

ORAL COMMUNICATIONS

**001
ENDOGLIN HAS A CRUCIAL ROLE IN BLOOD
CELL-MEDIATED VASCULAR REPAIR**

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Endoglin is highly expressed by active endothelial cells (ECs) and is causally associated with HHT1. Mutant endoglin (*Eng*^{-/-}) mice die at embryonic day (E)10.5 from defects in vessel and heart development. Vasculogenesis is normal but angiogenesis is impaired. We have used experimental myocardial infarction (MI) in wild-type and *Eng*^{-/-} mice to investigate the effects of the HHT1 mutation on angiogenesis and vasculogenesis, integral to remodeling after MI, in adult mice. Analysis of mouse hearts and cardiac biopsies from adult humans revealed that endoglin is upregulated in neoangiogenic vessels formed after MI. In *Eng*^{-/-} mice, endoglin levels and microvasculature within the infarct zone are strikingly reduced after MI compared to wild-type mice, which causes a significantly greater deterioration in cardiac function measured by Magnetic Resonance Imaging (MRI). Interestingly, defects in vessel formation and heart function in *Eng*^{-/-} mice were rescued by injection of mononuclear cells (MNCs) from healthy human donors but not from HHT1 patients, indicating defective vascular repair as contributing to the etiology of HHT1.¹ Since vascular damage/inflammation occur randomly, this may also explain disease heterogeneity. An additional factor in HHT may be intrinsic differences in the levels of endoglin between tissues and as a function of age as we observed in both wt and *Eng*^{-/-} mice. *In vitro* studies on ECs showed that threshold levels of endoglin are required for normal TGFβ signalling; natural decreases in endoglin expression with age may lead to subthreshold levels in some tissues in which vascular defects then develop.

Reference

1. Van Laake, et al. *Circulation* 2006;114:2288-97.

**002
IDENTIFICATION OF BMP9 AS THE SPECIFIC
LIGAND OF ALK1: A NEW CIRCULATING
ANGIOGENIC FACTOR**

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ALK1 is an endothelial-specific type I receptor of the TGFβ receptor family that is implicated in the pathogenesis of HHT2. Although TGFβ1 and 3 have been shown to bind ALK1 and activate the Smad1/5 pathway in certain cell types under certain experimental conditions, they may not represent the physiological ligands for this receptor. In the present study, we demonstrate that BMP9 induces the phosphorylation of Smad1/5/8 in microvascular endothelial cells and this phosphorylation lasts over a period of 24 h. BMP9 also activates the Id1 promoter-derived BMP response element (BRE) in a dose-dependent manner (EC₅₀ = 45±27 pg/mL) and this activation is abolished by silencing ALK1 expression or addition of ALK1 extracellular domain. Overexpression of endoglin increases the BMP9 response whereas silencing of both BMPRII and ActRIIA expressions completely abolishes it. Further, we demonstrate that BMP9 potently inhibits endothelial cell migration and growth, and stimulate endothelial expression of a panel of genes that were previously reported to be activated by the constitutively active form of ALK1. Finally, we show that the ALK1 activity present in the serum is completely inhibited by a neutralising BMP9 antibody. Taken together, our results demonstrate that BMP9 binds specifically to ALK1 in presence of BMPRII or ActRIIA and that its activity is increased by endoglin. Further, BMP9 is a circulating factor (2 ng/mL in human serum) that inhibits endothelial proliferation and migration. BMP9 is therefore a new circulating angiogenic factor that may play a role in the development of HHT.

**003
CANDIDATE GENE ANALYSES IN THE HHT3
INTERVAL ON CHROMOSOME 5**

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Three disease genes for hereditary haemorrhagic telangiectasia (HHT) have been identified to date, resulting in HHT type 1 (*endoglin*), HHT type 2 (*ALK-1*), or, JPHT a juvenile polyposis-HHT overlap syndrome (MADH4). *Endoglin* and *ALK-1* encode proteins expressed on vascular endothelial cells, and all three gene products modulate or transmit signals by transforming growth factor (TGF)-β, superfamily members. We recently described

the HHT3 locus in an HHT family unlinked to *endoglin*, *ALK-1*, or *MADH4*, with no mutations in the known HHT genes¹. A genome-wide linkage study identified the disease gene interval on chromosome 5 where a single haplotype was inherited by all affected members of the pedigree (Z_{\max} 3.45, $\theta=0$, fully informative markers). The remainder of the genome (including the newly described *HHT4* locus) was excluded to a 2-5cM resolution. Fine mapping narrowed the interval to a 5.4cM/6Mb region; additional polymorphic markers have now been studied. Candidate genes in the interval were initially selected based on known function and/or expression on vascular endothelial cells. cDNA library screening and 5'RACE were used to identify additional endothelial cell-expressed sequences in our interval genes. Sequencing has been undertaken in our *HHT3*-linked family, and other HHT families in which no mutations were present in *endoglin* or *ALK-1*. Analysis of sequence variations in candidate genes is ongoing. Identification of HHT3 disease-causing mutations is anticipated to lead to a better understanding of pathophysiological processes in HHT, and to offer more complete genetic testing for affected families.

This work is supported by the British Heart Foundation

Reference

1. Cole, Begbie, Wallace and Shovlin. J Med Genet 2005;42;577-82

004

CLINICAL FEATURES OF ENG- AND ALK1-MUTATION CARRIERS

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Hereditary hemorrhagic telangiectasia (HHT) is mainly caused by *Endoglin* and *ALK1* gene mutations, which are responsible, respectively, for HHT1 and HHT2. Genotype-phenotype correlations performed on *ENG* and *ALK1* mutation carriers have recently demonstrated that genetic heterogeneity largely accounts for the diversity of clinical features. However, studies show discrepancies regarding the prevalence of visceral localizations, most likely due to the diverse sensitivity of the techniques employed for instrumental investigation. In the present study, a total of 135 consecutive adult patients were subjected to genetic analyses and separately evaluated for the presence of AVMs with highly-sensitive instruments, such as chest-abdomen multislice CT, brain MRI/MRA, and upper endoscopy, independent of the presence of clinical symptoms and results of genetic testing. A genotype-phenotype correlation was established for the 122 patients with identified mutations. Our study evidenced a higher visceral involvement in HHT1 and HHT2 compared to previous reports. PAVMs and CAVMs were significantly more frequent in HHT1 (75% vs 44%, $p<0.0005$; 20% vs 0%, $p<0.002$) whereas HAVMs were more numerous in HHT2 (60% vs 84%, $p<0.01$). No age difference was found for PAVMs but HAVMs were significantly

more frequent in older patients in both HHT1 and HHT2. Furthermore, neurological manifestations secondary to CAVMs/PAVMs were found only in HHT1 patients, while severe liver involvement was detected only in HHT2. Respiratory symptoms were mainly noted in HHT1. In conclusion, the use of highly-sensitive methodologies and knowledge of the mutated gene will permit a better definition of the appropriate protocol for the follow up of HHT1 and HHT2 patients.

005

PREVALENCE OF LIVER INVOLVEMENT IN ITALIAN FAMILIES WITH ENG AND ALK1 MUTATIONS IN HEREDITARY HEMORRHAGIC TELANGIECTASIA

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Aim. to correlate prevalence of hepatic vascular malformations (VMs) in hereditary hemorrhagic telangiectasia (HHT), as estimated by family screening with Doppler ultrasonography (US), with family genotype, as detected by mutation screening. *Methods.* Four hundred and eighty-three subjects underwent our screening protocol including genetic testing and, besides other tests, clinical, biochemical, and Doppler US evaluation for detection of liver VMs; among them 296 subjects belonged to families in which a *ENG* or *Alk1* mutation was identified. Statistical associations were established by comparative analysis with χ^2 (with Yates correction) between the liver VMs prevalence, demographic characteristics, and mutation. *Results.* 194 out of 296 proved to be affected by HHT (96 males, mean age 39.09). In 143 subjects (71 males, mean age 41.3) belonging to HHT families with *ALK1* mutations liver VMs were detected in 56, 39.1% (14 males, 25%, mean age 52.5). In 51 subjects (25 males, mean age 33.4) belonging to HHT families with *ENG* mutations liver VMs were detected in 17, 33.3% (males 8, 47%, mean age 45.2). Prevalence of liver VMs, age distribution in subjects with liver VMs proved not significantly different between *ENG* and *Alk1* families, $p>0.5$. Sex distribution proved significantly different between *Alk1* subjects with liver VMs and all affected subjects, $p<0.01$, non significantly different with *ENG* mutated subjects with liver VMs, p 0.15. *Conclusions.* Liver VMs show different pattern of distribution in *ENG* and *Alk1* HHT families.

006

MULTIPLEX LIGATION PROBE AMPLIFICATION ANALYSIS IDENTIFIES ENG AND ALK1 DELETIONS IN HEREDITARY HEMORRHAGIC TELANGIECTASIA FAMILIES

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Hereditary Hemorrhagic Telangiectasia (HHT) or Rendu-Osler-Weber disease is an autosomal dominant, heterogeneous disorder. Sequencing revealed mutations in the *ENG* gene (HHT1) or the *ALK1/ACVRL1* (HHT2) in the majority of patients. In a few cases other genes have been identified to cause the disease. To date, we have tested 300 probands. It has however to be kept in mind that a possible relationship between probands cannot be excluded. The testing was performed by direct sequencing of all exons of both the *ENG* and *ALK1* gene. Probands that did not show a deleterious mutation, were tested using Multiplex Ligation-dependent Probe Amplification (MLPA). MLPA is a multiplex PCR based method designed to detect deletions/duplications of one or more exons of the genes. The specificity of the amplification products is determined by the site-specific hybridization of two adjacent primers and their ligation prior to amplification. Polymorphisms located in the recognition sequence will disturb the MLPA and were tested for. In the *ENG* gene 111 probands showed 70 different pathogenic mutations. Two probands showed deletions that could be identified by MLPA: a deletion of exon 3 respectively a deletion of the exons 3-14. In the *ALK1* gene 85 probands showed 50 different pathogenic mutations. One proband showed a deletion of exons 2-8. No abnormalities were identified in the remaining probands. The results indicate that larger deletions that include at least one exon are rare for both the *ENG* gene (2.8%) and the *ALK1* gene (2.0%) at least in this panel of HHT probands.

007

CLINICAL MOLECULAR SCREENING PROGRAM FOR HEREDITARY HEMORRHAGIC TELANGIECTASIA—A BIENNIAL UPDATE, 2007

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Hereditary hemorrhagic telangiectasia (HHT, Osler-Weber-Rendu) was initially associated with point mutations in Endoglin (*ENG*) and activin receptor-like kinase 1 (*ALK1*) genes. An additional gene, *SMAD4*, and loci on chromosomes 5 and 7 have been linked to clinically confirmed cases of HHT. Molecular screening can aid in the identification of individuals and their family members with mutations and provide surveillance and adequate treatment of AVMs. We screened 324 individuals with HHT and observed 192 (58.6%) coding sequence mutations in

ENG and *ACVRL-1* genes. This was followed by sequencing of the coding exons of *SMAD4* gene on 40 individuals. We identified 2 mutations, for which one individual was a mosaic. Next, we investigated the presence of intragenic deletion or duplication within the *ENG* and *ACVRL-1* using the techniques of Multiplex PCR and Multiplex Ligation-dependent Probe Amplification (MLPA). Forty-four coding sequence mutation negative individuals were scanned and 5 mutations were identified - one whole gene deletion and three intragenic deletions with multiple exons in *ENG*, and one single exon deletion in *ALK1*. Through the use of these two techniques an additional 5% of individuals were determined to have HHT because of intragenic deletions. Finally, southern blot analysis is being performed to detect any potential deletion of the coding exons of *SMAD4* gene. The results will be reported. Thus with this multi-step analysis, we can provide an in-depth clinical diagnostic test to aid individuals and their family members in deciding if future medical evaluations are needed.

008

HAPLOTYPE STUDY AND ESTIMATION OF THE AGE OF RECURRENT ACVRL1 MUTATIONS IN FRANCE AND ITALY

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Twenty years ago, a large scale French epidemiological study pointed out that the distribution of HHT could vary greatly from one area to another, some very high concentrations of patients being found in given areas. Although mutations in HHT patients are and are usually unique and family specific, some recurrent mutations have been reported, especially for *ACVRL1*. We report on the haplotype analysis in 112 French (n=102) and Italian (n=10) patients carrying 13 *ACVRL1* recurrent mutations. Results showed that in most cases, their frequency observed during mutation screening was related to both independent mutational events and small mutation hot-spots. Out of the five mutations common to French and Italian patients, three were associated to a partially common haplotype in some patients from both countries, suggesting a unique mutational event. For five mutations, we could estimate the time of their introduction in the respective populations. The c.1112dupG mutation is very likely to be responsible for the very unusually high frequency of HHT observed in the *Ain* and *Jura* departments in the former epidemiological study. This mutation is likely to have occurred in a common ancestor living in a valley of

the Haut-Jura Mountains more than three centuries ago and to have spread over the generations in the Rhône-Alpes region and outside.

009

LIKELIHOOD RATIOS TO ASSESS GENETIC EVIDENCE FOR CLINICAL SIGNIFICANCE OF UNCERTAIN VARIANTS IN HEREDITARY HEMORRHAGIC TELANGIECTASIA

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Clinical molecular genetic testing for hereditary hemorrhagic telangiectasia (HHT) relies heavily on gene sequencing and as a result laboratories frequently detect previously unreported and/or uncharacterized variants. Often these are missense or intronic mutations in which the contribution to disease cannot be reliably predicted and are thus reported as variants of uncertain significance (VUS). This uncertainty in test interpretation is a source of considerable frustration and concern to clinicians and HHT families alike. Follow-up to assess family concordance is recommended by the American College of Medical Genetics to give genetic evidence for clinical significance. Although family concordance studies show whether a variant tracks with disease in the family, the strength of evidence varies by number of family members available for testing and the degree of relatedness. We investigated a statistical model using the program MLINK to perform a Bayesian analysis which accounts for the pedigree, inheritance pattern, and age-related penetrance in HHT to determine a likelihood ratio. We will present and discuss our application of this analysis which yielded likelihood ratios of variants showing concordance ranging from 20:1 to over 1000:1. These ratios provide an objective measure of the strength of genetic evidence for causality and can be considered with other parameters such as amino acid severity predictions, ortholog and paralog comparisons and functional assays to determine the likelihood that a variant is the cause of HHT.

010

A STRATEGY FOR INTERPRETATION OF MISSENSE VARIANTS IN HHT

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A recent comprehensive survey¹ of reported mutations

associated with HHT revealed that 20% of *ENG* and 53% of *ACVRL1* mutations are single base pair substitutions leading to an amino acid replacement. These missense mutations must have some deleterious effects on protein structure and function in order to be disease-associated. On the other hand, several polymorphisms listed in the HHT Mutation Database are missense variants; these should not affect protein structure and function. Since missense variations comprise a large portion of the mutations in HHT, a strategy that can help distinguish disease-associated mutations from benign polymorphisms would facilitate application and interpretation of genetic analysis. Direct studies of the effect of variants on gene function are not practical in a clinical diagnostic setting, but indirect methods can be applied to increase the level of confidence in the assignment of disease-associated mutations. In several cases, our laboratory achieved better predictions by analyzing DNA from additional family members, performing protein modeling, and studying amino acid conservation across species using the SIFT program. Not all methods are appropriate in each case, and it is important to know the applicability and the predictive limitations of each method. We discuss our strategy as a clinical diagnostics laboratory to analyze missense variants and report results.

Reference

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011

SCREENING FOR PULMONARY ARTERIOVENOUS MALFORMATION USING TRANSTHORACIC ECHOCARDIOGRAPHY: A PROSPECTIVE STUDY

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Background. We prospectively evaluated the diagnostic value of non-invasive tests for screening PAVM, using the HRCT as the *golden standard*. **Methods.** All persons (>16 years), screened for HHT and presence of PAVM by HRCT between 05-2004 and 12-2006 were included. Screening was done by a chest radiograph (n=221), arterial oxygen pressure (P_aO₂) measurement (n=196), the 100% oxygen technique (n=66), and a transthoracic contrast echocardiography (TTE (n=208)), all on the same day. The sensitivity and negative predictive values (NPV) with their 95% confidence intervals (CI) were measured for each test using the HRCT as golden standard. **Results.** A HRCT was made in 225 subjects (65% female, mean age 44.5±14.7 years). Overall, a PAVM was present in 20%. The sensitivity and NPV of the different tests were respectively, for the chest radiograph: 29.5% (95% CI 24.5-60.9) and 85.1% (95% CI 79.5-89.6), for a P_aO₂<12 kPa: 73.7% (95% CI 56.9-86.6) and 90.0% (95% CI 82.4-95.1), for an abnormal RLS measurement (>5% shunt fraction): 82.6% (95% CI 61.2-95.1) and 90.5% (95% CI 77.4-97.3), for the combination of chest radiograph and 100% oxygen test: 48.9% (95% CI 33.7-64.2) and 88.3% (95% CI 82.9-92.4), and for TTE: 95.3% (95% CI 84.2-

99.4) and 98% (95% CI 94.6-99.8). In 37 patients (17%) a pulmonary shunt was suggested by TTE whereas the HRCT was negative for PAVM. *Conclusion.* In our prospective study, TTE is the best non-invasive diagnostic test for PAVM, probably even more sensitive in diagnosing a pulmonary shunt than the main *golden standard* a HRCT.

012 MIGRAINE CHARACTERISTICS IN PATIENTS WITH AND WITHOUT PULMONARY ARTERIOVENOUS MALFORMATIONS

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Background. migraine with aura (MA) seems to be associated with both cardiac and pulmonary right-to-left shunts (RLS). We prospectively evaluate the difference in migraine characteristics in patients with and without PAVM. *Methods.* All subjects (>16 years of age), who underwent a transthoracic contrast echocardiography (TTE) for the diagnosis of PAVM in our HHT screening program between 05-2004 and 11-2006, received a structured headache questionnaire. Two neurologists diagnosed migraine according to International Headache Society criteria. *Results.* one-hundred and ninety-nine patients (63% female, mean age 44.7±14.2 years) underwent a TTE and completed the questionnaire. Migraine was present in 23% and MA in 10%. A PAVM was present in 36%. In patients with migraine the prevalence of PAVM was 33% compared to 37% in those without migraine ($p=0.68$). In patients with MA a PAVM was present in 55% compared to 34% in those without MA ($p=0.06$). In the 44 patients with migraine (79.5% female, 44.1±12.7 years) there was no difference in frequency ($p=0.64$), severity (6.7 versus 6.0, $p=0.19$), and duration (13.9h versus 16.3h, $p=0.71$) between patients with and without PAVM. In the 20 patients (75% female, mean age 44.5±12.6 years) with MA there was no significant difference in frequency ($p=0.1$), severity (6.8 versus 5.6, $p=0.19$), and duration of headache (13.7h versus 8.3h, $p=0.52$) between patients with and without PAVM. Kappa coefficient interobserver reliability was 0.9 ($p<0.001$). *Conclusion.* In patients screened for HHT, MA tended to be associated with PAVM. However, there is no difference in migraine characteristics between patients with or without PAVM.

013 MATERNAL RISKS OF PREGNANCY FOR INDIVIDUALS WITH PULMONARY ARTERIOVENOUS MALFORMATIONS AND HEREDITARY HAEMORRHAGIC TELANGIECTASIA

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Introduction. Having reported two maternal deaths from haemoptysis, and pulmonary arteriovenous malformations (PAVM) deterioration during pregnancy¹, we sought to identify the frequency and associations of these complications. *Methods.* All 142 women with PAVMs in the 1999-2005 Hammersmith series were studied. 32 (22.5%) had presented with respiratory symptoms; 135 (95%) had HHT. Pre and post pregnancy imaging, and oxygen saturation (SaO₂, measured at 1 minute intervals after standing or lying for 7 minutes) were compared. Values for the most recent datapoint pre-pregnancy, latest timepoint during pregnancy, and post-partum measurements at 6-12 months, and 2-3 years (if no intervening embolisation) were compared using repeated measures ANOVA and paired t-tests. *Results.* 262 pregnancies occurred in 105 women either before PAVM assessment (239 pregnancies; 97 women) and/or post PAVM assessment (23 pregnancies; 16 women). 6/262 (2.3%) pregnancies were complicated by major haemoptysis or haemothorax requiring emergency embolization (n=3), thoracotomy (n=2) or induced delivery (n=1). Three first-degree relatives had died from massive haemoptysis. One individual with HHT required emergency preterm Caesarean section for pre-eclampsia. Overall, there was a significant fall in SaO₂ standing between pre-pregnancy and 6-12 months post partum values ($p=0.021$). PAVM growth was confirmed by CT scan or angiography in 7/9 cases. There was a spontaneous and statistically significant ($p=0.0033$) post-partum improvement in standing SaO₂ between measurements at 6-12 months and 2-3 years post partum. *Conclusions.* in this series, life-threatening PAVM bleeding occurred in 2.2% of pregnancies, or 5.7% of women. Pregnancy is associated with PAVM growth, and spontaneous improvement post partum. *We thank the families and friends of HHT patients whose donations supported this work*

Reference

1. Shovlin et al, Quart J Med 1995: 88:879-887

014 MANAGEMENT OF ASYMPTOMATIC PULMONARY ARTERIOVENOUS MALFORMATION IN HEREDITARY HAEMORRHAGIC TELANGIECTASIA: A DECISION ANALYTIC MODEL

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Although many experts recommend coil embolotherapy for patients with hereditary hemorrhagic telangiectasia

(HHT) who develop asymptomatic pulmonary arteriovenous malformations (PAVMs) with feeding arteries ≥ 3 mm, this approach has never been studied prospectively. We used decision analysis to evaluate survival and quality of life gains associated with embolotherapy compared to conservative management with observation.

We developed a Markov model, incorporating PAVM complications (cerebrovascular events, cerebral abscess, hemothorax and massive hemoptysis), and treatment effects and complications, and the possibility of new PAVM growth or reperfusion of successfully embolized PAVMs. The base case was a 40-year-old man with HHT and an asymptomatic PAVM with a 3 mm feeding artery. We modelled the natural history of HHT from review of the medical literature. We incorporated quality of life weights, derived from direct assessment of patient preferences (n=45) and literature review. Embolotherapy and conservative management were associated with survivals of 35.5 and 33.8 years, respectively. After adjusting for quality of life, the corresponding estimates were 33.9 and 29.7 quality-adjusted life years (QALYs). The outcome of the model was sensitive both to the probability of successful embolization and to the probability of a disabling cerebral stroke from embolization, but only outside of their expected plausible ranges. Subjects with HHT and a PAVM with a ≥ 3 mm feeding artery have improved life expectancy and quality adjusted life expectancy with embolotherapy, compared to observation. Our study suggests that embolotherapy should be the standard therapy for these patients. *Financial support: Canadian Lung Association, University of Toronto, Clinician Scientist Training Program (SG), Nelson Arthur Hyland Foundation (MEF)*

015 OUTPATIENT SINGLE-SESSION PAVM EMBOLIZATION

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Purpose. PAVM embolization is performed as an inpatient procedure in most institutions, often with sequential procedures to treat multiple PAVMs. In addition to increasing cost and inconvenience to the patient, this practice may exacerbate non-compliance with embolotherapy. In our HHT center we perform all PAVM embolizations in teenagers and adults as outpatients, treating all accessible PAVM in a single session if possible. We describe our results on an intent-to-treat basis. *Patients and Methods.* 45 patients, age 15-78 (mean 43), 41 with HHT, underwent outpatient embolization in 57 procedures. Multiple procedures were performed when PAVM were too numerous for single session treatment (n=5) or for PAVM recurrence or growth (n=7). Thus, single session embolization was achieved in 40/45 patients (89%). Planned multiple sessions were performed on consecutive days or at longer intervals at patient request. All procedures were performed using moderate sedation. Patients were discharged after 2 hour observation and scheduled for 30 day followup in the IR clinic. Embolization was perfor-

med with platinum coils and/or Amplatzer vascular occluders. *Results.* One patient with multiple (10) PAVMs was admitted overnight at her request to complete the embolization the next day. All others were successfully treated as outpatients. No patient required admission or clinic visit during the postoperative period. Mean 2.5 PAVMs were embolized per procedure (range, 1-9). Minor chest pain occurred after 13 procedures (23%). *Conclusion.* Outpatient single session PAVM embolization is achievable in nearly 90% of patients. This practice may reduce reluctance of asymptomatic patients to undergo much-needed PAVM embolotherapy.

016 THE AMPLATZER VASCULAR PLUG—PRELIMINARY RESULTS OF USE IN TREATING PULMONARY ARTERIOVENOUS MALFORMATIONS

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Purpose. Treatment modalities for pulmonary arteriovenous malformations (PAVMs) have included surgical excision, sclerotherapy, and embolotherapy. PAVMs treated by embolotherapy may recur as a result of recanalisation or the development of new feeding arteries. Transcatheter occlusion of PAVMs is technically challenging owing to the risks of paradoxical embolisation of embolic materials or thrombus. The Amplatzer Vascular Plug (AVP) has recently been approved for use in treating peripheral AVMs. As with other recoverable devices the AVP offers lower risks of paradoxical embolisation than using regular pushable coils. This study presents our preliminary results of their use in PAVMs. *Materials and Methods.* 9 patients with PAVMs (age range 20-63 years, M:F=2:7), 5 of whom were asymptomatic and detected on screening, and 4 of whom were symptomatic, were treated with AVPs over a 2 year period. Initial angiographic localisation of the PAVMs was followed by selective catheterisation, and deployment of the AVP as distally as possible in the feeding vessel. *Results.* A total of 10 PAVMs were deemed suitable for the AVP, and all were successfully occluded. There were no device or procedural complications and no repeat procedure has been necessary. 2 patients have undergone check angiography at 1 year. All patients remain well and symptom-free with respect to their PAVMs at a mean follow-up of 14months (range 1-28). *Conclusions.* Embolotherapy is the preferred treatment modality and the use of the AVP has added a new device to our armamentarium of embolic devices. We present the initial successful outcome of using this device.

017**NEW CLASSIFICATION AND NATURAL HISTORY OF PATIENTS WITH DIFFUSE PULMONARY ARTERIOVENOUS MALFORMATIONS: 29-YEAR EXPERIENCE**P. Pierucci, J. Murphy, K. Henderson, D. Chyun,¹ R.I. White Jr.*Yale University School of Medicine, ¹Yale University School of Nursing, New Haven, CT, USA*

Introduction. Patients with diffuse pulmonary arteriovenous malformations (PAVM), a small but important subset of the PAVM population, have significant morbidity and mortality. **Materials and Methods.** 35 patients (20 female, 15 male) with diffuse PAVM, from a cohort of 821 consecutive patients with PAVM were evaluated during 2006. Diffuse PAVM were categorized angiographically: involvement of one or more segmental pulmonary arteries in one or both lungs. Hereditary hemorrhagic telangiectasia (HHT) status, gender, presence or absence of large (equal to or greater than 3mm diameter artery) focal PAVM, initial oxygen saturations and latest or current oxygen saturations post embolization, development of hemoptysis and survival were tabulated. **Results.** HHT was present in 30/35 (86%) of the patients and diffuse PAVM were more commonly bilateral than unilateral. Focal PAVM were present in both groups but more commonly in those with bilateral involvement. All deaths occurred in those with bilateral involvement and were due to hemoptysis of bronchial artery origin (2), spontaneous liver necrosis (3), brain hemorrhage (1), brain abscess (1), and operative death during attempted lung transplant (1). **Conclusions.** Patients with diffuse PAVM are at a higher risk for complications than patients with focal PAVM and require yearly monitoring.

Table.

	UNILATERAL N=10	BILATERAL N=25	p-value
HHT	7 (70%)	23 (92%)	0.11
<i>Gender</i>			
Male	8 (80%)	7 (28%)	0.01
Female	2 (20%)	18 (72%)	
Focal PAVMs	2 (20%)	16 (64%)	0.02
Initial O ₂ Sat	0.87±.07	0.80±.08	0.02
Current O ₂ Sat	0.91±.09	0.84±.10	0.08
Hemoptysis	2 (20%)	6 (24%)	0.34
Death	0	8 (32%)	0.05

018**DIFFUSE PULMONARY ARTERIOVENOUS MALFORMATIONS IN HHT PATIENTS: LONG-TERM RESULTS OF EMBOLIZATION ACCORDING TO THE DISTRIBUTION AND EXTENT OF LUNG INVOLVEMENT**

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Objectives. To evaluate the clinical and morphological results of embolization for diffuse pulmonary arteriovenous malformations (PAVMs). **Materials and Methods.** Retrospective analysis of HHT patients with diffuse PAVMs (group 1: PAVMs supplied by all subsegmental arteries of at least one lobe and group 2: PAVMs supplied by all segmental arteries of at least one lobe) treated with embolization. Demographics, clinical presentation, number and characteristics of PAVMs, number of procedures and coils were recorded. After embolization, clinical and morphological results were evaluated. **Results.** 36 (19 and 17 patients in groups 1 and 2, respectively) patients (mean age 36 years) were treated. Neurological events were reported in 44% of patients before embolization. PAVMs were simple or complex in 32 and 4 patients respectively. In two cases a thrombus was found within the aneurysm. The feeding artery was >3mm in 361 PAVMs and ≤3mm in 972 PAVMs. A total of 576 PAVMs were embolized in 66 sessions using 1051 coils. Immediate complications included TIA (1 case), sudden deafness (1 case) and proximal migration of coils (2 cases). In two cases the feeding artery could not be catheterized. The mean follow-up after embolization was 44 months. In five patients (14%), recanalization of PAVMs was detected on CT and embolization was repeated. Of interest, a patent foramen ovale (2 cases) and/or liver involvement (5 cases) were present in these patients. **Conclusion.** Morphological results of embolization for diffuse PAVMs were similar to those reported for localized PAVMs. In both groups, clinical results were related to the number of lobes involved and, when only one lobe was involved, results were similar to those reported in patients with localized PAVMs.

019**RECONSTITUTION OF ENDOGLIN EXPRESSION IN ENDOTHELIAL AND SMOOTH MUSCLE CELLS IN VIVO CAN PARTIALLY RESTORE VASCULAR SMOOTH MUSCLE CELL RECRUITMENT**M.L. Mancini,¹ B.A. Conley,¹ T. Nicola,² L. Oxburgh,¹ C.P.H. Vary¹*¹Center for Molecular Medicine, Maine Medical Center Research Institute, Scarborough, Maine; ²Department of Cell Biology, University of Alabama, Birmingham, AL, USA*

Endoglin is a TGFβ co-receptor that is required for angiogenesis. It is predominantly expressed in the endothelial cells, however its expression has been detected in other cell types, including multiple stem cell populations, neural crest cells (see Mancini *et al.*, this meeting) and vascu-

lar smooth muscle cells (vSMCs). Endoglin null embryos die by E11.5 due to impaired angiogenesis characterized by a failure of vSMC recruitment. Based on the phenotype of the endoglin null mouse it is unclear whether impaired vSMC recruitment is secondary to a lack of endoglin expression in the endothelial cells or if endoglin expression is also required in the developing vSMCs. To investigate the role of endoglin in cell autonomous signaling, we generated a conditional transgene and subsequently overexpressed endoglin in a cell type specific manner utilizing smooth muscle (SM22-cre) and endothelial (Tie2cre) drivers. In both cases, we observed hemorrhaging in the dorsal aorta. Furthermore, using a genetic complementation assay we restored endoglin expression in null embryos in a cell type specific manner using the conditional endoglin transgene driven by SM22-cre and Tie2cre. Our results demonstrated that vascular smooth muscle cell recruitment to the dorsal aorta can be partially rescued using the Tie2cre driver, and to a lesser extent, using the SM22-cre driver. These results indicate that endoglin expression in endothelial cells alone is not sufficient to rescue the null phenotype, and suggest that endoglin expression is required in both endothelial and smooth muscle cell types for normal angiogenesis.

020
DIFFERENTIAL EXPRESSION PROFILES OF MULTIPLE TARGET GENES POTENTIALLY INVOLVED IN THE ONSET OF HEREDITARY HEMORRHAGIC TELANGIECTASIA

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Hereditary Hemorrhagic Telangiectasia (HHT) is an autosomal dominant vascular disorder clinically characterized by telangiectases and internal arteriovenous malformations. It is caused by mutations in elements of the TGF- β (Transforming Growth Factor- β) receptor complex: Endoglin, a co-receptor, responsible for HHT1, or ALK-1 (Activin Receptor-like Kinase 1), a type I receptor leading to HHT2. Recently, we were able to get cultures of HHT endothelial cells, primary targets of the disease. These cells had deficient TGF- β signaling and abnormal angiogenesis, representing an ideal human model to study the molecular mechanism of this disease. To understand the mechanism underlying HHT, whole human genome expression microarrays were used comparing cells from normal and HHT donors. Given the similarity of symptoms in HHT1 and HHT2, special interest has been put on the identification of common targets for both HHT types. A total of 277 genes were downregulated while 63 were found upregulated in HHT vs. control cells. Most of the differentially expressed genes found in the Microarray are involved in biological processes relevant to the HHT pathology, such as angiogenesis, leukocyte transmigration, cytoskeleton organization, cell migration, proliferation and vascular physiology. We

have studied these processes using HHT endothelial cells versus control cells leading us to propose a model of HHT pathogenesis, opening new perspectives to understand this disorder. Moreover, the results obtained in the Microarray may be also useful to find out targets of the TGF- β pathway in endothelium.

021
ALK1 AND ENDOGLIN EXPRESSION IN MURINE LUNG: INSIGHTS INTO LOCATION AND FREQUENCY OF PULMONARY ARTERIOVENOUS MALFORMATIONS IN HEREDITARY HEMORRHAGIC TELANGIECTASIA

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The lung is a major site of vascular disease in HHT, with pulmonary arteriovenous malformations (PAVMs) occurring in over 20% of HHT patients. The prevalence of PAVMs is higher in HHT1 (endoglin) patients compared with HHT2 (ALK-1) patients, suggesting endoglin is more important than ALK-1 for the correct formation of the pulmonary vasculature. Generally, PAVMs are more prevalent in the lower lobes, which is thought to relate to this being the major region of blood flow. Also, the location of PAVMs is usually distal, with the proximal vasculature less commonly involved. The reason for this is unclear. We undertook a detailed study of ALK-1 and endoglin expression in mouse lung to determine whether this might help explain the location and frequency of PAVMs. Immunohistochemistry and rtPCR from laser-microdissected vessels was used to evaluate gene expression. Our analysis showed that endoglin was uniformly expressed in pulmonary veins, whereas ALK-1 expression was restricted to the distal venous branches. Both Endoglin and ALK-1 were strongly expressed in capillaries. Interestingly, neither endoglin nor ALK-1 were detected in parenchymal arteries except in distal branches, where they were co-expressed at the branch level of the terminal bronchioles. Here, there was an associated activation of downstream SMAD1/5. In *eng*^{-/-} mice, ALK1 expression was unchanged, whilst endoglin levels were reduced approximately two-fold, as expected, with one intriguing exception – endoglin expression in some distal arteries was reduced to *undetectable* levels. This data points to distal arteries and pulmonary veins being important vascular regions in PAVM development.

022
A DISTURBED CXCR4/CD26 BALANCE IN ENDOGLIN DEFICIENT MONONUCLEAR CELLS RESULTS IN IMPAIRED HOMING

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Recently we showed that mononuclear cells (MNCs) from Hereditary Hemorrhagic Telangiectasia type-1 (HHT1)-patients were impaired in homing to the infar-

cted area in a mouse myocardial infarction (MI)-model. After MI, Stromal Derived Factor-1 (SDF-1) levels are elevated in the infarcted area. The SDF-1/CXCR4-axis is important for homing of cells to ischemic injury. TGF β , also induced after MI, is capable of shifting the SDF-1/CXCR4-axis towards more migration. CD26 can inhibit this axis by inactivating SDF-1. We hypothesize that impaired homing of HHT1-MNCs is due to their inability to increase CXCR4 and lower CD26 in response to increased TGF β levels after MI. We analyzed CXCR4 and CD26 expression by flow cytometry in MNCs from 8 HHT1-patients and controls without or stimulated with TGF β . TGF β stimulation increased CXCR4 expression in controls, but this was significantly less in HHT1-MNCs ($p=0.013$) and CD26 levels were higher ($p=0.01$). HHT1-MNCs showed less migration *in vitro*. Incubated HHT1-MNCs with Diprotin-A, a specific CD26 inhibitor, improved their migration. To demonstrate the significance of elevated CD26 expression for homing of MNC *in vivo*, we injected MNCs incubated with Diprotin-A, 24 hours post-MI in the tail vein of wildtype mice. Five days post-MI, significantly more MNCs homed to the infarcted area when pretreated with Diprotin-A. The inability of HHT1-MNCs to increase CXCR4 upon TGF β stimulation and elevated CD26 levels could explain the reduced homing to ischemic tissue. Therefore, inhibition of CD26 by incubating HHT-1-MNC with Diprotin-A might improve homing to sites of ischemic injury in HHT-1 patients and enhance tissue repair.

023 DEVELOPING HIGH THROUGHPUT SCREENS BASED ON ENDOGLIN MUTANT EMBRYONIC STEM CELLS UNDERGOING VASCULOGENESIS FOR TESTING POTENTIAL HHT DRUGS

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One of the most stressful symptoms for HHT patients is persistent and frequent nosebleeds. Treatments that could alleviate these symptoms would mean a significant increase in the quality of life of these patients. Thalidomide has been identified as a potentially interesting drug to test but is known to have severe effects on unborn children so would not be suitable for use by young women. Other drugs with a similar mechanism of action would be extremely welcome but often the cost of testing are often prohibitive for a relatively uncommon disease. Animal models that are available are also expensive and have the disadvantage that the symptoms are not always evident in those bearing the defective gene. We are developing an *in vitro* model based on embryonic stem (ES) cells from both wild type and endoglin mutant mice to test drugs like Thalidomide. ES cells are pluripotent and have the ability to form complex vascular structures in culture that include vascular endothelial cells closely associated with vascular smooth muscle cells. We have derived preliminary data showing that the ES model

system can faithfully report the effects of Thalidomide on blood vessel structure. Analysis is by morphology, markers and microarray. This is currently being extended to human cells and may represent an easy means of understanding the mechanism underlying Thalidomide action, and more importantly, may lead to new strategies for novel drug design.

024 IMPAIRED ANGIOGENESIS IN ADULT ENDOGLIN HETEROZYGOUS MICE IS BASED ON REDUCED NO SYNTHESIS

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Adult endoglin heterozygous ($Eng^{+/-}$) mice show altered angiogenesis and reduced NO synthesis. Our purpose has been to assess the role of reduced NO synthesis in this altered angiogenesis. Two *in vivo* models of angiogenesis were tested in the $Eng^{+/-}$ mice and in their control littermates ($Eng^{+/+}$): tumor-induced angiogenesis and reperfusion following hind-limb ischemia. Tumor was induced by injecting 10⁶ Lewis lung carcinoma (3LL) cells subcutaneously. For hind-limb ischemia, the left femoral artery was ligated 2-3 mm distal to the inguinal ligament. Hindlimb perfusion was measured every other day up to 28 days by laser Doppler. Four days after femoral ligation, the adductor muscle was rapidly excised from both ischemic and non-ischemic limbs. In both experimental models, NO synthesis was inhibited with L-NAME in the drinking water of some animals. Capillary density (CD31 immunohistochemistry), hemoglobin content and vascular cell adhesion molecule-1 (VCAM-1) expression were used to assess vascularization. The expression of endoglin, hypoxia inducible factor (HIF), endothelial nitric oxide synthase (eNOS) and vascular endothelial growth factor (VEGF) were determined by Western blot analysis. Tumor weight, capillary density, hemoglobin and VCAM-1 were reduced by about 30% in $Eng^{+/-}$ respect to $Eng^{+/+}$ littermates. eNOS and phosphorylated eNOS levels were significantly reduced and HIF expression was slightly reduced, whereas VEGF levels were slightly increased in $Eng^{+/-}$ respect to $Eng^{+/+}$. Differences in tumor vascularization disappeared in the presence of L-NAME. $Eng^{+/-}$ mice showed a delayed reperfusion following hindlimb ischemia compared to $Eng^{+/+}$ mice. Following ischemia, $Eng^{+/-}$ mice had a significantly lower adductor muscle vessel density than $Eng^{+/+}$ mice. eNOS levels were significantly lower in non-ischemic $Eng^{+/-}$ than in $Eng^{+/+}$ muscles and this difference was maintained despite a significant ischemia-induced increase in both groups. L-NAME significantly reduced hind-limb reperfusion to similar levels in both groups of mice, whereas a NO donor significantly increased reperfusion rate in $Eng^{+/-}$, whereas the

enhancing reperfusion effect in *Eng*^{+/+} mice was much lower. In conclusion, angiogenic abnormalities observed in adult *Eng*^{+/+} mice seem to be based on a reduced NO production.

**025
GENERATION AND ANALYSIS OF A CONDITIONAL
ENDOGLIN KNOCKOUT MOUSE**

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Endoglin is an essential gene in development. It is up-regulated in endothelial cells during angiogenesis and loss of endoglin expression in the mouse results in embryonic lethality at mid-gestation. This phenotype points to an important role of endoglin in new blood vessel formation but precluded analysis at later stages in development and in postnatal life. To bypass this limitation and allow further investigations of the function of endoglin we have generated a floxed endoglin allele in which loxP sites flank exons 5 and 6. Mice homozygous for this allele are normal and in the presence of appropriate Cre lines allow time and cell specific endoglin deletion for *in vivo* analysis of function. We have used the inducible Cre line, VE-Cadherin-Cre-ERT2 (in which Cre expression in endothelial cells is driven by the VE-cadherin promoter) to generate a conditional endoglin knockout mouse in which endoglin is depleted from vascular endothelial cells in a temporally controlled manner. In this line, Cre recombinase is efficiently activated by tamoxifen treatment. This can be done at any chosen stage of development or adult life, and is monitored using the Rosa26R reporter. When the endoglin gene is inactivated by Cre in late embryogenesis, at approximately E14.5, there is a rapid loss of endoglin protein and dramatic lethal changes in the embryo. In contrast, when endoglin is inactivated in adult life, animals appear to be viable. We will present data describing the vascular changes in conditional knockout animals focussing on changes relating to those seen in HHT.

**026
FUNCTIONAL STUDY OF ENDOTHELIAL CELLS
FROM LIVER OF TRANSPLANTED HHT2 PATIENTS**

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Hepatic lesions in HHT consist of diffuse arteriovenous malformations of various sizes that can lead to high-output cardiac failure or acute ischemic biliary necrosis, requiring liver transplantation. Severe liver involvement has been shown to be associated to mutations in *ACVRL1*.

The pathological mechanisms remain mostly unknown, but dysfunction of endothelial cells (EC) may play a key role in the initiation and progression of the disease. EC and fibroblasts (FB) were isolated from explanted liver of four HHT patients with different *ACVRL1* mutations. Control EC and FB were isolated from the peritumoral tissue of a cholangiocarcinoma. Cells were characterized by their morphology, immunofluorescent labeling with specific antibodies and RT-PCR. We searched for a second mutational event by dHPLC and QMPSF and performed different functional assays. No second mutation or loss of heterozygosity was found, consistent with the haplo-insufficiency model. A slightly higher proliferation rate was observed for EC compared to the control. EC were able to proliferate and behave normally in the wound healing assay. Tubulogenesis formation on Matrigel was not impaired. Western blot analysis showed normal expression of ALK-1. Activated TGF- β was found in the culture medium from EC of the patients, suggesting an autocrine secretion, probably related to the portal fibrogenetic process associated to the liver lesions. We are currently investigating the functionality of Smad1 and Smad2 in response to different stimuli.

**027
INTEROBSERVER AGREEMENT IN DIAGNOSING
LIVER INVOLVEMENT IN HEREDITARY
HEMORRHAGIC TELANGIECTASIA BY DOPPLER
ULTRASOUND**

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Aim. The purpose was to evaluate interobserver agreement on Doppler ultrasonographic (US) diagnosis of liver vascular malformations (VMs) in hereditary hemorrhagic telangiectasia (HHT) and on their severity grading. *Methods.* During the interobserver agreement study three observers, with at least 20 years of experience using Doppler US, judged about the presence/absence of liver VMs and their severity on a set of images and videoclips. Interobserver agreement was estimated with kappa statistics. 110 cases were reviewed during interobserver study (80 cases with liver VMs, 30 without). *Results.* The overall kappa coefficient for the three observers varied from 0.84 to 0.43. For two of the observers, it was statistically significant versus kappa of 0.60. The highest values were found in the ability to distinguish ill from diseased cases. All observers demonstrated excellent sensitivity and specificity in identifying HHT patients, with their respective

AUC areas ranging from 0.97 to 0.99. Interobserver agreement among the three investigators in staging liver involvement of HHT patients was fair (Kendall's coefficient of concordance =0.25), with significant correlation with the operator's experience in using Doppler US on HHT patients. **Conclusions.** Study results indicate that Doppler US diagnosis of liver VMs in HHT has a high degree of reproducibility among ultrasonographers; greater operator experience can improve staging ability as well.

028

DETECTION OF EARLY LIVER ARTERIOVENOUS MALFORMATIONS IN HEREDITARY HAEMORRHAGIC TELANGIECTASIA BY CONTRAST ECHOGRAPHY

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Aim. HHT is a multivisceral disease with liver arteriovenous malformations which are usually detected by color and pulse wave doppler ultrasound. Some of them are nevertheless normal. The objective of this study was to evaluate hepatic transit time (HTT) by contrast ultrasound to detect early liver malformations. **Materials and Methods.** 18 patients with HHT (mutation in the ALK1 gene) were investigated by echography doppler (Sequoia® Siemens USA) and contrast ultrasound (Sonovue® Altana-Bracco Italy). We compared HHT population with a healthy control group (8) by contrast echography. HTT was calculated between portal and hepatic vein with Quantif® (Noesis France). **Results.** 30 percent of HHT population without abnormal echography-doppler ultrasound exam had an hepatic transit time in the same range than the healthy control group. The others had a significant decreased hepatic transit time. **Conclusions.** Contrast echography can reveal earlier liver arteriovenous malformations which are no visualized by standard echography and doppler ultrasound. For HHT population with normal hepatic transit time, further investigations are needed to conclude either to absence of hepatic lesion, either to still undetectable arterio-venous malformations.

029

13C-METHACETIN BREATH TEST: A NEW DIAGNOSTIC TOOL FOR DIAGNOSIS OF LIVER INVOLVEMENT IN PATIENTS AFFECTED BY HEREDITARY HAEMORRHAGIC TELANGIECTASIA

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HHT is a systemic disease with visceral arteriovenous malformations and hepatic involvement in approximately 70% of patients and is diagnosed with multislice contrast-

enhanced computed tomography (MSCT). The 13C-methacetin breath test (MBT) permits the evaluation of liver functions and might be altered by vascular liver shunts. We aimed to assess the effect of hepatic vascular shunts on MBT in HHT patients. 45 HHT patients (22 males; mean age: 52±15 years) with systemic involvement, and 45 age/sex-matched healthy controls were enrolled in the study. All patients were subjected to MSCT. A MBT was performed according to the standard method: after administration of 75 mg 13C-methacetin dissolved in water, expired air samples were collected at baseline and every 15 minutes for two hours. The sample 13C-methacetin enrichment was measured with an isotope-ratio-mass-spectrometer and results expressed as cumulative dose percentage absorbed after 2 hours (CPD2). Statistical analysis was performed by Student's T test and ROC curve analysis. The CPD2 of controls and patients were 35.05%±4.1% and 23.7±8.9%, respectively ($p<0.00001$). Among HHT patients, MSCT evidenced vascular shunts in 34 cases. The CPD2 in controls was significantly higher than in HHT patients with (22.6±8.9%) ($p<0.000001$) and without (29.4±7.7%) ($p<0.01$) liver involvement. Furthermore, the CPD2 level in patients without hepatic shunts was higher than that observed in those with shunts ($p=0.036$). Patients with CPD2 <22% showed a 94% positive predictive value (PPV) to have hepatic shunts at MSCT (specificity 90%). The MBT showed a high PPV to identify hepatic shunts in HHT patients and could be proposed as screening tool to select patients in whom abdomen MSCT is warranted. The MBT appears to be able to detect even mild liver involvement in HHT patients, and could be used to avoid radiological screening of the about 30% HHT patients without HAVMs at first examination.

030

EVALUATION OF LIVER DISEASE IN HEREDITARY HAEMORRHAGIC TELANGIECTASIA: MAGNETIC RESONANCE IMAGING WITH FLOW QUANTIFICATION

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Purpose. To describe findings, including flow quantification, obtained with magnetic resonance imaging (MRI) of the liver in patients with hereditary hemorrhagic telangiectasia (HHT). **Materials and Methods.** MRI was performed in 35 patients [20 females; mean age, 49 years (range, 15–75 years)] seen in the HHT Clinic, during a 2-year period. The protocol was designed to evaluate the morphologic changes seen in HHT and evaluate flows. The presence of shunts, hepatic perfusion disorders, telangiectases, other vascular lesions, and flow abnormalities within the celiac and hepatic artery were evaluated by two radiologists in consensus. **Results.** Thirty-one/35 patients (89%) had a definite clinical diagnosis of HHT (*Curacao Criteria*). Genetic mutation was available in 16/35 (49%), of whom 75% had ALK-1 mutation. Nine out of 35 (25%) were symptomatic of hepatic vascular malformations. Hepatic vascular abnormalities were

found in 25/35 (74%) patients. Arteriportal shunts were present in 18/35 (51%) patients and arteriosystemic shunts in 5 of 35 (14%). In 17/35 (49%) patients, parenchymal perfusion disorders were detected. Telangiectases were found in 18/35 (51%) patients. In 14/35 (41%) patients, large confluent vascular masses were visualised. In 13/32 (41%) patients, the celiac artery flow exceeded 2L/min. The flows flow quantification allows for the characterization of the ranged from 2-7L/min. In 7/31 patients (23%) the hepatic artery flow exceeded 2L/min, with flows ranging from 2-4L/min. *Conclusion.* MRI with hepatic malformations in patients with hepatic involvement of HHT.

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**031
MULTI-SLICE-CT VS DYNAMIC TIME-RESOLVED
MR-ANGIOGRAPHY FOR EVALUATION OF
HEPATIC INVOLVEMENT IN HEREDITARY
HEMORRHAGIC TELANGIECTASIA**

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MultiSlice CT (MSCT) and time-resolved dynamic MR-Angiography (MR-A) were compared for evaluation of hepatic involvement in HHT patients. Thirty consecutive patients (17 males, 13 females) referring to the HHT Interdepartmental Center of Bari (Italy) underwent abdominal MSCT and MR-A. MSCT used a triphasic protocol after injection of 100-120 ml of iodinated contrast medium (370-400 mgI/mL) at 3,5-4 ml/sec flow. MR-A was performed with a 1.5 magnet (Philips Achieva). Unenhanced transverse SST2, SS-T2 spair, T1 images and coronal B-TFE images were obtained in all cases. Subsequently, eight consecutive 3D FFE-T1-dynamic sequences were produced during a 35-40 secs acquisition (4-5 secs/dynamic) in a single breath-hold in the coronal plane with the following parameters: TR/TE: shortest; flip angle 25°, thickness 2,5 mm; acquisition matrix 256. Scan delay was assessed using a bolus track technique after a 0.1 mL/kg bolus of GD-DTPA flushed with 20 ml of saline at 2.5 ml/sec. Images were reviewed and processed at a separate workstation (ViewForum; Philips Medical systems) to obtain MIP reformations of MR Angiograms. Hepatic vascular abnormalities were found in 21/30 (70%) patients with both techniques. Teleangiectases were evident in 19/30 (63%) cases with MSCT and 18/30 (60%) with MR-A. Arteriportal shunts were diagnosed in 6/30 (20%) and 8/30 (26%) cases, respectively, by MSCT and MR-A. Arteriovenous shunts were evident in 4/30 (13%) patients with both techniques. Mixed shunts were present in 5/30 (16%) case with MSCT and 3/30 (10%) with MR-A. Perfusion disorders were noted in 13/30 (43%) cases as transient-hepatic-attenuation-differences with both imaging modalities. Accuracy for diagnosis of HHT hepatic vascular altera-

tions was similar for MSCT and MR-A. Thanks to multiple dynamic scans, MR-A has an higher temporal resolution allowing for easier recognition of type of hepatic shunt. Due to the lack of radiation and any gadolinium-based contrast medium toxicity, MR-A might be preferred to MSCT to rule out hepatic involvement in HHT patients.

**032
OUTCOME OF SYMPTOMATIC LIVER VASCULAR
MALFORMATIONS IN HEREDITARY
HEMORRHAGIC TELANGIECTASIA**

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HHT patients with symptomatic liver AVMs can present with high cardiac output heart failure (type 1), portal hypertension (type 2) or biliary ischemia (type 3), however, their natural history remains poorly understood. The clinical course and factors related to mortality were evaluated at a single HHT center over a 12-year period. Of 2500 HHT patients, 48 had symptomatic liver AVMs, including 37 with type 1, 8 with type 2, and 3 with type 3 manifestations. Age at initial evaluation was 58±12 years and 38 were women. Of the type 1 patients, 4 subsequently developed biliary ischemia and were classified as type 3 cross-over patients. Over 5.6±3.5 year follow-up, 16/48 (33%) patients died, including 6/33 type 1 (18%, age 74±5), 5/8 type 2 (63%, age 68±6), 1/3 type 3 (33%, age 74), and 4/4 type 3 cross-over (100%, age 52±15). Kaplan-Meier survival was best in type 1 patients ($p=0.03$). In bivariate analysis, type at last visit ($p=0.004$), refractory GI bleeding ($p=0.02$) and ascites ($p=0.04$) were associated with mortality. In type 1, cross-over to type 3 ($p=0.003$) and refractory GI bleed ($p=0.01$) were associated with mortality. Thus, in symptomatic liver HHT, type of presentation has important prognostic implications. Type 1 has a favorable prognosis unless biliary ischemia or severe GI bleeding develops.

**033
HEPATIC ARTERY EMBOLIZATION IN
SYMPTOMATIC PATIENTS WITH HEREDITARY
HEMORRHAGIC TELANGIECTASIA**

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Purpose. To evaluate the role of staged hepatic artery embolization (HAE) in symptomatic patients with Hereditary Hemorrhagic Telangiectasia (HHT). *Methods.* In 19 patients, hepatic artery branches were selectively embolized in stages using polyvinyl alcohol particles (PVA) and microcoils. Clinical symptomatology and cardiac output were assessed before and after therapy as well as at the end of follow-up (median 29 months; range 8 - 184 months). *Results.* Following embolization, abdominal pain as well as symptoms arising from cardiac insuff-

iciency/failure or portal hypertension resolved completely in 11 and partially in 2 (one with encephalopathy) of the 19 patients. The mean cardiac output decreased significantly ($p < 0.001$) from 11.87 L/min pre-treatment to 7.91 L/min at the end of follow-up. Symptoms did not improve in one patient. One patient showed initial regression of symptoms; starting about 2 years following embolization she developed debilitating hepatic cirrhosis necessitating liver transplantation. After excellent regression of symptoms for 7 months following therapy, one patient returned with hematemesis; she died following an attempt to surgically ligate the hepatic artery. One patient with cardiac insufficiency and severe Parkinson's disease showed improvement of cardiac insufficiency after two embolizations, but succumbed to Parkinsonism and a complicated traumatic femoral fracture before completion of therapy. Two patients with pre-existing cirrhosis, encephalopathy and pulmonary hypertension died as a complication of the embolization. *Conclusion.* Staged HAE yields long-term relief of clinical symptoms in selected patients with HHT and hepatic involvement. Patients with pre-existing hepatic cirrhosis/fibrosis, hepatic encephalopathy or pulmonary hypertension may be poor candidates for HAE.

034

ECHO DOPPLER COMPARED TO RIGHT HEART CATHETERIZATION FOR DIAGNOSIS OF HIGH OUTPUT HEART FAILURE IN HEREDITARY HEMORRHAGIC TELANGIECTASIA

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High output cardiac failure (HOCF) from systemic arteriovenous malformations is a poorly studied complication of Hereditary Hemorrhagic Telangiectasia (HHT). Traditionally, right heart catheterization (RHC) is used to diagnose and evaluate this high output state. We report the first study of HOCF in HHT aimed at comparing transthoracic echo with RHC for detection of this high output state. *Methods.* Of 346 HHT patients (pts) referred to a large HHT center from 1997-2006, 15 with symptoms attributable to heart failure (4.0%) had both a high cardiac output by Fick and a transthoracic echo performed and were included in this study. Data was retrospectively collected. Two readers interpreted echo derived cardiac indices: stroke distance/left ventricular outflow tract (LVOT) time velocity integral (TVI) and calculated cardiac output. Data is reported as medians with ranges. *Results.* Median cardiac output by Fick was 10.5 L/min (range 7.1-14.8). Median heart rate was 80. Median LVOT diameter was normal at 2.0cm. Median LVOT VTI (stroke distance) was elevated at 29 cm (range 19-39.6), as was the subsequent calculated cardiac output by echo at 8.2 L/min. *Conclusions.* In HHT patients with symptomatic

clinical heart failure, a high output cardiac state was evident on echo as a high LVOT VTI and an elevated calculated cardiac output. Although the elevation in cardiac output is not as marked as by RHC, echo doppler with attention to LVOT VTI and subsequent calculated cardiac output is a noninvasive method to screen for HOCF in HHT patients.

035

STRUCTURE OF HUMAN ENDOGLIN, A RECEPTOR INVOLVED IN THE HEREDITARY HEMORRHAGIC TELANGIECTASIA

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Endoglin is a transmembrane glycoprotein expressed as a disulfide-linked homodimer whose haploinsufficiency is responsible for the Hereditary Hemorrhagic Telangiectasia type 1 (HHT1). Structurally, endoglin belongs to the zona pellucida (ZP) family of proteins that share a ZP domain of ~260 amino acids at their extracellular region. Unfortunately, little is known about its 3D structure. Here, we have determined for the first time the 3D structure of the ~140-kDa extracellular domain of endoglin at 25 Å resolution using single-particle electron microscopy (EM). This reconstruction provides the overall architecture of endoglin, which arranges as a dome made of anti-parallel oriented monomers enclosing a cavity at one end. In addition, we have obtained a high resolution structure of endoglin, modelling *de novo* its amino acid sequence using the Robetta server (<http://rosetta.bakerlab.org/>). The results of this analysis reveal that each subunit comprises three well defined domains, two of them corresponding to ZP regions, organised into an open U-shaped monomer. This domain arrangement was found to closely resemble the overall structure derived experimentally and the three modelled *de novo* domains were tentatively assigned to the domains observed in the EM reconstruction. Combined, these data provide the structural framework to interpret endoglin's functional domains and mutations found in HHT1 patients. Interestingly, most of the HHT1 missense mutations were non-randomly distributed throughout the extracellular region, but were located within the orphan domain or the juxtaposed ZP-N subdomain. Compatible with the haploinsufficiency model, these results suggest that the protein modules encompassing these mutated residues are critical structures for the correct expression of the mature endoglin protein at the cell surface.

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036

IN VIVO DISSECTION OF TGF- β SIGNAL TRANSDUCTION PATHWAYS FOR THE PATHOGENESIS OF HEREDITARY HEMORRHAGIC TELANGIECTASIA

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Heterozygous mutations in ALK1, ENG, or SMAD4 cause hereditary hemorrhagic telangiectasia (HHT), and TGF- β 1 has been considered as the ALK1 ligand pertinent to HHT. It has been shown that ALK1 and another TGF- β type I receptor ALK5 in endothelial cells form a balance for mediating TGF- β signal which controls angiogenesis. To assess the balance *in vivo* and to test whether ALK1 is a TGF- β receptor, we have compared the phenotypes of mice in which the Alk1, Alk5 or Tgfr2 gene was conditionally deleted in the endothelial cells of specific vascular beds using a novel Cre driver that we have generated. We demonstrate that endothelial Alk1 deficiency exhibits the hallmark of vascular malformations seen in HHT patients, i.e. dilation of lumen, thinning of vascular walls, loss of capillaries, and AVMs, in a consistent and predictable manner in late gestational and early postnatal mutants. Therefore these mice would not only serve as an *in vivo* model to study molecular and histological pathogenesis of HHT vascular malformation, but also to test preclinical therapeutic reagents to prevent progression or to reverse pathogenesis of the vascular malformations. We show that ALK5 is not required for ALK1 signaling, and suggest that the proposed balance of ALK1 and ALK5 in ECs has a limited role in vascular development. We also demonstrate that the ALK1 signaling does not require TGFBR2, indicating that HHT may not be a TGF- β disease.

037

ALK1 SIGNALING IN MICROVASCULAR ENDOTHELIAL CELLS

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The HHT1 and HHT2 genes endoglin and ALK1 are receptors for members of the TGF- β superfamily. It is thought that the mutations in Endoglin and ALK1 disrupt or deregulate signaling below a certain threshold, leading to cellular dysfunction of the endothelium. ALK1 mediates TGF- β 1, - β 3 and BMP9 signals. Data about ALK1's cellular function are contradictory, reported to be an inhibitor of proliferation and migration in one report and the opposite in another report. Aside from methodology differences the use of different endothelial cell types might also account for the diverging results. Since HHT is a disease predominantly affecting the microvasculature we used primary human microvascular endothelial cells (HDMEC) to investigate the TGF- β , BMP9 and ALK1 signaling pathway, cellular and gene expression responses. Low TGF- β 1 concentration (0.5ng/mL) induced cell proliferation whereas

higher concentrations were inhibitory. Results for BMP9 were not definite. We also tested the direct effects of ALK1 and ALK5 on cell proliferation. HDMECs were transfected by nucleofection with ALK1, ALK5 and constitutively active (ca) ALK1 and caALK5. ALK1, caALK1 and ALK5 enhanced proliferation in contrast to caALK5 inhibiting proliferation. Furthermore, we investigated the ALK1/ALK5 interdependent relationship on gene expression by treating HDMECs with siALK1 or siALK5. Knock-down of ALK1 caused dramatic downregulation of ALK5 and vice versa. In this way an ALK1 HHT mutant and an ALK5-Smad2/3 binding mutant were investigated for TGF- β 1 regulated endogenous ALK1/ALK5 expression. We are currently trying to establish whether Smad1 or Smad5 is activated by ALK1 upon TGF- β and BMP9. Results will be discussed.

038

A HIGH THROUGHPUT ANALYSIS OF PROTEIN NETWORKS SHARED BY ENDOGLIN AND TRANSFORMING GROWTH FACTOR- β RECEPTOR II

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Endoglin and ALK1, the protein products of the genes mutated in HHT1 and HHT2, are receptors of the TGF- β superfamily specifically expressed in endothelial cells, which signal via the TGF- β Receptor II (T_R-II). We hypothesize that protein networks shared by Endoglin, T_R-II and ALK1 are critical for endothelial cell function and defective in HHT. To date, we have used the LUMinescence based Mammalian IntERactome mapping (LUMIER) robotic screen to identify protein interaction networks of Endoglin and T_R-II. Their cDNA were fused to Renilla luciferase tag (RL) to generate baits, which were then individually tested by co-transfection with 600 different Flag-tagged cDNA preys. The bait-prey complexes were immunoprecipitated by an antibody to the Flag-tag (M2) and the light emitted, which is proportional to the extent of bait-prey binding, was measured. The z-score was calculated for each bait-prey complex to predict significant interactions; for high stringency, we first selected a z-score ≥ 19 . We found 24 proteins binding to Endoglin, 126 to T_R-II and 21 to both. We are currently performing bioinformatics analysis to define potential novel pathways emerging from these screens and will confirm selected interactions in HEK293T and endothelial cells. Our primary focus is to determine the role of these novel protein networks in normal endothelial cells and in the pathways defective in HHT. These studies may identify novel potential candidate proteins for future therapeutic interventions.

039

THERAPEUTIC ACTION OF TRANEXAMIC ACID IN HHT. REGULATION OF ALK-1/ENDOGLIN PATHWAY IN ENDOTHELIAL CELLS

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Recurrent epistaxis is the most frequent clinical manifestation of Hereditary Haemorrhagic Telangiectasia (HHT), a vascular dysplasia originated by mutations in *endoglin* or *ALK-1* genes. Our objective was to assess the use of Tranexamic Acid (TA), an antifibrinolytic drug, for the treatment of epistaxis in HHT patients, and investigate the *in vitro* effects of TA on Endoglin and ALK-1 expression and activity in endothelial cells. A prospective study was carried out on patients treated with oral TA in the HHT Unit of Sierrallana Hospital (Cantabria, Spain). The results showed that the fourteen HHT TA-treated patients improved decreasing their frequency and severity of epistaxis. No complications derived from the treatment were observed. Since endoglin or ALK-1 haploinsufficiency is at the basis of HHT pathogenesis, the effect of TA on endoglin and ALK1 expression and function was assessed. Cultured endothelial cells incubated with TA exhibited: i) increased levels of endoglin and ALK-1 proteins at the cell surface, as shown by flow cytometry; ii) increased mRNA levels of endoglin and ALK-1, as measured by real time PCR; iii) enhanced TGF- β signaling as determined with reporters for endoglin or ALK-1 promoters and for the ALK-1/endoglin pathway; and iv) improved endothelial cell functions like tubulogenesis and migration. In summary, oral administration of TA proved beneficial for epistaxis treatment in selected patients with HHT. In addition to its already reported antifibrinolytic effects, TA stimulates the expression of ALK-1 and endoglin, as well as the activity of the ALK-1/endoglin pathway.

040

ENDOGLIN IS ASSOCIATED WITH SMOOTH MUSCLE CELLS AND STABLE HUMAN ATHEROSCLEROTIC LESIONS

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Transforming growth factor- β (TGF β) is involved in vascular remodeling and plays a pivotal role in the maintenance of the balance between inflammation and fibrosis in atherosclerotic plaques. Endoglin, mainly expressed on ECs and important for their growth, was recently found to be expressed on smooth muscle cells (SMCs) in athero-

sclerotic plaques. However, the function of endoglin in plaque SMCs is not known. In this study, we investigated endoglin expression in relation to SMCs, plaque phenotype and the matrix metalloproteinase (MMP) pathway in human atherosclerotic lesions. *Methods and Results.* We analyzed phosphorylated Smad proteins, representing an active TGF β signal, in 50 protein samples from patients that underwent carotid-endarterectomy. pSmad2 positively correlated with EMMPRIN45kD ($p=0.024$), MMP2 activity ($p=0.024$) and SMCs ($p=0.044$). A positive correlation was also found between endoglin and pSmad2 ($p<0.001$), EMMPRIN45KD ($p=0.029$), MMP2 activity ($p=0.016$) and SMCs ($p=0.0036$). Interestingly, there was no correlation of endoglin with VEGF or Hif-1 α , excluding a neoangiogenic function for endoglin in the plaques. *In vitro* analysis revealed that cultured SMCs expressed Endoglin and down-regulation of endoglin expression using RNAi reduced their growth significantly while endoglin over-expression stimulates it. *Conclusion.* For the first time, we show a relation between endoglin, SMCs and MMP activation suggesting a function for endoglin in SMCs. The strong correlation of SMCs, EMMPRIN45KD, MMP2 activity and TGF β signaling in human atherosclerotic plaques suggests a role in matrix turnover, a key feature of plaque stabilization. The effect endoglin has on SMC growth *in vitro* further supports a function for endoglin in plaque stabilization.

041

SMAD-INDEPENDENT REQUIREMENT FOR ENDOGLIN IN THE MAINTENANCE OF THE MYOGENIC DIFFERENTIATION POTENTIAL OF NEURAL CREST STEM CELLS

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Genetic studies show that TGF β signaling is essential for vascular development, although the mechanism through which this pathway operates is incompletely understood. Here we demonstrate that the TGF β auxiliary coreceptor endoglin (*eng*, CD105) is required for the myogenic differentiation of neural crest stem cells (NCSCs). Overexpression of endoglin in the neural crest caused pericardial hemorrhaging, correlating with altered vascular smooth muscle cell investment in the walls of major vessels and upregulation of smooth muscle α -actin protein levels. Endoglin over expression combined with conditional deletion of Smad4 suggested that hemorrhage was independent of Smad4. Clonogenic differentiation assay of neural crest stem cells derived from neural tube explants demonstrated that neural crest stem cells expressing high levels of endoglin had myogenic differentiation potential, whereas those expressing low endoglin levels were deficient in the capacity for myogenesis. Restoration of endoglin expression in NCSC lacking endoglin restore their myogenic potential. Furthermore, myogenic potential was deficient in neural crest stem

cells obtained from endoglin null embryos. Expression of endoglin in neural crest stem cells declined with age, coinciding with a reduction in both smooth muscle differentiation potential and TGF β 1 responsiveness. These findings demonstrate a cell autonomous role for endoglin in smooth muscle cell specification contributing to vascular integrity.

042
ENDOGLIN MODULATES KERATINOCYTE CELL GROWTH AND INVASIVENESS DURING MOUSE SKIN CARCINOGENESIS

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We and others have provided evidence for a direct role of endoglin in malignant progression. Thus, reduction of endoglin expression in endoglin heterozygous (Eng^{+/−}) mice had a double effect on two-stage chemical skin carcinogenesis, by inhibiting the early appearance of benign tumors (papillomas), but increasing progression to spindle cell carcinomas (SpCC). The transition from squamous to spindle cell carcinomas (SCC-SpCC), which involves an epithelial-mesenchymal transition linked to enhanced tumor cell invasion and metastasis, is considered the latest stage in carcinogenesis. It can be induced *in vitro* or *in vivo* by TGF- β 1. We found that shedding of keratinocyte membrane-bound endoglin, that releases soluble endoglin into the stroma and vessels, is an event associated with the SCC-SpCC transition *in vitro* and *in vivo*. Furthermore, siRNA-mediated knockdown of endoglin allowed transformed keratinocytes to undergo a partial EMT, and sensitized these cells for TGF- β 1-stimulated conversion into the spindle phenotype. Downregulation of endoglin expression led to increased cell migration and invasiveness, but endoglin-downregulated cells showed reduced growth *in vitro* and *in vivo*. These results are consistent with the dual phenotype of Eng^{+/−} mice, and suggest a role for endoglin in carcinogenesis by modulating the TGF- β cell response.

043
COMPUTER MODELING OF PULMONARY HEMODYNAMICS ON WALL SHEAR STRESS IN PULMONARY ARTERIOVENOUS MALFORMATIONS

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Purpose. Exercise increases both pulmonary artery (PA)

flow rate and pressure. As patients with Hereditary Hemorrhagic Telangiectasia (HHT) are predisposed to developing pulmonary arteriovenous malformations (PAVMs), it is important to consider the effects of pulmonary hemodynamics, such as those of exercise, on the pulmonary blood vessels in this population. *Materials and Methods.* PAVMs in 2 HHT patients from our institution were modeled from CT scans with 1.25 mm slice thickness. Cross-sectional geometry for each AVM was converted to Cartesian coordinates in MATLAB and built into a mesh for Computational Fluid Dynamics (CFD) analysis in FloWorks™. The effect of blood velocity on vessel wall shear stress was simulated at 100%, 150%, 200% and 250% of typical flow rates. A similar study of PA pressure was made at 25 mmHg, 37.5 mmHg, 50 mmHg and 75 mmHg. Finally, both velocity and pressure were increased simultaneously by these increments. *Results.* Increase in blood flow velocity resulted in an approximately linear increase in shear stress, with a 250% increase in velocity ultimately resulting in 245% and 382% increases in shear stress in each PAVM respectively. Interestingly, increases in arterial pressure did not have a significant impact on vessel wall shear stress. Cumulative effects of increased velocity and PA pressure were minimal. *Conclusions.* This preliminary study suggests that when PA blood flow is increased, shear stress increases linearly. We speculate that this may contribute to PAVM development, growth, and/or rupture.

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044
ELEVATED PULMONARY PRESSURE, REDUCED VASCULAR DENSITY AND ABNORMAL VASOMOTOR FUNCTION IN A MURINE MODEL OF HEREDITARY HEMORRHAGIC TELANGIECTASIA

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Pulmonary hypertension (PAH) is a disorder caused by increased pulmonary vascular resistance due to remodeling and pruning of peripheral pulmonary vessels, and increased vasoconstrictor tone. Mutations in activin-like kinase 1 (ACVRL1) receptor have been found in patients presenting with PAH as well as in patients with HHT2. However, despite the close functional association between endoglin (Eng) and Alk-1 and their modulatory effects in TGF- β signaling, the role of Eng in normal pulmonary vascular homeostasis and PAH remains to be elucidated. Using invasive Millar pressure catheters, ultrasound bio-

microscopy, fluorescence microangiography (FMA) and standard light microscopy, we report that *Eng*^{+/+} mice display signs of PAH including increased right ventricular systolic pressure (RVSP), reduced RV cardiac output and increased pulmonary vascular resistance (PVR), with no evidence of RV hypertrophy. Changes in lung vascular remodeling characterized by dilated central vessels and reduction of peripheral vessel density were observed by FMA and quantified by microscopy. Vasomotor studies on isolated *Eng*^{+/+} resistance pulmonary vessels revealed significantly enhanced endothelial NO synthase mediated ACh-induced vasodilatation. This enhanced vasodilatory response, intrinsically associated with HHT, may serve as a compensatory/negative feedback mechanism to maximize ventilation-perfusion and alleviate the already compromised hemoglobin oxygen saturation in *Eng*^{+/+} mice. Moreover, it may simultaneously minimize PVR and prevent the progression of PAH in the murine model of HHT1.

045

NON INVASIVE EVALUATION OF PULMONARY ARTERY PRESSURES IN A LARGE COHORT OF HEREDITARY HEMORRHAGIC TELANGIECTASIA PATIENTS: PRELIMINARY DATA ON SPONTANEOUS MEDIUM TERM EVOLUTION

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Doppler transthoracic echocardiography (TTE) is a non invasive method suitable for obtaining reliable estimates of Pulmonary Artery Systolic (PASP) and Diastolic (PADP) Pressure. Our preliminary data (Olivieri *et al.*, 2006) showed that elevated PASP values may be found in HHT patients. To the best of our knowledge, no data are currently available on the natural course of PAH in these patients. Here we present our data on TTE screening of PAH in a large cohort of HHT patients (265; 131 males). *Aim* of our work was to evaluate prevalence of PAH and to study the evolution of PASP and PADP over time. *Methods and Results.* Non-invasive evaluation of pulmonary pressures was possible in 187 subjects (71%), 86 males; 22/187 (11.8%) showed values at or above 1 SD compared with normal age-corrected range according to Mc Quillian *et al.* (2001). In 12/22 (55%) subjects we identified a disease causing mutations in ACVRL1 gene. TTE follow-up (mean time 36±16 months) was available

in 12/22 cases (54.5%). Of these, 11 showed PASP and PADP values that were stable over the follow-up period and not associated with signs of right ventricular dysfunction. One patient showed significantly elevated PASP and PADP basal values (50 and 33 mmHg, respectively) that showed a 20% increase during follow-up and were associated with a deterioration of right ventricular function. *Conclusions.* TTE screening discloses a significant number of HHT patients with baseline high PAP values and permits a non-invasive monitoring of the natural evolution of this potentially harmful condition.

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EFFECT OF MUTATIONS IN THE TRANSFORMING GROWTH FACTOR- β RECEPTOR SUPERFAMILY ON THE FUNCTION OF CIRCULATING ENDOTHELIAL PROGENITOR CELLS: INSIGHT INTO THE RELATIONSHIP BETWEEN HEREDITARY HEMORRHAGIC TELANGIECTASIA AND IDIOPATHIC PULMONARY ARTERIAL HYPERTENSION

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Hereditary hemorrhagic telangiectasia (HHT) and idiopathic pulmonary arterial hypertension (IPAH) are associated with mutations in TGF β superfamily receptors: Endoglin (ENG), activin receptor-like kinase-1 (ALK-1) and bone morphogenetic protein receptor-2 (BMPR2). Characterization of circulating endothelial progenitor cells (EPCs), shown to play an important role in vascular homeostasis and repair, may help to understand how mutations which affect members of the same superfamily give rise to such different clinical phenotypes. *Methods.* EPCs were isolated from controls, HHT1, HHT2 and IPAH patients with/without known mutations in BMPR2. Circulating EPCs were quantified by flow cytometry while remaining cells were cultured on fibronectin-coated slides for 7 days. EPC differentiation was quantified by immunofluorescent staining for mature EC-markers. Migration was assessed using a modified-Boyden Chamber, apoptosis was measured by TUNEL and AnnexinV staining, and mRNA expression was determined by real-time-PCR. *Results.* Circulating CD34⁺ cells were elevated in both patients with HHT and IPAH compared to controls, while differentiation towards an endothelial-like phenotype was significantly reduced. EPCs derived from IPAH patients demonstrated a significantly increased rate of apoptosis and a reduced migratory capacity. In contrast, EPCs derived from HHT patients demonstrated more modest increases in apoptosis rate

and reduced migration, but a more significant reduction in gene expression (most notably eNOS). *Conclusions.* EPCs derived from patients with HHT and PAH responded to various functional assays in disparate fashion, demonstrating a clear dichotomy between patient groups. These results may help us understand how mutations in similar TGF β -receptor superfamily genes give rise to such different vascular disease phenotypes.

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RISKS AND ASSOCIATIONS OF PULMONARY EMBOLI AND DEEP VEIN THROMBOSES IN INDIVIDUALS WITH HEREDITARY HAEMORRHAGIC TELANGIECTASIA

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Risks of deep venous thromboses/pulmonary emboli (DVT/PE) are not generally considered for individuals with hereditary haemorrhagic telangiectasia (HHT), a condition often treated with prothrombotic agents such as hormones, tranexamic and aminocaproic acids. We addressed the frequency and associations of DVT/PE in our 1999-2005 series. 309 individuals had definite HHT. 207/309 (67%) had pulmonary arteriovenous malformations, though 164/207 (79%) had no reported respiratory symptoms before diagnosis. 1) DVT and/or PE was confirmed in 20/309 (6.5%) individuals, including five transfusion-dependent, at median age 61 (32-82)ys. Most had clinical risk factors, and Factor V Leiden heterozygosity was more common in the 15 tested DVT/PE patients than 33 age-matched unrelated HHT controls ($p=0.014$, Fisher's exact test). 2) Plasma proteins reflecting endothelium/coagulation perturbations were measured in 38 healthy individuals with HHT (no previous DVT/PE), and 38 age and sex-matched controls. Von Willebrand Factor and Factor VIII (FVIII) antigen concentrations were significantly elevated in the HHT group, findings not explained by disseminated intravascular coagulation (ISTH score), or ABO blood group. 3) Service laboratory FVIII:Ag was then measured in unselected clinic patients >4 months from any illness/intervention ($n=125$). Log-transformed FVIII:Ag was significantly elevated compared to the normal range, and inversely correlated with the synchronous activated partial thromboplastin time (APTT). In logistic regression analyses, baseline FVIII:Ag at an interval of 10-132 months from DVT/PE was associated with DVT/PE ($p=0.008$), odds ratio 2.41 (95% confidence intervals 1.254, 4.612) for unit increase in log-transformed Factor VIII:Ag. Age made no additional contribution. We suggest further consideration of thrombotic risks in HHT.

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INTERNATIONAL GUIDELINES FOR DIAGNOSIS AND MANAGEMENT OF HHT

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Background. International experts have identified a significant care gap in diagnosis and management of Hereditary Hemorrhagic Telangiectasia (HHT). *Objectives.* To develop evidence-based recommendations for the diagnosis and management of HHT. *Methods.* An internationally representative sample of HHT experts developed key questions reflecting the important aspects of HHT care using a modified Delphi process. Systematic searches of the medical literature were conducted to identify studies addressing these questions. Results from studies meeting inclusion criteria were extracted into evidence tables. Experts were polled to determine if any additional relevant literature was missing. Experts, other health professionals and patients with the disorder convened at a conference to partake in a structured consensus process using the evidence tables. With assistance from methodologic facilitators, small groups generated recommendations for the key questions. The small groups assembled afterwards to vote (anonymously) agreement for all recommendations. Those recommendations achieving <80% agreement were re-discussed, with a facilitator, and re-voted. *Results.* Fifty key questions were developed. Literature searches identified 2694 abstracts, of which 171 articles were found suitable for full review. Six groups representing expertise in the areas of HHT diagnosis, epistaxis, CNS vascular malformations, pulmonary arteriovenous malformations, gastrointestinal bleeding and liver vascular malformations generated 31 recommendations. Twenty-one/31 (67%) recommendations received $\geq 80\%$ agreement on first vote. Ten recommendations were further discussed and re-voted, resulting in a final count of 34 evidence-based recommendations, with $\geq 80\%$ agreement in 31/34 (91%). *Conclusion.* Thirty-four evidence-based consensus recommendations have been generated, with excellent agreement amongst international experts, for the diagnosis and management of HHT.

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PROSPECTIVE EVALUATION OF ISCHEMIC STROKE AND BRAIN ABSCESS IN INDIVIDUALS WITH PULMONARY ARTERIOVENOUS MALFORMATIONS AND HEREDITARY HAEMORRHAGIC TELANGIECTASIA

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Pulmonary arteriovenous malformations (PAVMs), present in $\approx 50\%$ of individuals with hereditary haemorrhagic telangiectasia (HHT), cause ischaemic stroke and brain

abscess. In order to address stroke/abscess risk factors, and risk reduction following PAVM embolisation, 219 consecutive individuals with PAVMs reviewed between May 1999 and May 2005 were studied. A history of focal cerebral deficit of rapid onset lasting >24 hr was recorded, and assigned to ischaemic (neurologist diagnosis), brain abscess (neurosurgical reports), haemorrhagic (imaging, post mortem) or uncertain aetiology. Six markers of PAVM severity, twelve patient or HHT-associated variables, and seven neurovascular risk factors were measured and/or recorded. In a predominantly asymptomatic population, excluding ascertainment bias, 60/219 (27.3%) individuals experienced at least one ischaemic stroke or brain abscess at median age 45 (Q₁ 38; Q₃ 53)yr. All had HHT. 39/60 (65%) had no pre-existing diagnosis of PAVM. Significant neurological defects persisted in 30/49 (62%) of abscess/stroke patients at median interval 8.0 (Q₁ 1.5; Q₃ 11.5)yr. In univariate analyses, and Cox proportional hazards models, none of the PAVM severity markers or neurovascular risk factors were associated with ischaemic stroke or brain abscess. One novel variable was significantly associated with the risk of ischaemic stroke ($p=2\times 10^{-5}$), and is under further study. PAVM embolisation significantly reduced ischaemic stroke and brain abscess rates. However, for patients diagnosed after 1985, the mean delay to referral and treatment was significantly longer when the risk of stroke/abscess was not recognised. Ischaemic stroke and brain abscess occur commonly in patients with PAVMs. Opportunities for preventative treatment are being missed.

We thank the families and friends of HHT patients whose donations supported this work

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A PILOT STUDY ON THE EFFECT OF ACETYLCYSTEINE ON EPISTAXIS AND QUALITY OF LIFE IN HHT

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Introduction. Precapillary sphincter abnormalities in arteries of *Eng^{+/+}* mice caused by free O₂ radicals may contribute to nosebleeds. Antioxydants, like acetylcysteine, may be a treatment modality for epistaxis in HHT. **Methods.** 456 Patients with definite HHT, over 18 years with at least 3 nosebleeds a week without transfusion needs and living in the Netherlands, were asked to participate in a trial with acetylcysteine. 62 Patients agreed to participate, but 19 dropped out. Of the remaining 43 patients 23 had HHT-1, 18 HHT-2 and 2 HHT-?. A diary about frequency, severity and duration of nosebleeds and quality of life (QoL), private and work-related, was completed during 6 weeks prior to treatment (week 0-6) and during 6 weeks in the last treatment period (week 13-18). Acetylcysteine, 600 mg t.i.d., was taken during week 7-18. Hemoglobin was measured before and after the trial. Each patient was his own control. **Results.** There was a significant improvement of frequency (<.005) and duration ($p<.05$) of nosebleeds during the day, as well for HHT-1 as HHT-2. This improvement was significant for the men, but not *although there was a trend* for the

women. Nocturnal epistaxis did not improve significantly. QoL related to work ($p<.005$) and to private life ($p<.07$) improved. Hemoglobin showed an insignificant rise. **Comment.** This pilot study was conducted to see whether animal experiments can be translated to humans with HHT regarding nosebleeds. The results with acetylcysteine are promising, as well for HHT-1 as HHT-2, and justifies a randomised trial.

Reference

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051

HHT ORO-FACIAL MANIFESTATIONS: HISTOPATHOLOGICAL AND CONFOCAL LASER SCANNING MICROSCOPY ANALYSIS

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Seventy-eight HHT patients who referred to the HHT Interdepartmental Center of the University of Bari during the period 2003-06 were enrolled in this study. The tongue represented the most frequent site of angiectatic lesions (57.5%) followed by the lips (47%); lesions were present in both sites in 42% of cases. The palatal location and other mucosal sites were less frequently affected (19%) as well as other mucosal sites. Bleeding lesions were noted in 46% of cases. Five telangiectatic bleeding lesions responsible for several haemorrhagic episodes, and affected by traumas due to their sites (tongue, cleft), underwent excision with margins of healthy tissue, followed by histopathological and confocal laser scanning microscopy (CLSM) analyses. In the literature, no CLSM-based studies on HHT lesions in the oral cavity have been conducted up to now. It was possible to identify three histopathological variants of the vessel morphostructure with autofluorescence, probably corresponding to progressive steps: a) telangiectases in an initial phase with angiogenic mesenchyma with poor collagen component in micronodular pattern around primordial vascular cavities; b) Fully developed non-bleeding telangiectases with the endothelial layer appearing stretched, with rare flat endothelial cells and with external layer with collagen morphological alterations in the perivascular collagen distribution, which is scattered and organized in microclusters; c) Telangiectases with traumatic haemorrhages: in addition to the vascular morphostructure subversion, there was a secondary reactive aspecific lymphoplasmacellular infiltrate. The lack of the elastic fibers is responsible for the elastic return loss during the sphygmic wave associated with vessel dilatation. The CLSM images permitted us to clearly define the marked capillary wall thinning which appeared as weak fluorescent lines, better detectable in comparison to traditional optical microscopy.

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HEMORRHAGIC HEREDITARY TELANGIECTASIA (RENDU-OSLER DISEASE) AND INFECTIOUS DISEASES: AN UNDERESTIMATED ASSOCIATION

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Introduction. Hereditary hemorrhagic telangiectasia (HHT) is an autosomal dominant disorder characterized by angiodysplastic lesions that affect many organs. Numerous case reports indicate that patients with HHT and pulmonary AVM are more susceptible to cerebral abscesses, however, only four cases of life-threatening extra-cerebral severe infections have been reported in the literature. **Objective.** To analyze incidence, type of infections and bacteriological features, risk factors for severe infections in HHT patients. **Methods.** We retrospectively analyzed a single centre cohort of patients from 1985 to 2005. **Results.** Three hundred and fifty three patients were included. Sixty seven severe infectious episodes were registered affecting 48 patients (13.6%). Extra-cerebral and cerebral infections account for 67% and 33% of all infections respectively. The mean age at the first severe infection was 44.3 years (range 12–80). Extra-cerebral severe infections were mainly septicemias (13.4%), arthritis and osteomyelitis (8.9%), skin infections (8.9%), muscular abscesses (7.4%), spondylodiscitis (6.3%) and hepatic abscesses (7.4%) and most involved *Staphylococcus aureus* species. On the contrary, cerebral abscesses were mainly due to multiple and anaerobic bacteria. Extra-cerebral severe infections were associated with prolonged epistaxis and with both, *ACVRL1* and *ENG* mutations. Cerebral abscesses were significantly associated with the presence of pulmonary arterio-venous malformations (AVM) ($p < 0.001$) and *ENG* mutations ($p < 0.001$). Surprisingly, the mean duration of epistaxis was significantly lower in patients with cerebral abscess than in those with other infections. **Conclusion.** The incidence of cerebral abscesses and extra-cerebral infections is high in HHT patients. Mechanical factors associated with moderate abnormalities of the immune system may explain this high rate of infections. However, prospective clinical studies to analyze epistaxis and the role of local infections, as well as, immunological investigations to highlight the possible role of abnormalities of the immune system are needed.

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MULTIDECTOR ROW COMPUTED TOMOGRAPHY IN THE EVALUATION OF PANCREATIC INVOLVEMENT IN PATIENTS WITH HEREDITARY HEMORRHAGIC TELANGIECTASIA

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Aim. To prospectively evaluate pancreatic involvement in patients with hereditary hemorrhagic telangiectasia (HHT). To report the types of pancreatic abnormalities identified in HHT patients. **Material and Methods.** During 12 months, all consecutive patients already diagnosed with HHT underwent contrast-enhanced 16-Slice multidetector row computed tomography (MDCT). All MDCT obtained at the arterial and portal phases were evaluated by experienced radiologists. The pancreas was carefully evaluated with special emphasis on telangiectases, arteriovenous malformations or perfusion defects. The size and location of identified telangiectases was recorded. The diameter of the splenic arteries was measured and the presence of arterial aneurysms was recorded. The presence of associated liver involvement (telangiectases, vascular masses and intraparenchymal arteriportal, arteriosystemic or portosystemic shunts) was also evaluated. **Results.** Thirty six patients (20 women and 16 men, mean age 48.1 y.o.) have been enrolled in the study. MDCT detected pancreatic vascular abnormalities in 11 patients (31%). All abnormalities were telangiectases. The mean diameter was 4 mm (2-11mm). Sixty nine percent of telangiectases were located in the corporeal part of the pancreas and 31% in the head. No arteriovenous malformation was found. In addition, 7 patients (19%) had a sacciform aneurysm of a branch of the splenic artery (mean size 8 mm). All but one patient with pancreatic telangiectases had associated liver involvement. **Conclusion.** Pancreatic telangiectases was considered as a rare finding in patients with HHT. With the use MDCT, pancreatic abnormalities have been identified in one third of HHT patients. We suggest that the presence of pancreatic telangiectases should be added in the list of visceral involvement used for the diagnosis of HHT.

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VIDEO CAPSULE ENDOSCOPY IN THE EVALUATION OF SMALL BOWEL TELANGIECTASIAS IN PATIENTS WITH HEREDITARY HEMORRHAGIC TELANGIECTASIA: A SINGLE CENTER PROSPECTIVE TRIAL

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Hereditary hemorrhagic telangiectasia (HHT) is an autosomal dominant disorder of vascular dysplasia causing telangiectasia formation which may bleed. In this study, video capsule endoscopy (VCE) was utilized to compare the small bowel findings in HHT and non-HHT patients.

Ninety-three HHT (38) and non-HHT (55) patients were evaluated for obscure/small bowel bleeding sources by VCE. Nine patients were excluded as the capsule failed to reach the cecum. The findings of 32 HHT and 48 non-HHT patients were recorded and compared. VCE detected telangiectasias evenly distributed throughout the small bowel in 26 (81%) HHT patients versus 14 (29%) non-HHT patients. When active bleeding was observed in HHT patients (4 patients), all had bleeding within reach of standard small bowel push enteroscopy. The presence of 5 or more GI telangiectasias by VCE had a sensitivity of 75% and a positive predictive value of 86% for diagnosing HHT. Unexpected findings of small bowel polyps and mass-like lesions were seen in both HHT (6.2 %) and non-HHT patients (2.1%). Small bowel telangiectasias were found in 81% of HHT patients and were evenly distributed throughout the small bowel by VCE. Actively bleeding small bowel telangiectasias in HHT patients were found in the proximal and mid-small bowel, all within reach of an enteroscope. Telangiectasias were observed in only a minority of non-HHT patients (29%). In non-HHT patients, multiple (≥ 10) telangiectasias were only observed in post-menopausal females. We propose using at least 5 GI telangiectasias as the diagnostic cutoff to support the diagnosis of HHT with wireless VCE.

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ENDOSCOPIC BAND LIGATION OF
ARTERIOVENOUS MALFORMATIONS IN PATIENTS
WITH HEREDITARY HEMORRHAGIC
TELANGIECTASIA

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Arteriovenous malformations (including telangiectasias) are a common cause of gastrointestinal (GI) bleeding. We present two hereditary hemorrhagic telangiectasia (HHT) patients with gastric arteriovenous malformations (AVMs) successfully treated with endoscopic band ligation. Patient 1: A 45 year-old male with anemia and fainting episodes related to severe GI bleeding from gastric AVMs required multiple blood transfusions and ICU admissions. Previous treatments were unsuccessful including repeated endovascular embolization at an outside hospital, argon plasma coagulation, injection therapy, and hormonal therapy. At our institution, endoscopy showed two large gastric AVMs. In view of previous failed therapies, the lesions were treated with endoscopic band ligation. Follow up showed endoscopic resolution of lesions and improved clinical status. Patient 2: A 63 year-old female with recurrent GI bleeding required multiple blood transfusions. Endoscopy disclosed approximately one hundred AVMs up to one centimeter in diameter involving the entire stomach. She received repeated sessions of APC with partial disappearance of smaller lesions. However, larger lesions persisted along with symptoms. Ultimately, endoscopic band ligation of the larger AVMs was performed. Follow up showed endoscopic resolution of treated lesions and improved symptoms.

This is the first report for the treatment of gastric AVMs using band ligation in patients with HHT. Band ligation should be considered as an alternative therapy in cases of refractory GI hemorrhage related to gastric AVMs. This modality provides superior endoscopic therapy for larger lesions which may contain ectatic sub-mucosal vessels.

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CIROCO: A DATABASE FOR CLINICAL SURVEY
OF HEMORRHAGIC HEREDITARY
TELANGIECTASIA PATIENTS AND A NEW WEB
BASED TOOL FOR THE EUROPEAN NETWORK

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Introduction. Registries of patients with rare diseases are important and represent valuable sources of information on epidemiology, natural history of a disease, treatment response and medical practices. A local database for HHT was built in 2001 on File Maker Pro® in order to collect clinical data from the French-Italian network. This database was recently modified in order to simplify its use. CIROCO (Clinical Investigations and Research for the Rendu-Osler Cohort) was developed in close cooperation with clinical partners and collects detailed information on HHT (e.g. symptoms, laboratory findings, therapy and genetic data). *Objective.* Our aim was to build a database which is (1) accessible on the internet, (2) secure, (3) easy-to-use, (4) capable of allowing each center to analyze its own data, (5) a tool used to document co-morbidity and mortality for multi-center analyses and (6) enables data comparison for clinical research. *Methods.* A new database was built using SQL software developed on an internet server. Previous data, entered on File Maker Pro® were transferred to the new database using SAS software to standardize the data. Special attention has been devoted to investigating the nature, frequency and severity of associated complications. Special emphasis has been placed on pulmonary, hepatic, digestive and neurological involvement. *Results.* The database is now accessible on the internet using username and password. Professionals can login to submit new cases, add information, and perform searches in the database. Four login degrees are possible (consulting, data entry, validation or administrator). The database was simplified. The 1290 items were replaced by 400 items, and 200 new items were added. Each center can easily import and analyze its own data using MS Excel® or Access® software. Rules have been defined for the use of this database to authorize multi-center analysis. Currently the database contains 600 patients from 12 participating centers (11 French and 1 Italian). Frequent addition of new data ensures the up-to-date quality of the collection. The rate of new patients added to the database such continue at a rate of around 100 new entries per year. *Conclusion.* This large database will help increase knowledge of HHT and ultimately lead to improved long-term management of HHT patients.

**057
TOPICAL NASAL PREPARATIONS IN HEREDITARY
HEMORRHAGIC TELANGIECTASIA**

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Aim. Epistaxis is the most frequent manifestation of hereditary hemorrhagic telangiectasia (HHT). Few data exists regarding the use of topical nasal preparations in HHT. **Methods.** Patients with HHT were asked by a questionnaire or by direct interview regarding the composition of topical nasal preparations and its effects. **Results.** 158 patients with HHT filled out the written questionnaire sufficiently. 101 out of these (64 %) stated that they use topical nasal preparations for the prevention of recurrent epistaxis. Additionally, 65 patients were interviewed orally. 262 reports on 99 different preparations, mostly lipid-based, were made including ratings in 153 cases, the majority being positive. Several active ingredients including antioxidants and antifibrinolytic substances were part of the preparations. **Conclusions.** This is the first systematic study assessing the common use of a large variety of topical nasal preparations among HHT patients. Similar to atrophic rhinitis avoidance of crust formation the consistency of lipid-based semi-solid preparations seems to be especially effective to prevent epistaxis. Especially promising active ingredients are antioxidants/sesame oil and female sex hormones. The data provided can be useful for clinical consultations and a rationale development of clinical trials.

**058
SURGICAL INTERVENTIONS FOR HEREDITARY
HAEMORRHAGIC TELANGIECTASIA IN A DAY
SURGICAL UNIT: A FIVE YEAR EXPERIENCE IN
PATTERNS OF INTERVENTION FREQUENCY**

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Introduction. A variety of modalities are available for the control of recurrent epistaxis in hereditary haemorrhagic telangiectasia (HHT). Laser ablation, in particular potassium-titanyl-phosphate (KTP), has gain popularity for vessel ablation with minimal peripheral tissue injury. Septodermoplasty and nasal closure can also be performed in the day surgery setting. Repeat interventions for new telangiectasia can be a deterrent for surgical management. However, estimating the number of interventions required is not well documented. **Aims.** The aim of this study was to determine the frequency of day surgical interventions for HHT patients. **Methods.** A retrospective case controlled review of day surgical procedures performed in a tertiary hospital unit. The period of study was from January 2002 to December 2006. The incidence of KTP laser, septodermoplasty and nasal closure were audi-

ted. **Results.** 302 procedures were under taken in the 60 months reviewed. Over 75% of this work was for 'definite' HHT. Only five patients required more than 5 interventions in the 60 month period. In HHT patients, 62% required 2≤ procedures, 32% 2 – 5 and 6% >5. This compared to 85% ≤2 and 15% 2 -5 for other KTP laser of non HHT nasal lesions. Peri-operative haemoglobin was also shown not to be a significant predictor for treatment frequency. **Discussion.** Surgical treatment, in particular KTP laser ablation, serves to alleviate the symptoms but not provide cure. This review provides a guide to educate patients on the frequency of intervention for symptom control.

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THALIDOMIDE FOR THE TREATMENT OF
EPISTAXIS IN HEREDITARY HEMORRHAGIC
TELEANGIECTASIA**

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Background. Hereditary hemorrhagic teleangiectasia (HHT) causes spontaneous recurrent epistaxis with severe impact on quality of life. Patients suffering from severe epistaxis are treated with lasertherapy or dermatoplasty with often disappointing long-term results. There is no effective registered drug. Thalidomide has anti-inflammatory, immunomodulatory and antiangiogenic properties. Case reports suggest a positive effect of thalidomide on epistaxis in HHT. We report the results of a pilot study using Thalidomide in patients with HHT and severe epistaxis. **Methods.** Six patients with severe epistaxis received thalidomide 100mg/day during at least 3 months. The number and severity of epistaxis, number of blood transfusions and the haemoglobin level were assessed before and after start of thalidomide. Side effects of thalidomide were recorded.

Gender	Follow-up Age	period	Number of nosebleeds (per week)		Severity of bleeding*		Median Haemoglobin Level (mmol/ml)		Number of blood transfusions	
			Before	After	Before	After	Before	After	Before (per year)	After (during follow-up)
M;	13	32	11	++	+	6.0	6.0	1	0	
48 yrs	months									
F	13	32	2	++	+	5.5	8.0	6	0	
58 yrs	months									
M	36	35	35	++	++	5.5	6.5	NA	NA	
70 yrs	months									
M	3	21	14	+++	+	6.5	7.5	0	0	
57 yrs	months									
F	9	35	3	++	+	5.0	5.5	NA	NA	
71 yrs	months									
M;	3	18	1	++	++	6.0	8.0	2	0	
75 yrs	months									

*+mild, ++moderate, +++ severe, NA =Not Available

Results. Decline in number of nosebleeds was reported in 5 patients and decline in severity of bleeding was reported in 4 patients after starting Thalidomide. In 3 patients, the number of needed blood transfusions was reduced. In 5 patients, the average haemoglobin level in peripheral blood increased, without change in iron medication. Reported side effects were: mild constipation (1 patient), loss of libido (1 patient) and dullness (1 patient stopped using Thalidomide because of dullness). One patient (F, 71 yrs) died during the follow-up period because of progressive pulmonary arterial hypertension. *Conclusions.* The results of this pilot study indicate that Thalidomide may have a positive effect on number and severity of epistaxis in hereditary hemorrhagic telangiectasia (HHT) and justify a randomized controlled trial.

**060
INCREASED NOSE BLEEDING HERALDS HIGH
OUTPUT CARDIAC FAILURE IN HEREDITARY
HEMORRHAGIC TELANGIECTASIA—RESULTS OF A
CASE CONTROL STUDY**

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High-output cardiac failure (HOCF) is a poorly studied complication of Hereditary Hemorrhagic Telangiectasia (HHT). Case reports suggest increased bleeding complications in the setting of new symptomatic HOCF. We report the first case-control study of HOCF in HHT patients to investigate a temporal relation of bleeding complications to HOCF. *Methods.* Of 346 HHT patients referred to our HHT center from 1997-2006, 17 (4.9%) patients with heart failure symptoms had a high cardiac output on right heart catheterization (cases) and were compared to 17 asymptomatic HHT controls. Worsening of bleeding was defined as an increase in bleeding frequency with an increased transfusion requirement or hospital admission. Data was retrospectively collected. Results are given as frequencies and medians (ranges). *Results.* At HOCF diagnosis, 94% of cases experienced worsening of stable nose and/or gastrointestinal bleeding (median of 2 years before HOCF diagnosis) compared to 12% of controls at the concurrent visit (OR=120, 95%CI=9.8-1464, $p<0.001$). During those 2 years, cases needed 3 (0-12) transfusions/month, significantly greater than controls (median=0, range 0-1.5, $p<0.001$). In particular, cases had higher nose bleeding related transfusion requirements (71% vs. 18%, OR=11.2, 95%CI=2.2-56.9, $p=0.002$), and embolization/surgical intervention rates (65% vs. 18%, OR=8.5, 95%CI=1.7-42.2, $p=0.005$). *Conclusions.* Patients with HHT experience an increase in transfusion and intervention requirements from nose bleeding in the setting of HOCF. Worsening of previously controlled bleeding, especially nose bleeding, may serve as a harbinger of symptomatic HOCF in HHT.

**061
LONG TERM COMPLICATIONS OF SEPTAL
DERMOPLASTY IN HEREDITARY
HAEMORRHAGIC TELANGIECTASIA PATIENTS**

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Objectives. Septal dermoplasty has been recommended as the treatment of choice for life-threatening epistaxis in patients with hereditary hemorrhagic telangiectasia. The purpose of this study was to evaluate retrospectively the long term complications of the septal dermoplasty procedure. *Methods.* Between 1994 and 2006, septal dermoplasty was performed on 106 consecutive patients with severe epistaxis attributable to hereditary hemorrhagic telangiectasia. The number of patients complaining of odor, changes in smell, crusting, sinusitis, and data on quality of life were obtained by phone interview. *Results.* Data was obtained in 50 of 109 (46%) patients with a mean age of 65.6 years and a mean follow-up of 3 years and 9 months. 39 of 50 (78%) experienced odor at some point and 22 of 50 (44%) said it was currently a main problem. 36 of 50 (72%) had problems with crusting at some point after surgery, 16 of 50 (32%) indicated it was a main problem currently. 44 of 50 (88%) could breathe through their nose some to all of the time. 29 of 50 (58%) had a decreased sense of smell. 15 of 50 (30%) had worsened sinus infections. 43 of 50 (86%) stated improvements in their quality of life. *Conclusion.* Although extremely effective in treating epistaxis, this analysis demonstrates that long term complications exist with septal dermoplasty procedure. Even with these complications, the procedure subjectively improves patients' quality of life.