



## 11th International HHT Scientific Conference

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**Akiko Hata, PhD**

*Professor, Cardiovascular Research Institute  
University of California, San Francisco*

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*Associate Professor of Medicine  
Director, Pulmonary Hypertension Program  
University of Chicago*

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**Edda Spiekeroetter, MD**

*Assistant Professor of Medicine, Pulmonary & Critical Care  
Medicine  
Stanford University*

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Hepatology, Nutrition  
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**Alan E. Guttmacher, MD**

*Director  
Eunice Kennedy Shriver National Institute of Child Health and  
Human Development  
National Institutes of Health*

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**Rong Wang, PhD**

*Mildred V. Strouss Endowed Chair in Vascular Surgery  
Director, Laboratory for Accelerated Vascular Research  
University of California*

## ORAL COMMUNICATIONS

### SESSION 1 MECHANISMS OF HHT I

**ORI Essential roles for transforming growth factor-beta superfamily proteins during endothelial cell-pericyte capillary tube co-assembly in 3-dimensional matrices**

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**Objectives:** Capillary network formation requires coordinated molecular signals that promote endothelial cell (EC) tubulogenesis, pericyte recruitment to EC tubes and vascular basement membrane assembly. Without pericytes, capillary networks can become unstable and undergo regression. Hereditary hemorrhagic telangiectasia (HHT) is a vascular disorder that arises due to capillary network abnormalities, and genetic mutations in the transforming growth factor (TGF)- $\beta$  family of ligands and receptors in ECs have been documented. We hypothesize that such mutations cause abnormal communication between ECs and pericytes, resulting in adverse effects on capillary formation and maturation, which leads to capillary regression.

**Methods:** We used siRNA transfection of ECs or pericytes, growth factor additions or chemical drug inhibitors to investigate TGF $\beta$  signaling, including TGF $\beta$  isoforms, ActivinB, bone morphogenic proteins (BMPs), their receptors and regulators. To test these molecules, we performed assays of capillary morphogenesis in 3-D matrices with ECs alone or EC-pericyte co-cultures under serum-free, growth factor-defined conditions.

**Results and conclusions:** In summary, we find that disruption of ligands and receptors essential for TGF $\beta$ , ActivinB and BMP signaling markedly alter EC tubulogenesis and/or pericyte recruitment, resulting in abnormal capillary formation and maturation. In fact, these factors promote regression of EC-only tubes (i.e., without pericytes) and, therefore, select for EC tubes with abluminally-polarized pericytes, leading to proper vessel maturation. Additionally, EC-derived TGF $\beta$  and ActivinB appear to play an important role in mediating BMP9-dependent tube regression in this system. This defined in vitro model shows great promise in elucidating the underlying pathogenic basis for vascular anomalies such as HHT.

## OR2 Association of polymorphisms in TGF $\beta$ modifier loci with disease severity phenotypes in hereditary hemorrhagic telangiectasia

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**Objectives:** Hereditary hemorrhagic telangiectasia (HHT) patients have variable phenotypes including chronic or severe bleeding and organ vascular malformations (VM), which lead to complications including intracerebral hemorrhage (ICH) from brain VM. Factors underlying the phenotypic heterogeneity of HHT are poorly understood and a role for genetic modifier effects has been hypothesized. Common polymorphisms in loci that modify phenotype severity in *Tgfb* knockout mice have been reported to be associated with pulmonary AVM in HHT. We sought to replicate these associations and investigated whether these polymorphisms are associated with other HHT disease severity phenotypes.

**Methods:** We genotyped 4 polymorphisms (*PTPN14* rs2936018, *USH2A* rs700024, *ADAM17* rs10495565, *ADAM17* rs12474540) in 749 Caucasian HHT patients enrolled by the Brian Vascular Malformation Consortium. Association of genotype with phenotype (pulmonary, liver, brain VM, ICH presentation of brain VM, gastrointestinal bleeding, anemia) was evaluated by multivariate logistic regression adjusted for age, gender and family clustering, and stratified by HHT mutation (*ENG* or *ACVRL1*).

**Results:** None of the 4 polymorphisms was significantly associated with any VM type or bleeding. *USH2A* rs700024 was associated with ICH presentation of brain VM (OR = 3.15, 95 % CI 1.21–8.19,  $p = 0.018$ ). Among *ENG* mutation patients only, *PTPN14* rs2936018, *ADAM17* rs10495565 and *ADAM17* rs12474540) were associated with liver VM (OR = 3.06–2.15,  $p = 0.002$ – $0.021$ ).

**Conclusions:** We did not replicate previously reported associations with pulmonary AVM in HHT. *USH2A* rs700024 was associated with ICH presentation of brain VM, while three polymorphisms were associated with liver VM among *ENG* mutation patients only, suggesting that these variants may modify different severity phenotypes in HHT.

## OR3 Endoglin is crucial for atorvastatin induced eNOS expression in endothelial cells in vitro

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**Objectives:** Endoglin was shown to be co-expressed with eNOS in aortic endothelium in atherosclerotic plaques and upregulated by atorvastatin (ATV) treatment in mice. In this study, we focused on endoglin and eNOS expression during inflammation and after ATV treatment. We hypothesized whether inflammation modulates ATV-dependent induction of endoglin and eNOS expression in vitro in endothelial cells and whether ATV-induced eNOS expression is regulated via endoglin.

**Methods:** Human umbilical vein endothelial cells (HUVECs) were exposed to TNF $\alpha$  or/and ATV treatment. In ATV pretreatment model, cells were treated 24 h by ATV, and then cultured with TNF $\alpha$  for 16 h. The protein expression of selected markers was examined by flow cytometry and Western blot analysis and soluble endoglin levels in medium were measured by means of ELISA. Gene expression of endoglin was examined by qRT-PCR.

**Results:** ATV treatment significantly increased endoglin and eNOS expression, and interestingly it was able to prevent its TNF- $\alpha$ -mediated down regulation. Suppression of endoglin using small interfering RNA (siRNA), but not inhibition of TGF- $\beta$  signaling with SB431542, abrogated ATV-induced eNOS expression. ATV treatment did not change the expression of p-Smad2.

**Conclusions:** Our results showed that inflammation results in reduced expression of endoglin and eNOS in HUVECs, which could be prevented by ATV treatment. Moreover, ATV induced eNOS expression seems to be dependent on endoglin expression, but not on Smad2. Possible implications of this finding might be reflected in pathological conditions characterized by reduced levels of endoglin and eNOS as for example in hereditary hemorrhagic telangiectasia or in other endothelial dysfunctions.

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## SESSION 2 TREATMENT OF HHT I

### OR4 Nosebleeds and migraines are precipitated by similar dietary and environmental factors in patients with hereditary hemorrhagic telangiectasia

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**Objectives:** To identify safe preventative and therapeutic options for nosebleeds in hereditary hemorrhagic telangiectasia (HHT): Little is known of why people with HHT have nosebleeds at particular times. When discussing the results of our earlier study [1], several patients spontaneously commented that their nosebleeds seemed to be provoked by similar foods as migraines. Since there are more therapeutic options to prevent migraines, our objective was to test objectively whether there were any similarities between factors provoking these two, very distinct, vascular pathologies.

**Methods:** Study participants were recruited online following advertisement by the HHT Foundation International. They completed a

nonbiased questionnaire in which paired questions on nosebleeds and migraines were separated by at least 17 other questions. Reported frequencies and precipitants of epistaxis and migraines were compared using numerical scales applied equally for each condition.

**Results:** The 220 HHT-affected respondents reported frequent nosebleeds, 153 (69.5 %) used iron tablets, and 39 (17.7 %) had received at least 10 blood transfusions. Migraines displaying typical features were reported by 51 (23.2 %), and were more common with pulmonary or cerebral arteriovenous malformations. Thirty of 51 (58.8 %) migraine sufferers reported that nosebleeds occurred at the same time as their migraines. More frequent migraines were reported by patients with more frequent nosebleeds ( $p = 0.007$ ), or transfusions ( $p = 0.004$ ). Premenstrual states, lack of sleep, stress, caffeine, cheese, alcohol, and chocolate precipitated migraines and nosebleeds in similar proportions of HHT patients.

**Conclusions:** Anti-migraine approaches may provide further preventative and therapeutic options for HHT nosebleeds.

## Reference

[1] Silva et al., *Laryngoscope* 2013; 124(7):1521–1528.

## OR5 Bazedoxifene: a new orphan drug for the treatment of bleeding in hereditary haemorrhagic telangiectasia (HHT)

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**Background:** Hereditary haemorrhagic telangiectasia (HHT), or Rendu–Osler–Weber syndrome, is a dominant genetic vascular disorder. In HHT, blood vessels are weak and prone to bleeding, leading to epistaxis and anaemia, affecting severely patient's quality of life. Development of vascular malformations in HHT patients is originated mainly by mutations in *ACVRL1/ALK1* (activin receptor-like kinase type I) or *Endoglin (ENG)* genes. These genes encode proteins of the TGF- $\beta$  signalling pathway in endothelial cells, controlling angiogenesis. Haploinsufficiency of these proteins is the basis of HHT pathogenicity.

**Objectives:** Study the efficiency of Bazedoxifene, a selective estrogen receptor modulator (SERM) in HHT, looking for a decrease in epistaxis, and understanding the underlying molecular mechanism.

**Methods:** Plasma samples of 5 HHT patients were collected before, and after 1 and 3 months of Bazedoxifene treatment. *ENG* and *ALK1* expression was quantified in activated mononuclear cells derived from blood, as well as VEGF plasma levels. Quantification of *endoglin* and *ALK1* mRNA was done in endothelial cells derived from HHT and healthy donors, after in vitro treatment with Bazedoxifene. Angiogenesis was also measured by tubulogenesis and wound healing assays.

**Results:** Upon Bazedoxifene treatment, haemoglobin levels of HHT patients increased and the quantity and frequency of epistaxis decreased. Bazedoxifene increased *endoglin* and *ALK1* mRNA levels, in cells derived from blood samples and in cultured endothelial cells, promoting tube formation.

**Conclusions:** Bazedoxifene seems to decrease bleeding in HHT by partial compensation of haploinsufficiency. These results are the basis

of a new orphan designation for HHT by the European Medicine Agency (EMA).

## OR6 An old drug for a new application: carbazochrome–sodium–sulfonate in HHT

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Hereditary hemorrhagic teleangiectasia (HHT, Rendu–Osler–Weber syndrome) is a dominantly inherited genetic vascular disorder in which epistaxis is the most frequent manifestation, responsible for high morbidity and very poor quality of life (1). Unfortunately, management of this symptom has no standard care and local treatments are often aggressive such as laser procedures, septodermoplasty intervention or surgical closure of the nostrils (2). Therefore, the most important efforts are inclined to minimize bleeding as well as blood transfusion therapy avoiding as much as possible invasive procedures. In this setting, blood and hormone therapy and antifibrinolytic treatment are used with unclear efficacy (3). Recently, experimental studies on anti-angiogenic drugs are ongoing, but their efficacy has not been proven.

We report a collection of HHT patients showing an impressive response during treatment with an old haemostatic drug with capillary stabilizing action, carbazochrome–sodium–sulfonate (AC-17), never tested before in such patients. Carbazochrome–sodium–sulfonate is a capillary stabilizer and used clinically for the treatment of hemorrhage due to capillary's fragility. Its mechanism of action is still entirely unknown, but seems that it may modulate blood's fibrinolytic balance through changing the endothelial cells function. Carbazochrome had various applications in bleeding disorders (4).

We successfully treated, orally, ten patients (3 male, 7 female; median age of 42.8) who had HHT with carbazochrome–sodium–sulfonate 50 mg twice per day for 2 months. Patients underwent epistaxis severity score (ESS) validate questionnaire pre and post treatment.

We observed a reduction of the scores in all patients and in particular the pre-treatment mean score was of  $6.4 \pm 2.1$  versus a post treatment value at 1 month of  $4.9 \pm 1.8$  and after 2 months of  $3.4 \pm 1.34$ , with a significant statistical difference ( $p < 0.05$ ). Furthermore the mean hemoglobin level increased after 1 months from  $9.04 \pm 1.6$  g/dL to  $9.92 \pm 1.31$  and after 2 months to  $10.93 \pm 1.05$  g/dL; the statistical analyses showed that the differences reached levels of significance ( $p < 0.05$ ).

To our knowledge, finally, this is the first report on the potential beneficial effect of carbazochrome–sodium–sulfonate in treating HHT. Our observation indicates that its effect on the endothelial

barrier dysfunction through inhibition of agonist-induced phosphoinositide hydrolysis could be involved in the pathogenesis of nose bleeding HHT and it raises the hope that this safe and harmless drug may be potential therapeutic agents for HHT.

## References

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4. McLean WM, Campolongo JT. Carbazochrome salicylate as a parenteral hemostat in tonsillectomy. *Can J Surg.* 1973; 16(5):333–334.

## OR7 Evaluation of pazopanib on bleeding in subjects with hereditary hemorrhagic telangiectasia: rationale and design of phase II trial

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**Rationale:** VEGF plays a role in development of vascular malformations in HHT, which cause characteristic chronic nasal and gastrointestinal bleeding. Pazopanib, an oral antiangiogenic agent which blocks multiple VEGF and PDGF receptors in a dose dependent fashion, is attractive as a potential therapy for HHT. However, molecules with anti-VEGF properties confer considerable toxicity at maximal oncologic exposures. The challenge for Pazopanib is to find an effective dose but with limited risk.

**Methods:** HHT experts, patient advocates and GSK scientists collaboratively developed a protocol to test proof of concept that Pazopanib reduces bleeding in HHT, and study dosing and drug safety in HHT. The protocol was approved by GSK, FDA and will be approved by institutional ethics boards.

**Study design:** Phase II, open label, non-randomized trial. Adult HHT patients with substantial epistaxis ( $\geq 3$  bleeds/week  $>15$  min total duration) who require iron supplementation, and/or subjects anemic ( $<11$  g/dL) despite iron infusion or blood transfusion, ( $<10$  g/dL) with low pre-infusion ferritin levels, will be entered into a study with two parts: Part A will recruit up to 24 subjects, 6 at a time with progressively higher dosing for each cohort (50, 100, 200 and 400 mg—1/16th to 1/2 of oncologic dosing) for maximum 3 months. Observed efficacy will truncate the study, and avoid risk of additional dose increases; and lead to Part B where the dose and duration efficacy are established. Primary endpoints are improvement in epistaxis, hemoglobin, ferritin, along with reduction in blood/iron requirements. There are also multiple safety endpoints related to known drug toxicity (hepatic toxicity, hypertension, etc.).

## OR8 Efficacy of topical timolol for the treatment of mucocutaneous telangiectasias in patients with hereditary haemorrhagic telangiectasia

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**Objectives:** Due to reports about antiangiogenic and apoptotic properties of systemic and topical beta blockers on hemangiomas and nose bleeding improvement with topical timolol, the aim is to assess efficacy and safety of the topical application of timolol for the treatment of telangiectasias in HHT patients.

**Methods:** Pilot study with application of a low dose of topical timolol b.i.d. (0.1 %) on mucocutaneous telangiectasias. 1 Month and 3 months follow up were performed in order to evaluate bleeding and size response of the treated lesions through interview and photograph respectively and to disclose side effects.

**Results:** 22 patients (9 men and 13 women), age range 41–79 were recruited. Nine patients (40.90 %) were HHT1, 12 (54.54 %) HHT2 and 1 had unknown mutation. In two cases the treatment was abandoned due to voluntary incompleteness. Seventeen patients received treatment on mucous lesions and 3 on skin. Photographic evolution was positive in 55 % and mild in 15 %. Of the 13 patients with bleeding lesions, 12 improved after administration considering bleeding and 9 considering photographic control. None of the three patients treated on skin lesions improved. Considering mucous telangiectasias 100 % of HHT2 patients improved (clinically and/or photographic) while only 75 % of HHT1 patients did. No adverse effects were observed in any case.

**Conclusions:** Topical timolol could be an option for the treatment of mucous telangiectasias in HHT patients. Efficacy and safety of higher doses and differences considering genetic type should be further evaluated.

## OR9 Topical propranolol treatment for severe epistaxis in HHT

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**Background:** Recent studies suggest that anti-VEGF (vascular endothelial growth factor) agents reduce epistaxis in HHT. Topical Propranolol was shown to be effective in infantile hemangioma, decreasing expression of VEGF gene and triggering apoptosis of capillary endothelial cells. Propranolol has also been shown to act as antiangiogenic in HHT human umbilical vein endothelial cells and to decrease migration and tubulogenesis. We hypothesized that topical propranolol might be effective in reducing epistaxis in HHT.

**Methods:** A pilot study to assess efficacy and tolerability of propranolol gel in patients with HHT and epistaxis. Six patients were recruited. Propranolol gel 1.5 % was applied to nasal mucosa via an introducer –0.5CC to each nostril twice daily.

**Table 1** Clinical data of patients treated with Propranolol gel

| Age/gender | ESS pre treatment | ESS after 2 months | BTs/month Pre treatment | BTs/month After 2 month |
|------------|-------------------|--------------------|-------------------------|-------------------------|
| 70/M       | 7.28              | 1.52               | 3                       | 0                       |
| 66/M       | 9.09              | 6.38               | 1                       | 0.5                     |
| 62/F       | 3.44              | 1.01               | 0                       | 0                       |

Epistaxis Severity Score (ESS), and number of blood transfusions (BTs) pre and post administration were recorded.

**Results:** The outcome of 3/6 patients is described here (full results will be presented at the conference).

Three patients with epistaxis (two-severe), were treated with Propranolol gel. Rapid improvement in epistaxis was observed with a decrease in epistaxis severity, frequency and the need for BTs. No side effects were observed (Table 1).

**Conclusion:** In a pilot study Propranolol gel showed promising results with improvement in epistaxis severity. A randomized trial is being conducted to assess Propranolol efficacy and safety treating epistaxis in HHT.

#### OR10 Oral doxycycline for the treatment of epistaxis in patients with hereditary hemorrhagic telangiectasia

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**Objectives:** Many agents have been used with varied success to control epistaxis in HHT patients. Our goal is to establish a safe and affordable medication to mitigate epistaxis.

**Methods:** Metalloproteinase 9 (MMP-9) is over expressed in AVMs. Doxycycline is an MMP-9 inhibitor with a well-established safety profile. We used oral doxycycline for treatment of epistaxis in patients with HHT.

A retrospective analysis of 16 patients with HHT undergoing treatment with oral Doxycycline was performed. Changes in epistaxis severity scores (ESS) from pre-to post-treatment was evaluated using a paired *t* test. Baseline ESS for those with and without follow-up was compared using a two-sample *t* test.

**Results:** All seven patients completing treatment demonstrated a decrease in ESS by an average (SD) of 2.4 (1.2) ( $p = 0.002$ ). Two patients did not complete 30 days of treatment. One patient stopped the medication reporting no effect and refused to complete the ESS. A second patient reported subjective improvement but stopped the medication after 2 weeks related to a rash. Four patients did not fill the prescriptions. Three were unable to be reached for follow-up ESS. Baseline scores for those with and without follow-up did not significantly differ ( $p = 0.554$ ). The median (interquartile range) follow-up between pre and post ESS was 43 days (36–72).

**Table 1** ESS pre and post doxycycline treatment in HHT patients,  $p = 0.002$ 

| Variable   | N | Mean | SD  | Median | Minimum | Maximum | Lower quartile | Upper quartile |
|------------|---|------|-----|--------|---------|---------|----------------|----------------|
| Pre-ESS    | 7 | 4.9  | 2.2 | 4.9    | 1.7     | 7.9     | 3.0            | 7.2            |
| Post-ESS   | 7 | 2.6  | 1.6 | 2.4    | 0.0     | 4.5     | 1.5            | 4.3            |
| Difference | 7 | 2.4  | 1.2 | 2.6    | 0.6     | 3.8     | 1.4            | 3.4            |

**Conclusions:** A significant decrease in the ESS was seen in patients completing treatment with Doxycycline. This pilot data provides a rationale for a future randomized controlled trial (Table 1).

#### OR11 Sodium tetradecyl sulfate (Sotradecol<sup>®</sup>) sclerotherapy for HHT-associated epistaxis: the Washington University experience

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**Objectives:** Sclerosing therapy for HHT-associated epistaxis is described in the literature as an effective and well-tolerated procedure, but it is offered only in a limited number of centers in the US. The objective of this study is to describe the patient experience at the Washington University HHT Center with sodium tetradecyl sulfate sclerotherapy treatment for epistaxis (STS-STE).

**Methods:** Medical records of patients who received STS-STE between January 1, 2009 and December 31, 2014 were reviewed. Abstracted data included age, gender, race, number of separate injections, setting for the procedure (office- or OR-based), STS preparation (saline or air foamed), and complications.

**Results:** There were 449 procedures performed on 133 unique patients between 01/14/2009 and 12/22/2014. Patients were evenly distributed among genders, had a median age of 54 years at time of first procedure with range 16–83 years. The number of procedures per patient ranged from 1 to 18, with a median of two procedures per patient. Forty-nine patients (37 %) had only one procedure; while 18 patients (13.5 %) had 7 or more procedures. The majority of the procedures [ $n = 316$  (70 %)] were performed in the ENT-office. The number of procedures increased from 50 (2009) to 60 (2010) to 77 (2011–2013), and increased to 108 in 2014. The largest number of new patients was observed in 2009 ( $n = 31$ ) and the lowest number in 2013 ( $n = 13$ ). Complications were infrequent.

**Conclusions:** STS-STE at WU is a commonly performed procedure and appears to be a safe, effective, and low-cost treatment.

#### SESSION 3 DIAGNOSIS OF HHT I

##### OR12 HHT mutation discovery in the era of whole exome/genome sequencing

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For decades, the establishment of genetic linkage to a chromosomal location was the necessary first step in the identification of genes causing Mendelian disease. This approach required the collection of large multi-generational families with the disease to obtain the requisite power to detect linkage. The advent of genome sequencing technologies has altered this historic approach to disease gene discovery. The new strategy is to collect and sequence as many unrelated individuals as possible. The challenge now is to correctly distinguish sequence variants causing disease from the multitude of sequence variation existing within each person. The cumulative list of genes harboring sequence variants will be large, including many with functions that are plausibly relevant to the disease. For HHT, a list including all genes related to the TGF-beta superfamily will be quite extensive. It is therefore critical to formally assess whether implicated

genes truly contain such variants more often in cases than in appropriately matched controls. Since each gene in the genome has its own unique tolerance of sequence variation, analysis must include both a variant and a *gene-based* comparison of all variants in both controls and in cases. We will show our analysis of 22 exome-sequenced HHT patients (18 probands) in light of these principles. We will also re-evaluate other recently published work in light of these same principles and other principles of population and molecular genetics. We conclude that in the era of genome sequencing, very high standards are required to formally label a gene mutation as causing HHT.

### OR13 Incidence of epistaxis and telangiectases in the general population

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Consensus diagnostic criteria for hereditary hemorrhagic telangiectasia (HHT) include “recurrent” nosebleeds and “multiple” telangiectases in characteristic locations (oral mucosa, hands, face), but with unspecified minimum frequency and number. The population frequency of nosebleeds and telangiectases is not known. This information would inform the judgment regarding the likelihood of HHT based on clinical evaluation.

**Objective:** To assess the general population incidence of epistaxis and telangiectases of the lips, face and hands.

**Methods:** Administer nosebleed questionnaire and targeted examination for telangiectasia similar to that used during patient evaluation in the Utah HHT Center, in healthy individuals without a previous suspicion of a bleeding or vascular anomaly disorder.

**Results:** The epistaxis survey was administered to 189 individuals (123 female/66 male); the telangiectasia exam to 151. Median nosebleed frequency was 1 per 2 years. 46/189 (24 %) had never experienced bright red drops from their nose without a direct blow to the face; another 43 % experienced nosebleeding less than annually. Of the 33 % experiencing nosebleeds at least annually, 19 % more than 4 nosebleeds/year and 12 % had more than 8 nosebleeds/year. Based on examination of the hands, face and lips, 71 % had no telangiectasia, 21 % had 1–2, 5 % had 3–5, and 2 % had 6–9. No one had more than 9. The combination of at least 3 telangiectasias and at least 4 nosebleeds/year was found in 3/151 (2 %).

**Conclusion:** This study provides information about what is “normal” with regards to nosebleeds and telangiectasia, which will help in the clinical assessment for HHT.

### OR14 Uncovering the role of non-coding regions mutations in HHT

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Approximately 15 % of individuals with suspected HHT do not have a mutation in *ACVRL1*, *ENG* or *SMAD4*. The bone morphogenic 9 gene (*BMP9/GDF2*) was recently shown to cause HHT findings; yet, the overall contribution is estimated to account for <1 %.

**Objective:** In order to identify a molecular mechanism of disease in individuals with HHT, without an identifiable causative mutation, *ACVRL1* and *ENG* were thoroughly evaluated for variants that may alter splicing or expression.

**Methods:** Rare variants in deep intronic or 5′ untranslated region (UTR) as well as coding variants of uncertain significance (VUS) (missense, silent, and in-frame deletions) identified through Sanger sequencing and next generation sequencing were evaluated from 142 patients. Multiple in silico programs were used to predict if the variant causes a splicing aberration by altering or creating a new splice site, branch point, and/or regulatory protein site.

**Results:** In total, 175 variants in *ACVRL1* and *ENG* were evaluated, the majority of which were predicted to alter the activity of splicing regulators. Importantly, several in silico-predicted splicing aberrations were shown to alter splicing in vitro using cDNA sequencing. We identified c.-33A>G in the 5′ UTR of *ENG*, which may generate a new initiation codon (ATG) as one of the mechanisms that leads to haploinsufficiency.

**Conclusion:** The impact of all VUS on splicing should be evaluated in HHT patients. Deep intronic or 5′ UTR of *ENG* and *ACVRL1* may harbor variants that affect splicing in cases in which traditional HHT molecular testing does not identify a molecular mechanism of disease.

### OR15 Asymptomatic pulmonary arteriovenous malformations in children with hereditary hemorrhagic telangiectasia

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**Objective:** Children with HHT may have pulmonary arteriovenous malformations (PAVMs), which can present with hemoptysis, cyanosis, shortness of breath and exercise intolerance, or may be asymptomatic. Some guidance suggests that only children with suggestive symptoms be screened for PAVMs. This practice could miss asymptomatic PAVMs, leaving patients at risk for stroke, brain abscess or pulmonary hemorrhage from untreated PAVMs.

**Methods:** Standardized interviews were performed with children and their families at the Washington University HHT center from July 2010–Nov 2014 with HHT and definite PAVMs for signs and symptoms suggestive of PAVMs, such as hemoptysis, cyanosis, exercise intolerance and shortness of breath.

**Results:** 41 children with PAVMs were included, aged 4 months to 17 years. 30 children (73 %) had no PAVM symptoms. 8 had coughing or shortness of breath, possibly due to asthma or allergy. Only 3 had classic PAVM symptoms not attributable to other causes: one each with hemoptysis, cyanosis, and claudication. Of the 30 asymptomatic children with PAVM, 21 underwent embolization for PAVM ≥ 3 mm; 2 had diffuse type PAVM; and 7 had PAVMs with feeding vessels smaller than 3 mm in diameter and treatment was deferred.

**Conclusions:** Most pediatric HHT patients with PAVMs are asymptomatic. Suggestive symptoms may also be caused by other conditions such as asthma or allergy. A small minority (3/41 or 2.4 %) have PAVM symptoms that would trigger an investigation. Symptom screening is not sufficient to capture pediatric HHT patients with PAVMs, including those large enough to warrant embolization.

## SESSION 5 TREATMENT OF HHT II

**OR16 Modeling hereditary haemorrhagic telangiectasia (HHT) with patient specific induced pluripotent stem cells (iPSCs)**

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**Objectives:** We have recently developed human system to model HHT1 based on patient specific induced pluripotent stem cells (HHT1-iPSCs). These stem cells can be derived by reprogramming from skin or blood and are unique in their capacity to form all cells of the body, including those of blood vessels.

**Methods:** We first established robust protocols for the differentiation of hiPSC lines towards endothelial cells (ECs) and pericytes (Orlova et al. ATVB and Nat Proto 2014). In the present study we examined HHT1-ECs from iPSC lines derived from individuals with two different ENG nonsense mutations (IVS 5 + 2 (T>C) and 1083delAA).

**Results:** HHT1-iPSC-ECs strikingly recapitulate reduced ENG and soluble-ENG expression observed in peripheral blood monocytes from HHT1 patients. We next examined functionality of HHT1-iPSC-ECs in depth. Interestingly, we found two different phenotypes in HHT1-patient hiPSC ECs. HHT1-iPSC ECs from one patient exhibited increased vascular sprouting and endothelial cells proliferation in a 2D vascular plexus model. The extensive proliferation was diminished by exogenous BMP9, or by overexpression of ENG. On the other hand, HHT1-iPSC ECs from the second patient exhibited defective endothelial-pericyte communication and defective downstream activation of NOTCH signaling in endothelial cells. Mechanistic aspects underlying these two phenotypes are currently under investigation.

**Conclusions:** Our data indicate that HHT1 can be modeled with patient hiPSCs and can help elucidate different aspects of ENG deficiency in HHT1 patient-specific ECs. These patient-specific HHT1-iPSCs thus represent a renewable and scalable source of ECs for in vitro disease modelling to understand underlying mechanisms of disease in the future.

**OR17 Establishment of robotized routine next generation sequencing screening facility for molecular genetic analysis of ENG, ACVRL1 and SMAD4: the first year**

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**Objective:** Evaluation of the results received after the first year upon establishment of a semi-automated robotized NGS based molecular genetic analysis facility.

**Materials:** Patients referred to the department with symptoms of HHT (n = 17) or relatives of mutation carriers (n = 18) during 2014.

**Methods:** DNA was extracted on a Maxwell robot. Enrichment was performed using Sureselect and library sequenced by paired-end sequencing on HiSeq 1500. Samples were simultaneously analyzed for mutations in *ENG*, *ACVRL1* and *SMAD4*. Each exon and a minimum of 20 bases have been sequenced at coverage of at least 30 times into the 5'- and 3'-introns, respectively. The entire analysis

procedure is robotized with pre- and a post-PCR procedures running on spatially separated Biomek 4000 pipetting stations. Data analysis was performed using GATK. Copy number analysis was achieved using the proportions of NGS reads analyzed by CONTRA software. Missense variants was evaluated using mutation databases; HGMD, ARUP and LOVD. Conceivable new variants were assessed using the EPS and ExAC and if uncharacterized analyzed using PolyPhen, SIFT, MutationTaster and 3D structure prediction. Splice site variants was evaluated using MaxEntScan, SpliceSiteFinder-like, NNSPLICE, GeneSplicer and HumanSplicingFinder. Validations and analysis of familial samples were performed using bi-directional Sanger sequencing.

**Results:** Of the 17 referred probands 10 were positive mutation carriers. Of these 3 mutations has previously been described in HGMD/ARUP mutation databases. Of these 6 mutations were found in *ENG* and 4 in *ACVRL1*. 44 % of familial samples were mutation positive.

**Conclusion:** We present a fast robust genetic screening system for mutations in *ENG*, *ACVRL1* and *SMAD4* including simultaneous analysis for genetic variations i.e. substitutions, minor deletions, duplications, insertions, and large deletions/duplications. The presented method is extensively automatized excluding sampling errors and cross contamination.

**OR18 Overexpression of ALK1 prevents the development of AVMs caused by ENG-deficiency**

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**OR19 The value of therapeutic enteroscopy in patients with a rare disease (Rendu–Osler–Weber): a single centre experience**

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**Introduction:** Eighty percent of patients with Rendu–Osler syndrome (HHT) develop gastro-intestinal angiodysplasias that may occur with gastrointestinal bleeding. Bleeding can be treated with hormone therapy, antifibrinolytic acid and endoscopic treatment. Actually there are few studies about feasibility and effectiveness of endoscopic treatment in the bleeding control and on rebleeding in this type of patients. **Aims:** To evaluate the feasibility and effectiveness of endoscopic treatment to control and reduce the bleeding events in patients with HHT.

**Materials and methods:** We retrospectively reviewed data from a preformed database of patients with HHT who consecutively underwent enteroscopy (single balloon enteroscopy or spirus enteroscopy or push enteroscopy) for obscure bleeding in a tertiary referral center from November 2008 to November 2014. All the vascular lesions were treated with Argon Plasma Coagulation or endoscopic clipping.

**Results:** Eighteen patients were included in the study, (8 male and 10 female), mean age was 62.3 years (CI 23–82), mean observation time 35.7 months (CI 10–72) and mean value of hemoglobin was 8.5 gr/dL (CI 3–14.7). Seventeen patients were submitted to endoscopic treatment, only one was negative for small bowel lesions; twelve had a

second treatment, seven of these didn't have another episode of bleeding; five patients needed a third treatment; just two patients had four treatment and only one underwent the fifth treatment. The number of lesions decreased after the treatments mentioned in the below table.

| Number of lesions | First treatment No. of patients | Second treatment No. of patients | Third treatment No. of patients |
|-------------------|---------------------------------|----------------------------------|---------------------------------|
| 1–10              | 3                               | 7                                | 3                               |
| 11–20             | 9                               | 3                                | 1                               |
| 21–50             | 2                               | 2                                | 1                               |
| >50               | 3                               | –                                | –                               |

The mean value of hemoglobin after endoscopic treatment was 10.7 gr/dL (SD 2, CI 7–14). We registered two complications related to the procedure, an episode of massive epistaxis during orotracheal intubation for anesthesia, and an episode of atrial fibrillation during endoscopy; both episodes were treated by medical therapy.

**Conclusions:** This is the first study, to our knowledge, suggesting that the number of patients who needing endoscopic treatment and number of lesions detected decrease over time in patients with HHT. Moreover the mean value of hemoglobin after endoscopic treatment was about two point higher than the previous value. The endoscopic treatment is safe, repeatable over time and poor of complications.

## SESSION 6 DIAGNOSIS OF HHT II

### OR20 Curacao criteria highly predictive of a mutation in *ACVRL1* or *ENG*

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**Objective:** To determine the detection rate of a mutation in *ENG*, *ACVRL1* or *SMAD4* in individuals who meet consensus (Curacao) criteria for the clinical diagnosis of HHT.

**Methods:** Review of the University of Utah HHT Center patient database from January 2004 until October 2014 for individuals with three or more diagnostic criteria for HHT, in whom sequencing and deletion/duplication analysis for *ENG* (to include 5' UTR), *ACVRL1* and *SMAD4* had been performed. 141 family probands were identified who met these criteria.

**Results:** A variant either known or suspected to be pathogenic was detected in *ENG* in 66/141 (46.8 %), *ACVRL1* 69/141 (48.9 %) and *SMAD4* 2/141 (1.4 %). 4/141 (2.8 %) family probands with definite HHT did not have a mutation in either the *ENG*, *ACVRL1* or *SMAD4* gene.

**Conclusion:** Previous reports of the mutation detection rate in *ENG* and *ACVRL1* in HHT patients have come from laboratories, which receive samples from clinicians with a wide range of expertise with regards to recognizing clinical manifestations of HHT. These studies suggested a significantly lower detection rate (~75 %) than we have found in patients who meet strictly applied consensus diagnostic criteria (96 %). Analysis of *SMAD4* adds an additional detection rate of 1.4 %. HHT as defined by the Curacao criteria is highly predictive of a mutation in either *ENG* or *ACVRL1*.

### OR21 Molecular diagnostics in the new era: clinical utility of a next generation sequencing panel in the diagnosis of HHT

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Hereditary hemorrhagic telangiectasia (HHT) is a multigenic autosomal dominant disorder caused by mutations in several transforming growth factor beta (TGF- $\beta$ ) signaling pathway genes (*ENG*, *ACVRL1*, and *SMAD4*). Recently, we discovered a bone morphogenetic 9 (*BMP9/GDF2*) mutation in patients suspected to have HHT. *RASA1* mutations which cause capillary malformation-arteriovenous malformation (CM-AVM) syndrome have also been identified in patients suspected to have HHT.

**Objective:** Because of the genetic complexity of HHT and the phenotypic overlap of disorders featuring capillary malformations and/or telangiectasia, we developed a custom next-generation sequencing (NGS) panel assay to rapidly identify mutations in 5 genes (*ENG*, *ACVRL1*, *SMAD4*, *BMP9*, and *RASA1*) that cause HHT and related disorders.

**Methods:** Hybridization capture was used to interrogate the HHT-causative genes in genomic DNA from 150 patients submitted for clinical testing. Enriched samples were sequenced (HiSeq2500), NGS data was aligned, and variants were interpreted. A custom comparative genomic hybridization (CGH) array was also used to detect large deletions/duplications in the same 5 genes.

**Results:** A mutation in one of the panel genes was detected in ~25 % of cases. Several interesting cases will be presented. Clinical findings and sensitivity will be compared to those who have undergone HHT molecular testing by Sanger sequencing or a 14-gene vascular malformations panel. A molecular testing algorithm based on the suspected clinical diagnosis will be proposed.

**Conclusions:** Unlike traditional molecular approaches such as Sanger sequencing, the HHT NGS assay offers a cost-effective, multi-gene approach for the molecular diagnosis of cases suspected to have a hereditary telangiectasia syndrome.

### OR22 Genotype- phenotype correlations in hereditary haemorrhagic telangiectasia: data from a large nation-wide French cohort

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**Introduction:** Hereditary haemorrhagic telangiectasia (HHT) is a dominantly inherited genetic vascular disorder characterized by recurrent epistaxis, cutaneous telangiectasia and visceral arteriovenous malformations (AVM) that affect many organs, including the lungs, gastrointestinal tract, liver and brain.

**Aims:** To analyse the genotype-phenotype relationship of a given HHT mutation (*ENG*, *ACVRL1*, or *MADH4*) in a group of patients representing the French HHT population.

**Method:** We used the French HHT database CIROCO, which contains clinical features registered from 2005 to 2015.

**Results:** A total of 2230 files of patients with a proved mutation were analysed. Among them, the mutated gene was *ACVRL1* in 57 %, *ENG* in 41.3 % and *MADH4* in 1.7 % of patients. Nose-bleedings and telangiectasia events appear to be the predominant characteristics, but no significant difference was found according to the mutation type. Pulmonary arteriovenous malformations (PAVM) were present in 26.7, 72.9 and 60 % in patients carrying *ACVRL1*, *ENG* or *MADH4* mutations respectively. Cerebral abscesses and stroke were more frequently observed in *ENG* patients ( $p < 0.0001$ ). PAVMs were associated with clubbing fingers in 41 % of patients with *MADH4* mutations. The hepatic malformations (HAVM) were more often related to the presence of the *ACVRL1* mutation (65.1 vs. 34.7 and 37.9 %, respectively). All liver transplants have been performed in patients carrying an *ACVRL1* mutation. A high prevalence of cerebral arteriovenous malformations (CAVM) was observed in patients carrying *ENG* mutations (22.5 %) compare to *ACVRL1* mutation (7.5 %) ( $p < 0.0001$ ). Digestive AVMs were not significantly different between the three groups.

**Conclusions:** This study on a large cohort shows major differences linking the syndrome and the mutated gene.

#### OR23 20 years follow-up of Danish HHT patients

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#### OR24 Spontaneous growth of pulmonary arteriovenous malformations in hereditary hemorrhagic telangiectasia

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AP-HP, Hôpital Ambroise Paré, HHT Center of Paris, France: Blondel Jean-Hugues, Bonay Marcel, Bourgault-Villada Isabelle, Charron Philippe, Chesneau Bénédicte, Cordier Alexandre, Coudert François, Fagnou Carole, Karam Carma, Lesur Gilles, Ozanne Augustin, Soubrier Florent

**Objectives:** To assess the growth of pulmonary arteriovenous malformations (PAVMs) during 4 years using CT scan. To identify predicting factors of growth.

**Methods:** From a monocentric cohort of 233 patients with PAVMs embolization, we retrospectively included 27 patients who presented other PAVMs not eligible for embolization. We analyzed the growth of the aneurysmal sac of non embolized PAVMs, by using CT scan and a GE Lung volumetry software. PAVMs were followed during 4 years. Evolution was defined arbitrarily when the growth and loss of volume were superior to 10 %. We analyzed the influence of predicting factors on PAVM growth (sex, age, pulmonary artery pressures (PAP) and liver involvement).

**Results:** 49 PAVMs in 19 women and 8 men were analyzed. 31 of them (63 %) progressed, 14 (29 %) were stable and 4 (8 %) decreased. Large initial volume ( $r = 0.82$ ;  $p < 0.00001$ ), as well as PAP ( $15.9 \pm 4.7 = 6-32$ ) ( $p = 0.045$ ) are both growing factors of PAVMs. Conversely, age ( $45.8 \pm 14.5 = 19.7-69.8$ ) ( $p = 0.47$ ), and diameter of hepatic arteries, as liver involvement, ( $6.9 \pm 2.2 = 3.8-14.0$ ), did not influence the PAVMs growth.

**Conclusion:** Most non embolized PAVMs are evolutive in patients with prior PAVM embolization. Volumetry growth of PAVM is strongly correlated with sex, initial size of the aneurysmal sac and PAP.

#### OR25 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 Toronto HHT Database from 1997 to 2009. Bubble echocardiography and CT chest reports from initial assessment were reviewed, as well as those from subsequent rescreening until 2014 for all

**Table 1** Yield of rescreening patients with definite HHT and initial negative screening

|   | Rescreened at 5 years or later | Rescreened at 10 years or later | Rescreened at 15 years or later |
|---|--------------------------------|---------------------------------|---------------------------------|
| Number of patients rescreened for PAVMs with previous negative screening      | 73                             | 38                              | 12                              |
| Mean age at rescreening (years)   | 56.92 ± 14.90                  | 58.51 ± 12.87                   | 62.09 ± 12.90                   |
| Mean time interval between initial negative screening and rescreening (years) | 9.72 ± 3.58                    | 12.68 ± 2.00                    | 15.18 ± 1.17                    |
| Positive bubble echo on rescreening (%)                                       | 33/73 (45.2 %)*                | 12/38 (31.6 %)*                 | 2/12 (16.7 %)                   |
| Positive CT confirmed PAVMs on rescreening (%)                                | 2/73 (2.7 %)                   | 0/38 (0 %)                      | 0/12 (0 %)                      |

\* All patients with a positive bubble echo had confirmatory CT except for 4 patients at year 5 and 2 at year 10

patients with initial negative PAVM screening. Negative screening was defined as a negative bubble echo, while a positive screen required confirmed PAVMs on CT. The frequency of PAVMs was calculated at initial screening and at 5, 10, and 15 years thereafter.

**Results:** 504 patients had a definite HHT diagnosis, of whom 129 (52.7 % female, mean age 48.86 ± 15.46 years) had negative initial screening and 194 (38.5 %) had CT confirmed PAVMs. Demographic information and the yield of rescreening patients with initial negative screening at 5, 10 and 15 years are found in Table 1. In total, two patients with initial negative screening were positive at 5 years, but these were clinically insignificant PAVMs (feeding arteries of 1 mm) and remained so 5 years later. No new PAVMs were detected at 10 and 15 years.

**Conclusion:** No new clinically significant PAVMs were identified in patients rescreened up to 15 years after initial negative screening. While sample sizes are too small to make definitive recommendations, the data suggests that longer time intervals beyond 5 years may be considered between screenings.

**OR26 Follow-up of the pulmonary right-to-left shunt with transthoracic contrast echocardiography in hereditary haemorrhagic telangiectasia**

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**Objectives:** Pulmonary arteriovenous malformations (PAVMs) are associated with severe neurological complications in patients with hereditary haemorrhagic telangiectasia (HHT). Transthoracic contrast echocardiography (TTCE) is the first-line screening technique for the detection of pulmonary right-to-left shunts (RLS) and only moderate and large shunts seem to have clinical implications. Five years after the initial TTCE we evaluated the evolution of the pulmonary RLS in a single centre cohort.

**Methods:** All HHT patients underwent a second TTCE 5 years after screening. Patients with a history of PAVM embolisation were excluded. Opacification of the left ventricle was graded with a three grade scale. The TTCE after 5 years was compared to the TTCE performed at screening.

**Results:** In total 162 patients (55 % female, 65.4 % HHT type 2, age at follow-up 50.6 ± 14.0 years) were included (expected patient number at presentation 190). The median follow-up time was 5.4 years (interquartile range 5.1–5.9 years). A pulmonary RLS was present in 93 patients (57.4 %) at screening and 104 patients (64.2 %) at follow-up. Increase in shunt grade was seen in 27 patients (16.7 %). A significant increase was seen in 15 of these patients (55.6 %) and embolisation was indicated in 6 patients (22.2 %). Embolisation was feasible in 3 patients (1.9 %) in whom the shunt increased within one grade but with increase of the PAVM on computed tomography.

There were no complications.

**Conclusions:** Even in patients with no treatable PAVMs at screening, after 5 years treatable PAVMs are present in 6 %. In this population there is a number-needed-to screen of 18.

**OR27 A survey of pulmonary arteriovenous malformation management and follow-up with attention to small (<3 mm) feeding arteries**

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**Objectives:** The management of PAVMs, particularly those with feeding arteries <3 mm in diameter, remains controversial. We surveyed practices in HHT Centers of Excellence to determine current practice and inform research.

**Methods:** This 32 question survey focused on imaging (contrast echo, CT, MRI), management and follow-up, particularly for untreated PAVMs and those with small feeding arteries. The survey was sent to 147 participants at HHT Centers worldwide.

**Results:** 33 responses were received (22 %), 60 % from IR and 20 % from pulmonologists; 67 % US, 21 % European, 6 % Asian; 80 % specifying a direct role in management. Representative responses include:

|   |                     |                     |                    |                    |
|---|---------------------|---------------------|--------------------|--------------------|
| Repeat echo after Grade 0 echo?                               | None—15 %           | 3 years—4 %         | 5 years—16 %       | 10 years—7 %       |
| Imaging after Grade 1 echo?                                   | Non-con CT—22 %     | Enhanced CT—41 %    | Echo 5 years—22 %  | Echo 10 years—4 %  |
| Imaging after Grade 1 echo and normal enhanced CT?            | None—7 %            | CT 5 years—37 %     | Echo 5 years—37 %  | Echo 10 years—7 %  |
| Imaging after CT showing multiple small (<2 mm feeder) PAVMs? | Echo in 3 years—7 % | Echo in 5 years—4 % | CT in 3 years—33 % | CT in 5 years—37 % |

Non-contrast was the preferred CT (41 %), opinion regarding bubble filters was evenly divided, the most common age to cease screening was 70 (30 %), and the most common imaging interval was 5 years (42 %). 80 % treat PAVMs with feeding arteries <3 mm and 52 % treat feeders <2 mm.

**Conclusion:** There is substantial disparity in practice regarding small PAVMs and PAVM follow-up, suggesting a need for further research and guideline development.

## SESSION 7B: MECHANISMS OF HHT II

### OR28 Bone morphogenetic protein 9 signaling regulates the hippo pathway effectors Fat1, Salvador and Zyxin: effects on endothelial cell matricellular and chemokine responses

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Endoglin is a type III TGF $\beta$  auxiliary receptor that is upregulated in endothelial cells during angiogenesis and, when mutated in humans, results in the vascular disease hereditary hemorrhagic telangiectasia (HHT). Though endoglin has been implicated in cell adhesion, the underlying molecular mechanisms are still poorly understood. Here we show endoglin expression in endothelial cells regulates subcellular localization of zyxin in focal adhesions in response to BMP9. RNA knockdown of endoglin resulted in mislocalization of zyxin and altered formation of focal adhesions. The mechanotransduction role of focal adhesions and their ability to transmit regulatory signals through binding of the extracellular matrix are altered by endoglin deficiency. BMP/TGF $\beta$  transcription factors, SMADs, and zyxin have recently been implicated in a newly emerging signaling cascade, the Hippo pathway. Our data suggest that FAT1 and Salvador are BMP9 targets. Downstream, the Hippo transcription coactivator, YAP1 (yes-associated protein 1), plays a crucial role in mechanotransduction and cell–cell contact. Data indicating BMP9-dependent nuclear localization of YAP1 in response to endoglin expression suggests multilevel crosstalk between the two pathways. Suppression of endoglin and YAP1 alters BMP9-dependent expression of YAP1 target genes CCN1 (cysteine-rich 61, CYR61) and CCN2 (connective tissue growth factor, CTGF) as well as the chemokine CCL2 (monocyte chemoattractant protein 1, MCP-1). These results suggest a coordinate effect of endoglin deficiency on cell matrix remodeling and local inflammatory responses. Identification of a direct link between the Hippo pathway and endoglin may reveal novel mechanisms in the etiology of HHT.

### OR29 Involvement of BMP9 in pulmonary arterial hypertension

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BMP9 has been shown to be the ligand of high affinity of the type 1 receptor ALK1 (activin receptor like-kinase 1). ALK1 acts in a complex composed of type 2 receptor that could be BMPR2, ActR2A or ActR2B and the co-receptor endoglin. Mutations in ALK1 and endoglin cause HHT. Interestingly, mutations in BMPR2 and ALK1 have also been linked to another rare vascular disease (15–50 patients per million) called pulmonary arterial hypertension (PAH). PAH is a life-threatening disease involving pulmonary endothelial dysfunction, smooth muscle hyperplasia, sustained inflammation and dysimmunity, leading to right-sided heart failure and death (mean survival of 2.8 years). Although the same signaling pathway seems to be involved in these two diseases, they present very different characteristics and one challenging aim is to understand how alteration of the same pathway can lead to two very different vascular diseases. As BMP9 is the most potent ligand of this impaired ALK1/BMPR2 signaling pathway, we sought to address its potential role in the pathophysiological mechanism of PAH. For this purpose, we have used *Bmp9*-KO mice that are viable and fertile and we have challenged them with two experimental models of pulmonary hypertension (the chronic hypoxia exposure model and the monocrotaline–pyrrole model). Surprisingly, our preliminary results suggest that *Bmp9*-KO mice are protected against the development of pulmonary hypertension in these two different in vivo models. Current work is ongoing to understand this unexpected observation.

### OR30 Role of oscillatory expression of bone morphogenetic proteins inhibitors in arteriovenous malformations formation

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During morphogenesis, oscillatory gene expression (ultradian rhythms) has been shown to be critical for morphogenetic processes such as somitogenesis, where bone morphogenetic proteins (BMPs) pathways, could play a central role. Hereditary hemorrhagic telangiectasia 2 (HHT2) is caused by mutations in a BMP receptor, the activin receptor-like kinase 1 (*Alk1*) gene, and characterized by an abnormal morphogenesis of the vasculature, the arteriovenous malformations (AVMs). The expression of ALK1 and its co-receptor endoglin are tightly regulated by BMP4 and 9 and their respective inhibitors, matrix Gla protein (Mgp) and Crossveinless-2 (CV2), through negative feedback regulation. Aberrant ALK1 signaling during endothelial cell differentiation is intimately related to vascular morphogenesis and the formation of AVMs, and depends on normal action of MGP and CV2. We demonstrated that the formation of pulmonary AVMs in a mouse model of HHT2 (*Alk1*<sup>+/-</sup> mice) is suppressed by increasing the MGP levels through the use of a MGP transgene. We hypothesized that oscillatory expression of BMP inhibitors might play a critical role in the regulation of vascular formation. Oscillations in the expression of MGP and CV2 were predicted by a dynamical mathematical model of oscillatory gene expression. Using human pulmonary artery endothelial cells we demonstrated that stimulation with BMP9 induced oscillatory gene expression of MGP, CV2 and endoglin genes, with the expected time delays. Such oscillatory regulation of BMP inhibitors could represent a mechanism in the organization of blood vessels, allowing for a sequential action of BMP receptors and inhibitors.

### OR31 Germline mutations in BMP9 are not identified in Danish patients with hereditary haemorrhagic telangiectasia

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Hereditary hemorrhagic telangiectasia (HHT), an inherited vascular disorder, is, in the majority of cases (85 %), caused by mutations in one of three genes (*ENG*, *ACVRL1*, and *SMAD4*). In the remaining group of individuals with clinical HHT, mutations have not been identified, suggesting yet undiscovered HHT causative genes. A new vascular-anomaly syndrome caused by mutations in *BMP9* has recently been published. Three patients suspected of HHT, with familial nose bleedings and dermal manifestations not characteristic for HHT, were described. Although, it was concluded that these patients probably had a different vascular-anomaly syndrome, the suspicion that *BMP9* mutations might cause HHT remained.

To evaluate if germline mutations in *BMP9* can be identified in HHT patients, we investigated the Danish cohort of mutation-negative HHT patients.

The 14 included patients (from 14 different families) had clinical HHT and no pathogenic mutations located in *ENG*, *ACVRL1* or *SMAD4*. Exons and exon–intron boundaries of *BMP9* were analyzed by bi-directional Sanger sequencing. No mutations of potential pathogenicity was identified.

This study does not suggest that *BMP9* mutations causes HHT, however, it cannot be excluded based on this study. A patient with both clinical definite HHT and a mutation in *BMP9* has still not been reported.

### OR32 B-type natriuretic peptide predicts high cardiac output

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Decompensated high-output heart failure is associated with high morbidity and mortality in hereditary hemorrhagic telangiectasia (HHT). B-type natriuretic peptide (BNP) is a sensitive biomarker of myocardial stress and aids in the diagnosis of heart failure. We hypothesized that an elevated BNP (level > 100 pg/mL) identifies patients with high-output at risk for heart failure.

**Methods:** We retrospectively studied adults seen in the Utah HHT Center from August 2013 to January 2015. These patients had a detailed cardiac history, a laboratory workup including BNP, and an echocardiogram. High-output was defined as a cardiac index (CI) >4 L/min/m<sup>2</sup> on either the echocardiogram or a right heart catheterization. Two-tailed Fisher exact test and sensitivity/specificity were calculated.

**Results:** 75 individuals meeting Curaçao criteria were evaluated. Eighteen individuals had elevated BNPs > 100 pg/mL with eight associated with an elevated CI. The patients with an elevated BNP secondary to high-output all had hepatic AVMs and had worse

epistaxis and lower hemoglobin than those with a high BNP but without high-output. Only one patient had high-output with a rising BNP that was still below the 100 pg/mL threshold.

**Conclusions:** High-output was found in 12 % of patients evaluated in our center. BNP was highly associated with high-output ( $p = 0.0001$ ) and an excellent screening tool for high-output heart failure with a level >100 pg/mL having a high sensitivity of 88.89 %, 95 % CI (51.75–98.16 %) and a specificity of 84.85 %, 95 % CI (73.89–92.47 %).

### SESSION 8 DIAGNOSIS OF HHT III

#### OR33 A grading scheme for liver arteriovenous malformations using computed tomography in patients with hereditary hemorrhagic telangiectasia

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**Objectives:** Hepatic arteriovenous malformations (AVMs) are commonly associated with hereditary hemorrhagic telangiectasia (HHT). These can place patients at risk of high-output cardiac failure, cardiac chamber enlargement with subsequent valvular regurgitation or arrhythmia, and cirrhosis/portal hypertension. We developed a classification scheme using computer tomographic (CT) imaging to help predict those HHT patients at highest risk of developing hepatic AVM-related complications.

**Methods:** Among 249 patients with confirmed HHT at our institution, we identified 139 patients who were over 18 years of age and had contrast-enhanced CT imaging which included the liver. Of these, 60 patients had CT evidence of liver AVMs. These were classified as follows: Grade 1 = one or few foci of abnormal enhancement (n = 9); Grade 2 = diffuse patchy or heterogeneous enhancement (n = 33); Grade 3 = frank liver AVMs (n = 18). Clinical notes and echocardiography reports were evaluated for symptoms and signs of heart failure.

**Results:** Of 18 patients with grade 3 liver AVMs, 11 had clinical symptoms of heart failure, 3 others suffered from atrial fibrillation, and one had cirrhosis. No patients with grade 1 liver AVMs suffered any hepatic AVM related complications. Patients with grade 2 liver AVMs were at intermediate risk.

**Conclusions:** A grading scheme for liver AVMs using contrast-enhanced CT is very useful for predicting the development of complications in HHT patients. Patients with grade 3 liver AVMs are at very high risk, and constitute a group in which early therapy may be indicated.

#### OR34 Correlation of oxygen saturation and serious adverse events in pediatric patients with documented or suspected pulmonary arteriovenous malformation

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**Objective:** To identify baseline demographics for pediatric patients who had a serious adverse event (SAE) related to pulmonary arteriovenous malformation (PAVM).

**Methods:** One hundred sixty-four patients up to the age of 15 were evaluated for PAVM over a 12-year period (2002–2014). Based on clinical criteria and genetic testing, patients were categorized as having hereditary hemorrhagic telangiectasia (HHT), probable HHT, or not having HHT. Clinical evaluation and pulse oximetry were performed. Additional studies were reserved for patients with exertional dyspnea or significant hypoxemia. Other information collected included the presence of migraine, epistaxis, telangiectasia, and cerebral AVM.

**Results:** The average age was 9.6 years (range 1–15), with 83 male (51 %). HHT was present in 101 (62 %) and probable HHT in 47 (27 %). Baseline resting pulse oximetry was available for 115 (70 %) with an average of 97 % (range 64–100 %). Fatal and non-fatal SAEs occurred in 9 (5 %). Non-fatal ones in 5 consisted of cerebral hemorrhage in 1 (11 %), seizure in 1 (11 %) and intestinal bleeding in 3 (33 %). Fatal events in 4 (36 %) consisted of high output cardiac failure from diffuse PAVM in 1 (11 %), presumed hemoptysis in 2 (22 %), and cerebral hemorrhage in 1 (11 %). Resting oximetry in patients with any SAE was 84 % (range 70–97 %, median 84 %), while in those with an SAE attributable to PAVM was 77 % (range 70–84 %).

**Conclusion:** SAE were more likely to occur in patients with low baseline pulse oximetry, with the majority of these attributable to PAVM. All PAVM related SAEs had an oxygen saturation  $\leq$ 84 %.

#### **OR35 Long-term embolotherapy treatment outcomes of pulmonary arteriovenous malformations in children with hereditary hemorrhagic telangiectasia**

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**Objectives:** To review the long-term outcomes of transcatheter embolotherapy for pulmonary arteriovenous malformations (PAVMs) in children with hereditary hemorrhagic telangiectasia (HHT) at a single HHT Center of Excellence and assess for trends with respect to age at time of first therapy.

**Methods:** Retrospective review of medical records and imaging was performed on all pediatric patients (age  $\leq$  18 years) with known diagnosis of HHT who underwent embolotherapy for PAVM from 1998 to 2014. 14 patients (11 male, 3 female) underwent embolization treatments. A total of 47 embolization treatments for 28 PAVMs were performed in 38 separate sessions. Mean age at time of first treatment was 11.9 years (age range 3–17 years). Twelve of 14 patients were available for follow up with 20 treated PAVMs having at least one follow-up exam. Mean interval between initial procedure and last available follow-up was 9.4 years (range 1–17 years). Data were compared to previous literature on outcomes of PAVM embolization in adults and children.

**Results:** Persistence of 70 % of treated PAVMs (14/20) was identified in 10 out of 12 patients with follow-up (83 %). Persistence associated with collateral reperfusion was more common in patients treated at a younger age (mean 8.5 years) compared to recanalization only (mean 16.4 years).

**Conclusions:** Persistence of PAVMs after embolotherapy in children with HHT are significantly higher than previously published data in adults. Consideration of patterns of persistence and re-intervention

should factor into risk–benefit analysis when deciding which patients to treat, particularly in the asymptomatic patient.

#### **OR36 Alegori study: efficacy of a bevacizumab nasal spray as a treatment for epistaxis in hereditary hemorrhagic telangiectasia (HHT). A randomized trial against placebo with dose selection**

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**Background:** Antiangiogenic drugs, such as bevacizumab, are a new treatment strategy in hereditary hemorrhagic telangiectasia (HHT). Its systemic administration in HHT patients improves epistaxis. To limit the systemic adverse effects of bevacizumab and to ease administration, a local administration seems suitable. A phase I study, with bevacizumab nasal spray revealed a good tolerance of the product. The aim of the study is to evaluate bevacizumab nasal spray efficacy in HHT.

**Objectives:** Primary objective is to evaluate, 3 months after the end of the treatment, the efficacy on the duration of the nosebleeds with three different doses of bevacizumab (25, 50 and 75 mg) administered as a nasal spray in a repeated manner (three administrations) in patients with HHT complicated by nosebleeds. Secondary objectives are to evaluate the tolerance, the efficacy at 6 months after the end of the treatment, and the efficacy on anemia and on clinical parameters (nosebleeds, quality of life, blood transfusions).

**Material and method:** A “seamless” phase II/III study: randomized multicenter double blind trial with adaptive dose selection at an interim analysis. The first step is a randomized, four-arm parallel

study with a placebo and three doses of bevacizumab administered by nasal spray. After interim analysis (3 months after the end of treatment), one dose will be selected and the second stage of the study will begin, where patients are randomized between placebo and the dose retained using an allocation ratio of 2:1. According to the sample size calculation, 80 patients are needed in the first phase and 60–120 in the second phase.

**Results:** A total of 80 HHT patients have been included between April 2014 and February 2015 (first step, phase II). Inclusions of patients for the phase III will start in July 2015 after intermediate analysis.

**Trial Registration:** ClinicalTrials.gov Identifier #NCT02106520.

## SESSION 9 MECHANISMS OF HHT III

### OR37 Macrophage recruitment rather than angiogenic stimulation is critical for wound-induced arteriovenous malformation

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### OR38 Consistent infiltration and pro-inflammatory differentiation of hereditary hemorrhagic telangiectasia monocytes contribute to the pathogenesis of arteriovenous malformation

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**Objective:** Arteriovenous malformations (AVMs) in patients with hereditary hemorrhagic telangiectasia (HHT) have a high number of macrophages. How these macrophages accumulate in AVM lesions is not completely understood. We hypothesize that persistent HHT monocyte infiltration and pro-inflammatory differentiation in angiogenic tissues increase the macrophage burden in AVM.

**Method:** An HHT1 mouse model and human monocytes were used. The HHT1 model was induced through global *Endoglin* gene deletion. Brain focal angiogenesis was induced through intra-brain injection of adeno-associated viral vector expressing vascular endothelial growth factor (AAV-VEGF). CD68 positive macrophages were quantified in the brain angiogenic region. An endothelial cell (EC) and vascular smooth muscle (SMC) transwell co-culture system was used to mimic the angiogenic niche to analyze HHT monocyte differentiation. CD34 positive monocytes were labeled with fluorescent and loaded on top of EC layer. Monocyte-derived ECs incorporated with ECs and macrophages migrated towards the SMC layer, were quantified 7 days after culture.

**Results:** HHT1 and wild-type (WT) mice had a similar number of CD68 positive cells in the brain angiogenic foci ( $367 \pm 40/\text{mm}^2$  vs.  $475 \pm 77$ ,  $P = 0.29$ ) at 2 weeks after AAV-VEGF injection. At 8 weeks, HHT1 mice had more CD68 positive cells than WT mice ( $562 \pm 182/\text{mm}^2$  vs.  $353 \pm 86$ ,  $P = 0.05$ ). Similar numbers of control and HHT cells survived in the co-culture ( $P = 0.15$ ). Among surviving cells, more HHT cells than control cells ( $80 \pm 7.1\%$  vs.  $64\% \pm 6.4$ ,  $P < 0.001$ ) differentiated into macrophages. HHT1 and HHT2 cells behaved similarly.

**Conclusions:** Consistent infiltration and pro-inflammatory differentiation of HHT monocytes contribute to AVM inflammation and lesion progression.

### OR39 Specific cancer rates may differ in patients with hereditary haemorrhagic telangiectasia compared to controls

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**Background:** Epidemiological studies show that patients with hereditary haemorrhagic telangiectasia (HHT) have a surprisingly good life expectancy (particularly for patients over 60 years), despite recognised mortality and morbidity due to arteriovenous malformations. We hypothesised that individuals with HHT may be protected against life-limiting cancers.

**Methods:** To compare specific cancer rates in HHT patients and controls, we developed a questionnaire capturing data on multiple relatives per respondent, powered to detect differences in the four most common solid non skin cancers (breast, colorectal, lung and prostate). Blinded to cancer responses, reports of HHT-specific features allowed assignment of participants and relatives as HHT-subjects, unknowns, or controls. Logistic and quadratic regressions were used to compare rates of specific cancer types between HHT subjects and controls.

**Results:** 1307 participants completed the questionnaire including 1007 HHT-subjects and 142 controls. HHT subjects recruited through the survey had similar demographics to controls, although the HHT group reported a significantly greater smoking habit. Combining data of participants and uniquely reported relatives resulted in an HHT-arm of 2161 (58 % female), and control-arm of 2817 (52 % female), with median ages of 66 years [IQR 53–77] and 77 years [IQR 65–82] respectively. Lung cancers were significantly less frequent in the HHT arm than controls (age-adjusted odds ratio 0.48 [0.30, 0.70],  $p = 0.0012$ ). Breast cancer prevalence was higher in HHT than controls (age-adjusted OR 1.52 [1.07, 2.14],  $p = 0.018$ ). Overall, prostate and colorectal cancer rates were equivalent.

**Conclusions:** These preliminary survey data suggest clinically significant differences in the rates of lung, breast and colorectal cancer in HHT patients compared to controls. For rare diseases in which longitudinal studies take decades to recruit equivalent datasets, this type of methodology provides a good first-step method for data collection.

### OR40 Moderate iron deficiency doubles the risk of ischemic stroke risk for patients with hereditary hemorrhagic telangiectasia and pulmonary arteriovenous malformations

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**Objectives:** To identify risk factors for ischemic stroke in patients with pulmonary arteriovenous malformations (PAVMs) and hereditary hemorrhagic telangiectasia (HHT). Ischemic strokes are a common complication of PAVMs. Surprisingly, for CT scan-visible PAVMs, a series of 219 patients suggested stroke risk was no greater in patients whose PAVMs were more severe [1].

**Methods:** 497 consecutive patients with CT-proven PAVMs were studied prospectively. Relationships between radiologically-confirmed ischemic stroke and patient-specific variables were determined using logistic regression and receiver operator characteristic (ROC) analyses.

**Results:** Sixty-one individuals (12.3 %) had acute, non-iatrogenic ischemic clinical strokes at a median age of 52 (IQR 41–63) years. In crude and age-adjusted logistic regression, stroke risk was associated not with venous thromboemboli or conventional neurovascular risk factors, but with low serum iron. The normal range of serum iron is 7–27  $\mu\text{mol/L}$  but for every single  $\mu\text{mol/L}$  rise, the risk of stroke fell by 0.96 [95 % confidence intervals 0.92, 1.00]. Thus for the same PAVM(s), the stroke risk would approximately double with serum iron 6  $\mu\text{mol/L}$  compared to mid-normal range. Other factors associated with higher stroke risk were low pulmonary artery pressure, and serum fibrinogen, the predominant circulating plasma protein for platelet adhesion. Platelet aggregation studies demonstrated that iron deficiency was associated with exuberant platelet aggregation to serotonin (5HT), correcting following iron treatment.

**Conclusions:** These data demonstrate that patients with pulmonary arteriovenous malformations are at increased risk of ischemic stroke if they are iron deficient, and that mechanisms are likely to include enhanced aggregation of circulating platelets.

#### Reference

1. Shovlin et al. *Thorax* 2008; 63:259–266.

#### OR41 Perfusion after PAVM embolization

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**Introduction:** The standard treatment of pulmonary arteriovenous malformations (PAVM's) is embolization of the feeding vessel with coils and/or vascular plugs. A large portion of these embolized PAVM's re-perfuse with the possible risk of paradoxical embolizations to the rest of the body.

**Objective:** To determine how many of the treated PAVM's in a single expert center have perfusion after embolization. And more important to determine the factors that lead to re-perfusion.

**Methods:** Retrospective the pre-embolization CT, the angiography during the embolization and the follow-up CT's of 299 PAVM's were analyzed. On follow-up CT-scans all PAVM's were evaluated for their perfusion status. 50 % had one follow up CECT 50 % had one or more.

**Results:** All but one of the PAVM's that showed re-perfusion were detected on the first follow up CECT. The one that showed re-perfusion on the second follow up CECT was a complex PAVM with bronchial artery involvement.

Of 293 embolized PAVM's 28 % showed perfusion.

Reasons for perfusion after embolization are; persistent enhancement on angiography directly after embolization, complexity of the PAVM, diameter of the feeding vessel, embolization more distally from the PAVM-sack. There is no significant difference in the use of plugs or coils.

**Conclusions:** Treatment success depends more on the location of embolization and the type of PAVM than on the material used. Future PAVM embolization might be more effective if the PAVM-sack is embolized directly instead of the feeding vessel. If, on the first follow up CT, the treated PAVM shows no perfusion further follow up seems unnecessary.

#### OR42 Manganese-related central nervous system injury and neurological consequences in HHT patients with hepatic vascular malformations: a case-control study

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**Introduction:** HHT patients with hepatic involvement may have manganese (Mn) deposits in basal ganglia (BG) probably due to a diminished Mn hepatic clearance and facilitated by iron deficiency anemia. There is uncertainty about clinical findings in these patients. **Aims:** Describe BG Mn deposit induced lesions (BGMnIL) frequency; determine their clinical consequences in a case control study and to describe its relationship with iron deficiency anemia (IDA) and hepatic vascular malformations (HVMs).

**Methods:** Case-control study of the Hospital Italiano de Buenos Aires HHT registry. Patients with 3–4 Curacao criteria were included. Cases were those with BGMnIL and controls patients without BGMnIL. A random sample of cases and controls underwent neuropsychological assessment. Individuals without MRI were excluded.

**Results:** We included 179 patients. The prevalence of BG lesions was 34.6 % (CI 95 % 27.69–42.09–62/179). 47 (75.8 %) were women within the cases and 77 (65.8 %) women within controls,  $p = 0.168$ . Cases mean age: 55.34 years (SD 14.11), controls mean age 43.10 (SD 17.33),  $p < 0.001$ . The prevalence of liver involvement in cases was 95.1 % (58/61), controls 71.4 % (70/98)  $p = 0.001$ . IDA prevalence was 56/62 (90.3 %) in cases, and 54/100 (54 %), controls  $p < 0.00$ . Mn deposits risk in IDA OR 7.95 (CI 95 % 3.13–20.13)  $p = 0.00$ , adjusted OR (adjusted for HVMs) OR 8.52 (CI 95 % 3.26–22.27)  $p = 0.00$ , OR adjusted for HVMs and age and gender: 6.32 (CI 95 % 2.32–17.2)  $p = 0.00$ . HVMs risk for BGMnIL, OR 7.73 (CI 95 % 2.23–26.73)  $p = 0.00$ , adjusted for IDA OR 6.49 (CI 95 % 1.75–24.02)  $p = 0.005$ ; adjusted for HVMs age and gender OR 5.31 (CI 95 % 1.39–20.32)  $p = 0.014$ . Cases performance went worse in all neuropsychological tests.

**Conclusion:** Prevalence of BG Mn deposits is high. IDA and HVMs increase risk of BG Mn deposit lesions. Patients with Mn lesions have neuropsychological symptoms for they might benefit from future therapeutic options.

#### OR43 Nonsense readthrough: a therapeutic strategy to correct bone morphogenetic protein signaling in inherited vascular disorders

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**Objectives:** Hereditary hemorrhagic telangiectasia (HHT) and pulmonary arterial hypertension (PAH) are two inherited vascular disorders caused by mutations in genes of the bone morphogenetic protein (BMP) pathway. Our hypothesis is that restoring normal BMP signaling in patients with these mutations may prevent or reverse vascular changes in HHT and PAH. We are testing this hypothesis using drugs that promote ribosomal readthrough of nonsense mutations to restore a full-length functional protein.

**Methods:** Restoration of functional BMP signaling is determined in primary endothelial cells isolated from the blood of patients with nonsense mutations. We also engineered a novel dual-tag reporter

assay to measure the efficiency of readthrough for different drugs across a range of disease-causing mutations.

**Results:** We initially focused on ataluren, a novel drug in clinical trials for two other genetic disorders. Ataluren produced full-length protein in 7 of 8 mutant reporter constructs, achieving up to 85 % of the wildtype expression level. Readthrough efficiency was highest for UGA stop codons. In primary endothelial cells, ataluren restored BMP signaling and corrected abnormal cell proliferation for 7 of 8 PAH mutations tested.

**Conclusions:** Nonsense readthrough is a promising approach to correct BMP signaling and restore normal endothelial cell function. We are now extending our analysis to HHT nonsense mutations, which account for at least 20 % of pathogenic mutations in *ACVRL1* and 15 % in *ENG*, and testing additional compounds that may be more effective against UAG/UAA codons. Our long-term goal is a personalized approach to BMP-related vascular disorders based on individual mutation characteristics.

#### OR44 Relationship between HHT genotype, epistaxis severity and response to antifibrinolytic therapy

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**Objectives:** To determine whether epistaxis severity scores (ESS) correlated with genotype and if antifibrinolytic therapy is effective in reducing ESS in patients with hereditary hemorrhagic telangiectasia (HHT).

**Methods:** Retrospective chart review was completed on patients seen at UNC from 2009 to December 31, 2014. ESS and demographics were documented. Genetic testing was offered to appropriate patients. Response to antifibrinolytic therapy was evaluated by comparing pre-post ESS. Values are reported as  $\pm$ SE of means.

**Results:** 145 patients were eligible for evaluation. 109 patients underwent genetic testing. 54 had an *ACVRL1* mutation while 39 had an *ENG* mutation. Six had a Variant of Uncertain Significance, two had *SMAD4* mutation, while eight patient had no mutation identified. Higher ESS correlated with *ACVRL1* genotype ( $p < 0.01$ ). Patients with an *ACVRL1* mutation had significantly higher ESS ( $4.66 \pm 0.3$ ,  $p < 0.05$ ) compared to patients with an *ENG* mutation ( $3.6 \pm 0.3$  and those without a mutation ( $2.3 \pm 0.8$ ). Forty-five HHT patients with ESS  $> 4$  were started on an antifibrinolytic agent (tranexamic acid or aminocaproic acid). Twenty patients had follow up visits with the average pre-treatment ESS score of  $6.1 \pm 0.43$ , which decreased to  $4.8 \pm 0.36$  with antifibrinolytic therapy ( $p < 0.05$ ).

**Conclusions:** We demonstrate that the ESS correlates with HHT genotype. Epistaxis is more severe in patients with *ACVRL1* mutations. Additionally, we confirm that chronic use of antifibrinolytic therapy is effective in improving the severity of epistaxis in individuals with HHT.

#### SESSION 10 TREATMENT OF HHT III

##### OR45 Targeting VEGFR2 signaling rescues arterio-venous malformations in a hemorrhagic human telangiectasia type 2 mouse model

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Mutations in the endothelial BMP9/10 receptor, Activin-receptor-like kinase 1 (*Alk1*) cause hereditary hemorrhagic telangiectasia type (HHT2). HHT2 is characterized by arterial-venous malformations (AVMs), in which blood flow is shunted from an artery directly into a vein. Clinical trials with Bevacizumab, a VEGFA blocking antibody, in a small number of HHT patients have reported encouraging results. However, VEGF blockade is associated with adverse effects on blood vessel integrity and function, suggesting the need for new targeted therapies. We have generated mosaic, tamoxifen inducible deletion of *Alk1* in endothelial cells using *Cdh5-CreERT2*; *Acrvrl1* Flox/Flox, and mT:mG reporter mice. Low dose of Tamoxifen administered in these mice produces deletion of *Alk1* in a subset of GFP positive endothelial cells. *Alk1* mutant endothelial cells engage in AVMs within 24 h after gene deletion, allowing us to understand dynamic cell behavior and cellular mechanisms initiating AVM formation. Using this model, we have addressed the hypothesis that targeting VEGFR2 and its downstream signaling components will rescue AVM formation. Our preliminary results show that specific blockade of PI3K-AKT signaling rescues AV shunt formation in *Alk1*/iEcko mice, indicating that targeting AKT1 may be a novel and powerful means to treat vascular malformations in patients. Results from these studies will allow rational drug design for *Alk1*-based AV shunt inhibition and define novel approaches to improve the lives of HHT2 patients.

##### OR46 North American study of epistaxis in HHT (nose trial)

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**Objectives:** Examine the safety and efficacy of three nasal sprays versus placebo in HHT related epistaxis.

**Methods:** In a double blind multicenter study, 121 patients with moderate to severe epistaxis were randomized to receive one of four nasal sprays (0.1 mL in each nostril bid) for 12 weeks: bevacizumab 1 % in saline (BEV), estriol 0.1 % in methylcellulose suspension (EST), tranexamic acid 10 % in saline (TA), and 0.9 % saline (placebo, P). Endpoints included frequency and duration of nosebleeds, ESS (epistaxis severity score), QOL, and treatment failure.

**Results:** Results are respectively reported for BEV, EST, TA, and P. Baseline characteristics were similar between the groups. The mean age was 48–57 years, 55–58 % were male, baseline nosebleed frequency was 9.7–10.9/week, and 88–97 % of patients were Caucasian. After 12 weeks of treatment, 68, 46, 65, and 80 % of patients stated their nosebleeds were “better”. The mean “% better” was 34, 13, 30, and 45 % with the EST group significantly worse than P ( $p = 0.04$ ). 13 patients dropped out before the end of the 12-week treatment period, with 10 due to treatment failure; 5 treatment failures were in the EST group. All drugs were well tolerated and no patient dropped out due to side effects.

**Conclusions:** Saline nasal spray alone was moderately effective in the treatment of HHT related epistaxis. Saline-based BEV and TA were slightly less effective than P (not statistically significant);

methylcellulose-based EST was significantly less effective than P. The differing vehicle of EST may have contributed to its lesser benefits.

#### OR47 Intravenous bevacizumab therapy in transfusion-dependent patients with hereditary hemorrhagic telangiectasia from refractory gastrointestinal bleeding and/or epistaxis

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**Introduction:** Treatment options for severe, transfusion-dependent epistaxis and GI bleeding in HHT are limited. Herein, we present a case series of such patients treated with intravenous (IV) bevacizumab, an angiogenesis (VEGF) inhibitor.

**Methods:** Nine consecutive patients with transfusion dependent anemia due to epistaxis alone (n = 5) or epistaxis plus GI bleeding (n = 4) received IV bevacizumab at 5 mg/kg every 2 weeks (4 doses) followed by either 1 mg/kg, or 2.5 mg/kg or 5 mg/kg every 2–4 weeks based on the initial treatment response.

**Results:** At a median follow up of 9 months (range 3–20); most patients (n = 8) experienced a marked reduction in duration and intensity of epistaxis. The median epistaxis severity score (ESS) before initiation of Bevacizumab was 9.09 (range 6.16–10). The 3- and 6-month post Bevacizumab median ESS scores were significantly lower at 2.97 (range 0.91–6.8) and 2.03 (range 0.72–6.27). Eight patients reported a complete cessation of RBC transfusions and one patient had a 50 % reduction in transfusion needs (from 4 PRBC's/week to 2 PRBC's/week). Patients experienced a significant improvement in quality of life (7 point Likert scale). No major side effects were observed except for transient hypertension (n = 1) and an infusion reaction (chills and rigors) (n = 1).

**Conclusions:** Intravenous Bevacizumab appears to be a safe and effective treatment option for refractory, transfusion dependent patients with HHT related GI bleeding and/or epistaxis.

#### OR48 Intravenous bevacizumab for complications of hereditary hemorrhagic telangiectasia: a review of the literature

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#### SESSION 11 DIAGNOSIS OF HHT IV

#### OR49 Comparative study of two different contrast agents used in transthoracic contrast echocardiograph for the screening of pulmonary arteriovenous malformations in HHT patients

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**Objective:** Agitated saline solution (ASS) and Gelofundine (GF) (Braun) can be used as contrast agents in transthoracic echocardiography (TTCE). The aim of this study is report our results comparing these two contrast agents.

**Methods:** 221 patients with definite criteria for HHT studied with ASS TTCE and CT; 120 of these 221 patients in addition to the study with SSA, had a TTCE using GF. With both contrasts, we used a semi quantitative 4-point graded scale to classify the pulmonary shunt size.

**Results:** Twenty-nine of the 120 patients had SSA TTCE grade 0. Of them, 10 had grade 0, 18 grade 1, and 1 grade 2 with GF. In 34 patients with SSA grade 1, 16 had grade 1, 15 grade 2 and 3 grade 3 with GF. In 30 patients with SSA grade 2, 12 had grade 2, 14 grade 3 and 4 grade 4 with GF. In 15 patients with SSA grade 3: 5 had grade 3 and 10 grade 4 with GF. Twelve patients with SSA grade 4 had grade 4 with GF. The degree of agreement quantified by Kappa statistic was 0.32 ( $p < 0.0001$ ). Sixty-three of the 221 patients had PAVMs in CT. Compared with CT, the sensitivity and specificity, PPV and NPV of TTCE were: 98.4 %; 36.1 %; 38 %; 98.3 % with SSA and 100 %; 13 %; 39.1 %; 100 % with GF.

**Conclusion:** The degree of agreement among the two contrast was low. While GF increased TTCE grade, the specificity of TTCE grade with PAVMs in CT was decreased with GF.

#### OR50 Is contrast echocardiography (CE) for screening of pulmonary arteriovenous malformations (PAVMs) a safe procedure?

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**Aims:** CE is being increasingly used for screening of PAVMs in HHT. This study is aimed to evaluate safety profile of CE for screening of PAVMs.

**Methods:** Adverse events related to CE have been prospectively recorded in 617 consecutive subjects (males 282, mean age 41, range 3–84) at risk for HHT who underwent our multidisciplinary screening protocol for HHT, including CE for detection of PAVMs. CE positivity: for PAVMs, if any bubble appeared in the left atrium after more than three cardiac cycles after initial opacification of the right chambers; for patent foramen ovale (PFO), with an earlier bubble appearance. Shunt grading: according to the contrast opacification of the left-sided chambers, from 0 to 3. All CE procedures were recorded for review. Patients were observed during and for 2 h after CE for detection of complications—defined as mild, severe, fatal—resulting from paradoxical air embolism. Complications were correlated ( $\chi^2$  with Yates correction) with CE grading.

**Results:**

|            | n (%)    |
|------------|----------|
| CE grade 1 | 208 (34) |
| CE grade 2 | 118 (19) |
| CE grade 3 | 61 (10)  |
| CE grade 0 | 171 (28) |
| PFO        | 59 (9)   |

Minor complications occurred in 15 patients (2.4 %): migraine in 8, associated with nausea and vomiting in 1 case, blurred vision in 3, numbness in 4; paresthesias, associated or not to migraine, occurred in 9. Complications occurred within 2–25 min after saline injection, and showed fast and spontaneous recovery in all cases. All complications occurred in affected subjects with PAVMs, in 11 with grade 3 shunt, and in 4 with grade 2. Correlation was found between the CE grading and complications ( $p < 0.0001$ ).

**Conclusions:** Our findings indicate CE is a safe diagnostic tool for suspected PAVMs.

#### OR51 Novel method for distinguishing intrapulmonary shunting from interatrial shunting based on the “ramping up” pattern of bubble appearance in the left heart

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**Background:** The ‘three beat rule’ on agitated saline contrast study (SC) has been traditionally used to differentiate inter-atrial shunting from pulmonary arteriovenous malformations (PAVM). In patients with PAVM we noticed a distinct pattern of bubble appearance, which we called the ‘ramping up’ pattern. We hypothesized that this “ramping up” pattern if present would suggest PAVMs irrespective of the timing of bubble appearance.

**Materials and methods:** SC studies from a total of 89 patients including 53 patients with known HHT and intra-pulmonary shunting and 36 patients with PFO/small ASD were selected and randomly arranged. Four echocardiographers were familiarized with the ‘ramping up’ pattern and were asked to review all 89 studies to identify the presence of ramping up pattern and categorize the timing of bubble appearance in the left heart.

**Results:** ‘Ramping up’ pattern was identified in 35/53 (68 %) patients with PAVM and in 4.5/36 (12.5 %) patients with PFO or small ASD. All four echocardiographers agreed on the presence of ramping up pattern in 26/53 (49 %) patients and at least 3 agreed on 31/53 (58.5 %) patients in the PAVM group. In the PFO/small ASD group only one patient (2.7 %) was identified as having the ramping up pattern by all four echocardiographers. In the PFO/small ASD group 25 % had ‘delayed’ appearance of bubbles while in the PAVM group 24 % had ‘early’ appearance of bubbles.

**Conclusion:** ‘Ramping up’ pattern on SC echo is associated with the presence of PAVM irrespective of the timing of the bubble

appearance. Identification of this specific pattern is a skill that can be easily taught and reproduced.

#### OR52 Prevalence of pulmonary hypertension in a large cohort of patients with hereditary hemorrhagic telangiectasia

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**Objectives:** Pulmonary hypertension (PH) is increasingly recognized as a severe complication of hereditary hemorrhagic telangiectasia (HHT), but its prevalence remains unknown. Therefore we studied the estimated prevalence of PH in HHT.

**Methods:** All consecutive persons screened for HHT between 2004 and 2012 underwent transthoracic contrast echocardiography (TTCE). All persons with mutation analysis and a reliable right ventricular systolic pressure (RVSP) measurement were included. PH was suspected when an RVSP  $\geq 36$  mmHg was measured.

**Results:** In 578 patients a TTCE and mutation analysis was available. A valid RVSP was measured in 383 (66.3 %) patients; 127 HHT type 1 (61.4 % female, mean age  $44.2 \pm 15.6$  years), 150 HHT type 2 (59.3 % female, mean age  $48.9 \pm 14.2$  years) and 106 none HHT controls (62.3 % female, mean age  $38.6 \pm 12.8$  years). The mean RVSP was  $27.0 \pm 7.4$  mmHg,  $28.8 \pm 9.6$  mmHg, and  $24.6 \pm 5.5$  mmHg respectively ( $p = 0.006$  and  $p < 0.001$  vs. controls). An RVSP  $\geq 36$  mmHg was found in 42 patients [11 (8.7 %) HHT type 1, 27 (18.0 %) HHT type 2 and 4 (3.8 %) controls]. HHT type 2, hepatic arteriovenous malformations (HAVMs) and age were predictors for an RVSP  $\geq 36$  mmHg (OR 3.4;  $p = 0.03$ , OR 10.5;  $p < 0.001$  and OR 1.1;  $p < 0.001$ , respectively). Two patients (1.3 %) with both HHT type 2, were diagnosed with pulmonary arterial hypertension (PAH).

**Conclusion:** The estimated prevalence of PH in HHT is increased, especially in HHT type 2 and associated with HAVMs. PAH was diagnosed in 1.3 %.

#### SESSION 12 MECHANISMS OF HHT IV

##### OR53 Cerebral vascular lesions in hereditary hemorrhagic telangiectasia

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**Objective:** In HHT, various vascular lesions may occur in the brain and contribute to the risk of intracranial hemorrhage. The purpose of this study was to analyze the spectrum of cerebral vascular manifestations in HHT, based on their MRI appearance.

**Methods:** 293 HHT patients underwent brain MRI/MRA between 2009 and 2014. All studies were retrospectively reviewed and classified based on imaging appearance into arteriovenous malformation (AVM), developmental venous anomaly (DVA), aneurysm, capillary telangiectasia, and hemosiderin deposits. The frequency of occurrence of vascular lesions was calculated and compared to reported literature values.

**Results:** 101 of 293 (34.5 %) HHT patients revealed cerebral vascular lesions. Of these, 43 (14.7 %) had AVMs, predominantly capillary malformations and micro-AVMs, compared to reported detection of 0.82–1.42 % in the unselected population. 32 (10.9 %) showed

DVAs, slightly higher than the general population (6.4 %). 15 (5.1 %) displayed aneurysms, and 1 (0.3 %) capillary telangiectasia; both similar to 3.2 and 0.4 %, respectively, in the general population. On susceptibility weighted images, 22 (7.5 %) showed hemosiderin deposits.

**Conclusions:** AVMs (particularly capillary malformations and micro-AVMs) are the most prevalent cerebral manifestation in HHT, occurring at a significantly higher rate than sporadic AVMs in the general population. DVAs are slightly more frequent in the setting of HHT; while the prevalence of aneurysms and capillary telangiectasias is similar to that of the general population. The appearance of hemosiderin deposits may be attributed to other existing etiologies; its occurrence in a younger population, however, suggests an underlying relationship with HHT, possibly representing old hemorrhage or vascular thrombosis [1].

#### References

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#### OR54 Nitric oxide synthase inhibition attenuates the formation of notch-mediated brain arteriovenous malformations

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**Objectives:** Brain arteriovenous malformations (BAVMs) occur in hereditary hemorrhagic telangiectasia (HHT) patients, and HHT2/Alk1 and Notch signaling pathways interact. Endothelial expression of constitutively-active Notch4 (Notch4\*) initiates BAVMs in mice through enlargement of microvessels without an increase in endothelial cell number or proliferation. Notch activation induces nitric oxide (NO) synthesis that regulates vascular tone. We hypothesized that Notch4\* initiates BAVMs by disrupting normal NO signaling and vascular tone, thereby permitting vessel enlargement and AV shunting.

**Methods:** To determine the effect of reduced NO on BAVM formation, Notch4\* mutants were treated with NO synthase (NOS) inhibitor NG-nitro-L-arginine (L-NNA) and evaluated for BAVM formation. To measure vascular tone, diameters of cannulated and pressurized cerebral arteries from Notch4\* and control mice were analyzed.

**Results:** L-NNA attenuated Notch4\*-induced AV shunt initiation: AV connection diameter in L-NNA-treated Notch4\* mutants was decreased versus saline-treated Notch4\* mutants at postnatal day 12, when vessel enlargement and AV shunting first become apparent in untreated mutants. L-NNA-treated Notch4\* mutants exhibited delayed BAVM-associated pathologies and mortality. In moribund L-NNA-treated Notch4\* mutants, AV connection diameter was decreased versus saline-treated Notch4\* mutants. Median survival time was increased in L-NNA-treated Notch4\* mutants. Furthermore, Notch4\* cerebral arteries exhibited decreased tone versus controls.

**Conclusions:** Our results suggest that vascular dysfunction contributes to Notch4\*-mediated BAVM formation and that inhibiting NOS attenuates the onset of BAVM formation. These data support a role for NO pathway in Notch4\*-mediated BAVM formation. As Notch and HHT2/Alk1 signaling interact, this work may open new directions in investigating mechanisms underlying BAVM formation in HHT.

#### OR55 Arteriovenous malformations in ALK1 mutants arise independently of perturbations in notch signaling

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**Objectives:** Notch and activin receptor-like kinase 1 (ALK1) have been implicated in arterial specification and angiogenic tip/stalk cell differentiation, and perturbation of either pathway causes arteriovenous malformations (AVMs). Furthermore, ALK1 can cooperate with Notch to upregulate expression of Notch target genes in cultured endothelial cells. These findings have led to the hypothesis that Notch and ALK1 collaboratively program arterial identity and prevent AVMs. The objective of this work was to investigate the interaction between Notch and Alk1 signaling in vascular development in vivo.

**Methods:** We modulated Notch and Alk1 activities in zebrafish (*Danio rerio*) embryos and examined effects on Notch target gene expression and vascular morphology.

**Results:** Results demonstrate that control of Notch targets is context-dependent, with gene-specific and region-specific requirements for Notch and Alk1. Alk1 and Notch cooperatively support *hey2* and *ephrinb2a* expression in the dorsal aorta, yet play opposing roles in *dll4* expression in the dorsal aorta and cranial arteries and in *hey2* expression in cranial arteries, with Notch inducing and Alk1 repressing expression of these genes. Although loss of *alk1* increases expression of *dll4*, which encodes a Notch ligand, AVMs in *alk1* mutants could neither be phenocopied by Notch activation nor rescued by Notch inhibition.

**Conclusions:** Alk1 is dispensable for acquisition and maintenance of arterial identity, and perturbations in Notch signaling cannot account for AVMs in *alk1* mutants.

#### OR56 Transcription factor KLF6 upregulates gene expression of metalloprotease MMP-14 and soluble endoglin release upon vascular injury

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It has been postulated that an impaired vascular remodelling in an endoglin haploinsufficient setting is at the pathogenic basis of hereditary hemorrhagic telangiectasia. After endothelial injury, the transcription factor Krüppel-like factor 6 (KLF6) translocates into the cell nucleus to regulate a variety of target genes involved in vascular repair and remodelling, including components of the membrane TGF- $\beta$  receptor complex such as endoglin and ALK1. While most of endoglin studies have focused on the membrane form, the regulation and role of soluble endoglin are poorly understood. The membrane metalloproteinase 14 (MMP14) targets membrane endoglin to release soluble endoglin and is involved in vascular inflammation and endothelial tube formation, but little is known about the regulation of MMP14 expression during vascular wounding. In vitro denudation of monolayers of human endothelial cells (ECs) leads to an increase in the KLF6 gene transcriptional rate, followed by an upregulation of MMP14 and soluble endoglin. Concomitant with this process, MMP14 co-localizes with endoglin in the sprouting endothelial cells surrounding the wound border. Moreover, after wire-induced endothelial denudation, *Klf6*<sup>+/-</sup> mice show lower levels of MMP14 in

their vasculature compared with their wild-type siblings. Overexpression of KLF6 results in an increased transcription rate of MMP14, and chromatin immunoprecipitation assays show that KLF6 interacts with MMP14 promoter in ECs, this interaction being enhanced during wound healing. Furthermore, KLF6 markedly increased the transcriptional activity of different luciferase reporter constructs of MMP14 gene promoter. These results suggest that KLF6 regulates MMP14 transcription and is a critical player of the complex gene expression network triggered during endothelial repair.

#### SESSION 13 TREATMENT OF HHT IV

##### OR57 Treatment of severe refractory epistaxis in hereditary hemorrhagic telangiectasia using a two-flap nasal closure method

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**Objective:** Nasal closure has been shown to effectively manage severe epistaxis refractory to other treatments in patients with hereditary hemorrhagic telangiectasia (HHT). The current nasal closure procedure may be underutilized due to its surgical complexity and flap breakdown. We characterize a typical nasal closure patient and compare outcomes based on our experience with the traditional three-flap closure and a simplified two-flap nasal closure procedure.

**Methods:** Retrospective review of a database of HHT patients treated for severe epistaxis with nasal closure between 2005 and 2013. Operating room (OR) time, need for revision surgery, pre- and post-procedure epistaxis severity score (ESS), complete blood count values, and Glasgow Benefit Inventory (GBI) questionnaire results were collected for each patient.

**Results:** Average decrease in ESS subsequent to nasal closure using the two flap method is 6.17 and mean GBI score is 56.3. The average candidate for nasal closure has an ESS of 7.42, Hgb of 8.9 g/dL, received multiple transfusions, iron therapy, and cautery/coagulation procedures. Comparison of six patients who underwent the traditional nasal closure procedure and eight patients receiving the simplified two flap nasal closure showed no statistically significant difference in postoperative ESS or GBI metrics. Mean OR times of the traditional and simplified methods were 3.12 and 1.44 h ( $p < 0.05$ ). Mean time to first revision for eight nasal closure patients is 21.5 months (1.8–59.4 months).

**Conclusion:** In short-term follow-up, the two-flap procedure has shown comparable effectiveness with significantly reduced complexity and operative time compared to the traditional nasal closure method.

##### OR58 Septodermoplasty using a porcine derived mucosal graft

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Nasal septum resurfacing techniques have long been a mainstay for surgical management of epistaxis related to HHT. While generally effective, the use of dermis to accomplish this is often associated with two particular problems. The first is the impact of replacing the mucous membrane with squamous epithelium. This leads to issues of drying and crusting requiring frequent debridement and irrigations. Secondly, there is the pain of the donor site.

Herein is presented three procedures performed in two patients using a porcine derived mucosal graft material. The material used is manufactured by Cook Medical and sold under the name Biodesign

ENT repair graft. It is FDA approved for nasal mucosal replacement procedures.

In all three procedures, the mucosal healing was complete with no exposed areas at the 6-week post-op follow-up. Pain was minimal for each patient. There were no complications such as postop bleeding, infection, or graft separation. The long-term goal of epistaxis control is still under review and will require more patients to make a meaningful comparison to traditional dermal grafting.

##### OR59 Detection and characterization of hepatic manifestation of HHT by means of magnetic resonance imaging

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**Objectives:** To evaluate Magnetic Resonance Imaging for screening and characterization of hepatic manifestation of HHT.

**Methods:** 316 patients (males 129; age range 3–86 years; mean  $46 \pm 17$  years) underwent unenhanced and contrast-enhanced MRI of the liver and hepatic vasculature including hepatobiliary phase and contrast-enhanced MR-angiography (Gadolinium-BOPTA 0.05 mmol/kg bodyweight; MultiHance, Bracco).

**Results:** Magnetic resonance imaging revealed 57 patients (males 13; mean  $56 \pm 11$  years) with hepatic manifestation of HHT. 18 patients also showed coexistent PAVM. Typical findings in hepatic HHT were hepatomegalia ( $n = 34$ ) or right-heart insufficiency (RHI) ( $n = 15$ ) in the absence of PAVM. Patients with an enlarged hepatic artery were usually associated with hyperplastic liver nodules similar to focal nodular hyperplasia (FNH) ( $n = 19$ ). Furthermore, signs of RHI were rare and the diameter of the portal vein was rather normal.

**Conclusions:** MRI has the ability for a detailed characterization of hepatic involvement of HHT. Hepatomegalia and nodular hyperplasia may represent sequelae of shunts at the sinusoidal level, which do not result in direct hemodynamical left-to-right shunting. In contrast, direct arterio-venous and arterio-portal shunts are likely associated with right-heart insufficiency.

##### OR60 Late manifestations and diagnosis of HHT

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**Objective:** To describe the epidemiologic and clinic variability of HHT and its potential impact on diagnostics.

**Methods:** After foundation of a new interdisciplinary treatment centre in 2007 data of visiting patients were collected by a standardized patient interview at the moment of the first patient visit.

**Results:** A total of 312 persons were referred to our HHT centre from 10/2007 to 1/2013. No clinical criterion was fulfilled in eight persons, the diagnosis could be confirmed in 254 patients (mean age/minimum age/maximum age in years (me/mi/ma): 52/7/92 years). First symptoms occurred at a mean age of 23 years and were in nearly all patients nosebleeds. However, according to some patients first manifestations were experienced at a much higher age ranging up to 87 years (me/mi/ma: 23/0/87 years,  $n = 135$ ). Average age of patients at the point of diagnosis was 41 years (me/mi/ma: 41/8/87 years,  $n = 121$ ). The average delay between first symptoms and

clinical diagnosis was 16 years but ranged up to 52 years (me/mi/ma: 16/0/52 years,  $n = 105$ ).

**Conclusion:** Our data confirmed the report of another study showing a relatively long diagnostic delay in HHT. Additionally, we report on a number of patients with a very high age at the moment of first manifestation. This adds a new aspect to the question if routine diagnostic procedures for HHT should not only include clinical criteria, but also molecular genetic testing as otherwise HHT might be passed on unknowingly even for 2 generations.

## POSTER PRESENTATIONS

### ASSORTED TOPICS OF HHT (P1–P10)

#### P1 C.A.R.D project for HHT patients: a 2 year activity report

**Manfredi G<sup>1</sup>; Alicante S<sup>1</sup>; De Grazia F<sup>1</sup>; Lupinacci G<sup>1</sup>; Menozzi F<sup>1</sup>; Brambilla G<sup>1</sup>; Londoni C<sup>1</sup>; Gazzaniga P<sup>2</sup>; Gandolfi S<sup>3</sup>; Forner PA<sup>4</sup>; Danesino C<sup>5</sup>; Olivieri C<sup>5</sup>; Ornati F<sup>5</sup>; Pagella F<sup>6</sup>; Grosso M<sup>7</sup>; Pongiglione G<sup>8</sup>; Boccardi E<sup>9</sup>; Buscarini E<sup>1</sup> and on behalf of HHT-NET**

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**Background and study aims:** In 2013 we started the C.A.R.D (Crisis Aid for a Rare Disease) project aimed to assist patient management in case of emergent/severe complications of HHT.

**Methods:** Every patient in our database with a definite HHT diagnosis and with a previous clinical and instrumental assessment in our HHT center, received a plastic card bearing on the front the patient data: name, surname, date of birth, fiscal code, the diagnosis of HHT, the presence/absence or lack of evaluation of: CAVM, PAVM, HAVM, GI telangiectases, high output cardiac failure; the need or not for antibiotic prophylaxis; the card back lists general recommendations regarding presentation and treatment of main and most severe HHT complications, and the phone numbers of our HHT center for

**Table 1** Reports the number and cause of phone calls received and patient outcomes

| Phone call causes/number of cases     | Phone call | Patient outcome   |
|---------------------------------------|------------|-------------------|
| Portosystemic encephalopathy/2        | 4          | Recovery          |
| Aortic dissection (focal)/1           | 5          | Patient stability |
| Cardiac arrhythmia/8                  | 12         | Recovery          |
| Deep vein thrombosis/3                | 5          | Recovery          |
| Takotsubo cardiomyopathy/1            | 7          | Recovery          |
| Acute myocardial infarction/1         | 22         | Death             |
| Emergent anticoagulation management/5 | 6          | Recovery          |
| Pneumothorax/1                        | 3          | Recovery          |
| Severe overt GI bleeding/2            | 7          | Recovery          |
| Severe nosebleed/3                    | 3          | Recovery          |
| Pre-surgery advice/6                  | 6          | Uneventful        |
| Total: 33                             | 80         |                   |

emergency calls. Physicians and nurses of our HHT center warranted a 24/7/365 availability to answer to phone calls regarding HHT-related urgent/severe conditions; the name and phone number of patient/physician calling is passed to the HHT-expert physicians who contacted the patient immediately. An appropriate recording of the number and causes of phone calls received, and of patient outcome has been scheduled.

**Results:** Since October 2012 to December 2014 572 cards have been sent to the patients (Table 1).

**Conclusions:** The HHT C.A.R.D. can offer greater timeliness and appropriateness in the treatment of HHT-related complications outside HHT centers. Either the number and severity of HHT related emergencies or the need for urgent advice are probably greater than expected, thus enhancing the importance of offering specialized advice.

#### P2 Comprehensive screening for arteriovenous malformations in hereditary hemorrhagic telangiectasia patients

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**Introduction:** Hereditary hemorrhagic telangiectasia (HHT), also known as Osler–Weber–Rendu syndrome or Osler–Weber–Rendu disease, is an autosomal dominant genetic disorder that leads to malformations of the blood vessel in the skin, mucous membranes, and often in internal organs such as lungs, liver, and brain. The phenotype of the disease has a wide variability.

**Method:** There were 55 cases examined in our clinic in a period of 8 years time. The evaluation was based on the results of the imaging investigations given by our screening draft. This contains: an abdominal ultrasound, a CT scan of the thorax (with contrast medium) and an MRI scan of the brain.

**Results:** Out of the total of 55 cases a complete staging took place in 45 of the cases. Endonasal telangiectasia was detected in 95 % of all the cases. Extranasal lesions were found in decreasing order in the area of the tongue, enoral, skin of the fingers, head and chest. The arteriovenous malformations showed a great variability in size and form. In 4 % of the cases there was a primary extranasal bleeding described from the epiglottis area. In 65 % of the total 45 cases we found occult arteriovenous malformations: 30 % in the lungs, 17 % in the liver, 13 % gastrointestinal and 4 % in the brain.

**Conclusion:** The imaging screening is very important especially in detecting the occult arteriovenous malformations and preventing their further dramatic outcomes.

#### P3 Creating a database for the study of the disease manifestations of hereditary hemorrhagic telangiectasia

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**Objectives:** The purpose of this study is to create a consortium, or group of centers that share information and research focusing on the evaluation and treatment of patients with HHT. The Brain and Vascular Malformation Consortium has successfully provided a large-scale collaboration to study risk factors for bleeding from cerebral AVMs. We propose to increase data collection of problems associated with HHT, specifically pulmonary AVMs, liver AVMs, anemia, epistaxis, and GI bleeding to create a more inclusive database.

**Methods:** After IRB approval and patient consent, patients will be followed and data will be collected and entered into a registry. Templates were constructed to mimic the BVMC collection sheets for cerebral AVMs.

**Results:** We have successfully created a database and have begun to collect data internally at UCSF on the above-described disease manifestations.

**Conclusion:** The availability of a more complete standardized data collection forum will assist clinicians in establishing best practices for improving HHT patient outcomes. We hope that the knowledge we obtain about the risk factors and treatment success in these patients from this larger study will help us improve the care of HHT patients. We welcome collaboration with other interested sites.

#### P4 Electronic medical records in hereditary hemorrhagic telangiectasia: implementation of monitoring flow sheets for physiology and iron stores

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**Introduction:** Documenting care for persons with hereditary hemorrhagic telangiectasia (HHT) can be complex and time-consuming. Electronic medical records (EMRs) can help reduce errors of omission but at the cost of increased informational burdens for clinicians unless specific customizations are built to facilitate efficient disease-specific function and outcomes tracking. Data digests must be easily integrated into succinct communications that facilitate continuity of care. Along with iron deficiency anemia, many patients with HHT have obstructive airways diseases (OAD), mandating consistent pulmonary physiologic monitoring so that treatment can be tailored for persons who may have both pulmonary arterio-venous malformations (PAVMs) and OAD.

**Methods:** The Edmonton HHT Center, part of Alberta Health Services, adopted the Epic ambulatory care EMR (“eCLINICIAN”), and has over 2 years’ experience using this system for HHT chronic disease management. A systematic consensus-building process was used to derive a disease-specific electronic flow sheet for PFT monitoring. A prototype was developed, then selectively deployed and iteratively modified over a 12-month period, following which review and optimization was performed on “test charts” prior to broader implementation.

**Results:** The HHT pulmonary physiology flow sheet has been successfully integrated into care management for HHT patients within the eCLINICIAN EMR.

**Clinical impact:** Use of the pulmonary physiology electronic flow sheet for persons with HHT has improved EMR workflows and clinician efficiency while improving the quality of HHT chronic disease management. Enhancements continue, including plans for documentation of PAVM localization and thoracic embolization treatment.

**Funding support:** EMR Innovation Grant, University of Alberta.

#### P5 Quality of life in patients with hereditary hemorrhagic telangiectasia (Rendu–Osler–Weber syndrome)

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HHT affects 1/5000–8000 individuals worldwide. Clinical manifestations depend on the presence, localization and severity of these alterations; with possible complications added, this disorder frequently alters quality of life and produces social isolation.

**Objective:** To identify the associated factors to a lower quality of life in our HHT adult patients.

**Methods:** Cross-sectional study of patients that belong to the institutional registry of HHT, 2010–2013, Hospital Italiano, Buenos Aires. All patients were evaluated prospectively by a HHT specialist. Quality of life was measured with the Euroqol and analogue visual scale validated for the country.

**Results:** The study included 127 patients. 42 (33 %) were men, with a mean age of 47 years (16). The median for quality of life measured by the analogue visual scale was 69 (ICR: 20.3). From the total of patients, 96.1 % (122) presented epistaxis, and from those patients the 33.9 % (33) had severe epistaxis; 76 (60.3 %) had anemia, 71 (65.1 %) pulmonary AVMs, from which 50 (39.4 %) presented related symptoms; 21 (19.6 %) had AVMs in CNS; 75 (73.5 %) had hepatic AVMs and 46 (63 %) had GI AVMs from which 34 (27.6 %) were symptomatic. Association was found between de means of quality of life and anemia.

**Conclusion:** Considering quality of life in HHT, may help to guide clinical evaluation and treatment, and make additional measures in order to improve quality of life in patients with anemia and severe epistaxis and symptoms associated to GI and CNS AVM.

#### P6 Strategies for navigating insurance denials for HHT treatments

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**Objectives:** (1) Outline the common HHT tests and treatments for which payment is often refused by insurance companies. (2) Provide examples of templates useable by the caregivers for HHT patients to request and/or appeal insurance approval for these tests and treatments. (3) Provide citations of the most relevant research articles supporting the use of these tests and treatments, which can be used to support insurance appeals and in peer-to-peer reviews. (4) Offer general tips and considerations in regards to successfully obtaining insurance approvals for HHT patients.

**Methods:** We have reviewed the insurance approvals and denials for HHT patients at a large HHT center over the last 5 years. Based on this experience, we have compiled templates, citations, and other strategies to help other HHT physicians obtain insurance approval for necessary tests and treatments.

**Results:** Depending on the exact insurance plan and prescription drug coverage, initial denial of coverage for such off-label medications as tranexamic acid and Avastin exceeds 50 %. Denial of genetic testing, necessary imaging tests and out-of-network visits to an HHT center of excellence occur at a lesser but still significant rate. Employing the proper strategy can help achieve insurance approval for these necessary tests and treatments.

**Conclusions:** HHT is a rare disease, and insurance companies are often unaware of HHT-specific tests and treatments, especially if being used in an off-label fashion. Having access to appropriate templates and research citations can help HHT physicians achieve insurance approval.

### P7 The HHTreat project: disease mechanism-driven drug screening

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The understanding of the molecular mechanisms underlying HHT has substantially progressed. Our group was first to identify the bone morphogenetic proteins 9 and 10 (BMP9/10) as the physiological high-affinity ligands of ALK1 and endoglin. The involvement of these two BMPs in HHT pathogenesis is supported by the recent identification of *GDF2* (encoding BMP9) mutations in three HHT families and by our characterization of two *ENG* mutations that suppress BMP9 binding to endoglin. These observations support the hypothesis that HHT is a BMP9/BMP10 signaling disease.

As most heterozygous *ACVRL1* or *ENG* mutations result in reduced functional cell surface expression of ALK1 or endoglin receptors, we reasoned that drugs able to potentiate the cellular response to BMP9 might improve the endothelial functions of HHT patients.

The goal of the HHTreat project is to identify novel drugs that could increase the physiological BMP9 signaling in endothelial cells of HHT patients. For this purpose, we intend to screen the Prestwick Chemical Library<sup>®</sup>. This commercial library contains 1280 molecules whose toxicological and pharmacological properties are well characterized. Being already FDA-approved, these drugs will present the advantage to be rapidly testable in a clinical trial. The screening will be performed using a miniaturized BMP9-response assay based on the ability of drugs to potentiate the Smad1/5 phosphorylation induced by picomolar doses of BMP9 in microvascular endothelial cells from human dermis. This project aims at identifying drugs that could correct the signaling pathway altered in HHT in contrast to current therapeutic approaches that treat the symptoms of the disease rather than its mechanistic cause.

### P8 University of California San Francisco's hereditary hemorrhagic telangiectasia center of excellence: first year clinical experience

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**Objectives:** To report the first year clinical experience at UCSF's HHT Center of Excellence in recruiting patients and characterize the spectrum of disease manifestations in our patients.

**Methods:** From January 2014 to January 2015, 79 patients have been recruited into our HHT COE, 36 males and 43 females with a mean age of 39 years. Clinical notes, imaging reports and labs were reviewed.

**Results:** Thirty patients have positively tested for a known HHT genetic mutation (16 with *ACVRL1* mutation and 14 with *ENG* mutation). The median Curacao score is 3. Forty-two patients have visceral AVMs, 35 have telangiectasia, 59 report recurrent spontaneous epistaxis and 39 report a family history of HHT or HHT symptoms. Patients were seen by self-referral or by referral from their primary care physician, a specialist, or the HHT Foundation. Forty patients had one or more additional family members counseled by a specialist in the HHT COE. Epistaxis was the most common first symptom of the disease. Within this inaugural year, patients have received the following treatments for diseases secondary to HHT: 8

pulmonary embolizations, 1 lung transplant, 2 surgical AVM resections, 1 radiosurgical procedure, 4 cauterizations of nasal telangiectasia and 2 nasal splints. Twenty-five patients have enrolled in the Brain Vascular Malformation Consortium.

**Conclusion:** We characterized patients on the basis of visceral AVMs, telangiectasia, epistaxis and family history. Patients received care according to HHT treatment guidelines regarding their vascular malformations and other manifestations of HHT.

### P9 Vaccination program for patients with hereditary hemorrhagic telangiectasia

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**Objectives:** Hereditary haemorrhagic telangiectasia (HHT) patients are suspected to be in a higher risk for infectious diseases with 28 % cases requiring hospitalization. A vaccination program has been developed in our HHT unit in order to help to prevent some of these infections.

**Methods:** Revision of the literature and study of co-morbidities in our series of patients to establish epidemiology of infectious diseases. Development of a consensus program between Infection Control (IC) and Internal Medicine departments. Design of a vaccination protocol and its approval by the Hospital's Committees; inclusion of new preparations for in hospital administration (Prevenar-13, Bexero) and finally consensus with Public Health for Ambulatory immunization in primary care units.

**Results:** Four vaccination categories were stated: (a) General: Pneumococcal conjugate, *Influenza*, and anti-*Haemophilus influenzae*. (b) Heavy bleeding (risk of transfusions): addition of anti-hepatitis B. (c) Anticipation of future needs of antiangiogenic drugs: addition of 23-valent pneumococcal polysaccharide, quadrivalent anti-meningococcal conjugate and anti-meningococcal B. (d) Children: child calendar using quadrivalent anti-meningococcal conjugate and adding anti-meningococcal B and 13-valent conjugate pneumococcal vaccines. A circuit of vaccination was established based on a network including Specialized Care (HHT Unit IC), Primary Care and reference hospital, to minimize number of hospital consultancies. Our aim is to achieve immunization of all patients attended without generating extra hospital visits for them and to prevent infectious complications.

**Conclusions:** Design of prevention strategies of infectious complications in HHT patients with vaccination protocols seem to be necessary. Efficiency, effectiveness and safety of the program need to be assayed.

### P10 Working together for the benefit of the patient? Organization of the health care services for the HHT patients at Oslo University Hospital

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Centre for rare disorders is an interdisciplinary, nationwide competence centre with a special responsibility for counseling, information and educational work. HHT is one of the Centre's designated diagnoses. When establishing the service for the HHT group, an important goal was to create a collaborating and integrated service. The Osler Team was established by involving representatives from central specialties at Oslo University Hospital. The team comprises of a geneticist, pulmonologist, radiologist, neuro-surgeon, oto-rhino-lyngologist and representatives from the Centre. Other relevant specialties are involved on a consultative basis. The team's first task was to establish guidelines and routines for treatment and follow up, make the service known to patients and health professionals throughout the country and to write and distribute information materials. Further, the team's work has concentrated on improving services, discussion on case and diagnose-related themes, research projects and updates.

The patient organization was founded in 2008, and has emphasized the importance of having a close connection with the medical specialists and Centre for rare disorders, especially regarding activities like planning and cooperation on courses, spreading knowledge and awareness on the disease, and how to cope. The organization's board meetings are held at the Center's location and hence contribute to contact and cooperation.

This three party collaboration; the patient organization, the clinic and the Centre for rare disorders, has been pivotal in developing a good medical and information service for the HHT group. The patient's voice is heard and heeded and thus is an example of shared decision-making at organizational level.

#### CLINICAL MANIFESTATIONS OF HHT (P11–P23)

##### P11 Overlap syndrome of hereditary hemorrhagic telangiectasia and juvenile polyposis: a case series

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**Introduction:** Hereditary hemorrhagic telangiectasia (HHT) and juvenile polyposis syndrome (JPS) are two clinical entities that can overlap when SMAD4 mutation is present, called overlap syndrome (OS). Its prevalence is very low and displays characteristics of both diseases.

**Objective:** To describe a case series of OS.

**Methods:** Cases diagnosed belong to HHT Institutional Registry (NCT01761981) of Hospital Italiano, Buenos Aires.

**Clinical cases:** Six cases were found, all women between 16 and 66 years. Two patients were first-degree relatives (mother and daughter). Four presented SMAD4 gene mutation, one had negative ENG and AKI-1 and one didn't had tests. Five presented AVMs in solid organs. All cases had gastrointestinal telangiectases and presented gastrointestinal bleeding (GIB). Three underwent successful endoscopic argon plasma coagulation sessions to control GIB. Three required total gastrectomy for multiple large polyps and persistent anemia. One case suffered pyloric syndrome due to large size polyps. After gastrectomy, low-grade dysplastic polyps were found in one patient (daughter) and high-grade dysplastic polyps were found in another (mother). Two underwent total colectomy and small-bowel resection.

**Discussion:** Previous studies show that around 2 % of HHT patients suffer JPS, however 20 % of JPS patients have HHT. OS patients develop AVMs earlier than those with isolated HHT. Moreover, GIB,

dysplastic lesions, gastrointestinal tract cancer and surgery requirement at an early age is characteristic. All patients developed GIB, five of them underwent surgery for gastric and colonic dysplastic lesions. Though OS is not a prevalent condition, it has important health implications and its recognition allows an appropriate surveillance.

##### P12 Aortic dilation in patients with hereditary haemorrhagic telangiectasia and a SMAD4 gene mutation

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**Objectives:** Aortic dilation is associated with life-threatening complications such as aortic dissection and rupture. Hereditary haemorrhagic telangiectasia (HHT) can be caused by a mutation in the SMAD4 gene, which is part of the TGF- $\beta$  signalling pathway. Mutations in several other genes within this pathway are associated with familial thoracic aortic aneurysm and aortic dissection. A few case reports and small case series describe patients with a SMAD4 gene mutation and aortic disease, but the true prevalence remains unknown.

**Methods:** Chest computed tomography (CT) of all HHT patients with a SMAD4 gene mutation were reviewed retrospectively. The aortic root, ascending aorta and descending aorta were measured.

**Results:** In total 15 patients (66.7 % female, mean age  $40.5 \pm 13.4$  years) were included. The mean dimension of the aortic root, ascending aorta and descending aorta was  $34.1 \pm 5.2$ ,  $32.9 \pm 6.4$  and  $23.4 \pm 4.5$  mm, respectively. Corrected for age and BSA, the aortic root was dilated in five patients (33.3 %). Corrected for age, the ascending and descending aorta were dilated in two patients (13.3 %). None of the patients required intervention.

**Conclusion:** Aortic dilation is common in HHT patients with a SMAD4 gene mutation and most frequently involves the aortic root. We advise to screen for aortic dilation in all HHT patients with a SMAD4 gene mutation.

##### P13 Exploring the contribution of hereditary haemorrhagic telangiectasia to the aetiology of nosebleeds and early strokes in ghana: a research proposal

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**Introduction:** The notion that hereditary haemorrhagic telangiectasia (HHT) is rare in sub-Saharan Africa (SSA) has been held for a long time. The criteria for HHT diagnosis is difficult to apply in dark-skinned populations. Case reports and studies among black populations outside of SSA suggest the need for a systematic approach to HHT inquiry among populations in SSA.

**Methods:** The presentation will outline a research proposal to explore the contribution of HHT to the aetiology of nosebleeds and strokes in persons younger than 40 years in Ghana. Through a network of health

facilities, patients will be enrolled into a survey that includes blood sampling for HHT genetic tests. The objective is to determine the prevalence of HHT genetic markers and to describe in detail the clinical manifestation of HHT in this population. The provisional sample size is 900 cases recruited over 15 months. Patients with HHT genetic markers will receive counselling. The goal of the presentation at this Conference is to solicit partnership from individuals and institutions with capacity for HHT genetic analysis. It is also intended to provide opportunity for critical feedback on the research design.

**Conclusion:** Holding on to the notion that HHT is rare in SSA in the absence of evidence from systematic inquiry is unethical and inequitable. The application of HHT genetic analysis should make it possible to this notion to be confirmed or debunked in favour of improved care for HHT-affected patients in SSA.

#### P14 High prevalence and multiple etiologies of heart failure in hereditary hemorrhagic telangiectasia

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Heart failure (HF) is under-recognized in HHT. We evaluated patients referred to our center to determine prevalence, etiology, and contributing factors of HF in this population.

**Methods:** We retrospectively studied adults seen in the Utah HHT Center from August 2013 to January 2015. These patients had a detailed cardiac history, a laboratory workup including BNP, and an echocardiogram. HF was defined as BNP > 100 pg/mL. We compared HHT patients with elevated BNP to sex and age matched HHT controls using univariate analyses.

**Results:** 75 individuals meeting Curaçao criteria were evaluated. Heart failure was seen with high prevalence (24 %). In patients over 50 years of age, HF prevalence increased to 42.5 %. Only 44 % (8/18) of HF cases had documented high-output. Identifiable causes of HF other than high-output (ischemic, HFpEF, valvular) were identified in 7 of the HF cases with remaining etiologies likely multifactorial. Cardiac EF was not different between groups. HF was associated with worse anemia ( $p = 0.0125$ ), more GI bleeding ( $p = 0.0229$ ), increased left atrial size ( $p < 0.0001$ ), a higher echo-derived cardiac index ( $p = 0.0226$ ), and more prevalence of atrial fibrillation ( $p = 0.0455$ ). Dyspnea was highly prevalent in both groups (78 % with HF and 56 % without) and was not a good predictor of heart failure. Neither were standard cardiac risk factors like DM, smoking history, HTN, or CAD.

**Conclusions:** HF is prevalent in the HHT population and the diagnosis may be masked by high prevalence of dyspnea in this population. Though a subset of HF patients had high-output, HF in HHT is more complex.

#### P15 Laryngeal telangiectatic lesions in a patient diagnosed with hereditary haemorrhagic telangiectasia

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**Objective:** To present a case of Laryngeal telangiectatic lesions in a 69-year-old female patient with hereditary haemorrhagic telangiectasia (HHT).

**Methods:** The Patient was clinically evaluated she was suffering from hoarseness. Fiberoptic laryngoscopy revealed a telangiectatic lesion on the right vocal cord. With the patient in general anaesthesia the lesion was treated with laser.

**Results:** After treatment her voice improved markedly. The improvement in voice was documented by phonetogram measurement.

**Conclusion:** Patients with HHT who complain of hoarseness should be referred to an otolaryngologist for evaluation. If telangiectatic lesions are identified at the vocal cords, these may explain the hoarseness and should be treated with laser surgery, in order to improve the voice and prevent re-bleeding. The treatment can be performed without any harm to the vocal mucosa, and a marked improvement of the voice may be noted.

#### P16 Left adrenal arteriovenous malformation growing during the 8-year follow-up of a patient with hereditary hemorrhagic telangiectasia: successful treatment by embolization

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**Objective:** To report the first case of an adrenal arteriovenous malformation (AAVM) in a patient with HHT who was successfully treated by selective embolization.

**Methods:** A 47-year-old man with HHT1 and hepatic and pulmonary involvements underwent serial enhanced CT-scans.

**Result:** Multiple pulmonary arteriovenous malformations (PAVM) had been revealed by a brain abscess, and previously treated by embolization. A left AAVM was also detected initially, but was too small to be embolized. The size of the aneurismal sac of the AAVM was 3, 3 and 16 mm, on the initial CT-scan, and 1 and 5 years later, respectively. The CT-scan performed 7 years later revealed exponential enlargement of the AAVM. The size of the aneurismal sac then reached, 20 mm. To prevent complication, i.e. retroperitoneal rupture and life-threatening hemorrhage, we decided to undergo an embolization procedure. The arteriography showed that the AAVM was fed by the left adrenal artery and drained into the left renal vein. A supraseductive embolization by microcoils was successfully performed. There was no immediate or delayed complication of the procedure.

**Conclusion:** In HHT patients, the involvement of the liver and the gastrointestinal tract leads to the development of visceral artery aneurisms. The present case, not previously reported, suggests that another unidentified mechanism of systemic vascular growth factor exists. Furthermore, we question about the contributing role of PAVM embolization as a growth factor of this AAVM.

#### P17 Managing venous thrombosis in hereditary hemorrhagic telangiectasia

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**Background:** Hereditary hemorrhagic telangiectasia (HHT) is characterized phenotypically by abnormal connections between arteries and veins resulting in spontaneous and recurrent epistaxis, telangiectasia, and arteriovenous malformations (AVMs). New evidence indicates that HHT patients also have clinical manifestations not directly attributable to the abnormal blood vessel assembly, including migraine headaches, defective immunity, and venous thrombosis (VT). Moreover, previous studies have shown that HHT individuals have higher incidence of VT than the general population. However, many physicians consider HHT an absolute contraindication to anticoagulation, and there is no consensus on how to anticoagulate these patients.

**Methods:** We present our experience managing 12 adult patients with HHT and VT at the University of Texas Southwestern Medical Center. Those considered for anticoagulation did not have history of severe active epistaxis or gastrointestinal bleeding, blood transfusion dependency, or untreated pulmonary or cerebral AVMs.

**Results:** All patients had a definite diagnosis of HHT by Curaçao criteria. Ages ranged from 23 to 74 years. Genders were equally represented. Pulmonary embolism (PE) was the primary presentation, 40 % having both deep vein thrombosis and PE, and 16 % having thrombosis in veins other than extremities. Risk factors for VT were identified in 58 %. Heparin, fundaparinux, or warfarin was used for anticoagulation. Anticoagulation was discontinued in 25 % of patients due to bleeding. 41 % received IVC filters. No anticoagulation-related deaths were observed.

**Conclusion:** HHT individuals have higher prevalence of VT than the general population. HHT patients can be effectively and safely anticoagulated when carefully selected. Thrombosis and anticoagulation in HHT warrants further investigation.

#### P18 New telangiectasia syndrome(s)?

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**Objective:** Describe findings in multigenerational families referred to a specialty clinic for HHT, with evidence of a telangiectasia syndrome, but who do not meet clinical or molecular criteria for HHT.

**Methods:** Retrospective pedigree and chart review of families who are negative for a mutation in *ENG*, *ACVRL1* or *SMAD4*, do not have solid organ involvement despite routinely recommended screening, yet are compelling for having a hereditary predisposition to form superficial telangiectases typical of HHT.

**Results:** 10 families were identified and will be described in which dermal telangiectases typical of HHT are present in multiple members and in which one or more members experience recurrent epistaxis. One family had a member with a large lower limb AVM, but AVMs were otherwise not reported in these families or detected in examined members.

**Conclusions:** Epistaxis and telangiectasias are hallmarks of HHT, yet these families that lack a mutation in one of the known HHT genes and lack solid organ involvement suggests that there is a spectrum of hereditary telangiectasia disorders including some with a more benign course. Clinical and molecular characterization of such families may

require either an expanded definition of HHT itself, or an expanded categorization of telangiectasia/AVM disorders which includes HHT.

#### P19 Obstructive airway disorders affecting individuals with hereditary hemorrhagic telangiectasia

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**Objectives:** Dyspnea is a common respiratory symptom with varied causes. Pulmonary vascular involvement in hereditary hemorrhagic telangiectasia (HHT) includes pulmonary arterio-venous malformations (PAVMs), and much less commonly pulmonary hypertension (PH), both of which may result in dyspnea. Additionally dyspnea is a common symptom in individuals who have an obstructive airway disorder (OAD) such as asthma, bronchiectasis or COPD. Sometimes individuals with HHT can have both pulmonary vascular disorders and a concomitant OAD, and recognition of this would be critical to effective management of each.

**Methods:** We conducted a retrospective database review of individuals seen in the Edmonton HHT clinic with a definite diagnosis of HHT (vs. “possible HHT”) to assess the proportion of patients with a concomitant OAD (bronchiectasis, asthma or COPD). Data was captured through our existing Edmonton HHT database, following institutional ethics approval. Use and type of inhalers was no included in this review.

**Results:** A total of 209 charts were included in the database, of which 80 charts of patients with definite HHT were reviewed. 39 % had at least one identified PAVM. As well 28 % had a documented OAD. Of the charts reviewed 11 % of the patients had both an OAD and a PAVM identified.

**Conclusions:** Concomitant diagnoses of obstructive airways diseases and HHT can complicate care, particularly when screening for PAVMs. Assessing dyspnea in these individuals needs to take both into account. Early recognition of these individuals and close management could result in fewer complications over time.

#### P20 Pulmonary arterial hypertension and hereditary haemorrhagic telangiectasia

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**Introduction:** The development of Pulmonary Hypertension (PH) in HHT patients can occur via two different mechanisms: PH associated with an increased cardiac output and heart failure due to systemic arterio-venous malformations (AVMs), mostly in the liver, and precapillary PH as a form of pulmonary arterial hypertension (PAH). Depending on which mechanism is involved, treatment will have opposite effects.

**Aims:** From a case we could observe, to discuss the primitive or secondary form of the PAH in these patients with HHT.

**Method:** to report the case medical history, results of pulmonary pressure measures and progression of the PH under treatment with either anti-idiopathic PAH or antiangiogenic drugs.

**Results:** A young woman was followed up for an “idiopathic” PAH. Pulmonary arterial pressure (PAP) was at 85 mmHg and she had a severe precapillary PH with pulmonary vascular resistance (PVR) at 5.7 WU. One week after beginning an anti-idiopathic PAH treatment (bosentan and tadalafil), she needed intensive care for an intestinal hemorrhage and liver failure. At that time, diagnosis of HHT was made based on familial history, presence of hepatic arterio-venous malformations (AVMs), and gastric telangiectasia. She had the familial mutation in the *ACVRL1* gene. As she did not improve with the anti-primitive PAHT treatment, we initiated a treatment with bevacizumab, thinking her PH could be consecutive to her hepatic AVFs. From that time, she improved with no more need of blood transfusion, and PAP moving from 94 mmHg before treatment to 45 mmHG 3 months later, and PVR from 5.3 to 4.2 WU.

### P21 Splenic artery aneurisms in hereditary hemorrhagic telangiectasia

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**Objectives:** To assess the frequency and CT features of splenic artery aneurisms (SAA) in HHT patients and to identify factors associated with the development of SAA.

**Methods:** 210 CT-scans of HHT patients followed in our center were compared to 210 CT-scans of age-matched controls. We described SAA (size, number and location) and recorded the genotypic status, the diameter of the splenic and hepatic arteries, the pulmonary, hepatic, pancreatic and splenic parenchymal involvements (telangiectasia, arterio-venous malformations, hemangioma, focal nodular hyperplasia). We compared the frequency, size and number of SAA between groups and determined, with uni- and multivariate analysis, the relationship with age, sex, genotypic status and visceral involvement.

**Results:** SAA are more frequent (27.1 % vs. 4.3 %,  $p < 0.001$ ) and numerous (mean  $\pm$  SD =  $1.9 \pm 1.3$  vs.  $1.1 \pm 0.3$ ,  $p = 0.03$ ) in HHT patients than in the general population. The size of the aneurisms and the diameter of the splenic artery do not differ between groups. Factors associated with the development of SAA are advanced age ( $p < 0.0001$ ; OR: 1.04 [1.02–1.06]), female gender ( $p < 0.0001$ ; OR: 5.64 [2.84–11.18]) and pancreatic parenchymal involvement ( $p < 0.0001$ ; OR: 4.65 [2.46–8.80]). We found no statistically significant relationship between development of SAA and genotypic status, pulmonary or hepatic involvement and size of hepatic or splenic arteries.

**Conclusions:** Given the life-threatening risks of rupture, screening for SAA should be performed in HHT patients, especially in elderly patients, in female patients and when there is pancreatic parenchymal involvement.

### P22 *Staphylococcus aureus* spondylodiscitis in three hereditary hemorrhagic telangiectasia patients

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**Objective:** Severe infections have been reported in patients with hereditary hemorrhagic telangiectasia (HHT). Pulmonary arteriovenous malformations (AVMs) have been associated with cerebral infections. Risk factors for extra-cerebral infections have not been studied. We describe three cases of spondylodiscitis in our HHT population, a rarely reported complication to identify possible risk factors.

**Methods:** Chart and imaging review.

**Results:** Case 1: 61-year-old woman (ENG mutation), untreated lung AVMs, asymptomatic liver vascular malformations (VMs), epistaxis, chronic GI bleeding, and anemia presented with 2 months of back pain. She was afebrile, leukocyte count normal and epistaxis severity score (ESS) of 7.68 (severe). MRI confirmed L4/L5 spondylodiscitis and epidural abscess, which cultured methicillin sensitive *Staphylococcus aureus* (MSSA). Case 2: 59-year-old man (ENG mutation), epistaxis and anemia, with no lung or brain AVMs, presented with 2 months of back pain, night sweats and ESS of 6.05 (moderate). He was afebrile with an elevated leukocyte count ( $18 \times 10^{-9}/L$ ). Blood cultures grew MSSA. MRI confirmed L4/L5 spondylodiscitis. Case 3: 62-year-old woman (ALK1 variant), treated lung AVMs, severe liver VMs (on TPN, with central line), epistaxis and anemia, presented with fever and back pain. Blood cultures grew MSSA. After initial antibiotic therapy, back pain persisted. MRI confirmed L5/S1 spondylodiscitis. Leukocyte count was elevated ( $8.55 \times 10^{-9}/L$ ) and ESS was 0.70 (mild).

**Conclusion:** We report three cases of MSSA spondylodiscitis in HHT patients. We speculate that moderate-severe chronic epistaxis may have provided entry portal in the first two cases. The relationship between recurrent epistaxis and severe infections requires further study and may have implications for nasal care.

### P23 Telangiectases anatomic location related to visceral arteriovenous malformations in hereditary haemorrhagic telangiectasia

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**Objective:** To establish a relationship between anatomic location of telangiectases in HHT patients and visceral arteriovenous malformations (AVM).

**Methods:** Retrospective review of 72 adults HHT patients at the HHT unit. Data was obtained from the institutional record of HHT (clinicaltrials.gov NCT01761981). Telangiectases anatomic location and AVM were analyzed.

**Results:** At least one AVM was present in 64/72 patients. Telangiectases were located in lips in 79.1 %, face 63.8 %, trunk 58.3 % upper extremities 58.3 % and lower extremities 14.2 %. Patients with AVM presented the same frequency of telangiectasia location: lips 81.2 %, face 64 %, trunk 59.3 %, upper extremities 59.3 % and lower extremities 17.1 %. Patients with hepatic AVM (46/64) presented

telangiectases in lips in 84.7 %, face in 65.2 %, ears in 50 %, trunk in 63 %, upper extremities in 65.2 and 21.7 % in lower extremities; patients with lung AVM (33/64) presented respectively 78.7, 60.6, 36.6, 63.6, 63.6 and 9 %; Patients with gastrointestinal AVM (25/64) presented telangiectases in lips in 92 %, facial in 92 %, in ears in 65.2 %, in trunk in 68 %, upper extremities in 72 % and lower in 12 %. Telangiectases in patients with central nervous system AVMs were detected in the lips in 78.5 %, in the face in 5, in ears 42.8 %, trunk 64.2 %, upper extremities 71.4 %, lower extremities 21.4 %.

**Conclusion:** The most frequent anatomic location for telangiectasias was the lips. Patients with gastrointestinal and hepatic AVM present the greatest amount of telangiectasias in most of the evaluated anatomic areas.

**EPISTAXIS AND GI BLEEDING IN HHT (P24–P30)**

**P24 Common bleeding disorders affecting individuals with hereditary hemorrhagic telangiectasia**

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**Objectives:** Hereditary hemorrhagic telangiectasia (HHT), is an autosomal dominant disorder affecting vasculature in different organ systems, seen in approximately 1:5000 in North America. Complications with significant increases in health service utilization arise from bleeding and shunts particularly problematic in the lung and liver. Although these patients tend to chronically bleed from the GI tract and nasal cavities, a single bleed from AVMs in the lungs (PAVM) or brain (BAVM) can be disastrous, and sometimes fatal. Bleeding seen in HHT patients can be further complicated with a concomitant bleeding disorder.

**Methods:** We sought to determine the proportion of patients seen in the Edmonton HHT center with a concomitant bleeding disorder, as assessed by blood test results for Factor VIII and related factors (Ristocetin Cofactor), along with Factor IX and Factor XI.

**Results:** Of 77 individuals with HHT, 4 had below normal values of either von Willebrand Factor, ristocetin cofactor or factor VIII values. Two patients had laboratory parameters diagnostic for a bleeding disorder, accounting for 2.6 % of confirmed HHT subjects. This indicates that establishing screening for bleeding disorders in HHT centers is important in managing bleeding symptomatology.

**Conclusions:** Given the significant clinical implications of common bleeding disorders in some HHT patients, further research incorporating other HHT Centers will be helpful to assess the health service utilization impact of this sub-group, as well as development of best practice protocols for HHT patients seen locally and at other Centers.

**P25 Daily Nasal Hygiene and ESS: marker of severity or causal relationship?**

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**Objectives:** Most patients present with recurrent epistaxis, the severity of which has potential for anemia-related complications and functional impairment. We review our patient’s management

**Table 1** Epistaxis severity score survey results

| Normalised EES |      |     |        |         |         |                |                |
|----------------|------|-----|--------|---------|---------|----------------|----------------|
| N              | Mean | SD  | Median | Minimum | Maximum | Lower quartile | Upper quartile |
| 49             | 4.3  | 2.3 | 3.7    | 0.5     | 8.5     | 2.4            | 6.0            |

**Table 2** Epistaxis severity score with daily hygiene

| Normalised EES according to daily hygiene |    |      |     |        |         |         |                |                |
|---|----|------|-----|--------|---------|---------|----------------|----------------|
| Daily hygiene                             | N  | Mean | SD  | Median | Minimum | Maximum | Lower quartile | Upper quartile |
| No  | 22 | 3.4  | 2.0 | 2.6    | 0.5     | 8.1     | 2.1            | 4.6            |
| Yes                                       | 27 | 5.1  | 2.2 | 5.1    | 1.0     | 8.5     | 2.9            | 7.1            |

*P* = 0.006 (Wilcoxon rank sum test)

strategies for epistaxis and their daily nasal routine improve treatment algorithms.

**Methods:** A telephone survey was administered to 57 patients and consisted of current epistaxis severity score (ESS) and daily nasal hygiene. The normalized ESS was evaluated for an association between the above-mentioned variables (Tables 1, 2).

**Results:** Epistaxis was reported in (n = 49, 86 %) of patients, with mean normalized ESS of 4.3 (SD = 2.3, n = 49). A significant difference in normalized ESS is noted between the absence or presence of a daily hygiene regimen (*p* = 0.006).

**Conclusion:** A significant difference in normalized ESS was observed between those reporting daily hygiene versus those not endorsing a daily hygiene regimen. This finding could represent a need for improved symptom control in the setting of severe epistaxis or a causal relationship between some component of daily hygiene and worsening epistaxis. Detailed analysis comparing components of patients’ daily routine is ongoing with results available in June 2015.

**P26 Endonasal sclerotherapy treatment in patients with hereditary hemorrhagic telangiectasia: our experience in HHT center—Argentina**

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**Objective:** Describe our experience with office-based sclerotherapy for the treatment of epistaxis in HHT and report improvement, safety and patients’ satisfaction after it.

**Methods:** Between July 2012 and January 2015, medical records of all patients with HHT treated with local sclerotherapy (polidocanol 0.5 %, injected submucosally) were retrospectively reviewed. We assessed bleeding with Sadick scale according to intensity and frequency. Patients’ satisfaction and quality of life (QoL) were analyzed with a questionnaire.

**Results:** Twenty-two out of 294 patients (16 females) underwent sclerotherapy with a total of 45 procedures. Median age was 52 years (range 15–82 years). Before treatment, 14 (63.63 %) patients had a bleeding frequency grade III, 6 (27.27 %) grade II and 2 (9.09 %) grade I. In terms of bleeding intensity, 8 (36.36 %), 7 (31.81 %) and 7

(31.81 %) patients were grade III, II and I, respectively. One month after the first injection, 2 (9 %) patients remained with a grade III of frequency, 8 (36.36 %) achieved grade II and 11 (50 %) grade I. Talking about intensity, 18 (81.8 %) patients achieved a Grade I and three (13.63 %) Grade II. One patient had no bleedings.

### P27 Gastrointestinal bleeding in patients with hereditary hemorrhagic telangiectasia: a cross sectional study from a referral center in Latin America

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**Introduction:** Nearly 25 % of HHT patients experience gastrointestinal bleeding (GIB) and 80 % have gastrointestinal telangiectases. In patients with anemia and mild epistaxis, the gastrointestinal tract is a potential source of bleeding.

**Objectives:** The main objective was to describe the prevalence of GIB. Secondary objectives were to estimate the association between unexplained iron deficiency anemia (UIDA) and overt GIB, and assess quality of life in HHT patients with GIB.

**Methods:** Patients from the HHT Unit of our hospital were included. Iron deficiency anemia in the absence of epistaxis or with a mild severity was defined as UIDA. Occult GIB was defined as UIDA in the absence of overt GIB. Quality of life was assessed by EuroQol visual analog scale.

**Results:** In all, 146 patients were included. Prevalence of GIB was 40.4 %, mean age was 54 years (SD 14.5 years) and 72.9 % were women. Prevalence of overt and occult GIB were 24.7 and 15.8 %, respectively. Telangiectases were located mainly in the stomach (54.2 %) and duodenum (40.7 %). Twenty-four percent were admitted to the hospital for GIB. Median EuroQol score was 6 (IQR = 3) and 8 (IQR = 2.25) in patients with and without GIB ( $P = 0.02$ ). UIDA was present in 28 % of the patients. Prevalence of overt GIB was higher in patients with UIDA (44 vs. 17 %;  $P = 0.001$ ; OR 3.78; 95 % CI 1.7–8.4).

**Conclusions:** Prevalence of overt GIB was similar to previous studies and significantly higher among patients with UIDA. Patients with history of GIB reported a poorer quality of life.

### P28 Impact of heat source on epistaxis severity score

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**Objectives:** Most patients present with recurrent epistaxis, the severity of which has potential for anemia-related complications and functional impairment. We review our patients' management strategies for frequent epistaxis and the utility of environmental factors to further patient education and improve treatment algorithms.

**Methods:** A telephone survey was administered to 57 patients and consisted of: [1] current Epistaxis Severity Score (ESS), [2] personal epistaxis management for symptom control, [3] type of home heat

**Table 1** Normalized ESS according to humidifier and heat source

|                | n  | Mean (SD) | P value |
|----------------|----|-----------|---------|
| Heat source*   |    |           |         |
| Electric       | 34 | 4.0 (2.2) | 0.078   |
| Gas            | 10 | 4.1 (2.6) |         |
| Wood           | 7  | 6.1 (1.7) |         |
| Humidifier use |    |           |         |
| Yes            | 13 | 4.4 (2.2) | 0.830   |
| No             | 36 | 4.3 (2.3) |         |

\* Note:  $p = 0.024$  for electric versus wood;  $p = 0.132$  for gas versus wood;  $p = 0.923$  for electric versus gas

source, and [4] humidifier use over the previous 3 months. Normalized ESS was evaluated for an association with heat source and humidifier use.

**Results:** Epistaxis was reported in ( $n = 49$ , 85.9 %) of patients, with the common management strategies being external pressure ( $n = 26$ , 53.1 %), packing ( $n = 16$ , 32.6 %), head positioning ( $n = 8$ , 16.3 %), and cold packs ( $n = 8$ , 16.3 %). Mean normalized ESS was 4.3 (SD = 2.3,  $n = 49$ ). Mean ESS score was significantly lower for electric as compared to wood burning sources ( $p = 0.024$ , Table 1). There was no significant difference when comparing ESS according to humidifier use.

### P29 Nasal self-packing for epistaxis in HHT increases quality of life

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**Background:** Hereditary haemorrhagic telangiectasia is characterised by recurrent epistaxis which can lead to a feeling to loose control. We examined whether the use of high volume low pressure nasal packings is a secure and practical method to improve patients' quality of life.

**Method:** A total of 10 patients were instructed to use a high volume low pressure nasal packing (i.e. Rapid Rhino<sup>®</sup>). They were evaluated with a structured interview including the Glasgow Benefit Inventory (GBI).

**Results:** Having learned the self-packing 8 patients regularly use high volume low pressure packings (i.e. Rapid Rhino<sup>®</sup>), one patient used Tabotamp<sup>®</sup> and one patient used handkerchiefs. All patients could stop their epistaxis sufficiently, abbreviating the length of the nasal bleeding and decreasing the number of medical consultations. A total of three patients reported minor and temporary complications (secondary bleeding, pressure pain, nasal dressing was too long). One used smaller nasal dressings. Due to lack of manual ability the other two patients preferred using Tabotamp<sup>®</sup> and handkerchiefs. In general, patients were more confident and satisfied, having a feeling of losing control less often ( $t$  test: 9.22;  $p < 0.001$ ;  $n = 6$ ). Overall the quality of life improved (average GBI-score = +20.6, from -100 to +100).

**Conclusion:** Having a low rate of complications and being very user-friendly, the self-packing is a secure method to treat patients with HHT. Patients are more self-confident and independent, and the number of medical consultations decreases whereas quality of life increases.

### P30 Tempo: efficacy of a timolol nasal spray as a treatment for epistaxis in hereditary hemorrhagic telangiectasia (HHT). Randomized trial against placebo

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**Background:** Timolol is a nonselective  $\beta$ -blocker commonly used in the treatment of glaucoma. Recently it has been used topically for the treatment of superficial hemangiomas. Because of its potential mechanism of action, it is possible that timolol could also be useful for the treatment of epistaxis in HHT. Moreover a case was reported in 2012 showing an improvement of nosebleeds with the use of topical nasal timolol. The aim of the study is to evaluate timolol nasal spray efficacy in HHT.

**Objectives:** The main objective of this trial is to evaluate, 3 months after the end of the treatment, the efficacy on the duration of nosebleeds of a 4 weeks timolol intranasal treatment in HHT patients with nosebleeds ( $>20$  min/month). Secondary objectives are to evaluate the tolerance, the efficacy at 6 months after the end of the treatment, and the efficacy on anemia and on clinical parameters (nosebleeds, quality of life and blood transfusions).

**Material and method:** This is a prospective double blind phase II study, randomized against placebo using an allocation ratio of 1:1. A total of 58 patients will be included. The product (solution with timolol at 0.5 % or placebo) is self-administered by the patient with a posology of one spray (50  $\mu$ L) in each nostril three times a day for 28 consecutive days.

**Results:** Inclusions of patients will start in 2015.

### PULMONARY AVM DIAGNOSIS (P31–P39)

#### P31 A description of non-classic pulmonary arteriovenous malformations in patients with HHT

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**Objective:** Well-established categorization schemes described pulmonary arteriovenous malformations (pAVMs) in patients with HHT as simple, complex, or diffuse. The categorization has implications for treatment with embolization. When utilizing a dedicated pAVM protocol multidetector CT (MDCT), other pAVM findings may also be observed.

**Methods:** Adult and pediatric patients from our HHT center with HHT defined as 3–4 Curacao criteria or a causative mutation were selected. New referrals after 01/01/2005 in whom a new diagnosis of

HHT was made who had no prior pAVM embolization or surgery were included. For the cases with images available, the first MDCT in these patients was prospectively reviewed by an expert reader. pAVM findings were coded; discrete pAVMs were coded as simple, complex, fissural vessel, groundglass, or telangiectatic. A segmental artery involved with diffuse pAVMs was represented once for counting purposes.

**Results:** 150 new referrals in the study period were given a new diagnosis of HHT. 107 of these patients had their initial CT available to review. The first 16 cases were reviewed. 121 pAVM findings were made, an average of 7.6 findings per patient (range 3–18). There were 48 simple, 7 complex, and 1 telangiectatic pAVM. There were 32 groundglass pAVMs, 12 of which were diffusely involved segments. 31 fissural vessels were found. The right lung was more commonly involved (69/121 findings). The lower lobes were most commonly affected (47/121 findings) followed by the upper lobes (27/121).

**Conclusion:** Groundglass pAVMs and fissural vessels are pAVM findings in HHT patients and represent over half of the total pAVM findings in a prospectively reviewed cohort. Additional cases are being reviewed.

#### P32 Arterial blood oxygen content reflects anemia/hemoglobin more than commonly measured indices of oxygenation in patients with hereditary haemorrhagic telangiectasia

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**Objectives:** To evaluate factors that reduce arterial oxygen content ( $\text{CaO}_2$ ) and therefore demand higher cardiac outputs to maintain tissue oxygen delivery. This is important for clinical practice, and for clinical trials that use cardiac index (*cardiac output/surface area*) as a primary outcome measure for changes in hepatic arteriovenous malformations (AVMs) due to hereditary haemorrhagic telangiectasia (HHT).

**Methods:** Data from 497 consecutive HHT patients with pulmonary AVMs (PAVMs) described in [1] were evaluated. Presentation  $\text{CaO}_2$  could be calculated in 440 patients by *oxygen saturation* ( $\text{SaO}_2$ , %) \* *haemoglobin* (gram/decilitre[dL]) \* 1.34 mls/g.

**Results:** There was a fourfold difference in  $\text{CaO}_2$  across the 440 patients (range 7.6–27.5, median 17.6) mls of oxygen per deciliter of arterial blood.  $\text{SaO}_2$  ranged from 59 to 100 % (median 94.8 %), but  $\text{CaO}_2$  did not change appreciably across the  $\text{SaO}_2$  quartiles (median  $\text{CaO}_2$  17.1; 18.1; 17.7; 17.8 mls/dL;  $p = 0.34$ ). In contrast,  $\text{CaO}_2$  was primarily determined by hemoglobin: hemoglobin ranged from 5.9 to 21.8 g/dL (median 14.1 g/dL). The median  $\text{CaO}_2$  across quartiles of haemoglobin were 14.1; 16.7, 18.5; and 20.5 mls/dL ( $p < 0.0001$ ). For each 1 g/dL rise in hemoglobin, there was a 10 % increase in mls of oxygen per unit blood volume.

**Conclusions:** We have previously shown that patients with PAVMs maintain  $\text{CaO}_2$  [2], and deliver the same amount of oxygen per heart beat (oxygen pulse) before and after correction of hypoxemia by PAVM embolization [3]. Further attention should be given to minor incremental increases in hemoglobin that substantially increase arterial oxygen content.

### References

- [1] Shovlin et al., PLoS One 2014; 19; 9(2):e88812.
- [2] Santhirapala et al., PLoS One 2014; 17; 9(3):e90777.

### P33 Bibliometric mapping and review of literature on mechanisms of pulmonary vascular malformations

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**Objectives:** Hereditary hemorrhagic telangiectasia (HHT) manifests in numerous organ systems throughout the body, including arteriovenous malformations (AVMs), which present a bleeding risk. Pulmonary AVMS (PAVMs) present a further risk of hypoxemia, dyspnea, stroke, TIA and seizure. To further the overall understanding of PAVMs, we proposed to review the literature relating to mechanisms by which PAVMs form.

**Methods:** A comprehensive literature search was conducted using Embase and Medline to identify studies examining mechanisms by which PAVMs form in HHT January, published between January 1994 and September 2014. These papers were analyzed for authorship and MeSH term networks and were visualized using bibliometric mapping. A full review was conducted for eleven studies that were most relevant in the context of cellular or structural mechanisms of PAVM formation.

**Results:** Four major authorship groups were evident, with only a small number of linkages between them. The MeSH term analysis identified a wide range of research topics, that clustered into several subtopics, including signaling pathways, hemodynamic forces, physical remodeling of vessels or the sequence/timing of events that occur during PAVM formation. A few papers also indicated the impact of hemodynamic forces, nitric oxide, and the timing of vascular changes involved in PAVM formation.

**Conclusions:** This analysis underscores the need for future research to address mechanisms of PAVM formation in the lesser-studied types of HHT including Juvenile polyposis/HHT syndrome, HHT3 and HHT4. Research focus related to PAVMs in HHT within the scientific community needs to incorporate existing research, which would be aided by collaboration between researchers.

### P34 Detection of reperfused pulmonary arteriovenous malformations using ultrafast contrast enhanced magnetic-resonance-angiography

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**Objectives:** To evaluate time-resolved contrast-enhanced MR-Angiography for detection of reperfused PAVM.

**Methods:** 34 patients in which treatment of PAVMs by means of coil embolization or implantation of Amplatzer vascular plugs was performed underwent follow-up studies for detection of reperfused PAVM by contrast enhanced MRA. First, a time-resolved MRA-study was performed with injection of a small contrast medium bolus [0.025 mmol/kg BW (Gd-BOPTA) MultiHance, Bracco]. The temporal resolution of the sequence was 3 s/dataset with a total number of 72 slices. Thereafter a high resolution CE MRA (0.075 mmol/kg BW MultiHance) with a timing based on the findings from the time resolved study was performed. Images were evaluated regarding enhancement of the draining vein. Recanalization was considered when a simultaneous enhancement of feeding artery and draining vein or aneurysm sac was observed.

**Results:** Time-resolved MR-Angiography was technically adequate in 31 of 34 cases. In 7 out of the 31 patients diagnosis of reperfused PAVM was made based on both time-resolved and high-resolution MRA. In cases in which diagnosis of reperfused PAVM was unclear in the high resolution images, evaluation of the enhancement kinetics of the draining vein was helpful to make the diagnosis. All reperfused PAVM were confirmed by DSA and underwent reembolization. Of the seven cases of reperfusion 6 had complex PAVM with multiple feeding vessels.

**Conclusions:** Time resolved contrast-enhanced MR-Angiography is a helpful adjunct to standard anatomic imaging, allowing for the evaluation of the enhancement kinetics of the draining vein as an indicator of recanalization of PAVM.

### P35 Graded contrast echocardiography in the screening of pulmonary arteriovenous malformations in HHT patients. A single center experience

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**Objective:** Several studies have shown the usefulness of graded transthoracic contrast echocardiography (gTTCE) in the screening for pulmonary arteriovenous malformations (PAVMs) in patients with HHT. The aim of this study is to report the results obtained in our HHT Unit.

**Methods:** 221 patients (mean age 46.8 years old, 52.9 % women) with definite criteria for HHT (89 HHT1, 128 HHT2) underwent both, graded TTCE and chest CT for PAVM screening, with an interval between both studies inferior to 180 days. A semi quantitative 4-point graded scale was used to classify the pulmonary shunt size on TTCE.

**Results:** A total of 163 (73.8 %) patients had positive gTTCE: 73 (33 %) grade 1, 43 (19.5 %) grade 2, 25 (11.3 %) grade 3 and 22 (10 %) grade 4. Sixty-two patients (28.1 %) had PAVMs on CT (24 (38.7 %) single and 38 (61.3 %) multiple). Two (2.7 %) in grade 1; 18 (41.9 %) in grade 2, of which 11 (61.1 %) with multiple fistulae; 20 (80 %) in grade 3, of which 11 (55 %) were multiple, and 22 (100 %) in grade 4 16 (72.7 %) multiple. Only 6 (14 %) patients with grade 2 TTCE had afferent arteries big enough to be treated with embolotherapy; 17 (68 %) in grade 3; 21 (95.5 %) in grade 4.

**Conclusions:** gTTCE is a useful tool in the screening and selection of patients for embolotherapy. Only a small proportion of patients with grade 2 are susceptible for embolization. Probably, a better cut off between grades 1–2 is necessary. From grade 2–4 multiple PAVMs are predominant.

### P36 Preliminary results of the PIRANA trial

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**Introduction:** Currently the standard imaging technique used for the evaluation of pulmonary arteriovenous malformations (PAVM) is

computed tomography (CT). The sensitivity and specificity of CT are very high but it has the disadvantage of ionizing radiation. Because there is a considerable risk of persistent or re-perfusion after embolization multiple follow-up contrast enhanced (CE) CT scans are required. Although a single CT scan carries a negligible risk of adverse health effects, repeat scans increase this risk, especially in young people. The PIRANA trial; PAVM Imaging Research and Analysis, is a study that investigates the sensitivity and specificity of MRA using (CE) CT as the gold standard for the detection and follow-up of PAVM's.

**Objective:** The goal of the PIRANA trial is to compare MR Angiography (MRA) with CT or CTA for the detection of PAVM's. A total of 100 patients either suspected of having a PAVM on contrast echocardiography or who have been previously embolized and are scheduled for follow up CECT will be included. Until now 60 persons, with 150 PAVM's in total have been included. Although the spatial resolution of the MRA is less than the (CE) CT, most of the PAVM's that need to be embolized according to the international guidelines found on the (CE) CT were detected on the MRA.

**Conclusion:** The preliminary results show that MRA is technically challenging but feasible for the detection of PAVM's. Although CT is superior in depicting vascular anatomy and detecting small avm's MRA might be interchangeable as a screening tool for PAVM's that need to be treated.

Until June 2015 we expect to have enrolled 15 extra patient bringing the total to 75 patient having an estimate of 200 PAVM's. We expect to present more definite data at the HHT congress.

### **P37 Pulmonary arteriovenous malformations commonly lead to acute falls in blood oxygenation on standing (orthodeoxia) and chronotropic compensatory responses**

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**Objectives:** To evaluate how commonly patients with pulmonary arteriovenous malformations (PAVMs) display a fall in blood oxygen levels on standing (orthodeoxia), and evaluate hemodynamic compensatory mechanisms.

**Methods:** 258 consecutive patients with PAVMs due to hereditary haemorrhagic telangiectasia (HHT) were evaluated prospectively, and compared to 40 controls. Self-reported exercise tolerance was assigned to the MRC dyspnea scale. Pulse oximetry was performed for 10 min lying and standing, to evaluate mean oxygen saturation (SaO<sub>2</sub>) and pulse between minutes 7–10. Arterial oxygen content (CaO<sub>2</sub>) was calculated by  $1.34 * \text{haemoglobin} * \text{SaO}_2$ . Relationships were evaluated using logistic regression.

**Results:** The mean SaO<sub>2</sub> change on standing was +0.3 % (95 % CI –0.15, 0.74 %) in 40 controls but –1.3 % (95 % confidence intervals –1.6, –0.91 %) in the 257 patients completing the test. 75 (29 %) PAVM patients demonstrated an SaO<sub>2</sub> fall of at least 2 % on standing. None described platypnea (dyspnea on standing). In controls, the heart rate was higher when standing (mean 11.6 (95 % CI 8.4, 14.8 beats per minute (bpm)). Orthostatic tachycardia was more pronounced in PAVM patients than controls ( $p < 0.001$ ). For PAVM patients, a greater percentage fall in CaO<sub>2</sub> was associated with a more exuberant postural tachycardia, with a further 5 % increase in pulse rate for every 1 % fall in CaO<sub>2</sub> ( $p < 0.001$ ). In contrast to the postural orthostatic tachycardia syndrome, more pronounced orthostatic tachycardia was associated with better exercise tolerance ( $p = 0.007$ ).

**Conclusion:** Acute falls in SaO<sub>2</sub> on standing are common in patients with PAVMs. Exuberant postural tachycardia appears to offset these falls, and limit inotropic cardiac demands.

### **P38 Pulmonary arteriovenous malformations (PAVM) in hereditary hemorrhagic telangiectasia (HHT): is there a correlation between genotype and the imaging based phenotype?**

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**Objective:** To define if there is a relationship between the genetic mutations and the morphologic appearance of the PAVMs based on imaging data.

**Methods:** In a retrospective study of 255 HHT patients with PAVMs from the Louis Pradel Hospital (Competent Center for HHT in Lyon, France), we studied the imaging presentation of the PAVMs. The genetic findings for ALK1, ENG or SMAD4 were available for the overall population, from Ciroco HHT database.

**Results:** 255 CT-Scans or X-ray angiographies were analyzed independently by two observers. The number of PAVMs, their distribution, localization, and type (localized, diffuse, or disseminated form), as well as the main afferent artery size were collected. Preliminary results on 113 patients showed 10 diffuse and 17 disseminated PAVMs forms. The identification of potential correlation between imaging based phenotype and the genotype is in progress.

**Conclusion:** With this study, we expect to demonstrate a possible relationship between the genetic mutation type and the PAVMs appearance based on imaging criteria.

### **P39 Updated prevalence of pulmonary arteriovenous malformations in HHT patients based on multi-detector CT with dedicated screening protocol**

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**Objective:** The reported prevalence of pAVMs in an HHT population ranges from 15 to 50 %, but this is primarily based on earlier experiences with pulmonary angiography. When using MDCT with a dedicated screening pAVM protocol at baseline in all HHT patients, the frequency of pAVMs may be higher than previously reported, as MDCT is expected to have greater contrast resolution than pulmonary angiography.

**Methods:** New patients seen at our HHT center after 1/1/06 with 3–4/4 Curaçao criteria or causative mutations were included in this analysis. Patients with prior therapy for pAVM were excluded. All included patients underwent a contrast-enhanced MDCT with a dedicated pAVM protocol during their initial evaluation. Several

patients had a recent CT done at another facility and were not re-imaged and excluded. Radiology reports from included MDCT examinations at our facility were coded as negative, simple pAVM, complex pAVM, diffuse pAVM, or other pAVMs.

**Results:** 198 newly diagnosed adult HHT patients were seen during the study period. 183 of them had MDCT at our facility and 62/183 (33.9 %) were unequivocally negative. 121/183 (66.1 %) had identifiable pAVMs. 89/183 (48.6 %) demonstrated at least one simple AVM; 12/89 patients with simple AVMs also had a complex AVM. 2/183 had only a complex AVM. 30/183 (16.4 %) had other pAVMs including prominent vessels in the fissures, groundglass-type pAVMs, and telangiectatic pAVMs.

**Conclusion:** Using contrast-enhanced MDCT with a dedicated pAVM protocol at baseline evaluation, the frequency of pAVMs in HHT patients is ~66 % and more than previously reported.

#### PULMONARY AVM TREATMENT (P40–P46)

##### P40 Assessment of early and late results of pulmonary arteriovenous malformations embolization using the amplatzer vascular plugs

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##### P41 Clinical significance of pulmonary arteriovenous malformation reperfusion

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**Purpose:** To assess the clinical significance of reperfused pulmonary arteriovenous malformations (rPAVM) after embolization.

**Materials and methods:** This study is a retrospective review of patients from a single institution who underwent PAVM embolization between January 1, 2006 and December 30, 2007. Patients with diffuse PAVM's were excluded. Clinical history, manifestations compatible with PAVM (hypoxic related events, transient ischemic attack/stroke, septic emboli, hemoptysis, hemothorax, anemia, and migraines), and imaging findings were reviewed at the time of embolization as well as during follow-up through December 30, 2013, including note of any reperfused PAVMs. Symptoms were graded as mild (hypoxic related events, anemia, migraine) and severe (TIA/stroke, septic emboli, hemoptysis, hemothorax).

**Results:** During the 2-year treatment period, 101 patients underwent PAVM embolization. Twenty seven had reperfusion of previously embolized PAVM and they underwent repeat embolization. None of these developed recurrent rPAVM during follow-up, but three other patients developed new rPAVM, giving a total of 30 patients with rPAVMs. All of the rPAVM patients were coil embolized with the exception of one who had had a detachable balloon placement. Eighteen patients (60 %) were found to be symptomatic at the time of presentation with rPAVM. Three of the symptomatic rPAVM patients were excluded on the basis of respiratory manifestations that were not felt to be PAVM-related. Of the 15 remaining symptomatic rPAVM patients, 13 had at least one other significant PAVM at the time of

diagnosis of reperfusion. Only 2 symptomatic patients having just rPAVM, both had mild manifestations. A Fisher exact test showed a statistically significant difference ( $p = 0.004$ ) between symptomatic patients who only rPAVM and those with rPAVM and other PAVMs. **Conclusion:** The majority of symptomatic patients with rPAVM had additional PAVMs that required embolization. None of the symptomatic patients with rPAVM alone had severe manifestations. This suggests that rPAVM plays a small role in patients with recurrent symptoms after embolization.

##### P42 Complications of transarterial embolization of pulmonary AVMs in hereditary hemorrhagic telangiectasia

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**Objectives:** The aim of our study was to evaluate the safety of transarterial embolization of pulmonary arteriovenous malformations (PAVMs) in patients with hereditary haemorrhagic telangiectasia (HHT).

**Materials and methods:** From December 2001 to February 2015, we embolized 279 PAVMs in 124 procedures performed on 98 inpatients (46 m, mean age 45, range 13–76), referred by the HHT centre of Crema, and enrolled in a screening programme of HHT families. All patients underwent clinical evaluation, contrast-enhanced ultrasound (CEUS) and spiral computed tomography (CT). 54/279 PAVMs were treated using Amplatzer plug IV, 225/279 with coils. Procedure related complications/procedure were recorded; patients were to be discharged 24 h after procedure completion. Instructions regarding potential symptoms/signs and phone numbers for after-discharge assistance were given to the patients.

**Results:** One patient developed an ischemic stroke at the end of the procedure with functional recovery within 6 months. Two patients had a transient ischemic attack during the procedure, without CNS lesion at CT. Three patients had intraprocedural chest pain, with no modification of electrocardiogram, and which did not entail procedure interruption. Altogether we recorded a 0.8 % of major complications, 4 % of minor complications.

**Conclusions:** Our experience shows percutaneous embolisation is a safe treatment option in PAVMs.

##### P43 Propranolol treatment for diffuse pulmonary arteriovenous malformations

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**Background:** Diffuse pulmonary arteriovenous malformations (DPAVMs) present a treatment challenge. Propranolol is the treatment of choice for infantile hemangioma, acting by decreasing expression of *VEGF* and *bFGF* genes and triggering apoptosis of

capillary endothelial cells. Propranolol has been shown to act as antiangiogenic in HHT human umbilical vein endothelial cells and to decrease migration and tubulogenesis. We hypothesized that propranolol might be effective in decreasing right to left shunt in DPAVMs.

**Case report:** A 20-year old male with HHT1, with severe DPAVMs had multiple embolotherapies with over 20 coils inserted into multiple small PAVMs resulting in only transient improvement. His O<sub>2</sub> saturation was 56 % in room air and PaO<sub>2</sub> breathing 100 % O<sub>2</sub> was 46 mmHg. He also suffered from severe epistaxis and recurrent episodes of hematemesis. He was started on propranolol 20 mgX2/day for 3 months increasing to 40 mgX2/day for another 3 months. Blood pressure and heart rate were monitored initially daily and later weekly. A significant reduction in epistaxis was observed and he had no episodes of hematemesis. He experienced improved exercise tolerance but with only mild improvement in 6 min walk test—175–210 m. There was a slight improvement in O<sub>2</sub> saturation from 56 to 67 % but minimal improvement in PO<sub>2</sub> on 100 % O<sub>2</sub>—from 46 to 51 mmHg. No side effects were observed.

**Conclusion:** Propranolol might have some effect on abnormal blood vessels formation in HHT. Larger studies are required to assess efficacy and safety of propranolol in HHT.

#### **P44 Pulmonary arteriovenous malformations: percutaneous embolisation using amplatzer plug IV**

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**Objectives:** Since 2009 Amplatzer plug IV has become available for transarterial embolization; this device seems time and cost-saving compared to coils. The aim of our study was to evaluate effectiveness and safety of Amplatzer plug IV for transarterial embolization of high-flow pulmonary arteriovenous malformations (PAVMs) in patients with hereditary haemorrhagic telangiectasia (HHT).

**Materials and methods:** From December 2001 to February 2015, we performed transarterial embolization of 279 PAVMs in 124 procedures performed on 98 patients, referred by the HHT centre of Crema, and enrolled in a screening programme of HHT families. All patients underwent a previous clinical evaluation, with contrast-enhanced ultrasound (CEUS) and spiral computed tomography (CT). Procedure related complications were recorded.

**Results:** 54 of 279 PAVMs (19 %) in 38 patients were treated using Amplatzer plug IV (from April 2010 to July 2013). Amplatzer plug IV obtained the immediate exclusion of all 54 PAVMs. One patient (2.6 %) developed hemiparesis completely resolved in 12 h. At 6 months spiral-CT follow-up, we demonstrated exclusion from circulation of all 54 PAVMs.

**Conclusions:** Percutaneous embolisation of PAVMs with Amplatzer plug IV seems an effective and safe initial treatment option in PAVMs.

#### **P45 Safety, technical success, and short term outcomes of the microvascular plug for feeding artery embolization of pulmonary arteriovenous malformations**

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**Objective:** To evaluate the safety, technical success, and short term outcomes of the MicroVascular Plug (MVP) for arterial embolization of pulmonary arteriovenous malformations (PAVM).

**Methods:** Patients with PAVMs treated by MVPs between September 2014 and February 2015 were retrospectively reviewed. All patients met clinical criteria for HHT. Data collected included age, gender, prior symptoms, number and type of PAVM, feeding artery size, and migration after device deployment. Technical success was defined as immediate angiographic occlusion of the feeding artery beyond the plug. Periprocedural anticoagulation was administered. Two plug sizes were used to embolize the feeding arteries as distal as possible.

**Results:** In the 6 patients identified, 4 were female and 2 were male with an average age of 38. Two patients had prior TIAs. 12 MVP-3 and 6 MVP-5 plugs were deployed without migration to 15 simple and 1 complex PAVMs. Average feeding artery size was 3.0 mm. Technical success was 94 %. One patient required subsequent coil embolization proximal to the plug to achieve stasis due to feeding artery tortuosity. One patient had a subclinical microembolus to the toe. No major complications developed.

**Conclusion:** Embolization of PAVM feeding arteries with the MicroVascular plug is safe and results in a high rate of immediate angiographic occlusion. MVP is advantageous due to its resheathability, superselective distal microcatheter deployment, lack of need for proximal coil deposition, and immediate occlusion despite anticoagulation.

#### **P46 Should surgery be front-line therapy for diffuse-type pulmonary arteriovenous malformations limited to one lobe?**

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**Objectives:** Diffuse-type PAVMs respond poorly to embolotherapy. Although initial response is favorable, progressive development of bronchial to pulmonary collaterals is common and incurs late morbidity and mortality. When PAVMs are limited to one lobe, surgery can be curative and prevents the long-term sequelae of collateral formation and resultant hemoptysis.

**Methods:** Three patients ages 8, 13 and 19, two with HHT and all with diffuse-type PAVMs limited to one lobe, were treated by lobectomy. Two patients had multiple embolotherapy treatments at two HHT centers of excellence prior to surgery. The third had lobectomy without embolization. The outcomes of embolotherapy and surgery were analyzed retrospectively.

**Results:** The initial symptom was hypoxemia in all patients. Embolotherapy was performed four times in one patient and five times in another using conventional techniques (coils, microcoils) starting as peripherally as possible and working centrally as previously described. Initial response to embolotherapy in these patients yielded improvement in O<sub>2</sub> sat to >90 %. Failures of embolotherapy were declared for hemoptysis (n = 1) and intractable hypoxemia (n = 1). Lobectomy via thoracotomy was successful in all patients with normal postoperative O<sub>2</sub> saturation at mean 4-year FU (range 2–5.5). Hemoptysis has not occurred post surgery.

**Conclusion:** Surgery is successful in treating diffuse-type PAVMs limited to one lobe. With careful patient selection, curative lobectomy may be considered front-line therapy in this rare patient population as it avoids repeated radiation exposure and eliminates the risk of bronchial collateral formation.

## ANTIANGIOGENIC THERAPY (P47–P51)

**P47 BABH study: a double-blind, randomized international phase 2 study to evaluate efficacy and safety of bevacizumab for the treatment of severe bleedings in hemorrhagic hereditary telangiectasia (HHT)**

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**Background:** Antiangiogenic drugs, such as bevacizumab, a humanised monoclonal antibody targeting vascular endothelial growth factor (VEGF), are a new treatment strategy in hereditary hemorrhagic telangiectasia (HHT). Patients with very severe haemorrhages related to nose bleeds or digestive bleedings need multiple blood transfusions. Many case reports showing dramatic improvement of HHT bleedings after bevacizumab treatment have been published; however, no randomized phase II clinical trial to study bevacizumab efficiency and safety has been performed. For patients with severe bleedings, as this patient population is very small and dispersed, an international study is needed.

**Objectives:** The main objective of BABH Study is to repurpose bevacizumab in HHT on severe bleedings. Secondary objectives are to assess the role of maintenance therapy in HHT, the safety of bevacizumab in HHT patients with severe haemorrhages, to evaluate the efficacy of bevacizumab on associated vascular malformations (liver, lung, cerebral), to assess whether circulating concentrations of VEGF and VEGFR1 could be used as predictive angiogenic biomarkers of response to bevacizumab and to study the pharmacokinetics of bevacizumab in these patients in order to develop a pharmacokinetic–pharmacodynamic model.

**Material and method:** *Treatment:* Two consecutive randomized, double blind phase II study; the first one to test the efficacy of an induction treatment (1 bevacizumab injection every 14 days with a total of 6 injections) including 26 HHT patients with severe bleedings and then, a second one to test maintenance therapy (1 bevacizumab injection every 2 months with a total of 4 injections) including 54 patients will be carried out. 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 mean Hb level <9 g/dL before blood transfusion during 45 consecutive days and despite appropriate care and iron supplementation. *Endpoint and analysis:* Increase in mean hemoglobin level between baseline (before the beginning of the treatment) and after treatment.

**Results:** After obtaining the orphan drug designation for bevacizumab in HHT, an application for funding has been requested from the European call H2020 by 9 worldwide HHT centers to conduct this study.

**P48 Bevacizumab rescue from hepatorenal syndrome complicating high output heart failure**

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**Case presentation:** A 49-year-old woman with HHT type 2 was transferred to our hospital in decompensated heart failure with 50 lb weight gain, congestive hepatopathy and hepatorenal syndrome. She had transfusion dependent epistaxis despite septal dermoplasty. A pulmonary hypertension clinic documented elevated pulmonary artery pressures with high cardiac output and high normal pulmonary vascular resistance and prescribed ambrisentan. Her symptoms progressed and she developed acute liver failure complicated by dialysis dependent hepatorenal syndrome requiring inotropic support and ICU care due to distributive shock. On presentation her MELD score was 37. She was evaluated by transplant hepatology and considered for liver and kidney transplantation.

**Hemodynamics:** Cardiac index was 5.3 L/min/m<sup>2</sup> with CVP of 13 mmHg, pulmonary artery pressure of 79/17 (35) mmHg and pulmonary capillary wedge pressure of 20 mmHg. Her systemic resistance was 3.8 Wood units and pulmonary resistance was 1.9 Wood units.

**Treatment:** Soon after presentation she underwent Young's nasal closure. Her epistaxis and anemia resolved, yet she remained dialysis dependent in ICU. In preparation for listing for liver transplant she was given IV bevacizumab (5 mg/kg every 14 days × 6). After two doses she was able to tolerate intermittent outpatient dialysis and was discharged. After the six doses her cardiac output normalized and her liver and kidney function recovered to normal. Pulmonary vascular resistance increased and tadalafil was prescribed. Her quality of life markedly improved.

**Conclusion:** We report the successful therapy with bevacizumab for advanced high output heart failure complicated by hepatic and renal failure.

**P49 Followup of thalidomide treatment in patients with hereditary haemorrhagic telangiectasia**

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**Objectives:** HHT patients often have major restrictions in their daily lives because of severe epistaxis. The serendipitously discovered drug Thalidomide had beneficial effects on the disease symptoms in several of a small group of HHT patients: epistaxis and the incidence of anemia were reduced and patients required fewer blood transfusions. In addition, they reported a better quality of life. However, Thalidomide has significant negative side effects, including neuropathy and fatigue.

**Methods:** Here, we followed up all HHT patients in the Netherlands who had been taking Thalidomide at the time the original study was completed to find out (a) how many had continued taking Thalidomide and for how long, (b) the nature and severity of any side-effects and (c) whether side-effects had influenced their decision to continue taking Thalidomide.

**Results:** The results showed that only a minority of patients had continued taking the drug despite its beneficial effects on their symptoms. Four patients were still using Thalidomide after respectively 3, 27, 30 and 50 months. One patient stopped because of the

lack of effect on symptoms. However, side effects were the primary reason to stop and the remaining seven patients discontinued use because of side-effects.

**Conclusions:** Despite primary symptom reduction, alternative treatments are still necessary for epistaxis in HHT patients because of negative side effects and a large-scale clinical trial is not justified although incidental use in the most severely affected patients can be considered.

#### **P50 Is systemic bevacizumab economical for select hereditary hemorrhagic telangiectasia cases? A cost estimate**

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<sup>1</sup>Department of Hematology and Oncology, Medical College of Wisconsin, Milwaukee, WI, USA

**Objectives:** Hereditary hemorrhagic telangiectasia (HHT) is an inherited disorder characterized by multisystem arteriovenous malformations and bleeding. Bevacizumab results in decreased bleeding and transfusion-independence in patients with severe HHT. We estimated the cost benefits of Bevacizumab in a select HHT patient.

**Methods:** A 57-year-old-female with severe HHT required 2–4 units of PRBC transfusions/month with worsened quality of life (QoL) prior to intervention. She was started on systemic bevacizumab at a dose of 10 mg/kg every 2 weeks. Epistaxis, melena, oral mucosal telangiectasias improved, and transfusion needs decreased. Bevacizumab dose and frequency were gradually reduced to 7.5 mg/kg every 3 weeks to reduce the risk of toxicities and costs of therapy. Shander et al. reviewed the costs of blood transfusions including processes involved in acquisition, testing and administration. They estimated that the mean direct and indirect cost for each transfused PRBC unit was \$761 ± \$294. We compared the cost of HHT therapy in the years before and after bevacizumab.

**Results:** The patient needed a total of 39 PRBC transfusions in 1 year prior to initiation of systemic Bevacizumab, for an estimated cost of \$29,679 (\$20,358–\$46,137) while the cost for Bevacizumab infusion (chemotherapy, administration, and lab tests) was \$44,336.

**Conclusion:** Bevacizumab has been shown to increase transfusion-independence and QoL in HHT. Cost of bevacizumab infusion for severe HHT seems comparable to PRBC transfusion with the additional benefit of decreased hospitalizations and improved QoL. We believe that there is both a clinical and economic rationale to consider Bevacizumab in the management of HHT.

#### **P51 Prolonged benefit in hereditary hemorrhagic telangiectasia with bevacizumab maintenance**

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**Objectives:** Hepatic arterio-venous malformations (HAVMs) can be a serious complication of HHT. They can cause severe shunting and lead to high output cardiac failure in a subset of HHT patients. Orthotopic liver transplantation (OLT) may be a definitive therapy but carries a significant perioperative mortality (17 % in one series) and the need for long term immunosuppressive therapy. Bevacizumab in some case reports and a small series may show benefit in decreasing the shunting and improving cardiac parameters. However, long term benefit and need for maintenance therapy is not clear from published reports.

**Methods:** Our case, MC, 61-year old female presented 8/2010 with severe dyspnea, fluid overload, ascites, massive hepatomegaly, atrial fibrillation. She had bibasilar rales, edema. Cardiac output of 10.5 L/min. She was treated with bevacizumab for an initial six infusions at 3-week intervals.

**Results:** Patient had rapid improvement of CHF. Her liver returned to normal size. Her cardiac output decreased to 8.4 L/min and her LV ejection fraction improved from 50 to 65 %. After discussion with cardiology we extended treatment through 12/2011. After a 6-month respite from bevacizumab, she had increased bleeding, anemia and shortness of breath. She was restarted on bevacizumab with again significant improvement and has remained on treatment.

**Conclusion:** Bevacizumab with inhibition of VEGF can have significant activity in HAVMs of HHT and improve cardiac and liver function. Reinitiation and possibly long term maintenance therapy may prolong the benefit with limited side effects and may obviate the need for liver transplant.

#### **PEDIATRICS (P52–P59)**

#### **P52 Development of pulmonary arterio-venous malformations in children with HHT**

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**Objectives:** Pulmonary arterio-venous malformations (PAVMs) appear during growth in children with HHT. However, data on the development of PAVMs in children are scarce.

**Methods:** We reviewed the files of all HHT patients aged 15 or less referred to our center for evaluation between 2010 and 2014. Our routine work-up includes contrast echocardiography. A CT-scan of the chest is often performed after the age of 5, and in younger patients only in case of high suspicion of large PAVMs. The patients were divided into three groups according to their age: 0–4 years (group 1), 5–9 years (group 2), 10–14 years (group 3). PAVMs were considered present in case of a Barzilai grades 2–4 shunt on contrast echocardiography or direct visualization on CT-scan.

**Results:** The ages of patients were 2.6 ± 1.1 (mean ± SD; n = 17) in group 1, 7.0 ± 1.5 (n = 28) in group 2 and 12.1 ± 1.5 (n = 24) in group 3. Mutations of the endogline gene were identified in 8 children in group 1, 14 in group 2 and 10 in group 3. Mutations of the avscr11 gene were identified in 8 children in group 1, 13 in group 2 and 12 in group 3. Mutations of the madh4 gene were identified 1 child in group 1 and 1 in group 2. PAVMs were diagnosed in 4 children (24 %) in group 1, 16 (57 %) in group 2 and 13 (54 %) in group 3.

**Conclusions:** In HHT children, PAVMs usually develop before the age of 5.

#### **P53 Pediatric HHT: epistaxis doesn't predict the presence of organ arteriovenous malformations**

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**Objective:** To describe the clinical characteristics of children with HHT and whether the presence of epistaxis in children predicts the presence of organ AVMs.

**Methods:** The clinical characteristics of the first 100 children recruited to the multicenter Brain Vascular Malformation Study (BVMC) are reported and includes HHT patients with and without brain arteriovenous malformations (AVMs).

**Results:** 43 girls and 57 boys were recruited, with a mean age of 10.2 years (SD = 5.3, range 1 month–18 years). An endoglin mutation was present in 27, an ALK1 mutation in 35 and a SMAD4 mutation in 7. Epistaxis was present in 85 (85 %) with mean age of onset of epistaxis of 5.0 years (SD = 3.7, range from birth to 18 years) and telangiectases were present in 49 (49 %). Pulmonary AVMs were present in 30 (30 %), brain AVMs in 29 (29 %) (BVMC recruitment targets 25 % patients with brain AVMs), 6 % had a history of HHT—related GI bleeding and 4 % had been diagnosed with hepatic VMs. Epistaxis was not significantly associated with the presence of pulmonary AVMs, nor with the presence of brain AVMs, nor with the presence of either “lung or brain AVMs”.

**Conclusions:** No significant association between the symptom of epistaxis and the presence of organ AVMs was detected amongst 100 children with HHT. As in adults, the presence of epistaxis in a child with HHT does not appear to be a useful indicator of the presence of organ AVMs.

#### **P54 Prevalence and treatment of cerebral vascular malformations in pediatric hereditary hemorrhagic telangiectasia**

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**Objective:** Hereditary hemorrhagic telangiectasia (HHT) is characterized by mucocutaneous telangiectasias, frequent nosebleeds, and visceral arteriovenous malformations. Few reports have outlined the prevalence of the various cerebral vascular malformations found in pediatric patients and looked specifically at the effective treatments in this population.

**Methods:** A retrospective review of 167 pediatric patients (<21 years of age) referred to our HHT center was performed. Data regarding history, physical examination, and radiographic studies were reviewed. Numbers and kinds of cerebral vascular malformations were tabulated with specific attention to characteristics and treatment of brain arteriovenous malformations (AVM).

**Results:** Seventy six patients (46 %) were diagnosed with HHT by modified Curacao criteria out of the 167 pediatric patients. Screening MRI/MRA of the brain with contrast administration and gradient echo sequences were performed on 68 (89.5 %) of these 76 patients. Cerebral vascular malformations were present in 11 (14.5 %) patients. AVMs were identified in 7 (9.2 %) patients, developmental venous anomalies in 2 (2.6 %) patients, cavernous malformation in 1 (1.3 %) patient, and capillary telangiectasia in 1 (1.3 %) patient. Five AVMs were Spetzler–Martin Grade 1, and two were Grade 2. Five AVM patients were treated with single modality therapy (embolization, surgery, or radiosurgery).

**Conclusions:** Few studies have characterized the prevalence of cerebral vascular malformations in the pediatric HHT population. Current consensus guidelines call for MRI/MRA screening examinations of the brain in patients diagnosed with definite HHT. Given the small size and superficial location of HHT AVMs, single modality therapy can be a safe and efficacious treatment providing good patient outcomes.

#### **P55 Screening at a pediatric HHT center**

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**Objective:** The pediatric HHT center at Washington University Medical Center at St. Louis Children’s Hospital was established in 2002 and offers a dedicated multidisciplinary pediatric HHT team with one of the few pediatricians treating HHT. The process of screening a child with possible HHT will be presented here.

**Methods:** Children under the age of 18 years are referred to our center by their medical care team or family members. Patient information is obtained during initial phone interview with an experienced pediatric HHT nurse and an evaluation appointment is made based on review of family history and medical records. Screening appointments include comprehensive history and exam, pulse oximetry, bubble echocardiogram and brain MRI/MRA. The evaluation is usually completed in 1 day with results and treatment recommendations communicated to family when available. Appointments are coordinated for other family members with our adult HHT program.

**Results:** Since 2002, over 300 children ages 3 weeks to 18 years have been screened for HHT at the Washington University Center. Children from 24 states have visited our center and our unique resources include numerous amenities and focus on pediatric care through our equipment and décor.

**Conclusions:** A coordinated appointment with our adult physician colleagues provides families opportunity to have all members evaluated and screened with one scheduled trip to the center. Family centered care is evident throughout the patient experience. Patient satisfaction scores reveal excellent marks in areas including compassion, knowledge, concerns addressed, ease of appointment and recommendation of future care.

#### **P56 Screening of pediatric and adolescent patients for cerebral, hepatic and pulmonary manifestation of HHT by magnetic resonance imaging**

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**Objectives:** To evaluate Magnetic Resonance Imaging for screening of pediatric and adolescent patients with clinical signs of HHT or first degree relatives of patients with confirmed HHT for manifestation in the brain, liver or lung.

**Methods:** 24 patients (age range 3–18 years; mean 13 ± 5 years) underwent unenhanced and contrast-enhanced MRI of the brain, liver and pulmonary vasculature for detection of AV-malformations. Younger patients underwent imaging in general anesthesia. First T2w, FLAIR, SWI, DWI and T1w imaging of the brain was performed, followed by unenhanced T1w and T2w imaging of the liver. Additionally, flow-sensitive GRE-sequences of the abdomen were acquired to detect increased vessel density. Afterwards, time-resolved and high-resolution contrast-enhanced MR-angiography of the thorax was performed, followed by a final contrast-enhanced scan of the brain.

**Results:** In one patient a complex cerebral AV-malformation was detected. Since this patient had a family history of relatives with proven HHT and massive cerebral hemorrhage the patient underwent embolization of the cerebral AVM. Beforehand, embolization of a large pulmonary AVM detected on CE-MRA was performed. 5 other patients showed PAVM, of which 2 patients had 4 respectively 5 PAVMs, the remaining patients only had one PAVM each. All lesions were confirmed by DSA. In none of our 25 patients liver involvement was detected, however we studied a 19-year-old patient with massive liver involvement of HHT resulting in a pseudocirrhosis.

**Conclusions:** MRI is a feasible radiation-free imaging alternative to established methods like CT in screening of children and adolescent patients for visceral manifestation of HHT.

### **P57 Should surgery be considered for large complex pulmonary arteriovenous malformations (PAVMS) in young HHT patients?**

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**Objectives:** PAVMs expose HHT patients to a high risk of severe complications. Guidelines recommend preventive embolization of PAVMs. However, during follow-up, recanalization and/or reperfusion of PAVMs from pulmonary or systemic arteries may occur and need additional embolization procedures. We here report two cases of patients with large complex PAVMs who died from lung hemorrhage despite several embolization procedures.

**Methods:** Case report.

**Results:** The first patient was diagnosed with HHT1 at the age of 17. CT-scan revealed a large complex PAVM in the right lower lobe and small PAVMs in other lobes. He underwent four embolization procedures between 2001 and 2007. At the age of 30, he presented with severe pneumonia of the right lower lobe. The CT-scan revealed bronchial dilatations upstream to the proximal coils and systemic supply to the right lower lobe without reperfusion of the PAVMs. He received a 6-week antibiotic course and his condition improved. Unexpectedly, he died at home 2-month later from massive pulmonary hemorrhage. The second patient was diagnosed in 2005 with a large complex PAVM of the right lower lobe and HHT1 was confirmed in 2006. He underwent embolization procedures in 2006, 2007 and 2009. A CT-scan in 2011 revealed small reperfusion from systemic arteries. In 2013, he suddenly died at the age of 14 from massive pulmonary hemorrhage. Both patients never coughed up blood.

**Conclusion:** These patients' clinical courses raise the question of surgery for the treatment of large complex PAVMs especially in children and young adults.

### **P58 Utility of SaO<sub>2</sub> (pulse oximetry) in predicting PAVMs in children**

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**Objective:** Hereditary hemorrhagic telangiectasia (HHT) is an autosomal dominant blood vessel dysplasia that is characterized by recurrent epistaxis, mucocutaneous telangiectases and arteriovenous malformations (AVMs) that affects adults and children alike. Current international HHT guidelines recommend genetic testing at birth if family mutation is known or clinical screening with bubble echocardiogram and brain MRI/MRA within the first year of life. Often the recommended screening of children is postponed and vital signs with SaO<sub>2</sub> are performed. We reviewed our center's database SaO<sub>2</sub> values in relation to evidence of intrapulmonary shunting or PAVMs.

**Methods:** A database of 192 children, ages 3 weeks to 18 years with possible or definite HHT at a pediatric HHT center over a 12-year

period was reviewed. Of the patients enrolled, 53 had positive bubble echocardiogram and/or positive chest CT.

**Results:** Of the 53 children with evidence of intrapulmonary shunting, 15 had SaO<sub>2</sub> < 97 % and 38 had SaO<sub>2</sub> ≥ 97 %. Of the 34 children with positive bubble echocardiogram, 10 had SaO<sub>2</sub> < 97 % and 24 had SaO<sub>2</sub> ≥ 97 %. Fifteen of the 48 patients with PAVMs identified on chest CT had SaO<sub>2</sub> < 97 %, while 32 had SaO<sub>2</sub> ≥ 97 %.

**Conclusions:** The review of our patient population reveals that some patients with SaO<sub>2</sub> ≥ 97 % have intrapulmonary shunting, confirmed by bubble echocardiogram or chest CTA and are at risk for stroke, hypoxemia, hemorrhage and brain abscess. SaO<sub>2</sub> as a single screening tool cannot predict presence or absence of PAVMs. Children should have genetic and/or clinical screening.

### **P59 What constitutes optimal imaging for children with hereditary hemorrhagic telangiectasia?**

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**Objective:** To outline our approach to screening children suspected to have HHT, compare with literature and foster discussion with other centers.

**Methods:** Pediatric patients who present with 2/4 Curacao criteria are recommended to have the following screening: genetic testing, high-resolution brain MRI to assess for brain AVMs, echocardiogram-m with agitated saline to assess for pulmonary AVMs, a skin exam for cutaneous telangiectasia and a nasal exam for telangiectasia causing epistaxis. If the echocardiogram shows shunting of >30 bubbles, then structural images are obtained with noncontrast chest CT. GI lesions are not screened unless the child has had GI bleeding or is anemic for unknown reasons.

**Results:** Twenty-two pediatric patients are enrolled in UCSF HHT COE, 11 females and 11 males with an average age of 10 years. Eight patients have had visceral AVMs in the brain (4), lungs (2), both brain and lungs (1) or spine (1). Two of the brain AVMs presented with hemorrhage. Thirteen patients report nosebleeds and three have cutaneous telangiectasia. Thirteen patients have a family history of HHT or symptoms of HHT. Five have genetically confirmed HHT (4 with ACVRL1 mutation and 1 with ENG mutation).

**Conclusion:** We characterized our pediatric patient cohort and the diagnostic modalities used to screen for lesions of HHT. We are interested in collaborating with other HHT specialists to optimize the screening of children who may be affected by HHT.

### **BRAIN MANIFESTATION OF HHT (P60–P65)**

#### **P60 Another case of diagnostic delay brings about a serious brain abscess with complex clinical management**

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**Case report:** A 27-year-old woman presented to the local ER with headache. Despite recurrent nosebleeds and iron-deficiency-anemia, as well as cerebral haemorrhaging occurring in her sister, HHT had never been diagnosed. Cranial CT showed two brain abscesses, which were treated with anti-bacterial, anti-mycotic, and anti-edema drugs. The sudden onset of hypoxaemia and seizures prompted total-body CT investigation, which disclosed multiple pulmonary arterio-venous malformations (PAVMs), and antiepileptic therapy. The patient was referred to our Center, where HHT diagnosis was confirmed based on epistaxis, PAVMs and telangiectases. Stupor state, visual loss and lesion expansion required surgical removal of the abscesses. Meanwhile, the patient developed increased functional hepatic parameters, likely secondary to long-lasting multi-drug regimen.

#### P61 Assessment of T1 hyperintensity in basal ganglia on magnetic resonance brain imaging in patients with hereditary hemorrhagic telangiectasia

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**Objectives:** To determine the frequency of increased T1 signal within basal ganglia in patients with Hereditary hemorrhagic telangiectasia (HHT). Manganese deposition is known to increase T1 signal on MRI in basal ganglia, usually seen in patients with liver failure. This has occasionally been reported in patients with HHT who may not have overt evidence of liver dysfunction. Purpose of our study was to determine the frequency of this abnormality in patients with HHT.

**Methods:** Between March 2009 and April 2013, 54 definite HHT patients with brain MRI study were identified from our database. Presence of increase in T1 signal in the basal ganglia was assessed on sagittal T1WI.

50 MRI brain studies in age and gender matched controls referred for headache were also assessed for abnormal T1 signal in the basal ganglia. Pallidal index was used as a semi quantitative measure of T1 signal in the basal ganglia. Liver function tests, Hemoglobin and Ferritin levels of all patients were also recorded and any known porto-systemic shunts was noted.

**Results:** Out of 50 HHT patients, 18 had symmetric increased T1 signal in the basal ganglia. None of the 50 controls demonstrated this abnormality. Only one of the 18 HHT patients with abnormal T1 signal had evidence of abnormal LFT. We found statistically significant difference between mean pallidal index of controls (104.03; 95 % CI 103.15–104.91) compared to cases (111.06; 95 % CI 108.67–113.45) ( $P < 0.001$ ).

**Conclusions:** Increase in T1 signal in basal ganglia is seen in a significant percentage of HHT patients likely reflecting increased deposition of Manganese. The mechanism is not clearly known but may relate to asymptomatic porto-systemic shunting which may not be evident on imaging. Co-existing iron deficiency may contribute to increased absorption of Mn in these patients, as both these metals share common receptor for uptake. Adequate management of iron deficiency may help reduce the risk of manganese deposition in these patients. MR imaging of the brain may be valuable to detect manganese deposition in HHT patients, even in the subclinical stage.

#### P62 Brain MRI. Results from the evaluation of 201 consecutive HHT patients

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**Objective:** According to clinical guidelines, brain MRI should be performed in all patients with HHT. The aim of this study is to report our findings with this technique in 201 consecutive patients with definite criteria for HHT.

**Materials and methods:** 201 patients (mean age 47 years old SD 15.5; 54.7 % women) with definite criteria for HHT studied in our HHT Unit in between January 2004 to December 2013. Seventy-three HHT1; 114 HHT2. All studies were performed in a 1 or 1.5 T unit and included TI, T2, FLAIR, diffusion, 3D TOF and 3D T1 sequences without and with the administration of a contrast agent.

**Results:** In 131 (65.2 %) the study was considered normal. Cerebral arteriovenous malformations (cAVM) were observed in 13 (6.5 %) patients (53.8 % women and 69.2 % HHT1): 5 (2.5 %) were arteriovenous malformations, 7 (3.5 %) developmental venous anomalies and 1 (0.5 %) a cavernous malformation. Other findings were: basal ganglia hyperintensity 24 patients (12 %): 62.5 % women, 83.3 % HHT2; ischemic encephalopathy 4 cases (2 %): 75 % women, 50 % HHT1; polymicrogyria 2 patients (1 %): 50 % women, 100 % HHT1. Twenty-seven (13.4 %) relatively young patients (mean age 43.2 years old, SD 13.2) had multiple hyperintensity white matter foci on T2 and FLAIR images: 51.8 % women, 59.3 % HHT2.

**Conclusion:** Apart from cAVM other findings such as hyperintensities in the basal ganglia or hyperintense foci in the white matter can be seen especially in HHT2 patients. Whether they correspond to or are incidental findings, this should be studied. Two patients had images compatibles with polymicrogyria and both were HHT1.

#### P63 Clinical features of brain arteriovenous malformation and arteriovenous fistula in hereditary hemorrhagic telangiectasia

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**Object:** We aimed to report the clinical features of brain arteriovenous malformation (AVM) and arteriovenous fistula (AVF) in hereditary hemorrhagic telangiectasia (HHT).

**Methods:** Clinical and imaging data of 26 cases with brain AVM/AVFs among 138 HHT patients who were diagnosed in Osaka City General Hospital between 2004 and 2014 were reviewed retrospectively. AVM/AVFs were classified as those with nidus of 1 cm or less (micro-AVMs), those with nidus of over 1 cm (AVMs), and those of the fistulous type (AVFs).

**Results:** A total of 55 AVM/AVFs in 26 patients were found (male 17, female 9, age 2–78 years old, mean 27.7 years old), and 13 patients had multiple lesions. 22 patients were analyzed by genetic test and *endoglin* mutations were found in 20 cases, *ALK1* mutations in two cases. 22 AVMs were presented in 15 patients (symptomatic 6 patients), 25 micro-AVMs in 15 patients, and eight AVFs in eight patients (symptomatic 6 patients). Most of the lesions (94.5 %) were located superficially. 15 AVMs were treated by surgical resection in 3 lesions, endovascular treatment in 2 lesions and stereotactic radiosurgery in 10 lesions to obtain the obliteration of 3, one and 3 lesions by each treatment. Seven of eight AVFs were treated by endovascular

embolization to obtain obliteration of 6 lesions. All micro-AVMs were asymptomatic and 7 lesions were treated by stereotactic radiosurgery to demonstrate the absence of one lesion. There was no evidence of novel hemorrhage or neurological deficit during the follow-up period (3 months to 10 years and 5 months, mean 46.2 months).

**Conclusion:** Brain AVM/AVFs with HHT tend to be located superficially and have often multiplicity.

#### **P64 Prevalence of cerebrovascular malformations and associated intracranial hemorrhage in Latin American HHT adult patients**

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**Objectives:** To report the prevalence of the different types of cerebrovascular malformations, to assess their associated bleeding prevalence and to report the treatments performed.

**Methods:** We reviewed 172 clinical records of HHT patients seen at a single institution (Hospital Italiano de Buenos Aires, ClinicalTrials.gov # NCT01761981) who underwent a contrast MRI of the head for cerebrovascular malformation (CVM) screening. Patients demographic data, the evidence of a CVM, MRI characteristics of the CVM, Digital Angiography characteristics of the CVM, treatment of the CVM and the presence of associated intracranial hemorrhage to the CVM were recorded.

**Results:** Cerebrovascular malformations overall prevalence was 19.18 %. 5 patients had more than one type of CVM. 47 CVM were found in 33 patients. 84.8 % were women (women/men ratio of 4:1). The mean age was 45, 21 (20–77 range). 31 of the CVM were arteriovenous malformations (AVMs), found in 24 patients: 6 small AVMs and 25 microAVMs. 2 patients had multiple microAVMs. 14 developmental venous anomalies were observed in 14 patients, 1 vein of Galen malformation was present in 1 patient and 1 cavernoma was found in 1 patient. No intracranial hemorrhage was recorded. Imaging findings in 6 patients studied both with MRI and Digital Angiography, were similar between the two methods. Two patients underwent neurosurgery, a 66 years old female patient with a microAVM and a 71 years old female patient with a venous anomaly.

**Conclusions:** MicroAVMs were the most frequent CVM type observed. In this series there was no intracranial hemorrhage associated to CVM.

#### **P65 Stroke occurrences in hereditary hemorrhagic telangiectasia**

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**Objectives:** Strokes are an important manifestation of hereditary hemorrhagic telangiectasia (HHT). Many individuals may be unaware they have HHT, even after a stroke, due to its rarity. Our goal was to assess the occurrence of stroke among patients with HHT compared to the general population.

**Methods:** Population-based administrative health data on inpatient and ambulatory admissions were extracted for the period 1997–2012 in Alberta using ICD-9 and ICD-10 codes. We observed overall occurrence of strokes in the HHT population, compared to the general population, and analyzed the data by gender, specific age groups, and stroke subtypes.

**Results:** The age-standardized occurrence rate of stroke in HHT was about 450 [95 % CI 276.4, 622.6] per 100,000 PY compared to 260 [95 % CI 259.3, 262.1] per 100,000 PY in the general population. Less than 3 % of strokes occurred under 30 years old in both groups. Although the majority of strokes occurred after 60 years of age, 23 % of strokes in the general population occurred in the middle-aged group (31–60). The prevalence of HHT in Alberta is 1:3800 and thrice as many women were diagnosed with HHT than men over the study period.

**Conclusions:** The prevalence of HHT in Alberta was considerably higher than the North American estimate (1:5000). Individuals diagnosed with HHT were 1.73 times more likely to have a stroke than average. Among the general population, a substantial number of strokes occurred in the middle-aged group. There is a higher probability of an underlying genetic component in strokes occurring before 60 years.

#### **IRON AND SUPPLEMENTS (P66–P70)**

##### **P66 Food supplements and epistaxis in hereditary hemorrhagic telangiectasia**

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**Objectives:** To examine plausible mechanisms for reported associations between dietary supplements and nosebleed severity in patients with hereditary haemorrhagic telangiectasia (HHT): Dietary factors have been reported to precipitate nosebleeds in patients with HHT patients. Mechanisms remain speculative. Recognising antiplatelet agents can provoke HHT bleeding, we hypothesised that dietary supplements may exacerbate nosebleeds in part, by modifying platelet aggregation.

**Methods:** 52 patients with HHT were recruited into the NRES-approved study which assessed nosebleed severity by the epistaxis severity score (ESS), dietary supplement intake by the EPIC Food Frequency Questionnaire, and platelet aggregation to ADP by a Helena Aggregometer. Statistical analyses were performed using GraphPad Prism v6.0 and STATA IC v12.

**Results:** 25 patient used supplements, 27 did not. The three most commonly ingested supplements were multivitamins (N = 15), iron tablets (N = 13), and fish oils (N = 8). The groups were generally matched for age, haemoglobin, and haematocrit. Supplement users tended to have higher ESS scores (mean 4.3 ± 3.4 vs. 3.4 ± 1.8), higher iron, lower ferritin and lower platelet counts. In the subgroup of 11 undergoing platelet aggregation studies, supplement users had trends to slower and lesser platelet aggregation compared to non users. Epistaxis differences were most pronounced in iron tablet users, who displayed significantly higher ESS (5.2 ± 1.7, *p* < 0.01). Adjusting for their higher serum iron (22.6 ± 17.1 vs. 14.5 ± 7.4),

and lower ferritin and platelet counts enhanced the significance of the higher ESS in iron users ( $p < 0.0002$ ;  $r^2$  41.3 %).

**Conclusion:** This pilot study suggests supplement use (particularly iron) is associated with greater nosebleed severity. Platelet aggregation deserves further attention in HHT.

#### **P67 Hypophosphatemia induced by intravenous administration of iron in patients with hereditary hemorrhagic telangiectasia. A new clinical issue?**

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**Introduction:** HHT is an autosomal dominant disease that presents epistaxis and gastrointestinal bleeding that often leads to iron deficiency anemia (IDA). Usually these patients require intravenous iron treatment (IVI). It has been shown that different IVI formula induces hypophosphatemia due to impaired renal tubular phosphate reabsorption and low vitamin D3 levels secondary to high FGF23 levels. **Objective:** To evaluate hypophosphatemia prevalence in HHT patients that usually receive intravenous iron.

**Methods:** Cross-sectional study, adults patients from the Institutional Registry of HHT, the Hospital Italiano de Buenos Aires, with IDA treated with IVI were selected and included through simple random sampling. Phosphatemia, urine phosphate and bone mineral density was random measured and after IVI.

**Results:** Thirteen patients were evaluated. 76.92 % (10) were female. 53.85 % (IC 95 %: 25.13–80.7 %) presented hypophosphatemia, with a mean value of 1.73 mg/dL (SD 0.33). From these patients, 85.71 % (IC 95 %: 42.13–99.64 %) received more than 5 IVI in the last 6 months. 24 h urine phosphate had a median value of 593 urinary sodium excretion (IQR: 963) in this group. Besides, four patients presented an unjustified low bone mass density, one develop severe osteomalacia, all of these patients had hypophosphatemia. Four patients claimed for weakness and paresthesia independent of normal iron stores.

**Conclusion:** There is a high prevalence of hypophosphatemia among patients that receive IVI This represents clinical relevance because hypophosphatemia may produce neuromuscular symptoms similar to IDA, clinical implications and therefore be underdiagnosed. HHT patients who require IVI might be assessed for detection of hypophosphatemia and their clinical implications.

#### **P68 Hypophosphatemia: adverse effect of intravenous iron supplementation in hereditary hemorrhagic telangiectasia**

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Intravenous (IV) iron supplementation is frequent in patients with hereditary hemorrhagic telangiectasia (HHT). Ferric carboxymaltose (FCM) and saccharated oxyde ferric (SFO) often used, provide asymptomatic hypophosphatemia. A secondary osteomalacia may occur.

**Objective:** To evaluate hypophosphatemia and bone related diseases in patients with HHT treated with IV FCM or SFO.

**Method:** We evaluated phosphocalcic metabolism in HHT patients receiving IV iron in 2014.

**Results:** 19 HHT patients were studied. Sex ratio was 0:9; mean age was 64 years. Fourteen patients received FCM, 5 SFO with a mean dose of 5.5 and 2.6 g, respectively. Mean time between measures and the last IV iron infusion was 3 months (range 0.5–9). Hypophosphatemia occurred in 37 % of patients, calcemia was always normal, vitamin D deficiency was found in 35 %. Parathormon was high in 16 %. Six patients had bone pain related to iron infusion. Two hypophosphatemic patients had bone fractures. IV FCM or SFO infusions cause transient, asymptomatic hypophosphatemia in 18–60 % of patients. Our results are in accord to literature. Bone consequences are little known but seem not rare. Mechanism of hypophosphatemia could be mediated by fibroblast growth factor 23, a phosphate regulating hormone which induces phosphaturia.

**Conclusion:** Patients with HHT can receive a very large amount of IV iron. As hypophosphatemia and related symptoms are quite frequent in such circumstances, we advise to measure periodically phosphatemia and be aware of bone pain. Further studies are necessary to precise recommendations in IV iron management for HHT.

#### **P69 One in twenty patients with hereditary hemorrhagic telangiectasia report iron treatments precipitate nosebleeds**

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**Objectives:** To use replicate large surveys to evaluate nosebleed responses to iron treatments in patients with hereditary hemorrhagic telangiectasia (HHT): Iron treatments are commonly required in HHT to treat iron deficiency anemia. In the clinic, we were surprised by clinic patients who spontaneously volunteered that iron treatments precipitated nosebleeds.

**Methods:** An unbiased international survey that did not specify precise study foci was designed to capture acute responses to iron treatments. To confirm and extend, relevant questions were included in a further international survey, and evaluations of primary endothelial cells were performed.

**Results:** The first survey was completed by 1433 individuals, including 621 HHT users of iron tablets and 190 iron infusions users. There was no difference in childhood or trauma-induced nosebleeds in iron users. 4.9 % of HHT patients using iron tablets (28/574), and 6.8 % using iron infusions (13/190) reported nosebleeds appeared to be worse following the respective treatments. Seven iron infusion users spontaneously reported transient deterioration commencing initially/immediately, then settling over a few days. The second survey captured data on a further 188 iron tablet users, and 107 iron infusions users. Data corroborated the findings of the first survey. In addition, no individual agreed that iron treatments could stop a nosebleed, but a further 5/188 (2.7 %) iron tablet users, and 2/107 (1.9 %) iron infusion users reported that the treatments started a nosebleed. Endothelial studies provide plausible mechanisms.

**Conclusions:** These data raise the challenging issue that for some patients, iron treatments for anemia may exacerbate HHT nosebleeds in a vicious circle.

### P70 Predominant dietary iron sources in patients with hereditary hemorrhagic telangiectasia

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**Objectives:** To evaluate which iron-containing foods are best tolerated in hereditary haemorrhagic telangiectasia (HHT). Iron requirements are higher in HHT because of hemorrhagic iron losses [1], and iron deficiency carries particular hazards including anemia, deep venous thrombosis, ischemic stroke, and high output cardiac failure. However, HHT patients frequently report avoiding certain dietary items that can trigger nosebleeds [2, 3].

**Methods:** Gold standard, prospective 7-day weighed food diaries determined predominant dietary sources of iron in 25 adults with HHT. Data analyses were performed using UK Nutrient Databank software.

**Results:** The study group comprised 13 (52 %) males; 2 (8 %) premenopausal females and ten (40 %) post-menopausal females, with median age 56 years (IQR 52.5, 67.5). Breakfast cereals alone provided up to 90.3 % of the recommended dietary allowance (RDA) for men. Iron intake was higher from boxed, fortified cereals than from porridge (94 vs. 6 % maximal contribution to male RDA). Dedicated vegetarian meals provided similar proportions of dietary iron to meat (medians 12.9 and 12.6 mg/day, respectively). Bread, eggs, fish and other vegetables (especially potatoes, beans and lentils) also provided high contributions. Large volumes of dietary iron absorption inhibitors were also ingested, particularly polyphenol-containing tea. Furthermore, due to nosebleeds, the cohort's median hemorrhage adjusted iron requirement (HAIR[1]) was 4-fold higher than the male RDA.

**Conclusions:** These data reflecting both iron/inhibitor contents and quantities easily ingested, provide opportunities for rapid assessment and improvement of dietary iron intakes in HHT.

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### EPIDEMIOLOGY (P71–P74)

#### P71 First prevalence report of hereditary hemorrhagic telangiectasia in a Latin American population

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**Introduction:** Hereditary hemorrhagic telangiectasia is an autosomal dominant systemic disorder that affects 1/5000–8000 individuals worldwide and underdiagnosis is common. Sometimes the definitive diagnosis may delay 10–30 years. Is characterized for mucocutaneous

telangiectases and arteriovenous malformations in solid organs. In some places like Curaçao a very high prevalence was reported. There are few reports of prevalence, none in Latin American population.

**Objective:** Estimate period prevalence of HHT patients from 2010 to 2015 in Hospital Italiano de Buenos Aires.

**Methods:** Retrospective Cohort Study. We evaluate every electronic record of patients affiliated to an HMO in Buenos Aires. We used standardized vocabulary from electronic records to detect potential cases based on symptoms and classical manifestations of HHT. All potential cases were evaluated by a specialist in HHT in order to certified cases. We estimate period prevalence and report 95 % CI and express adjusted rates for gender and age.

**Results:** There were 55 HHT cases, Period prevalence: 3/10.000 affiliated patients (55/185270, 95 % CI 2.3–3.9). Specific prevalence in women 3.6/10,000 patients (39/109459, 95 % CI 2.6–4.9) and in men 2.1/10,000 patients (16/75811, 95 % CI 1.3–3.4). Female prevalence was 71 % (39/55), median age 58 (IC 95 % 21–99).

**Discussion:** This report expresses period prevalence of HHT, showing similar prevalence worldwide. The higher prevalence in women could be explained for they could be more likely to attend to the health system. Median age of these patients could refer to the delay between onset of symptoms and diagnosis. This is the first prevalence report in Latin American population.

#### P72 Gender differences in hereditary hemorrhagic telangiectasia

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**Objectives:** To determine the association between gender and clinical manifestations in patients with hereditary hemorrhagic telangiectasia (HHT).

**Methods:** All consecutive patients referred to our HHT Unit in a university hospital from September 2011 to October 2014 with a 'definite' diagnosis according to Curaçao criteria and/or a positive genetic mutation for HHT were included. Blood test, epistaxis severity score (ESS), transthoracic contrast echocardiography (TTCE) (using 4 grades) and thoraco-abdominal 64-slice computed tomography (CT) were performed.

**Results:** 111 out of 150 patients attended at our HHT Unit during the study period were included. Mean age was 50.7 (14–81; SD 15.3) years and 55 % were women. There were no statistically significant differences between women and men in mean age (50.5 vs. 50.8 years), ESS (3.9 vs. 4.1), hemoglobin levels (12.2 vs. 12.5 g/dL), treatment for iron deficiency (67.2 vs. 66 %), red blood cells transfusion (32.8 vs. 30 %) or non-hepatic vascular malformations (VMs) by abdominal CT (29.6 vs. 26 %). However, women showed statistically significant higher proportion of Grades 2–4 TTCE (42 vs.

14 %), pulmonary arteriovenous malformations (PAVMs) by thoracic CT (36.1 vs. 18 %), hepatic involvement with VMs by abdominal CT (60.7 vs. 42 %) and treatment of PAVMs with embolization (21.3 vs. 4 %). The four patients with PAVMs that had a medical history of ischaemic stroke or brain abscess were women. One female patient underwent successful liver transplantation due to extended biliary necrosis.

**Conclusions:** Female patients with HHT show a higher proportion of pulmonary and hepatic VMs than men, and need a more aggressive treatment.

### **P73 Multi-source approach permits a better ascertainment and provides more reliable estimates of hereditary haemorrhagic telangiectasia: a population-based study in Apulia, Italy**

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**Background:** Hereditary haemorrhagic telangiectasia (HHT) is typically under recognized due to a long diagnostic delay and enrollment of inappropriate cases. Both clinical data based on recruitment by tertiary referral Centers and administrative data based on health-insurance-system surveys may lack specificity and/or sensitivity. To date, no reliable data of HHT prevalence are available in Italian population.

**Aims:** To estimate the prevalence of HHT in the Italian Apulia Region by a multisource approach.

**Methods:** Four informative sources were employed in this study: (a) Administrative collection of hospital discharge records containing HHT ICD-10 code (448.0). (b) The administrative database of fiscal advantages for rare diseases (Italian law: DM 279/2001, HHT code: RG0100). (c) Recruitment by our HHT Center on outpatient access. (d) Patients with positive genetic testing for an ENG or ALK1/ACVRL1 mutation. Patients with only suspected clinical HHT diagnosis were excluded. Patients detected by the four informative sources were then cross-checked to estimate prevalence rate and to estimate specificity of administrative data in HHT diagnosis.

**Results:** A total of 187 patients were retrieved by the two administrative sources (period: 2001–2009). Cross-checking with data contained in the Bari HHT Center patients’ database identified case misclassification and confirmed HHT diagnosis in 100/187 (53.4 %) patients. Diagnosis of HHT was clinically/genetically ruled out in 11/187 cases (false positive rates: 5.9 %). Recruitment of patients who referred to our Center but who were not captured by administrative data permitted to add 102 patients with certain HHT. Multisource approach yielded an estimated prevalence of 1:16,474. Considerable gradient effect around our Center was evident, as the highest prevalence was observed in Bari province (1:10,799).

**Conclusions:** The multi-source approach permits to reduce under-ascertainment and to estimate specificity of administrative data.

### **P74 Population with hereditary hemorrhagic telangiectasia of the Winnipeg HHT Clinic**

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**Introduction:** The objective of this study is describing clinical features of patients seen at the Winnipeg HHT Clinic.

**Methods:** Medical information of patients seen at the Winnipeg HHT Clinic is continuously collected in the Winnipeg HHT Database after obtaining patients’ consent. Patients seen between January 2011 and January 2015 are included in this study.

**Results:** Seventy-five patients are included: 48 women and 27 men, average age 53.2 years. Forty-seven patients (63 %) have a definite clinical and/or genetic HHT diagnosis, 12 patients (16 %) have possible HHT and 16 patients (21 %) unlikely have HHT. Genetic testing has been done in 32 patients: 5 (16 %) have a mutation in the Endoglin gene, 15 (47 %) have an ACVRL-1 mutation, 12 (38 %) have no known HHT-related mutations. Screening for pulmonary arteriovenous malformations (PAVMs) occurred in 68/75 patients; 46 have a definite HHT diagnosis. Screening for the presence of PAVMs was positive in 20/46 (43 %) patients: 10/46 (22 %) had a PAVM on CT chest, 10/46 (22 %) had a negative CT chest, but a shunt on transthoracic contrast echocardiography (TTCE). Screening for PAVM in 7 patients with a possible HHT diagnosis revealed 1 patient with PAVM on CT chest, 1 with positive TTCE and 5 were negative. Screening for cerebral vascular malformations (CVM) occurred in 66/75 (88 %) patients. Seven/66 (11 %) had CVM; 5/7 have a definite HHT diagnosis and 2/7 have a possible HHT diagnosis.

**Conclusion:** The population at this clinic shows varied manifestations of HHT and follows trends seen in other population studies of patients with HHT.

### **GENETICS (P75–P82)**

#### **P75 A genetically proven endoglin mutation in hereditary hemorrhagic telangiectasia type 1 in a 29 year-old Filipina: (Osler–Weber–Rendu disease: a case report and review of literature)**

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**Background:** The autosomal-dominant trait hereditary hemorrhagic telangiectasia (HHT) affects 1 in 5–8000 people. Genes mutated in HHT (endoglin or activin receptor-like kinase (ALK1)) encode proteins that modulate transforming growth factor (TGF)- $\beta$  superfamily signalling in vascular endothelial cells leading to the development of fragile telangiectatic vessels and arteriovenous malformations wherein complications from bleeding or shunting may be catastrophic. In the authors’ knowledge, there has been no documented case of genetically proven HHT in the Philippines.

**Objectives:** To confirm the clinical diagnosis by genetic study the presence of HHT in a Filipina and to describe the treatment outcome and follow-up care for patients with genetically proven HHT.

**Case report:** A 29-year-old, right handed female, who has been having recurrent nosebleeding since childhood and was previously diagnosed with multiple pulmonary arteriovenous malformations, was admitted in our institution in active labor and was referred to our service because she developed generalized tonic-clonic seizure 3 days after her normal vaginal delivery. Diagnostics showed multiple cerebral aneurysms. She fulfills the Curacao Criteria for HHT. A genetic study was performed to confirm our clinical diagnosis. It showed a frameshift mutation in exon 6 of the *Endoglin* gene (chromosome 9q34.1). This confirms the diagnosis of hereditary hemorrhagic telangiectasia type 1.

**Conclusion:** Genetic testing for endoglin, ALK1/ACVRL1 and Smad4 is available and can confirm or refute the diagnosis in family

members. Management however remains highly challenging for even for experienced specialists.

### P76 Long non-coding rna expression profiles in hereditary haemorrhagic telangiectasia

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Hereditary haemorrhagic telangiectasia (HHT) is an autosomal dominantly inherited vascular disease characterized by the presence of mucocutaneous telangiectasia and arteriovenous malformations in visceral organs. HHT is predominantly caused by mutations in *ENG* and *ACVRL1*, which both belong to the TGF- $\beta$  signalling pathway. The exact mechanism of how haploinsufficiency of *ENG* and *ACVRL1* leads to HHT manifestations remains to be identified. As long non-coding RNAs (lncRNAs) are increasingly recognized as key regulators of gene expression and constitute a sizable fraction of the human transcriptome, we wanted to assess whether lncRNAs play a role in the molecular pathogenesis of HHT manifestations. By microarray technology, we profiled lncRNA transcripts from HHT nasal telangiectasial and non-telangiectasial tissue using a paired design. The microarray probes were annotated using the GENCODE v.16 dataset, identifying 4810 probes mapping to 2811 lncRNAs. Comparing HHT telangiectasial tissue with HHT non-telangiectasial tissue, we identified 42 lncRNAs that are differentially expressed ( $p < 0.001$ ). Using GREAT, a tool that assumes cis-regulation, we showed that differently expressed lncRNAs are enriched for genomic loci involved in key pathways concerning HHT. Our study identified lncRNAs that are aberrantly expressed in HHT telangiectasia and indicates that lncRNAs may contribute to regulate protein-coding loci in HHT. These results suggest that the lncRNA component of the transcriptome deserves more attention in HHT. A deeper understanding of lncRNAs and their role in telangiectasia formation possesses potential for discovering therapeutic targets in HHT.

### P77 Molecular screening for BMP9 mutations in a French cohort of HHT patients

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Mutations of the *endoglin* and *ACVRL1* genes are known to be the major genetic factors of hereditary hemorrhagic telangiectasia (HHT). Mutations in *SMAD4*, causing HHT in association with juvenile polyposis have also been described. However, mutations are not found in about 15 % of HHT cases. Recently, mutations in *BMP9* (alias *GDF2*) were identified by exome sequencing and sanger

sequencing in 191 individuals clinically suspected to have HHT. Three missense variants were identified in *BMP9*. Among them one is a rare variant previously reported in dbSNP with a frequency of 0.004. No clearly deleterious mutation was identified. The three individuals for whom *BMP9* variants were identified had epistaxis and dermal lesions that were not typical of HHT but more similar to those observed in *RASA1*-related disorders (capillary malformation-arteriovenous malformation syndrome). To investigate whether germline *BMP9* mutations could be a genetic factor for typical HHT cases, we screened, by sanger sequencing, 23 index cases from French or European origin, and negative for *ENG*, *ACVRL1* and *SMAD4* mutations. All selected subjects meet at least three of the Curaçao diagnosis criteria. Mutation screening revealed no mutation in *BMP9* in our cohort. These results show that in our cohort of typical HHT cases without molecular diagnosis, mutations of the *BMP9* gene are not involved. However, it will now be necessary to perform extensive screening of the *BMP9* gene in a larger HHT cohort in order to estimate its implication in the genetic architecture of HHT.

### P78 Mutation analysis in a national cohort of Norwegian families with hereditary hemorrhagic telangiectasia: results of variant evaluation using clinical information, in silico protein prediction and mRNA analyses

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**Objectives:** To present the results of genetic testing from a comprehensive Norwegian series of patients with HHT, and to describe our method of systematic variant analysis and evaluation.

**Methods:** Families with suspected HHT were classified according to the Curaçao criteria and analyzed for variants in *ENG/ACVRL1* by Sanger sequencing and MLPA. Variants were classified according to recommendations from the Association for Clinical Genetic Science into five classes (from 1 = benign variant, 3 = variant of uncertain significance, 5 = pathogenic variant) supplemented by in-house criteria. The latter combine information from clinical sources, in silico comparative analyses of conservation of protein domains in the serine/threonine protein kinase family and structure predictions. The effect of splice site variants was systematically tested with mRNA-analyses.

**Results:** Variants in *ENG* and *ACVRL1* were found in 105/113 index persons with Curaçao 2–4. Eight families had variants of uncertain pathogenic significance. Forty-two families had variants in *ENG* and 63 in *ACVRL1*. Sixty unique sequence variants were identified (33 in *ENG* and 27 in *ACVRL1*) including 32 novel variants (19 in *ENG* and 13 in *ACVRL1*). There were more missense variants in *ACVRL1* and more nonsense, frameshift and splice site mutations in *ENG*. The preponderance of *ACVRL1* mutations was due to founder mutations, specifically: c.830C>A, found in 24 families from the same geographical area of Norway.

**Conclusions:** We detected class 4–5 pathogenic mutations in 97/113 families (86 %) with HHT in a clinical setting. We propose to supplement the ACGS criteria for variant evaluation by a set of standardized criteria.

### P79 Mutation analysis of “*endoglin*” and “*activin receptor-like kinase*” genes in argentinean patients with hereditary hemorrhagic telangiectasia

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Hereditary hemorrhagic telangiectasia is an autosomal dominant disorder characterized by telangiectasia and arteriovenous malformations. Genetic diagnosis of HHT type 1 or 2 is made by mutations in *ENG* or *ACVRL1* genes. Samples: 50 Argentinean patients (48 unrelated subjects and 2 family with ¼ curacao-criteria). *ENG* and *ACVRL1* genes were analysed through DNA-sequencing. Unreported variants (ensemble nor HHT mutation databases) were analysed with PolyPhen-2, SIFT, ESE finder and BDPG softwares. *ACVRL1*: We identified 15 mutations in 19 patients: (c.203\_204insG; c.673\_674delAG; c.773-2A>G; c.925G>A; **c.1004A>G**; c.1080\_1099dup19; c.1120C>T; c.1126A>G; c.1231C>T; c.1232G>A; c.1261T>G; c.1270C>A; c.1427C>T; c.1435C>T; c.1451G>A). One mutation (6.7 %) was not previously reported (in bold). 93.4 % were found in the kinase-domain, mainly affecting exón 8. No mutations were detected in the trans-membrane domain. *ENG*: Eight mutations were identified in 12 patients (**c.523 + 1G>A**; c.618insG; **c.1109T>A**; c.1195delA; **c.1248\_1260del13**; c.1346\_1347delCT; **c.1427A>T**; c.1678C>T). Four mutations (50 %) were novels (in bold). All of the mutations were detected in the extracellular protein-domain and 75 % in Ser/Thr domain. No mutations were detected in the intracellular domain. In our sample of Argentinean patients, the rate of success in molecular diagnosis was 62 %. We found a preponderance of mutations in *ACVRL1* gene (61 vs. 39 %), which is in agreement with epidemiological data from mediterranean populations (Spain, Italy and France). From the detected mutations, 22 % were novels: 3 missense, 1 deletion and 1 splice-site. PAVMs and CAVMs were present more frequently in HHT1 than HHT2 patients (59 vs. 33 % and 5 vs. 16 %, respectively) while HAVMs was more common in HHT2 patients (60 vs. 29 %). There is still 38 % of patients with HHT clinic diagnosis but no genetic findings; additional approaches like MLPA and sequencing *SMAD4* should be used.

### P80 Sharing a diagnosis of hereditary hemorrhagic telangiectasia outside of family: a qualitative analysis

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Much is known about the communication of genetic information within families. However, less is known about how individuals with a genetic condition share their diagnosis with social groups outside of their biological family. Previous studies have focused on three disorders: hereditary breast and ovarian cancer, Huntington disease, and

alpha-1 antitrypsin deficiency. HHT is distinct because it has chronic mild manifestations as well as potentially severe and fatal complications across the lifespan, yet surveillance and management are effective.

**Objective:** To understand the lived experience of HHT, and to learn how individuals with HHT share their diagnosis with friends, romantic partners, employers and co-workers.

**Methods:** Adult patients at least 6 months from diagnosis of HHT, who had visited the University of Utah HHT Clinic between January and August 2014, were recruited by mail. Patients completed telephone interviews regarding the physical and emotional impact of living with HHT, as well as the process of sharing their diagnosis. Qualitative content analysis of interview transcripts was performed.

**Results:** 19 patients completed the interview and analysis suggests that individuals with HHT have varied emotional responses to living with the condition. Attitudes toward and patterns of sharing information with non-family members were also identified. Detailed findings will be presented.

**Conclusion:** This study offers a perspective on the lived experience of HHT for genetic counselors and health care providers. Altogether, the findings of this study should be generalizable to other rare genetic disorders with limited public recognition, and distinct morbidity and mortality.

### P81 Targeted locus amplification for targeted re-sequencing of the *ENG*, *ACVRL1* and *SMAD4* genes in hereditary hemorrhagic telangiectasia

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In DNA-diagnostics of hereditary hemorrhagic telangiectasia (HHT) sequencing of the *ENG* (HHT1) and the *ACVRL1* (HHT2) genes reveals mutations in the large majority of patients. In a few cases a mutation in the *SMAD4* gene has been identified to cause the disease. The test currently consists of the amplification of the exons and their immediately flanking sequences using PCR. In addition, detection of deletions and duplications is performed by multiplex ligation-dependent probe amplification (MLPA). Still, several probands remain unsolved although they fulfill the Curaçao criteria. To investigate whether they harbor potentially harmful non-coding genetic variants, in particular rearrangements, in or near the *ENG*, *ACVRL1* or *SMAD4* genes we applied TLA (targeted locus amplification). TLA enables the selective and comprehensive enrichment of entire genes and surrounding sequences by the use of only one or a few (inverse) PCR primer pairs. This technique therefore offers the opportunity to detect the full spectrum of genetic variation, including chromosomal rearrangements, in and around genes of interest, which can give patients the desired certainty about the presence or absence of mutations in the genes of interest. Ten probands that were negative by traditional sequencing of the *ENG*, *ACVRL1* and *SMAD4* gene but fulfilled the Curaçao criteria, were chosen for TLA analysis. None of the patients carried a rearrangement or other obvious disease-causing variant in these genes, strongly suggesting that their clinical manifestations are caused by genetic alterations in, yet to be discovered, other genes than *ENG*, *ACVRL1* and *SMAD4*.

## P82 Targeted next generation sequencing panel for molecular diagnosis of hereditary phenotypically related vascular diseases

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Next-generation sequencing-based methods are being widely adopted for genetic diagnostic testing. This approach is of interest for molecular diagnosis of hereditary vascular disorders because these diseases often have overlapping phenotypes and can be difficult to distinguish clinically. This is the case for hereditary hemorrhagic telangiectasia (HHT) and *RASA1*-related disorders (capillary malformation-arteriovenous malformation syndrome). This is also the case for pulmonary arterial hypertension (PAH) and pulmonary veno-occlusive disease, a rare form of PH for which the genetic cause was recently identified. Furthermore some of these diseases are tightly connected at the molecular level, such as HHT and PAH, since they are both linked to genes belonging to the TGF- $\beta$ /BMP receptor and they sometimes co-occur in a patient harbouring an *ACVRL1* mutation. Finally, genetic heterogeneity for these diseases is rapidly growing since exome sequencing identified pathogenic mutations in several new genes these last years. We developed a targeted exon-capture strategy using SeqCap EZ (Roche Nimblegen) coupled with multiplexing and high-throughput sequencing using the MiSeq system (Illumina). Twelve genes were targeted in total, including *ACVRL1*, *ENG*, *GDF2* (*BMP9*), *TEK*, *RASA1*, *SMAD4*, *BMPR2*, *CAV1*, *KCNK3*, *EIF2AK4*, *SMAD9*, *TBX4*. We screened 27 patients with known mutations as *proof-of-principle*. This strategy allowed the reliable detection of causative mutations (including heterozygous exon deletions) in all the 27 *proof-of-principle* samples. This first step demonstrates that this strategy guarantees a quality of coverage on coding sequences of target genes suited for diagnosis purpose and is highly efficient and cost effective for the molecular diagnosis of hereditary phenotypically related vascular diseases.

## MEDIATORS OF HHT (P83–P91)

### P83 Distinct angiostatic lung profile in endoglin and *ALK1* mouse models of hereditary hemorrhagic telangiectasia

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**Objectives:** (1) To investigate the angiogenic/angiostatic factors implicated in rarefaction of pulmonary peripheral microvascular density (MVD) in heterozygous *Endoglin* (*Eng*<sup>+/-</sup>) and *Alk1*<sup>+/-</sup> mice and (2) to measure the effects of anti-VEGF therapy on lung MVD and angiogenic/angiostatic factors in these mice.

**Methods:** MVD was quantified in peripheral lung sections of adult *Eng*<sup>+/-</sup> and *Alk1*<sup>+/-</sup> mice treated or not with 4 weekly injections (5 mg/kg) of G6-31 antibody to VEGF. Angiogenic/angiostatic factors were measured in mouse lung lysates by Western blot and ELISA. Thrombospondin-1 (TSP-1) levels were compared in embryonic E8.5 endoglin-deficient and control endothelial cells (EC).

**Results:** TSP-1 was fourfold higher in lungs of *Eng*<sup>+/-</sup> than WT mice. TSP-1 was also increased in *Eng*<sup>+/-</sup> and *Eng*<sup>-/-</sup> versus *Eng*<sup>+/+</sup> embryonic EC, suggesting that endoglin and TSP-1 were inversely correlated. Surprisingly, anti-VEGF therapy normalized pulmonary TSP-1 and increased the rarefied peripheral MVD in *Eng*<sup>+/-</sup> mice. *Alk1*<sup>+/-</sup> lungs had normal levels of TSP-1 but a significant rise in Angiopoietin-2 (Ang-2) versus WT. G6-31 treatment restored pulmonary Ang-2 levels and peripheral MVD in *Alk1*<sup>+/-</sup> lungs.

**Conclusions:** The rise in the major angiostatic factor TSP-1 in lungs of *Eng*<sup>+/-</sup> mice and in embryonic *Eng*<sup>+/-</sup> EC suggests that TSP-1 contributes to HHT1. The increase in the vascular destabilizing factor Ang-2 in *Alk1*<sup>+/-</sup> mice indicates that Ang-2 may be implicated in HHT2. The beneficial effects of anti-VEGF treatment in *Eng*<sup>+/-</sup> and *Alk1*<sup>+/-</sup> lungs occurred through decrease in pulmonary VEGF and normalization of TSP1 and Ang-2 levels respectively. However, caution is warranted before translating these results to clinic.

### P84 Endoglin is required to maintain normal cardiac function in adult life

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**Objectives:** Endoglin is a co-receptor for members of the transforming growth factor- $\beta$  superfamily of ligands, and regulates angiogenesis. Patients carrying mutations in endoglin develop hereditary haemorrhagic telangiectasia (HHT). Endoglin is mainly expressed in vascular endothelial cells, and plays a critical role in vascular remodelling. This work aims to investigate its role in adult life and assess its effects on cardiac function.

**Methods:** We used a transgenic Cre line (*Cdh5* (PAC)-CreERT2) to generate a mouse model with endothelial specific depletion of endoglin (*Eng*-iKOe). Cardiac magnetic resonance imaging (MRI), vascular casting, immunohistology and qPCR were used to evaluate cardiovascular changes after endoglin knockdown.

**Results:** Loss of endoglin leads to an enlarged heart and cardiomyocyte hypertrophy within 5 weeks, and cardiac ventricles continue to enlarge substantially thereafter. Cardiac output initially increases, progressing to high output heart failure (HOHF) associated with increased cardiac expression of brain natriuretic peptide, atrial natriuretic peptide and  $\alpha$ -skeletal actin. As HOHF in HHT may result from anaemia or more frequently from large hepatic AVMs, we first evaluated these phenotypes in endoglin deficient mice. However, we have not detected any AVMs in major organs or found evidence of anaemia that could potentially account for the rapid increase in cardiac output. On the other hand, we did observe enlargement of the pulmonary distal vasculature consistent with a defect in regulating

vascular tone and/or vessel architecture that is currently under investigation.

**Conclusion:** These results describe a novel phenotype and highlight the importance of endothelial endoglin in the maintenance of cardiac structure and function.

### P85 Endoglin-mediated angiogenic responses are regulated by its cytosolic domain

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**Objectives:** Underlying the pathogenesis of HHT, an impaired angiogenesis has been postulated, leading to HHT vascular defects. Endoglin proper functions are key for an accurate angiogenesis. There are two physiological membrane-bound Endoglin isoforms, full-length Endoglin (*L-Eng*) and S-Endoglin (*S-Eng*) lacking most part of the intracellular domain. Endoglin isoforms have been proposed to have different effects in angiogenesis. Here, we have assessed a role for Endoglin cytosolic domain in the modulation of angiogenesis, and specific endothelial processes affected.

**Methods and results:** We used mice that ubiquitously overexpress either *L-Eng* (*L-Eng*+), or *S-Eng* (*S-Eng*+).

*S-Eng*+ mice presented a delayed and impaired reperfusion after hindlimb ischemia compared with *L-Eng*+ and WT mice. Moreover, subcutaneous Matrigel<sup>®</sup> implants were less invaded by vascular endothelial cells when engrafted in *S-Eng*+ mice, compared with *L-Eng*+ and WT mice. We also assessed in vitro cell invasion of Matrigel<sup>®</sup>-covered transwells and capillary-like structures formation in Matrigel<sup>®</sup> using endothelial cells overexpressing *L-Eng* or *S-Eng*. We observed that *S-Eng*+ cells show a reduced motility compared with *L-Eng*+ or WT cells. On the other hand, we found an enhanced sprouting initiation in aortic rings from *S-Eng*+ mice, although this did not lead to more mature capillaries.

**Conclusions:** S-Endoglin, lacking most part of the cytosolic domain, results in an impaired angiogenic response in vivo, whereas L-Endoglin overexpression has no effect on angiogenic rate, compared with WT animals. S-Endoglin effect is caused, at least in part, by a reduction of cell motility and invasion of the extracellular matrix, so we can hypothesize a role of Endoglin cytosolic domain in angiogenesis modulation.

### P86 Endothelial and vascular function in mice overexpressing human soluble endoglin

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**Objectives:** A soluble form of endoglin (sEng) circulating in plasma and its increased levels has been detected in various pathological conditions related to cardiovascular system where endothelial dysfunction plays an important role. High concentration of sEng was also proposed to contribute to the development of endothelial dysfunction, however there is no evidence that this happens in atherosclerotic prone vessels. Therefore, in the present study we analyzed whether high sEng levels induce endothelial dysfunction in mouse aorta.

**Methods:** Four to 6-month-old transgenic mice with high expression of human sEng (*Sol-Eng*+) and age-matched transgenic littermates that do not develop high levels of human sEng (control animals) on chow diet were used. Analysis of vascular function in isolated aorta, Western Blot analysis and ELISA were performed.

**Results:** As expected, *Sol-Eng* +transgenic mice showed higher levels of plasma concentrations of human sEng as well as increased blood arterial pressure, as compared to control animals. Functional analysis either in vivo or ex vivo in isolated aorta demonstrated that the endothelium-dependent vascular function was similar in *Sol-Eng*+ and control mice. In addition, Western blot analysis showed no differences between *Sol-Eng*+ and control mice in the protein expression levels of endoglin, eNOS and pro-inflammatory ICAM-1 and VCAM-1.

**Conclusions:** Our results demonstrate that high levels of sEng alone do not induce endothelial dysfunction in *Sol-Eng*+ mice. However, these data do not rule out the possibility that sEng might contribute to alteration of endothelial function in combination with other risk factors related to cardiovascular disorders.

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### P87 High soluble endoglin levels and high fat diet effects on mouse aorta

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**Objectives:** Soluble endoglin (sEng) is a plasma protein, a cleavage product of the extracellular domain of membrane endoglin, which is strongly expressed by vascular endothelium. sEng was supposed to be

a biomarker in several cardiovascular pathologies, including preeclampsia, hypertension, hypercholesterolemia and diabetes mellitus. However, the specific role of sEng in these pathologies is still poorly understood. Therefore, we hypothesized whether high fat diet in combination with high levels of sEng may affect aortic endothelium in vivo.

**Methods:** Six-month-old transgenic female mice overexpressing human sEng on CBAXC57BL/6J background were fed high fat diet for the following 3 months. Mice were divided into two groups according to plasma levels of sEng determined by ELISA (*Sol-Eng+* group vs. control group). Cholesterol levels were measured and Western blot analysis of eNOS, peNOS, P-selectin, ICAM-1, pNFkB, iNOS, HO-1, NOX-2 expressions in aorta were performed. Functional parameters of aorta were assessed by means of wire myograph 620M.

**Results:** Cholesterol levels did not differ between *Sol-Eng+* group and control group of mice. The expression of P-selectin, ICAM-1, pNFkB, iNOS, HO-1 and NOX-2 were significantly higher in *Sol-Eng+* group than in control group. Endothelium-dependent response induced by acetylcholine was more profoundly impaired in control group than in *Sol-Eng+* group.

**Conclusions:** Results of the study demonstrated that high plasma levels of sEng might induce a pro-inflammatory and oxidative stress phenotype of aorta, which is however compensated by an improved endothelial function in *Sol-Eng+* mice. Potential mechanism of this compensatory response is now under investigation.

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#### P88 Loss of endoglin expression in embryonic PAX3-positive vascular progenitor cells impairs intersomitic vessel formation

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**Overview:** Endoglin-null (*eng*<sup>-/-</sup>) embryos die at E10.5–E11.5 due to defects in angiogenesis. In part, this is due to an absence of vascular smooth muscle cell differentiation and vessel investment. Prior studies from our lab and others have shown the importance of endoglin expression in embryonic development in both endothelial cells and neural crest stem cells. These studies support the idea that endoglin may play cell-autonomous roles in endothelial and vascular smooth muscle cell precursors. However, the role of endoglin in vascular cell precursors remains poorly defined.

**Objective:** Our objective was to generate conditional deletion of endoglin in the neural crest lineage using Pax3Cre and Wnt1Cre to understand its roles in vascular progenitor cell differentiation.

**Methods:** Pax3Cre and Wnt1Cre mice were crossed with *Eng*<sup>+/-</sup> mice to obtain the desired Cre: *Eng*<sup>+/-</sup> mice. These mice were then crossed with homozygous endoglin LoxP-mutated (*eng*<sup>F/F</sup>) mice to

knockout endoglin in specific lineages that contribute to endothelial and smooth muscle constituents of vessels.

**Results:** The Wnt1Cre: *Eng*<sup>F/-</sup> mice were born at the expected Mendelian frequency. Interestingly, the Pax3Cre: *Eng*<sup>F/-</sup> mice showed a variety of vascular defects at E10.5, and none of these mice survived past E12.5. Embryos analyzed at E10.5 showed marked vascular malformations and leakiness of the intersomitic vessels. The dorsal aorta showed significant dilation and disorganization of the invested vascular smooth muscle cells. This was associated with upregulation of several smooth muscle differentiation proteins, including smooth muscle actin.

**Conclusion:** These results suggest that Pax3-derived precursor cells require endoglin for formation of the early embryonic vasculature.

#### P89 Potential association of aberrant microRNA biogenesis with HHT

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#### P90 Profibrotic role of endoglin is dependent on its intracellular domain

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**Introduction and aims:** Endoglin, a 180 kDa membrane glycoprotein, is a TGF- $\beta$  co-receptor which is overexpressed in several models of chronic kidney disease but its specific function in renal fibrosis remains still undefined. Two membrane isoforms generated by alternative splicing have been described, full length Endoglin (L-Eng, the most abundant isoform) and S-Endoglin (short) that differ from L-Eng in the absence of the cytoplasmic tail. We have recently demonstrated that L-Eng overexpression enhances fibrosis in the unilateral ureteral obstruction (UUO) model of kidney fibrosis. The aim of the present study was to assess the effect of S-Endoglin overexpression in renal tubulo-interstitial fibrosis induced by unilateral ureteral obstruction (UUO).

**Methods:** For this purpose, a transgenic mouse which ubiquitously overexpresses human S-Endoglin (*S-ENG+*) was generated. UUO was performed in *S-ENG+* mice and their wild type littermates. Kidney Fibrosis was determined by morphometric techniques and by the expression of fibrosis-related molecules (collagen I, fibronectin) by western blot.

**Results:** Obstructed kidneys from *S-ENG+* mice showed reduced tubulo-interstitial fibrotic area and lower amounts of collagen I and fibronectin than obstructed kidneys from WT mice. Moreover, western blot analysis showed that levels of p-Smad1 and p-Smad3

were higher in obstructed kidneys, being these increase significantly lower in obstructed kidneys from *S-ENG+* than in those from WT animals.

**Conclusions:** The overexpression of *S-ENG+* reduces kidney fibrosis. These results are exactly the opposite of those obtained in *L-ENG+* mice. Therefore, we conclude that the intracellular domain of endoglin plays a major role in regulating its fibrotic effects.

### P91 Soluble endoglin, hypercholesterolemia and endothelial alteration

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**Objectives:** A soluble endoglin (sEng) plasma levels are increased in various pathological conditions including atherosclerosis and endothelial dysfunction. Several studies demonstrated that sEng levels fluctuate during hypercholesterolemia and sEng might contribute to the development of endothelial dysfunction, so far tested only in vascular bed not related to atherogenesis. Thus, we performed series of in vivo and in vitro experiments in order to elucidate relation between sEng, cholesterol levels and possible development of endothelial dysfunction in atherosclerosis prone aorta and endothelial cells.

**Methods:** ApoE-deficient mice and apoE/LDLr—deficient mice were fed with various types of atherogenic diet. Mice overexpressing human sEng on CBAx57BL/6J background were fed either chow or high fat diet. HUVEC cells were incubated with recombinant sEng. Cholesterol and soluble endoglin levels were measured, functional assessment of mice aorta and Western blot analysis of selected markers of endothelial function/dysfunction were performed.

**Results:** Hypercholesterolemia but not plaque progression correlated with high levels of sEng in mouse models of atherosclerosis. High levels of soluble endoglin alone were not able to affect aortic function and expression of markers of endothelial dysfunction in mice and HUVECs. Combination of high-fat diet and high sEng resulted in proinflammatory and pro oxidative changes in mice aorta with concurrent and surprising improvement of aortic vasodilation properties.

**Conclusions:** We propose that soluble endoglin might be interesting biomarker of alteration of endothelium, hypercholesterolemia and possible target of drugs preventing endothelial dysfunction. However, precise mechanism of its effect or relation with endothelium is still under investigation.

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### CELLULAR MECHANISMS (P92–P96)

#### P92 Endoglin haploinsufficiency in endothelial cells from Rendu–Osler–Weber patient derived induced pluripotent stem cells leads to upregulation of MTUS1 gene expression

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**Objectives:** Endoglin (ENG) is an accessory receptor for the transforming growth factor  $\beta$  (TGF $\beta$ ) receptor complex, mainly expressed by endothelial cells (ECs) and mononuclear cells (MNCs) in peripheral blood. Mutations in ENG in humans causes hereditary hemorrhagic telangiectasia type 1 (HHT1). We are interested in downstream targets of Endoglin.

**Methods:** Using human induced pluripotent stem cells (hiPSC) from HHT1 patients, we derived ECs in which ENG expression was constitutively reduced and examined ligand induced signaling. Some features of ECs are affected by cell culture density. We therefore first examined TGF $\beta$  downstream Smad activation upon BMP-9 or TGF $\beta$ -3 ligand binding as a function of cell density.

**Results:** At low cell densities, ligand stimulation resulted in the expected canonical TGF $\beta$  downstream signal activation of Smad2/3 and Smad1/5/8 respectively, whereas at high cell density, cross-reactivity with other TGF $\beta$  superfamily signaling pathways was observed. ENG expression itself was cell density dependent. Consistent with this, we showed that ENG had a prominent role in both canonical BMP9/Smad1/5/8 and TGF $\beta$ 3/Smad2/3 phosphorylation under low cell culture densities, whereas at high densities ENG was only crucial for TGF $\beta$ 3/Smad1-5-8 cross-reactivity. Global gene expression in HHT1 hiPSC-ECs compared with controls showed several differentially expressed genes, most strikingly MTUS1. Upregulation of mRNA and protein in response to siRNA knockdown and examination of vessels in HHT1 patient skin confirmed the inverse relationship between ENG expression and MTUS1 in vitro and in vivo.

**Conclusions:** We identified MTUS1, a microtubule-associated tumor suppressor gene as upregulated as a potential ENG target.

#### P93 Endovascular sampling of endothelial cells from pulmonary arteriovenous malformations in hereditary hemorrhagic telangiectasia: a new methodology for elucidating the molecular mechanisms underlying arteriovenous malformation development

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**Objectives:** To describe a novel methodology for studying endothelial cells (ECs) in pulmonary arteriovenous malformations (pAVM) in situ.

**Methods:** Cooke et al. recently described an endovascular technique for reliable EC harvesting in situ. Detachable coils were deployed and retrieved within porcine iliac arteries. Viable EC populations were obtained. Microfluidic single-cell quantitative PCR allowed analysis of transcriptional levels of 96 genes from a single EC. In our pilot study, 5 patients with HHT-1 and HHT-2 with pAVMs will undergo EC sampling prior to embolization. Gene targets will be chosen from published experience with TGF-beta signaling pathways, microarray studies with HHT cell lines, and available drug targets.

**Results:** This is a concept in preparation to expand our group's previous experience with EC sampling. Cooke has established the efficacy of this technique in iliac arteries and carotid aneurysms in rabbits. These studies established a reproducible protocol for EC purification and single cell gene expression analysis.

**Conclusions:** HHT murine models and human umbilical venous EC cultures have demonstrated differences in gene expression that may be involved in AVM development, growth, and persistence. However, no study to date has systematically assessed gene expression in ECs in AVMs in situ in humans. Elucidating the mechanisms underlying AVM formation, development, and response to treatment may enable development of targeted molecular therapies resulting in cessation of growth, reduction in sequelae, and/or involution. Establishing an in vivo gene expression database will further our understanding of HHT and other vascular diseases and enable novel collaboration between HHT centers.

**P94 Exaggerated inflammatory response associated with impaired pericyte recruitment in airways of hereditary hemorrhagic telangiectasia (HHT) type 1 mouse model**

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HHT is an autosomal dominant vascular disorder characterized by vascular anomalies, which range from small telangiectases in the nasal septum, oral mucosa and gastrointestinal tract to large arteriovenous malformations (AVMs) in major organs. The majority of HHT individuals will have HHT1 due to mutations in endoglin (*ENG*) or HHT2 due to mutations in *ACVRL1* (ALK1, activin receptor-like kinase 1). Both are receptors for Transforming Growth Factor- $\beta$  that are expressed in endothelial cells and share functions in signaling. Accumulating data indicate that excessive angiogenesis is implicated in the pathogenesis of HHT and may contribute to the formation of AVMs. However, the question of what explains the variability in clinical manifestations and severity is unresolved. Here, we present in vivo evidence that inflammation is required for blood vessels to develop vascular anomalies in HHT. Sustained airway inflammation in adult *Eng*<sup>+/-</sup> induced an inappropriate and excessive vessel sprouting with the formation of multiple AVMs. We also provide evidence that the excessive angiogenesis response in inflamed *Eng*<sup>+/-</sup> mice might be partially attributed to pericyte dysfunctions. Our data provide new insights into the pathology of HHT that may be useful as therapeutic strategies for the treatment of vascular malformations.

**P95 Pericytes have an important role in the pathogenesis of hereditary hemorrhagic telangiectasia (HHT)**

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Pericytes are obligatory constituents of blood capillaries and important regulators of vascular morphogenesis during development, vascular homeostasis and disease. They maintain the stability of the

vasculature and regulate endothelial cell proliferation and survival. They control blood capillary diameter, local blood flow and vessel permeability to solutes, molecules and cells. Due to their important roles and their special characteristics, in particular those related to cell plasticity, pericytes might be potential drug targets for future therapies. In this context, we have recently reported the potential benefit of thalidomide in HHT individuals and provided evidence that strengthen the case of pericytes as potential target for the treatment of vascular malformations. Nevertheless, the mechanisms underlying impaired pericyte-endothelial cell interaction are only partially understood and more importantly, how pericyte deficiencies affect vessel functionalities remain unresolved. Using a combination of unique transgenic mice expressing fluorescent pericytes, high-resolution confocal imaging and neat electrophysiology approaches, we provide insights into pericyte functions in HHT and importantly, valuable tools to screen drugs targeting pericytes that have fewer side effects than thalidomide, leading to new therapeutic options for HHT individuals.

**P96 Reduction of mutated endothelial cells mitigates intestinal bleeding and mortality in hereditary hemorrhagic telangiectasia type 2 mice**

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**Objectives:** Gastrointestinal bleeding is a devastating symptom for patients with hereditary hemorrhagic telangiectasia (HHT) due to arteriovenous malformation (AVM). Using an HHT2 mouse model, we tested whether reduction of *Alk1*-null endothelial cells (ECs) could mitigate severe bleeding.

**Methods:** *Pdgfb-iCreER*; Ai14; *Alk1*<sup>2/2f</sup> mice were treated with various doses of tamoxifen (TM, 1.25 mg/25 g, 0.75 mg/25 g, and 0.01 mg/25 g of body weight) to delete *Alk1* gene in ECs. TM treatment also activated the Ai14 gene. Using single cell-RT-qPCR, we found that 68 % of Ai14<sup>+</sup> bone marrow cells were *Alk1*-null. Therefore, Ai14 (red fluorescent) was used as a surrogate marker for *Alk1* deletion. Brain AVM was induced through intra-brain injection of an adeno-associated viral vector expressing vascular endothelial growth factor. The *Alk1*-null ECs were analyzed by quantifying Ai14<sup>+</sup> ECs, and AVMs in organs were visualized by latex vascular casting.

**Results:** All mice died within 2 weeks after receiving one dose of 1.25 mg/25 g of TM due to intestinal bleeding. Mice treated with 0.01 mg/25 g of TM survived beyond 4 weeks. Reduction of TM doses from 1.25 to 0.01 mg/25 g significantly reduced *Alk1*-null ECs in the lung (77 vs. 26 %) and the brain angiogenic foci (96 vs. 58 %). AVMs were still present in the brain and intestines of mice treated with 0.01 mg/25 g of TM.

**Conclusions:** Reduced *Alk1*-null ECs in an HHT2 mouse model mitigated intestinal bleeding and mortality. Therefore, decreasing the number of mutant ECs could be a potential therapeutic strategy to lessen the severity of bleeding in patients afflicted with HHT.