, in the case of HHT-1, to reduced endoglin levels. Consequently, enhancement of the expression of this protein has been suggested as a potential therapeutic approach. Here, we study whether increased endoglin actually improve angiogenesis.
Methods: We analyzed several models of angiogenesis in mice that ubiquitously overexpress human endoglin (*ENG*$^+$) and *WT* mice. Moreover, we performed in vitro experiments to assess the effect of enhanced endoglin expression on cellular processes implicated in sprouting and pericyte adhesion.
Results: Overexpression of endoglin does not increase post-ischemic revascularization or improve angiogenesis. A delayed angiogenic front was found in the retinal vasculature of *ENG*$^+$ mice. Furthermore, the vessels formed in these animals are more branched and tortuous than those of *WT* mice, allowing in some cases erythrocyte extravasation. In vitro experiments demonstrated that overexpression of endoglin in endothelial cells reduces proliferation while enhances cellular migration and matrix invasion. Moreover, high levels of endoglin in the endothelium inhibits pericyte adhesion.

Conclusion: Our results demonstrate that continuous overexpression of endoglin produces alterations in angiogenesis that seems to particularly affect to the vessel maturation. We hypothesize that the expression of endoglin is enhanced in endothelial cells of the angiogenic front, but its levels should decrease for adequate endothelium stabilization and mural cell recruitment. For this reason, we can conclude that a continuous increase of endoglin expression does not seems to be a good therapeutic approach for HHT patients.

Circulating microRNAs in hereditary hemorrhagic telangiectasia: preliminary results identify significant differences among patients

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Objectives: We are investigating the role of circulating microRNAs (miRNAs) in HHT as potential disease biomarkers. The main goal is to define an HHT-related miRNAs signature. Particular attention has been paid on miRNAs-genotype and miRNAs-phenotype correlations. Here we present the preliminary results of this study.
Methods: We performed a circulating miRNAs profiling in 15 subjects: 5 HHT1, 5 HHT2 Patients, and 5 controls, age and gender matched. miRNAs profile was analysed by qPCR, using serum/plasma microRNA PCR Panel (I + II), V4.M (Exiqon). Each panel contains 752 LNA™ primer sets of human miRNAs, including different controls. Statistical analyses were performed using parametric and non-parametric methods. miRNAs with a p value < 0.05 were considered statistically significant and underwent enrichment analysis.
Results: The overall result was the detection of 18 deregulated miRNAs. We observed differences between: HHT Patients versus controls; either HHT1 or HHT2 versus controls; HHT1 versus HHT2 Patients and also comparing Patients' subgroups showing different clinical features.
The enrichment analysis identified the top predicted target genes and the related pathways. Among these, we highlighted different pathways already described in association with HHT or angiogenesis.
Conclusions: We obtained a preliminary "HHT signature" for circulating miRNAs, underlying, for the first time, differences between the two disease subtypes and a more peculiar miRNAs profile in HHT2. We also described miRNA-PAVMs (Pulmonary Arteriovenous Malformations) correlations. Confirmation of these results in a larger cohort of patients is therefore mandatory, and Patients enrolment for the second step of this study is ongoing.

Study of the crosstalk between VEGF and BMP9 pathways: implications in hereditary hemorrhagic telangiectasia

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Aim/Method: Several methods are used especially Proximity ligation assay and immunoprecipitation to study receptors interactions; lipid raft extraction, flow cytometry, co-localization, biotinylation assays, antibody feeding, western blotting to study receptors trafficking and phosphorylation status; but also functional angiogenesis assays like proliferation (by BrdU incorporation), migration (by Scratch Assays) and tubulogenesis (by Matrigel assays).
Results: We found that VEGF induces complexes on the surface of HUVECs between VEGFR2/ALK1/Endoglin. Interestingly BMP9 alters the formation of those complexes. We observed that BMP9 downregulates angiogenesis induced by VEGF and bFGF. Using HUVEC cells from HHT2 patients we showed that ALK1 is crucial for BMP9 mediated effects on endothelial cells as without this receptor, BMP9 can't regulate angiogenesis anymore. Western blot analyses allowed us to understand that BMP9 upregulates phosphorylation of ERK induced by VEGF whereas on the opposite, VEGF is not able to modify phosphorylation of Smad1/5/9 induced by BMP9. Other pathways are currently under investigation. Recently we observed that depending of the treatment (VEGF or BMP9 alone and combination) VEFR2, ALK1, Endoglin and uPAR are differently internalized and their membrane localization is also affected. Notably when cells are treated with VEGF and BMP9 together, the internalizations of VEGFR2, Endoglin and uPAR are decreased. In HUVECs harbouring ALK-1 mutation we saw that these phenomenon are disrupted.
Conclusion: We propose that BMP9 plays an important regulatory role in healthy cells by avoiding excess of angiogenesis due to VEGF and bFGF process. This regulatory function is impair in cells from HHT2 patients in which ALK1 is not functional. This regulation involves modifications at the level of receptor interactions and trafficking that further alter protein phosphorylation and gene expression.

Endoglin regulates the immune response

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Objectives: Besides the vascular symptoms, arteriovenous malformations and severe bleedings, HHT patients also have persistent inflammation. The aim of this study is to elucidate endoglin involvement in the impaired inflammatory response in HHT1. Inflammatory response was studied in mice that ubiquitously overexpress human L-endoglin (L-ENG +). To assess if the effect is mediated by the extracellular domain, S-endoglin (S-ENG +) mice were used. Mice were nebulized with LPS as inflammatory agent. Leukocyte recruitment was analyzed in bronchoalveolar lavage (BAL) and by lung histological examination. Inflammatory cytokines in the tissue were determined. Vascular permeability was measured by the amount of proteins and FITC-Dextran in the BAL and lung-wet-to-dry weight.
Results: After LPS exposure, leukocyte recruitment and infiltration to the lungs was significantly lower in S-ENG + than in WT and L-ENG + mice, without differences between these two groups. Furthermore, inflammatory cytokines, IL1β and IL6, analyzed in lung tissue from LPS-nebulized mice, were significantly lower in S-ENG + mice than in WT and L-ENG + , without differences between these two groups. No vascular permeability differences were found between WT, L-ENG + and S-ENG + mice.
Conclusion: These results show that increased endoglin expression do not improve the inflammatory response to LPS. The decreased inflammatory response observed in the S-ENG + mice suggest that intracellular domain of endoglin plays an active role, modifying key

processes such as leukocyte recruitment to the site of inflammation and release of inflammatory cytokines. Thus, impaired inflammation in HHT patients could be due to altered endoglin intracellular signaling.

Co-injection of mesenchymal stem cells with endothelial progenitor cells accelerates muscle recovery in hind limb ischemia by an endoglin dependent mechanism

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Background: Endothelial colony-forming cells (ECFCs) are progenitor cells committed to endothelial lineages that have robust vasculogenic properties. Mesenchymal stem cells (MSCs) have been described to support ECFCs angiogenic process in different kind of matrices.
Aims: MSCs potential interaction with ECFCs in hind limb ischemia (HLI) remains largely unknown. Therefore, we assessed whether co-administration of ECFCs and MSCs can support vasculogenic properties in HLI of nude mice.
Methods: We previously described endoglin as a key adhesion molecule; we thus evaluated its implication in ECFCs/MSCs interaction. We examined the effect of ECFCs + MSCs injection after HLI in athymic nude mice. Immunohistochemistry for human and mouse CD31 and in situ hybridization (ISH) for ALU sequence were performed.
Results: Foot perfusion was increased after ECFCs injection in day 7 and was even better 14 days after injection. Co-administration of MSCs significantly increased vessel density and foot perfusion at day 7, although this difference was no longer significant at day 14. Capillary density was enhanced in ECFCs + MSCs mice by analysis of mouse CD31, human CD31 incorporation and ISH detecting human ALU sequence. We then examined injection of ECFCs silenced for Endoglin + MSCs and found a decreased vessel density and foot perfusion at 7 and 14 days ($p < 0.001$). Silencing endoglin in ECFC did not block MSC differentiation potential in perivascular cells or other mesenchymal lineages. However, silencing endoglin in ECFC dramatically decreased their adhesive properties on MSCs.
Conclusion: We demonstrate that MSCs in combination with ECFCs accelerate muscle recovery by an endoglin dependent mechanism. Our data suggest the systematic use of MSC as a means to improve ECFCs engraftment in hind limb ischemia.

A novel HHT mouse model generated by BMP9 and BMP10 immunoblocking

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Objectives: HHT is a potentially life-threatening genetic vascular disorder resulting from aberrant endothelial cell-driven hypervascularization, and caused by loss-of-function mutations in the ALK1/endoglin signaling cascade. Injections of mouse neonates with blocking antibodies directed against the ALK1 ligands, BMP9 and BMP10, lead to HHT-like vascular defects in the postnatal retinal angiogenesis model. Mothers and newborns share immunity through the transfer of maternal antibodies during breastfeeding. Here, we investigated whether the transmammary delivery route could improve the ease and consistency of administering anti-BMP9/10 antibodies in this angiogenesis model.
Methods: Lactating dams were intraperitoneally injected once with antibodies on post-natal day 3 (P3). The retinal vasculature of P6 pups breastfed for 3 days by antibody-treated dams were then analyzed using histology techniques and gene expression analyses (RNA-seq, qRT-PCR, and Western blots).
Results: Anti-BMP9/10 antibodies injected into dams were efficiently transferred into the circulation of breastfed pups. Strikingly, these pups displayed consistent and robust vascular pathology in the retina, which included hypervascularization, defects in arteriovenous specification, and presence of multiple arteriovenous malformations. RNA-seq analyses performed on whole retinas isolated from treated pups revealed that transmammary delivery of anti-BMP9/10 antibodies and vascular pathology are accompanied by a significant increase of the gene expression of the key pro-angiogenic factor, angiopoietin-2.
Conclusion: Transmammary delivery of anti-BMP9/10 antibodies causes a retinal vascular pathology characterized by the development of key HHT vascular defects. The presented HHT model is practical, noninvasive, reliable and robust, and is suitable for the evaluation of pharmacological and genetic approaches aimed at preventing HHT pathogenesis.

Endovascular sampling and targeted gene expression profiling of single endothelial cells from pulmonary arteriovenous malformations in hereditary hemorrhagic telangiectasia

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Objective: To establish the feasibility of isolating endothelial cells (ECs) from endovascular devices deployed in pAVMs for profiling target EC gene expressions on single cell level.
Methods: A cohort of 13 patients undergoing pAVM embolization was prospectively studied. Pulmonary AVMs were sampled using platinum coils, Microvascular plugs (MVP), or guidewires. The device was removed after at least 15 s in contact with the wall of the targeted vessel. ECs adherent to the devices were isolated using FACS by four EC markers (CD31, CD34, CD105 and CD146). Control ECs were harvested from the right iliac vein during each procedure. Single cell qPCR was used to quantify expression of 48 genes implicated in pAVM pathogenesis. Bioinformatics software was used to analyze the gene expression profiles.
Results: All sampling were finished without complications. The distal most section of the feeding artery of the pAVM was sampled in 8 patients (2.3 ± 0.66 mm) and the sac was sampled in 5 (18 ± 9.7 mm). 0–7 pAVM ECs (3 ± 2.8) were isolated from 12 cases except 24 from one sac by guidewire. Compared with iliac vein EC controls, 16 genes were upregulated (fold change > 2) and 2 genes downregulated (IL-6 and IFN-gamma, $p < 0.05$) in the pAVM ECs, but principal component analysis and heat map-based hierarchical clustering did not separate these two EC phenotypes.
Conclusion: Endovascular device sampling is safe and feasible for targeted EC collection from pAVMs. Sampling efficacy depends on

the AVM size and endovascular device type. Target gene expression profile indicated some functional EC genes' potential roles in pAVM pathogenesis.

Endoglin mediates vascular maturation by promoting vascular smooth muscle cell migration and spreading

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Objective: Endoglin, a TGF-β superfamily co-receptor, is predominantly expressed in endothelial cells and has essential roles in vascular development. However, whether endoglin is also expressed in the VSMCs, especially in vivo, remains controversial. Further, the roles of endoglin in vascular smooth muscle cells (VSMC) biology remain largely unknown. Our objective was to examine the expression and determine the function of endoglin in VSMCs during angiogenesis.
Approach and Results: Here we establish that endoglin is robustly expressed in VSMCs. Using CRISPR/CAS9 knockout and shRNA knockdown in the VSMC/endothelial co-culture model system, we determine that endoglin in VSMCs, but not in endothelial cells, promotes VSMCs recruitment by the endothelial cells both in vitro and in vivo. Using an unbiased bioinformatics analysis of RNA-SEQ data and further study, we determine that, mechanistically, endoglin mediates VSMC recruitment by promoting VSMC migration and spreading on endothelial cells via increasing integrin/FAK pathway signaling, while endoglin has minimal effects on VSMC adhesion to endothelial cells. In addition, we further determine that loss of endoglin in VSMCs inhibits VSMC recruitment in vivo.
Conclusion: These studies demonstrate that endoglin has an important role in VSMC recruitment and blood vessel maturation during angiogenesis and also provide novel insights into how discordant endoglin function in endothelial and VSMCs regulates vascular maturation and angiogenesis.

MODELS OF DISEASE

Genetic background-dependent vascular alterations of BMP9-KO mice

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Objectives/Methods: Our team previously identified bone morphogenetic proteins BMP9 and BMP10 as physiological high affinity ligands of the endothelial receptors ALK1 and Endoglin, whose genes are mutated in 85% of HHT patients. Bmp9 mutations have also recently been described in HHT. In order to understand the role of BMP9 in HHT development, we generated *Bmp9* knock-out (*Bmp9*-KO) mice in the C57/Bl6 genetic background. These mice were viable but presented alterations of their lymphatic vasculature, whereas their blood vasculature was normal (unless BMP10 was simultaneously neutralized with antibodies). Because *Eng* ± mice have been shown to present a more pronounced phenotype in the 129/Ola background, we wanted to study the phenotype of B*mp9*-KO mice in this background.
Results: Follow-up of 129/Ola *Bmp9*-KO mice viability revealed an abnormal gender-dependent mortality. *Bmp9*-KO male mice in the 129/Ola background had a mean survival age of 28 weeks whereas 77% of females were still alive at this age. In males, deaths were preceded by a rapid weight loss (− 20% within 1 week). Analysis of their vasculature suggested enlarged blood and lymphatic vessels. Interestingly, *Bmp9*-KO mice in 129/Ola background often presented macroscopic alterations of their kidneys (53% of males and 25% of females) and liver (23% of males and 75% of females).
Conclusion: Taken together, these observations suggest that the 129/Ola genetic background is prone to major physiological alterations when the B*mp9* gene is deleted whereas this is compensated in other genetic contexts. This observation paves the way for the search of susceptibility genes that can protect or worsen the clinical alterations observed in HHT patients.

Soluble endoglin decreases plasma cholesterol and bile acids levels in mice

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Objectives: Increased plasma levels of soluble form of endoglin (sEng) were observed in patients with hypercholesterolemia, but sEng effects on cholesterol metabolism in liver have not been published so far. Therefore, the aim of the study was to describe the effects of sEng on the cholesterol turnover in liver.
Methods: Six-month-old transgenic male mice overexpressing human sEng on CBAxC57BL/6 J background (sEng) and control mice underwent in vivo study with bile collection for 45 min, with subsequent biochemical and expression analyses. Cholesterol, bile acids, and liver tests were determined in plasma. Expressions of enzymes, transport proteins and nuclear receptors responsible for cholesterol and bile acids homeostasis in the liver were assessed by qRT-PCR and Western blot.
Results: Mice with increased sEng demonstrated decrease in plasma cholesterol levels. This effect paralleled upregulation of hepatic Sr-b1 and Ldlr proteins responsible for cholesterol uptake into hepatocytes, and increased mRNA levels of *Abcg8* and *Abcb4,* the transporters required for biliary excretion of cholesterol, and phospholipids, respectively. Net bile flow was also increased in sEng mice in association with increased gene expression of *Abcc2,* the major transporter mediating bile acid independent bile flow. Bile acids, the major metabolites of cholesterol, were reduced in plasma of sEng mice.
Conclusion: Results of the study demonstrated that high plasma levels of sEng reduced plasma concentrations of cholesterol and bile acids as a consequence of complex changes in the expression of

responsible transporters in the liver. Nevertheless, the exact mechanism of the observed changes remains to be further studied.
Grants: The study was supported by Czech Science Foundation No. GA15-24015S, Dean Foundation No. 150/11/1106-2 and UNCE No. 204019/304019/2012. Transgenic mice were kindly provided by Prof. Lopez-Novoa from University of Salamanca in Spain.

Perfusable blood network formation on a chip: an in vitro platform to investigate HHT

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Aim/Method: A reproducible, in vitro approach to form perfusable 3D microvascular networks on a microfluidic chip is reported.
Results: Blood vessel networks can be formed by angiogenesis or vasculogenesis by co-culturing endothelial cells with supporting cells such as fibroblasts. These engineered blood vessels exhibit morphological and biochemical markers of tight junctions, apical-basal polarity, basement membrane deposition, and permeability. Perfusable microvascular networks formed within the microfluidic device reproduce the 3D cellular niche, facilitating high-resolution, live cell imaging of angiogenesis, vasculogenesis and anastomosis.
Conclusion: This approach provides a platform for developing physiologically relevant model to investigate vascular malformation in HHT. Identification of the genetic factors contributing to different vascular malformations as well as an understanding the role of these factors in the pathogenesis of vascular malformations can be investigated using human cells.

Automatic detection and quantification of pulmonary arteriovenous malformations in hereditary hemorrhagic telangiectasia

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Objectives: Angiography multidetector computed tomography (AMDCT) provides substantial information about Pulmonary Arterio-Venous Malformation (PAVM) angioarchitecture. Currently, no predictive model or grading system is described for PAVM measurement. In daily practice, PAVM is ranked arbitrarily according to the feeding artery diameter (< 3 and > 3 mm), a threshold determinant for embolization. It is also crucial to follow small PAVMs with a confident method to depict time for treatment. We propose an automatic framework for PAVM detection and quantification on AMDCT, relying on mathematical morphology.
Methods: 20 AMDCT were reviewed. The proposed image processing pipeline performs successively the segmentation of lungs and vascular network using geodesic operators. Local caliber computation of the lung vessels is estimated by means of a granulometric measure and detection of local caliber increase is detected by the h-domes operator applied in a hysteresis scheme.
Results: PAVM detection performance was evaluated in terms of true positive rate (TPR) and precision (PR). We achieved a TPR of 81.25% and a PR of 47.7% with average false positives (FP)/scan of 2.85. The TPR value is explained by several PAVMs near the mediastinum. The low PR value is explained by the presence of ground glass mistaken as vessels. An adaptation of the vessel segmentation parameters would increase the PR value. Increasing the TPR would be possible by limiting the mediastinal region included in the caliber computation map.
Conclusion: Automatic detection and quantification of PAVMs at MDCT based on 3-D mathematical morphology is possible and accurate, to be considered in HHT management.

Impaired hemostasis in HHT animal models due to alterations in thrombus stabilization

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Objectives: HHT patients present recurrent and difficult to stop bleedings that compromise patients' life. The aim of this study is to determine whether hemostasis mechanisms in animal models of HHT are altered.
Methods: Two different murine models of HHT ($Eng^{+/-}$ and $ALK1^{+/-}$ mice) were used. Tail bleeding assay was used to determine bleeding and rebleeding time. Primary and secondary hemostasis were studied by analyzing activation and aggregation of platelets by flow cytometry, in vivo drug-induced thrombosis experiments and determining clotting times. Furthermore, thrombus stabilization was measured by using Transonic® flowmeter in the ferric chloride model of occlusive carotid thrombosis.
Results: Our results demonstrate that bleeding time is increased in both animal models of HHT. Data obtained from primary and secondary hemostasis assays show normal activity. However, experiments of thrombus stabilization reveal that $Eng^{+/-}$ mice need more time to reach artery closure, whereas ALK1 $+/^{-}$ show the same tendency. Mice overexpressing endoglin show an increased capacity of thrombus stabilization, what suggests that endoglin could be directly involved in this process.
Conclusion: Our results show that HHT murine models have a deficient hemostasis. Since platelet functionality seems to be unaltered, we propose that diminished hemostasis is attributable to an impaired interaction between platelets and endothelial endoglin. Lack of endoglin could involve a defective thrombus stabilization that could result in more severe hemorrhages. This hypothesis is based on other studies showing that endothelial endoglin RGD domain interacts with integrins. Accordingly, endothelial endoglin could also interact with platelet integrins having a role in hemostasis.

Endoglin protects against high output cardiac failure

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Objectives: Endoglin function is disrupted in the vascular disorder hereditary haemorrhagic telangiectasia type I. It is also required for normal vascular development and angiogenesis, but little is known about endoglin's role in quiescent adult vascular endothelium.
Methods: To investigate this role, tamoxifen was administered to adult Cdh5(PAC)-CreERT2;$Eng^{fl/fl}$ mice to generate endothelial-specific depletion of endoglin (Eng-iKO^{e}). Cardiac magnetic resonance imaging, myography, vascular casting, microsphere injection, immunohistology, qPCR and aortic telemetry were used to evaluate cardiovascular changes after endoglin knockdown.

Results: Endothelial specific loss of endoglin leads to an enlarged heart and cardiomyocyte hypertrophy within 5 weeks, progressing to high output heart failure (HOHF). HOHF could result from arteriovenous malformations (AVMs), however we have not detected any AVMs to account for the rapid increase in cardiac output.
Vasomotor function was altered in the aortas of Eng-iKO^{e} mice showing an increased contraction response to phenylephrine ex vivo. In addition, in vivo aortic telemetry revealed differences in aortic pressures between these mice and controls.

Having observed an increase of VEGF-A protein in tissues from Eng-iKO^{e} mice, we also found that inhibition of VEGFR2 was protective against enlargement of the heart and dilatation of the ventricles.
Conclusion: Our results showed the essential role of endoglin in the maintenance of adult cardio-vasculature through crosstalk with the VEGF signalling pathway.

Aortic endoglin expression and soluble endoglin levels are changed during early development of endothelial dysfunction in mouse model of atherosclerosis

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Objectives: Endothelial dysfunction is the first and key step in the development of atherosclerosis. Our previous studies suggested potential role of endoglin in endothelial function and dysfunction. Aortic endoglin was suggested to regulate eNOS expression and soluble endoglin (sEng) was demonstrated to be cleaved from membrane endoglin in various cardiovascular pathologies. Increased levels of sEng were found in patients with preeclampsia, type II diabetes, hypertension and hypercholesterolemia. The aim of this study was to evaluate the changes in aortic endoglin expression and sEng levels in blood during early development of endothelial dysfunction in mice.
Methods: Two-month-old female double knockout ApoE/LDLR$^{-/-}$ mice and age-matched female mice C57BL/6 J (control mice) were fed chow diet for 2 months. Western blot analysis of aorta and Luminex analysis of inflammatory and oxidative stress markers in blood were performed.
Results: The aortic expression of endoglin was significantly reduced in ApoE/LDLR$^{-/-}$ group as compared to control group. The same reduced expression was also demonstrated for p-eNOS (active form of eNOS) mediating NO-dependent vasodilation and pSmad2/3, which was shown to regulate eNOS expression. In addition, levels of sEng and soluble P-selectin levels (marker of inflammation) in blood were significantly higher in ApoE/LDLR$^{-/-}$ group.
Conclusion: Our results suggest that early development of endothelial dysfunction is accompanied by reduced expression of aortic endoglin and increased levels of sEng. Prospective studies are focused on the potential impact of reduced endoglin expression on the development of endothelial dysfunction and the mechanism of sEng cleavage from aorta.
Grants: The study was supported by grant from The Grant Agency of Charles University in Prague number 884216/C and 1284214/C, grant SVV/2016/260293 and by grant from Czech Science foundation GACR number 15-24015S.

PEDIATRICS

Pediatric probands: symptomatic presentations of HHT in children

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Objectives: To identify those pediatric patients who presented as the proband of their families, and to characterize their presentations and genotypes.
Methods: This was a retrospective review of records for patients seen in our HHT Center, who presented with symptoms of HHT before the age of 30 IN THE ABSENCE of a known family history of HHT.
Results: We identified 16 patients who proved to be probands, ages 1 day to 26 years. Fifteen of these patients presented with AVMs prior to HHT diagnosis, including one patient symptomatic from both brain and pulmonary AVMs. One patient presented with pulmonary arterial hypertension and was subsequently found to have an ACVRL1 mutation. Of the 10 brain AVMs, 8 presented with rupture and acute neurological signs, with one death. There were 6 pulmonary AVMs, 5 of which presented with hypoxia or fatigue and clubbing. Of the 16 patients, only 5 met clinical criteria at the time of their presentations (nosebleeds + telangiectasias), 3 met criteria for possible HHT (nosebleeds alone), and 8 had no other signs or symptoms. Twelve patients had mutations identified: 10 ENG (1 VUS), 1 ACVRL1, 1 SMAD4 VUS. Three patients had negative testing but ultimately met clinical criteria, and 1 is still pending.
Conclusion: Children with HHT can present with complications of HHT, even prior to meeting Curaçao criteria or identification of a family history of HHT. Therefore, it is crucial to have a high suspicion of and low threshold for evaluation for HHT in pediatric patients with symptomatic AVMs.

Results of HHT screening in pediatric patients: identification of AVMs and indications for treatment

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Objectives: The objective of this study was to identify pediatric patients (< 30yo) who were found on HHT screening to have AVMs, and to determine the frequency with which these patients where symptomatic and/or required intervention(s) and their genotypes.
Methods: This was a retrospective chart review of patients followed in our HHT center. Those patients who were diagnosed and screened before age 30 were included for analysis.
Results: 78 patients were identified as "at risk" based on family history and/or genetic testing. 52 underwent screening for brain and pulmonary AVMs. Overall, there were 24 positive screens in 21 children from 17 different families; in addition, 1 case of PAH was

identified. Three brain AVMs were identified, 2 of which were treated in patients with ENG mutations while the third (a microAVM) had an ACVRL1 mutation. 21 patients had lung AVMs, 8 with multiple AVMs identified. 4 of these patients, all with multiple AVMs, were treated due to size and/or symptoms including fatigue and headache; identified mutations include 12 ENG, 3 ACVRL1, 1 SMAD4, and 5 had negative genetic testing but meet clinical criteria.
Conclusion: Of our 52 screened patients under 30yo, 21 (40%) had at least one AVM. Of the 21 AVMs identified by screening, 7 required intervention, including 2 of 3 brain AVMs (67%) and 4 of 21 lung AVMs (19%) based on the initial imaging, and the rest are being followed closely for development of symptoms of growth of the AVM that would signal need for intervention.

Screening a pediatric population with brain AVMs for HHT

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Objectives: The objective of this study was to identify patients who may need additional screening for HHT in a pediatric brain AVM population.
Methods: The genetic counselor involved with the HHT Center screens patients with brain AVMs for genetic conditions by taking a targeted medical and family history during their follow-up appointments in Cerebrovascular Clinic with neurosurgery and interventional radiology, as schedules allow. A retrospective chart review for the 12 patients screened by the genetic counselor was conducted.
Results: Patients ages ranged from 5 weeks to 17 years at the time of initial evaluation. Of the 12 patients screened, 10 pursued some type of additional work-up for HHT. Nine patients completed genetic testing, with 7 negative results and 2 variants of uncertain significance identified. Five patients pursued an appointment in the HHT Center, including a physical exam. Five patients, one of whom was not evaluated in the HHT Center, pursued imaging screening for pulmonary AVMs. No patient had a first degree relative with a previous diagnosis of definite HHT. Of the 10 patients who completed additional screening for HHT, 2 eventually met criteria for definite HHT (ages 3 and 17) and 2 for possible HHT (ages 2 and 10). Because this is a pediatric population, additional patients may meet criteria in the future.
Conclusion: A standard algorithm for evaluating patients in the pediatric brain AVM setting, which incorporates the age-related nature of symptoms of HHT, should be developed to identify patients who are at risk for symptoms elsewhere.

Surgical removal of large complex PAVMs in paediatric HHT patients

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Objective: Percutaneous transcatheter embolization of pulmonary arteriovenous malformations (PAVMs) is recommended by current guidelines. During follow-up, recanalization and/or reperfusion of PAVMs from pulmonary or systemic arteries may occur and lead to severe hemoptysis. Surgery could therefore be considered in selected patients.
Methods: We report three cases of children with HHT and large complex PAVMs who were treated by surgery (lobectomy or bi-lobectomy).
Results: These patients were aged 3, 4.5 and 11.5 years at the time of surgery. All had an *eng* mutation. The 11.5 year old boy presented with large complex PAVMs of the right lower lobe previously treated by embolization (7 procedures). He was admitted in our center for severe hemoptysis. The CT scan revealed systemic supply and recanalization of the PAVMs. He was transferred for surgery in emergency. A bi-lobectomy was performed and the follow-up 7 years later was uneventful. The 3 year old boy had undergone embolizations of a large complex PAVM in the left upper lobe twice before. Because of recanalization and persistent hypoxemia, he was referred to the surgical department for lobectomy. The follow-up at 3 years was uneventful. The 4.5 year old boy presented with a large complex PAVM of the left upper lobe with large feeding arteries during the initial evaluation. Embolization was deemed difficult because of large size of the feeding arteries. A lobectomy was performed without complication with a follow-up of 6 months.
Conclusion: Surgical removal of PAVMs is a therapeutic option in selected children with HHT and large complex PAVMs.

Pneumonectomy in a pediatric patient with Rendu-Osler-Weber sÚndrome with severe pulmonary arteriovenous malformations. Case report

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Objective: To report a case of a 7 year-old patient with hemoptysis and multiple pulmonary arteriovenous malformations (PAVMs) who underwent unilateral pneumonectomy.
Results: We present a 7 year-old HHT girl, who suffered from self-limited hemoptysis episodes after previous pulmonary embolization. Past medical history includes episodes of respiratory distress and cyanosis, misinterpreted as pneumonia, but because of being unresponsive to treatment, a chest CT was performed, reaching the diagnosis of PAVMs in the right lung. Two consecutive pulmonary embolization (PE) were performed, within 2 years. At the age of five, she arrives to our HHT unit with cyanosis, polyglobulia, acropachy, fatigue and headache. The basal pulse oxygen saturation was 83%. The pulmonary angiography showed very large complex-ramified and recanalized PAVMs and an isolated small PAVM (1.5 mm feeding artery) in her left lung. We achieved partial embolization of these, however the oxygen saturation post-procedure was 98%. One year post-PE she reported several self-limited hemoptysis episodes during the last week. On physical examination, she looked well, with normal breathing without symptoms of acute bleeding. An urgent angiography was performed. Multiple bronchopulmonary AV fistulae along the right lung were observed. Due to the characteristics (extension and location) of the fistulae, the artery embolization was not feasible. The patient was discussed, reaching the decision of a right pneumonectomy to avoid life-threating pulmonary bleeding. Right pneumonectomy was performed successfully and she was discharged 7 days later.
Conclusion: Bronchopulmonary fistula may lead to life-threatening hemoptysis in young patients underwent previous pulmonary

embolization. The pneumonectomy may represent an option in selected cases.

PULMONARY AVM DIAGNOSIS

Pulmonary arteriovenous malformations in hereditary hemorrhagic telangiectasia: cross sectional study of 398 patients from Argentine HHT Reference Center

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Objective. To describe Pulmonary Arteriovenous Malformations in patients with Hereditary Hemorrhagic Telangiectasia in our population.
Methods. Cross sectional study from HHT institutional Registry (NCT01761981) of Hospital Italiano de Buenos Aires. We included patients with confirmed HHT based on Curaçao criteria and/or genetic test. Pulmonary shunt was screened with contrast echocardiography (CE) and defined as positive if grade 1 (20 bubbles) or more, after 3 beats. If CE was positive or symptoms were present, PAVMs were screened by means of contrast computed tomography (CT). If any PAVMs was present, the one with the biggest feeding artery was used to classify patients as (1) large PAVMs ($\geq$ 2 mm) or (2) small PAVMs ($<$ 2 mm). Complications were defined as stroke, brain/spinal abscesses and hypoxemia if saturation by pulse oximeter was below 95%.
Results. 398 patients had confirmed HHT, 64% (256) were women. Median age was 45 years (IQR25–75 31–60). 60% (240) of patients had CE performed and was positive in 62% (150). CT was performed in 59% (237) of patients and 45% (107) were positive for PAVMs, of which 38 were large. 21% (18) of the study population presented stroke, 17% (14) brain abscesses and 47% (39) hypoxemia.
Conclusion. Our study does not seem to differ from descriptions of HHT patients from other reports. Embolic complications and hypoxemia were common, therefore reinforcing the need for screening and eventually treating PAVMs.

Comparison of Ferumoxytol MR and CT for the detection of arterial venous malformations (AVMs) in HHT: initial results

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Objective: Screening for AVMs in HHT is predominantly performed using CT, with limited knowledge about the role of MRI. We hypothesize that MR Angiography with Ferumoxytol can serve as an alternative to CT, without exposure to radiation, nephrotoxic contrast agents or gadolinium. In this feasibility study, we compare the technical quality and diagnostic accuracy of Ferumoxytol MR with CT for pulmonary AVM detection.
Methods: Full IRB approval and informed patient consent was obtained. Ten patients with pulmonary artery AVMs who had prior chest CT within 12 months underwent MRI of the chest and abdomen with Ferumoxytol at 3.0T at a dose of 4 mg/kg. Two independent radiologists reviewed MR and CT images to assess AVM site, sac size, feeding artery diameter, draining vein diameter, image quality, artifact grade, vessel visibility of the main, lobar, segmental and subsegmental pulmonary vasculature. Incidental involvement of abdominal organs was also assessed on MRA.
Results: All studies were performed safely with high technical quality and a diagnostic score $>$ 3. Pulmonary arteries and veins were visualized to subsegmental level by CT and MR (score $>$ 4). No difference was found between CT and MR in AVM size, feeding artery and draining vein diameter ($p < 0.05$). Mean AVM sac size is 6.7 mm, feeding artery size 1.8 mm, draining vein size 4.5 mm. Degradation from coil artifact was more significant in CT (score $>$ 3). Multiple liver lesions were identified in two patients on MRA.
Conclusion: Initial results suggest that Ferumoxytol MR will be a practical alternative to CT with equal diagnostic accuracy for the screening of AVMs in HHT, while avoiding repeated exposure to radiation, nephrotoxic contrast or gadolinium.

"Preoperative" assessment of patients with pulmonary arteriovenous malformations and hereditary hemorrhagic telangiectasia

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Objective: Surgery, as required during the lifetime of most individuals with hereditary haemorrhagic telangiectasia (HHT), represents a premeditated injury to the body, resulting in a physiological stress response. During this phase of increased metabolic demand, oxygen consumption typically increases by approximately 5 ml O_2/kg/min: In the general population, an anerobic threshold (AT) $<$ 11 ml/kg/min is considered to indicate "high risk" patients recommended to have critical care organised post-operatively. Our goal was to evaluate anesthetic risk status of patients with HHT and pulmonary arteriovenous malformations (PAVMs) at risk of both hypoxemia and anemia.
Methods: Progressive incremental cardiopulmonary exercise tests (CPET) were performed breathing room air on an unselected cohort of 21 patients with PAVMs and HHT. Subjects were encouraged to achieve their perceived maximum.
Results: Males were aged 24–77 (median 57 years), with hemoglobin 10.8–18.3 (median 16.2) g/dL, and SaO_2 at peak exercise 81–96% (median 91%). Their AT ranged from 4.9 to 33 ml O_2/kg/min: 6/15 (40%) had AT $<$ 11 ml O_2/kg/min. Females were aged 27–68 (median 51 years), with hemoglobin 13.4–16.6 (median 15.9 g/dL), and SaO_2 at peak exercise 74–93 (median 84%). Their AT ranged from 6.0 to 14.3 ml O_2/kg/min: 4/6 (66%) had an AT $<$ 11 ml O_2/kg/min. The patients with AT $<$ 11 ml O_2/kg/min had significantly lower resting SaO_2 ($p = 0.0058$) with less marked difference in resting CaO_2 ($p = 0.073$), peak exercise SaO_2 ($p = 0.044$), and peak exercise CaO_2 ($p = 0.085$).
Conclusion: 10/21 (48%) of patients performing CPET fell into a category considered "high risk" for surgery. Resting SaO_2, reflecting severity of right-to-left shunting, was a better predictor than arterial oxygen content.

Cerebral abscess associated with odontogenic bacteremias, hypoxemia, and iron loading in immunocompetent patients with right-to-left shunting through pulmonary arteriovenous malformations

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Objectives: Cerebral abscess is a recognised complication of pulmonary arteriovenous malformations (PAVMs) that affect at least 50% of people with hereditary hemorrhagic telangiectasia (HHT). Our goal was to identify patients at higher risk of cerebral abscess.

Methods: Between June 2005 and December 2016, at a single institution, 445 consecutive adult patients with CT-scan confirmed PAVMs (including 403 (90.5%) with hereditary haemorrhagic telangiectasia) were recruited to a prospective series. Multivariate logistic regression, and detailed peri-abscess histories were evaluated to identify potential associations with cerebral abscess. Rates were compared to an earlier non-overlapping series.
Results: Thirty-seven (8.3%) of the 445 patients experienced a cerebral abscesses at 19–76 (median 50) years. The rate adjusted for ascertainment bias was 27/435 (6.2%). 29/37 (78.4%) patients had no PAVM diagnosis prior to their abscess, a rate unchanged from earlier UK series. 21/37 (56.7%) cerebral abscess patients suffered residual neurological deficits, most commonly memory/cognition impairment; hemiparesis, and visual defects. Isolation of periodontal microbes, precipitating dental and other interventional events emphasised potential sources of endovascular inoculations. In multivariate logistic regression, cerebral abscess was associated with low oxygen saturation (indicating greater right-to-left shunting); higher transferrin iron saturation index; intravenous iron use; male gender; and venous thromboemboli. There were no relationships with anatomic attributes of PAVMs, risk factors for ischemic stroke in the population (serum iron, fibrinogen, pulmonary artery pressure), or red cell indices often increased due to secondary polycythemia.
Conclusion: Definable subgroups of patients with PAVMs appear to be at higher risk of cerebral abscess.

Age penetrance of pulmonary right-to-left shunt in patients with hereditary haemorrhagic telangiectasia

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Objectives: The clinical presentation of hereditary haemorrhagic telangiectasia (HHT) is age-dependent and varies among patients. Additionally, growth of pulmonary arteriovenous malformations is described in patients with and without treatment. Therefore, we aimed to describe the prevalence of pulmonary right-to-left shunt (RLS) in HHT patients of different ages.
Methods: All consecutive patients with a definite HHT diagnosis based on DNA testing or clinical criteria, screened for HHT between 2004 and 2011 were included. Patients were divided in 4 groups based on age at screening (group 1: 15–29 years, 2: 30–44 years, 3: 45–59 years, 4: > 60 years). Pulmonary RLS were graded with a 3-point scale.
Results: In total 679 patients (57.3% female, age 46.2 ± 15.3 years) were included (N = 119, N = 197, N = 225, N = 138 for age group 1–4, respectively). There was a decrease in presence of pulmonary RLS with increasing age (79.8, 70.1, 62.7, and 58.0% for age group 1–4 respectively, $p < 0.001$). There was a decrease in presence of moderate and large pulmonary RLS with increasing age (pulmonary RLS grade 2 27.7, 19.8, 16.9, 15.9% and pulmonary RLS grade 3 24.4, 24.4, 22.2 and 16.7% for age group 1–4 respectively, $p = 0.015$).
Conclusion: At HHT screening, there is a decrease in prevalence and grade of pulmonary RLS with increasing age. Potentially, patients who are screened at younger age are more severely affected by HHT.

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

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Objectives: 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 center 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 randomly 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 variability for both the presence and grade of pulmonary RLS.

PULMONARY AVM TREATMENT

Endovascular kissing-stenting technique for pulmonary arteriovenous malformations (PAVM) with a short feeding artery at the bifurcation to preserve branch patency

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Aim: Occasionally, hereditary hemorrhagic telangiectasia (HHT) patients present with pulmonary arteriovenous malformations (PAVM) that can be challenging for endovascular interventional therapy. Successful utilization of a covered stent for PAVM with a short feeding artery has been reported. However, a kissing-stenting technique for a bifurcation PAVM lesion with a short feeding vessel to preserve branch vessel patency has not been previously described to our knowledge.
Method/Results: A 71-year-old male with HHT presented with shortness of breath, hypoxemia, epistaxis, and remote episodes of cerebrovascular accidents associated with multiple bilateral PAVMs. He had undergone staged embolotherapies of the bilateral PAVMs and presented for treatment of the remaining PAVMs. Selective left lower lobar pulmonary angiograms demonstrated a PAVM arising from the proximal aspect of the left medial basilar segmental artery, with a short feeding vessel arising immediately beyond its bifurcation with the posterior basilar segmental artery. The feeding artery was too short to accommodate coils or vascular occluder devices. Therefore, a covered balloon-expandable stent was deployed across the feeder

vessel mouth to exclude the PAVM and preserve parent vessel patency. An additional uncovered balloon-expandable stent deployed in the proximal posterior basilar segmental artery using a kissing balloon technique maintained patency of this normal branch vessel. Post-intervention pulmonary angiogram showed complete exclusion of the PAVM sac and preservation of the normal branch vessel.
Conclusion: Use of a stent graft and kissing stent implementation technique is a useful tool for treatment of a bifurcation PAVM lesion with a short feeding vessel in patients with HHT.

Outcomes of sac embolization of pulmonary arteriovenous malformations

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Objective: To evaluate the outcomes of PAVMs treated with sac embolization.
Methods: At a single institution, patients with HHT and PAVMs treated with sac embolization between October 2014 and January 2017 were retrospectively reviewed. Data collected included type of coil used, concurrent feeding artery embolization, sac size, and evidence of persistence on follow up CTA or conventional angiography. Successful treatment was defined as no PAVM persistence (no perfusion of the PAVM sac) on longest available interval follow up contrast-enhanced imaging.
Results: Seven PAVMs in 6 patients were treated with sac embolization. The average preembolization longest dimension sac diameter was 12.8 (range 6.36–20.6) mm. Intraprocedural stasis was achieved in 100%. Feeding artery plus sac embolization was performed in 6 of 7 PAVMs. One patient underwent sac embolization alone. Detachable coils were used in all patients. One patient developed post-procedure transient pleuritic chest pain. Follow up imaging was available in 6 of 7 PAVMs (mean = 216 (range 34–584) days): CT pulmonary angiogram in 4 PAVMs and conventional pulmonary angiograms in 2 PAVMs. There was no persistence on follow up imaging.
Conclusion: Advances in detachable helical and framing platinum coil technology have made sac embolization technically feasible with a low risk of paradoxical device migration. There was no reperfusion of PAVMs treated with sac embolization in our small series. Given the high rate of persistence of PAVMs treated with feeding artery embolization, larger studies should investigate feeding artery plus sac embolization.

Accuracy of a CTA vessel analysis tool for measurement of pulmonary arteriovenous malformation feeding arteries compared to conventional CT and angiography

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Objective: To assess the accuracy of a CTA Vessel analysis tool for measurement of feeding arteries of PAVMs compared to angiography and conventional CT.
Methods: A retrospective review of patients with HHT who underwent pulmonary angiography and embolization was conducted. Feeding artery diameters were measured during embolization using catheter-based calibration (gold standard). We compared these measurements to preprocedure chest CT using calipers in 2D PACS software, and a 3D-based CT measurement tool which measures the average diameter over a region encompassing the site of subsequent embolization (Advantage Workstation 4.6, General Electric Medical Systems). Statistical analysis compared vessel diameter from angiography, CT analysis, and 3D reconstruction. Linear mixed modeling was conducted using absolute difference between modalities and two-tailed *t* tests were conducted for each group (angiography vs. CT, angiography vs. 3D) to determine if the absolute difference was significantly different.
Results: Nineteen PAVM feeding arteries measured by angiography had a mean diameter of 2.94 mm (range 1.69–7.14 mm). By conventional CT measurement, mean diameter was 3.18 mm (range 2.00–5.90), and mean diameter from 3D measurement was 3.09 (range 1.60–6.40). 3D measurements were 0.297 mm more accurate than CT compared to angiography ($p = 0.002$).
Conclusion: Accurate measurement of pulmonary AVM feeding artery diameter is important for determining if a feeding artery of a PAVM is above or below the 2 mm treatment threshold. Our study revealed that the presented 3D analysis tool is significantly more accurate than traditional CT measurements of pulmonary AVM feeding arteries.

Clinical efficacy of hydrogel-coated coils in embolization of pulmonary arteriovenous malformations

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Purpose: The aim of this study is to evaluate the efficacy of hydrogel-coated coils for preventing recanalization after coil embolization of pulmonary arteriovenous malformations (PAVMs).
Materials and Methods: Forty patients with 64 untreated PAVMs underwent coil embolization between May 2011 and October 2016. The mean age was 50 years (range 9–83), and there were 9 male and 31 female patients. The median size of the feeding artery was 3.2 mm (range 1.4–6.1), and the median size of the venous sac was 8.8 mm (range 2.6–36.6). In 15 PAVMs, coil embolization was performed without hydrogel-coated coils using 0.010–0.018-inch bare platinum coils, fibered platinum coils, or both (conventional group), while 0.018-inch hydrogel-coated coils were used in combination with 0.0135–0.018-inch bare platinum and fibered platinum coils in 49 PAVMs (hydrogel group). All patients were followed-up with time-resolved magnetic resonance angiography and/or pulmonary angiography. Recanalization rates were compared between the conventional and hydrogel groups.
Results: Recanalization occurred in 4 PAVMs of the conventional group during a mean follow-up period of 24.5 months (range 1–53). There was no recanalization in the hydrogel group during a mean follow-up period of 16.7 months (range 2–45). The recanalization rates at 3, 6, 12, 24, 36, and 48 months were 7, 21, 21, 28, 28, and 28%, respectively, in the conventional group; the 3-, 6-, 12-, 24-, and 36-month recanalization rates were all 0% in the hydrogel group ($p = 0.0010$).
Conclusion: Hydrogel-coated coils appear to be useful for preventing recanalization after coil embolization of PAVMs.

Outcomes of patients with pulmonary arteriovenous malformations considered for lung transplantation

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Objectives: Pulmonary arteriovenous malformations (PAVMs) may not be amenable to treatment by embolization or surgical resection, and many patients are left with significant hypoxemia. Lung transplantation has been undertaken, though there is no guidance on selection criteria.
Methods: Following prospective recruitment at a single UK institution between 1985 and 2016, characteristics and outcomes for the six cases who were formally referred for lung transplantation assessment were evaluated retrospectively.
Results: Of 755 individuals with PAVMs assessed and treated at our institution since 1985, **six** (0.8%) patients were formally considered for lung transplantation purely for PAVMs. One underwent lung transplantation and died peri-operatively. The other five were not transplanted, in four cases at the patients' request. Their current survival ranges from 16 to 27 (median 21) years post transplant assessment compared to median survival figures of 5.7 years for adults, 5.4 years for children in the latest International Society for Heart Lung Transplantation (ISHLT) figures ($p < 0.01$), and the 10 year survival rates (48%) reported at one UK transplant centre ($p < 0.01$). The 4 "transplant-considered" cohort who had declined lung transplantation all confirmed their decision on multiple occasions, including at their most recent follow-up assessment. All stated a strong preference for their existing health state, despite additional PAVM complications since their transplantation evaluations. One noted that they had outlived all of their original cohort at the lung transplant clinic.
Conclusion: In view of non-transplanted patients' longevity, a very strong case must be made before lung transplantation is considered.

ASSORTED TOPICS OF HHT

Vascern HHT survey 2: drug registry-part 1

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Objectives: A recommendation of the auditing authority to European Reference Network for Rare Multisystemic Vascular Diseases (VASCERN), was to foster information on safety standards for rare disease patients. The HHT working group prioritized antiangiogenic drugs Thalidomide (TH) and Bevacizumab (BZB, Avastin), that have been increasingly used in the latest decade in HHT patients.
Methods: A VASCERN HHT Survey (Drug Registry-Part 1) was proposed to members of VASCERN HHT (patients, scientists and health care professionals (HCPs) who were asked to summarize their experience, if any, with these drugs.
The online questionnaire included 4 questions dedicated to patients, 2 to scientists, and 13 to HCPs focused on direct or indirect experience with BZB and TH, ranges of treated patients, efficacy, and safety evaluation.
Results: Of 15 respondents, 14 completed the questionnaire: 3 patients, 9 HCPs, 3 scientists. No patient had experience of either BZB or TH. 2 scientists reported awareness of effects of BZB or TH in HHT patients. HCPs reported direct experience of treatment with BZB and TH, in < 5, 6–20, 20–50, > 50 patients in 4, 1, 1, 0 centers, and in 2, 1, 3, 0 centers respectively. Table 1 summarises reported direct and indirect experience with BZB/TH.
Conclusion: Within VASCERN HHT there is a greater experience with TH than with BZB. Greatest agreement regarding BZB was on reduction of transfusion dependency, and for TH, is on its ability to reduce epistaxis. For safety, TH scored worse than BZB. The follow-up Drug Registry-Part 2 will be reported at the HHT International Conference.

Emergency department National Statistics for Hereditary Hemorrhagic Telangiectasia

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In the United States of America, outcomes of hospitalization of hereditary hemorrhagic telangiectasia (HHT) patients include high rates of bleeding-related complications: anemia, epistaxis, and gastrointestinal bleeding. Nationwide Inpatient Sample between 2000 and 2012 have shown potentially life-threatening manifestations and morbidities of HHT associated with AVMs in lungs, brain, liver, and spine. Rates of bleeding complications accounted for two-thirds of HHT-related complications. Up to 40% of HHT patients required hospital admissions for one or more transfusions of a blood product. The objective of this study is to present trends of emergency department (ED) visits and admissions from ED to the same hospitals using a large, multihospital inpatient database in the U.S. (Healthcare Cost and Utilization Project or HCUP).
Methods: Specifically, data from 2006 to 2014 Nationwide Inpatient Sample were evaluated using the ICD-9 code (448.0) for HHT in the U.S. Weighted national estimates from HCUP National (Nationwide) Emergency Department Sample (NEDS), [2006–2014], Agency for Healthcare Research and Quality (AHRQ), based on data collected by individual States and provided to AHRQ by the U.S. Total number of weighted visits in the U.S. based on HCUP NEDS = 120,033,750 (2006); 122,331,739 (2007); 124,945,264 (2008); 128,885,040 (2009); 128,970,364 (2010); 131,048,605 (2011); 134,399,179 (2012); 134,869,015 (2013); undefined (2014). Statistics based on estimates with a relative standard error (standard error/weighted estimate) greater than 0.30 or with standard error = 0 in the nationwide statistics (http://www.hcup-us.ahrq.gov).
Results: The demographic features of the participating hospitals were categorized into teaching ($\sim$ 2/3)/non-teaching ($\sim$ 1/3), rural ($\sim$ 10%)/urban ($\sim$ 90%), and trauma center/non-trauma center (variable). Private, not for profit type hospitals have taken up for up to $\sim$ 75%, private for profit type and government type hospitals $\sim$ 10–15% each. The review indicated that the trends of ED visits and admissions to the same hospitals for HHT patients increased over time in the U.S. from 2006 to 2013 and dropped in 2014 (attached Fig. 1). The total ED visits were 3751 in in 2006 and 5034 in 2014. The total number of admission from ED to the same hospitals were 2531 in 2006 and 3108 in 2014. The rates of discharged ED visits have increased from 2006 to 2013 and plateaued in 2014.

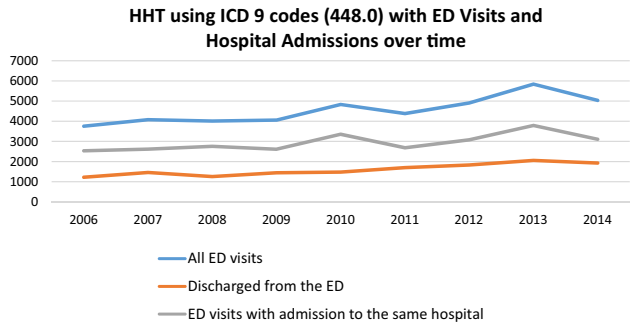

Fig. 1 HHT using ICD 9 codes (448.0) with ED Visits and Hospital Admissions over time

Conclusion: These findings have important clinical implications, as clinical providers should be aware of the epidemiology of health care system utilization of HHT patients in the U.S. Bleeding-related complications are likely the most common causes of these ED visits and hospital admissions. Due to the natural history and trends of increased attention to healthcare in our presenting data, a further study is required to analyze the indications for ED visits, discharged patients from ED visits and admission. This may help to improve the care of HHT patients and reduce the healthcare burden.

Alterations of the innate and adaptive immunity in HHT patients

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Objectives. Hereditary hemorrhagic telangiectasia (HHT) is associated with increased risk of bacterial infections. Literature data concerning the impairment of innate or adaptive immune responses in HHT are conflicting. Therefore, we aimed to reveal the immune system modulation in HHT patients.
Methods. Main WBC populations and lymphocyte subpopulations were analyzed in peripheral blood of non-leucopenic 30–70 years old HHT patients (n = 29) versus healthy controls (n = 16) using flow cytometry. Activation status of isolated neutrophils (CD11b, CD63, CD62L expression), ROS production and chemokine (IL-8, MIF, MIP1b) release in response to *P. aeruginosa* (PA14), migration to LPS and *P. aeruginosa* supernatant were estimated.
Results. Increased neutrophil and monocyte amounts and decreased lymphocyte amounts as well as the disproportion in lymphocyte subpopulations with relative decrease in T cells, especially CD4+, and increase in NK cells are observed in HHT patients compared to controls (all $p < 0.05$). Above-mentioned alterations increase with age and are not associated with iron or RBC (erythrocyte count, hemoglobin level) changes. Surprisingly, we observe increased antibacterial activity of neutrophils in HHT (enhanced CD11b expression, ROS production, chemokine release in response to bacteria and higher migratory capacity) compared to controls ($p = 0.04$–0.09).
Conclusion: Our results suggest that both innate and adaptive immunity are affected in HHT individuals. Surprisingly, neutrophils in HHT show enhanced response to bacterial stimuli while such individuals have increased susceptibility to bacterial infections. The contribution of observed neutrophil pre-activation in immune 'exhaustion' and impairment during infection is an interesting aspect for further studies.

RiTHHa registry: a national computerized regisry of patients with hemorrhagic hereditary telangiectasia in Spain

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Aim: To create a computerized broad database of patients with Hemorrhagic Hereditary Telangiectasia (HHT) attended at spanish hospitals which might be helpful in the clinical practice and to describe the clinical characteristics and manifestations of first patients introduced.
Methods: The ongoing RiTHHa (Registro Informatizado de la Telangiectasia Hemorrágica Hereditaria) is a computerized, observational registry that provides a prospective cohort of patients with HHT, derived from multiple Spanish hospitals since June 1st, 2016. All patients provided written consent for participation in the RiTHHa Registry, in accordance with Ethics Committee of the Hospital Universitari de Bellvitge, Barcelona, Spain. Data were recorded on a computer-based case report form at each participating hospital through a secure website.
Results: As of December 2017, 96 patients with HHT were included (34.4% male), mean age 52.4 ± 17.4 years. Initial symptom was nosebleed in 87 (90.6%) patients. Regarding to Curaçao Criteria, 91 (94.8%) patients had recurrent epistaxis, 84 (87.5%) had muco-cutaneous telangiectasia, 62 (64.6%) had visceral involvement and 80 (83.3%) had family history of HHT. HHT was considered "Definite" according to Curaçao Criteria in 79 (82.2%) patients and with a positive genetic test in the remainig patients. Genetic test was undergone in 39 patients, 11 were positive for ENG and 28 for ALK1. Anemia was present in 50 (52.1%) patients and 22 (22.9%) required red blood cell transfusion and 35 (36.5%) required attention at Emergency Department for recurrent epistaxis. Transthoracic contrast echocardiography (TTCE), were positive in 33 (60%) out of 55 patients. Of these, 25 patients had pulmonary arteriovenous malformations (PAVM) at thoracic CT. Out of 45 patients with abdominal CT, 33 (74%) had hepatic involvement, mostly widespread small diffuse liver telangiectasia (25 patients) and arteriovenous shunts (in 10). Focal nodular hyperplasia were seen in 3 patients and nodular regenerative hyperplasia in one. Eight (8.3%) patients had cancer, 4 (4.2%) atrial fibrillation, 2 (2.1%) mechanical valves, 6 (6.3%) history of venous thromboembolism and 9 (9.4%) ischaemic stroke or brain abscess.
Conclusion: The ongoing RiTHHa Registry will provide data on patients with HHT in a real-world situation. Data from RiTHHa will be hypothesis-generating and provide feedback from real-world clinical situations, such as HHT and cancer or needing for anticoagulant therapy.

How much do physicians know about HHT? A survey-based cross sectional study

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Objective: To estimate the level of medical information on HHT in Buenos Aires.

Methods: We performed a cross-sectional study in public and private hospitals in Buenos Aires city. HHT-majored physicians were excluded. We carried out an anonymous, self-administered paper questionnaire with 6 items: inheritance patterns (sex-linked or not, dominant or recessive), diagnosis method, Curaçao criteria (CC), the prevalence range and possible affected organs. Answering four or more questions correctly was considered appropriate information on HHT.
Results: We included 150 physicians. Median age was 34 years (IQR 29–44), 70 (47%) were female, the median time since graduation was 7 years (IQR 3–19). One hundred and twenty-three (82%, 95% CI 75–87%) had never treated HHT patients. Concerning general information, 69 (46%, 95% CI 38–54%) knew about: the gender predominance, 37 (25%, 95% CI 18–32%), the inheritance transmission, 35 (23%, 95% CI 17–30%), the prevalence and 53 (35%, 95% CI 28–43%), the diagnosis. Regarding the level of information on CC, 54 (46%, 95% CI 28–43%) knew about: epistaxis as one of the diagnostic criteria, 48 (32%, 95% CI 24–39%) family history, 74 (49%, 95% CI 41–57%) arteriovenous malformations, 96 (64%, 95% CI 55–71%) telangiectases. Concerning organ affection, 31 (20%, 95% CI 14–28%) knew about: the hepatic affection, 48 (32%, 95% CI 24–40%) the nose being affected, 89 (59%, 95% CI 51–67%) skin and mucosae affection and 50 (33%, 95% CI 22–38%) the digestive tract being affected. Knowledge on HHT was 11% (17, 95% CI 7–17%). In our center, home to an HHT Unit, knowledge was 8.5% (5, 95% CI 3–19%).
Conclusion: Appropriate information on HHT was lower than that on other rare diseases with equal or less prevalence, even at the institution home to an HHT Center.

Pathological spectrum of hepatic involvement in hereditary hemorrhagic telangiectasia (HHT): cross-sectional study at Argentine Reference Center

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Objectives: To describe the spectrum of hepatic disease in patients with HHT.
Methods: Cross-sectional study of the Institutional HHT Registry, Hospital Italiano de Buenos Aires (2010–2016).
Results: Three hundred and thirty-six patients (336) with confirmed HHT were included, 71% (239) had liver assessment performed, 54% (129/239) by ultrasound and 60% (145/239) contrast-multislice computed tomography. The prevalence of hepatic vascular malformations (HVMs) was 63% (151, 95% CI 56%–69%), including 57 (38%) cases with vascular fistula (VF) of which 19 (33%) had more than one type. The most common VF were the arteriovenous fistulae (75%). One hundred and forty-four 144 (95%) presented telangiectases, 56 (37%) confluent vascular masses and 47 (31%) dilated hepatic artery. Thirteen patients (5.4%) had nodular hyperplasia and 9 (6%) bile duct dilatation (BDD). One hundred and thirty-three (88%) underwent echocardiography assessment, 10 (7.5%) exhibited right ventricular enlargement, 9 (6.8%) pulmonary hypertension and 28 (21%) patients exhibited abnormal liver function test.
Symptoms were observed in 31/151 (20%), 10/31 (32%) suffered from hepatic pain, 16/31 (51%) congestive heart failure, one presented symptomatic biliary necrosis and 10/31 (32%) patients suffered from atrial fibrillation. Sixteen symptomatic patients received treatment with cardiotonics or diuretics and 4 patients received bevacizumab. None of them underwent surgical or interventional procedures. Three patients were assessed for liver transplantation and one of them died due to severe hepatic affection.
Conclusion: The prevalence of hepatic involvement in our population is close to the reported. We found a higher rate of symptomatic hepatic involvement.

A new kind of digestive arteriovenous malformation after endoscopic treatment in HHT patients. A second hit consequence? A case report

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Aim: To report atypical vascular gastric lesions after argon plasma coagulation (APC) treatment.
Case: We present a 50-year-old HHT female patient who suffered from mild epistaxis and longtime of severe refractory anemia (mean hemoglobin 6 g/dl). The first upper endoscopy (UE) we performed showed multiple and large bleeding gastric telangiectases. All of them were treated with adrenaline and APC. Three months later, a second UE showed recurrent multiple gastric telangiectases (diameter 4–10 mm) which were treated also with APC. After endoscopic treatment, she received 3 g daily of tranexamic acid without adherence. The hemoglobin levels never improved despite aggressive iron therapy. Four years later she was admitted due to hematemesis. Urgent UE evidenced gastric large, multiple, elevated and ulcerated bleeding vascular lesions with flat pigmented spots and clean base. No endoscopic treatment was applied but we added propranolol (40 mg/bid), esomeprazole (40 mg/bid) and bevacizumab (5 mg/kg dose). A biopsy ruled out other cause of gastric ulcers. In an early control, the skin and tongue telangiectases became smaller and the hemoglobin levels increased to 11 g/dl. No recurrence of epistaxis or overt digestive bleeding occurred. She could not complete the remaining bevacizumab sessions and after 2 months of the single dose, hemoglobin levels decreased to 9 g/dl.
Conclusion: As far as we know, there are no previous reports of late atypical vascular lesions after endoscopic treatment with APC in HHT patients. This case shows the importance of carefully consider an aggressive endoscopic treatment in patients with severe gastrointestinal affection.

Association between iron deficiency and leukopenia in HHT

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Objective: To assess the association between iron deficiency (ID) and leukopenia in HHT adult patients.
Methods: Cross sectional study from Hospital Italiano de Buenos Aires HHT Institutional Registry (NCT01761981) which is prospectively filled. We included HHT confirmed patients by available laboratory test. We evaluated all the results of ferritin and hemograms of the included patients between 2010 and 2016.
We excluded laboratory tests associated with transfusion, diagnosis/treatment of cancer, infections, proinflammatory states, bevacizumab treatment and pregnancy.

We define leukopenia when the white blood cells count was below $< 4000/mm^3$ and ID as ferritin dosage < 20 ng/dl. We used a linear regression model of mixed effects considering the ordinate to the origin as random.

Results: We analyzed 610 blood tests corresponding to 162 patients (1–36 tests per patient) of which 77% were women. The mean age was 55 (SD 18).
The prevalence of leukopenia was 16% (95% IC 13–19). The median of leukocytes count was 5360/mm^3 (IQR 25–75 4330–6810). Twenty-seven patients (16.6%) developed, at least, a leukopenia event. The median of ferritin was 17 ng/dl (IQR 25–75 7–35). Ninety-two patients (56.7%) presented, at least, a determination of iron deficiency.

The evaluation of the association between iron deficiency and white cells count presented a coefficient of −52 leukocytes/mm^3 (CI 95% − 258 to − 157, p 0.637). The OR of iron deficiency for leukopenia was 1.19 (95% CI 0.799–1.8, p 0.380).
Conclusion: We found no statistically significant association between the status of iron deficiency and leukopenia. However, the research on other causes of leukopenia in HHT must continue.

Dose dependent effect of 7-ketocholesterol treatment on the expression of the membrane endoglin and cell adhesion molecules in human aortic endothelial cells: pilot study

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Objectives: Changes of cell adhesion molecules and membrane endoglin expression in endothelium were proposed to reflect hypercholesterolemia in vivo. P/E-selectins, vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1) mediate adhesion and transmigration of leukocytes through endothelium in early endothelial dysfunction development. Endoglin (CD105, TGF-βRIII receptor), expressed by vascular endothelium, was shown to be affected by cholesterol levels in mice suffering with atherosclerosis. In this pilot study, we tested the effect of 7-ketocholesterol (7-K, oxidized cholesterol) on the expression of P/E-selectins, VCAM-1, ICAM-1 and endoglin in Human aortic endothelial cells (HAECs) in order to mimic situation during early endothelial dysfunction development caused by oxidized cholesterol.
Methods: (HAECs) were exposed to 7-K (1, 5, 10 μg/mL) for 12 h. Protein expression of ICAM-1, P/E-selectins, VCAM-1 and membrane endoglin was evaluated by immunofluorescence flow cytometry analysis.
Results: Significantly stronger basal expression of ICAM-1 was detected when compared with P/E-selectins and VCAM-1 expression in HAECs. 7-K in dose 10 μg/mL significantly increased expression of ICAM-1, P/E-selectins and membrane endoglin after 12 h treatment when compared to non-treated cells. In addition, 5 μg/mL 7-K treatment significantly increased expression of ICAM-1 and endoglin when compared to non-treated cells.
Conclusion: In this study, we demonstrated that 7-K induced pro-inflammatory reaction in endothelial cells and increase membrane endoglin expression. We propose that membrane endoglin might be involved in early development of endothelial dysfunction. Moreover, these results suggest that this experimental design is suitable for our prospective experiments focusing on endothelial dysfunction and endoglin.
This work was supported by grants from Czech Science Foundation (GACR 15-24015S, GAUK 1158413C and SVV/2016/260293).

A retrospective review of a hereditary hemorrhagic telangiectasia center's first year experience

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Objectives: This is a retrospective study of a new HHT center's patient population. The study reviewed the prevalence and severity of HHT diagnostic consensus criteria. The prevalence of past screening was reviewed, as well as patient adherence to further screening. Past IR therapeutics for AVMs were quantified. The prevalence and variety of subspecialty care was reviewed.
Methods: Retrospective review of medical records occurred for patients referred to our HHT Center and appeared for consultation from 01/01/2016 to 01/31/2017. Data was collected from patient history, examination, and imaging reports.
Results: 50 patients were included. 96% suffered from epistaxis, with a mean Epistaxis Severity Score of 5.17. 58% received epistaxis medical therapy while 52% received laser, cauterization, or IR/surgical intervention. 80% exhibited mucocutaneous telangiectasias and 98% reported familial involvement. 40% reported or displayed AVMs on imaging, with an overlapping 20% involving the brain, 70% involving the lungs, and 25% involving the liver. 24% reported positive bubble echocardiograms or had confirmed late shunting while 10% exhibited an RVP > 39. After consultation, 26–32% of patients without a component of AVM screening opted for additional screening. On serial imaging, 8% exhibited growth or appearance of new pulmonary AVMs. 20% underwent pulmonary angiography of which half underwent PAVM embolization. 8% underwent facial artery embolization and 2% underwent cerebral embolization. 42% had current subspecialty care for epistaxis and anemia while 56% requested further subspecialty referral.
Conclusion: Patients predominantly underwent management for epistaxis while screening for visceral AVMs were relatively low. However, after HHT consultation, patient screening rates substantially increased.

Epidemiology of hereditary haemorrhagic telangiectasia in Spain: experience of the HHT unit in hospital Sierrallana

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Objectives: To describe epidemiological characteristics of a wide cohort of Spanish patients with hereditary hemorrhagic telangiectasia (HHT)/Rendu-Osler-Weber disease.
Methods: Between 1 January 2002 and 31 December 2013, 667 Spanish patients with suspected HHT were evaluated in the reference HHT Unit in Hospital Sierrallana and 449 were diagnosed by clinical Curaçao criteria and/or genetic test. Screening protocol to disclose organ affectation and genetic test was performed.
Results: The diagnostic sensitivity of Curaçao clinical criteria in the population studied was 94.59%. Prevalence was 1:5936 people and lethality rate of 0.16%. Type 2 HHT was the most prevalent and in total 147 different mutations were identified. Epistaxis was the most

prevalent symptom (96.88% of cases) while 95.18% of patients showed typical telangiectasias. No significant differences were observed regarding epistaxis frequency considering sex and genetics with 96.88% of patients affected. HHT2 patients have a later onset of epistaxis than HHT1 ones. Nasal telangiectases were more complex in women and elderly patients. Pulmonary involvement was present in 28.25% of patients (regarding computed tomography) mainly in women and HHT1 cases while liver affectation was more prevalent in HHT2 cases. Brain involvement was disclosed in 28.35% of cases. Telangiectasias in conjuctival mucose were very frequent mainly in HHT1 elderly patients.
Conclusion: This series shows results about prevalence, genetic distribution and organ affectation of a broad non-previously evaluated population based on patients studied in the HHT Unit of Hospital Sierrallana. Some new observations can help guide the diagnostic and screening procedures for these patients.

Euroqol 5D test to evaluate quality of life in patients with hereditary haemorrhagic telangiectasia (HHT) in Spain

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Objectives: To assess the quality of life in a population of Spanish patients with HHT and compare it with the general population.
Methods: Between January 1st 2005 and December 31st 2013, 187 adult patients diagnosed with HHT who were admitted to the HHT Unit in Hospital Sierrallana, completed on their first visit, the EuroQol 5D-3L quality of life descriptive test and the visual analog scale (VAS). The numerical social index value was also determined and the subjective effect of the nasal epistaxis on their quality of life was estimated classified as mild, moderate or severe.
Results: Patients with HHT had greater problems than general population in the five dimensions of the EuroQol 5D-3L, above all, considering pain/discomfort and anxiety/depression. In the VAS and the social index value, patients with HHT also scored lower than the general population, particularly older patients, males, and patients with HHT2. They also had values similar to those of populations with chronic diseases. The subjective perception of the severity of epistaxis correlated strongly with the VAS and social index values.
Conclusion: The quality of life of patients with HHT, estimated using the EuroQol 5D-3L scale, is affected across all dimensions. The scores are similar to those observed in chronic diseases. Older patients, males and carriers of the ACVRL1 mutation generally have worse scores on these scales. The EQ-VAS and the social index value are index that correlate well with the severity of the clinical symptoms associated mainly with epistaxis.
(*Health Qual Life Outcomes* 2017 Jan 23;15(1)).

High content imaging and structural profiling to identify repurposable small molecules for the treatment of HHT

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Objectives: At Recursion Pharmaceuticals, we have developed an unbiased, target-agnostic drug screening technology that is ideally suited for rare monogenic loss of function diseases that have failed to benefit from traditional drug discovery techniques. Genes associated with loss of function monogenic diseases are knocked down with multiple independent siRNAs in multiple human cells, and structural phenotypes are identified using multi-parametric fluorescence image analysis.
Methods: Thousands of measurements are obtained at the single-cell level for tens of thousands of cells per model, and on-target shifts in specific features (e.g. size, shape, texture of organelles) among the cell population are identified. We used this technology to create screenable assays based on the phenotypic fingerprint ('phenoprint') composed of cellular features unique to the hereditary hemorrhagic telangiectasia (HHT) disease state upon ACVRL1 and SMAD4 loss of function.
Results: A screen of our partner libraries revealed a small number of repurposable hits for both genes and we are currently progressing the hits through our validation pipeline.