Clinical features of Hereditary Haemorrhagic Telangiectasia

Anna Ellen Hosman

Thesis University of Utrecht

ISBN: 978-94-6332-156-3

Cover design & lay-out: Kelly E. Hosman

Print: GVO Drukkers & Vormgevers, Ede

This research was performed with funding by TopZorg and SWORO foundation.

Financial support by the Dutch Heart Foundation for the publication of this thesis is gratefully acknowledged. Publication of this thesis was further supported by Actelion, TEVA Nederland BV and Westfalen.

Clinical features of Hereditary Haemorrhagic Telangiectasia

Klinische kenmerken van Hereditaire Hemorragische Teleangiëctastieën

(met een samenvatting in het Nederlands)

Proefschrift ter verdediging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof.dr. G.J. van der Zwaan, in gevolge van het besluit van het college voor promoties in het openbaar te verdedigen op dinsdag 4 april 2017 des middags te 2.30 uur

door

Anna Ellen Hosman

geboren op 11 oktober 1989 te Amsterdam

Promotoren:

Prof.dr. J.C. Grutters

Prof.dr. M.J.T.H. Goumans

Copromotoren:

Dr. J.J. Mager

Dr. M.C. Post

Dedicated to my grandfather

Contents

Chapter one

General introduction

History

In July 1876, doctor Wickham Legg was visited by a 65-year- old musician suffering from copious nosebleeds (or epistaxis), which he had been subject to since boyhood and seemed to be triggered by traumatic events, drinking beer and violent fits of anger. His mother had died of "blood loss and dropsy", of his seven siblings one sister suffered from epistaxis as did his 27-year- old son and 22-year- old daughter. Physical examination revealed numerous small naevi on his face and various parts of his torso which had appeared around the age of twenty-four and had further progressed since then. Dr Legg concluded that the symptoms must be caused by haemophilia as a consequence of congenital weakness of the vessels.[1]

Similar symptoms had been described a decade earlier by Henry Gawen Sutton in 1864, and in 1865 Benjamin Guy Babington had published a description of epistaxis in five generations of one family.[2, 3]

It was not until 1896 that the condition was differentiated from haemophilia by Henri Jules Louis Marie Rendu, publishing a case report on a 52-year old male with frequent epistaxis, describing telangiectases on the skin of the patient's face, lips, tongue, soft pallet and torso, and noting that the patients family members suffered the same symptoms. He also speculated that the epistaxis resulted from lesions in the nose.[4] In 1901, Sir William Osler published a paper on characteristic lesions in the digestive tract and the hereditary nature of the disorder and in 1907 Dr Frederick Parkes Weber wrote a case series further contributing to description of the disease as we know it today.[5,6] Although the disease was named "hereditary haemorrhagic telangiectasia" (HHT) as early as 1909, the term Rendu-Osler-Weber disease is still accepted internationally to the present day.[7]

Almost one century later, the first genetic mutations causing HHT were identified.[8] In the year 2000, the international scientific advisory committee of HHT Foundation International published the Curacao criteria that included the following list of symptoms: (1) recurrent and spontaneous epistaxis; (2) visceral localization; (3) a first-degree relative with a definite HHT diagnosis; and (4) the presence of multiple mucocutaneous telangiectases at typical anatomical sites (mainly lips/mouth and fingertips).[9] When an individual shows three or four of these Curacao criteria, they are considered to have HHT. When they meet two criteria they might have HHT, but no definite diagnosis is made, and with only one or none of these criteria, HHT is considered unlikely.

Clinical presentation

Affected individuals suffer from multi-systemic vascular lesions, known as telangiectases, characterised by focal dilation of post-capillary veins, that are susceptible to rupture and haemorrhage because of weak vessel walls and high perfusion pressures, leading to major morbidity and mortality.[9-12] Telangiectasia in the nasal mucosa and gastro-intestinal tract frequently haemorrhage, leading to chronic iron deficiency anaemia which may even be transfusion-dependent.[13,14]

Ninety to ninety-five percent of patients suffer from spontaneous and recurrent epistaxis, varying in severity between patients. Increasing age is associated with increasing severity and prevalence of telangiectasia (80%), gastrointestinal bleeding (25-30%), and comorbidities.[15] Pulmonary (50%), cerebral (10-20%), spinal (<1%) and hepatic (30-70%) arteriovenous malformations (AVMs) affect a high proportion of patients with HHT and commonly cause complications.[16-18] Pulmonary arteriovenous malformations create a right-to-left shunt bypassing the normal filter function of the lungs. This leaves a path open for septic and sterile emboli, which would usually get lodged in the pulmonary capillary bed but instead can now potentially travel straight to the brain, causing ischemic strokes and brain abscesses.[18-20] Furthermore, the aberrant communication between pulmonary arteries and veins in PAVM can lead to decreased oxygen saturation. Depending on the severity of altered blood flow through these right-to-left shunts, hypoxemia may be profound. However, small studies have suggested that patients with PAVMs tolerate hypoxemia due to right-to-left shunt well.[21] Acute desaturation appears to be compensated by increased heart rate and stroke volume and chronic hypoxaemia by erythrocytosis. [22]

Other severe complications include major haemorrhage and maternal death in pregnancy.[23,24] Hepatic AVMs may result in high output cardiac failure, and portal hypertension which ultimately may require liver transplantation.[25,26] Additional HHT-related pathologies include (i) pulmonary arterial hypertension (PAH), associated with ACVRL1 mutations, the prognosis of which appears worse than for patients with PAH due to BMPR2 mutations, (ii) a higher risk of venous thromboemboli (enhanced in the setting of iron deficiency), and (iii) for patients with SMAD4 mutations, colon cancer and other gastrointestinal cancers related to their juvenile polyposis.[27-32] Life-long monitoring and treatment are often needed.

Genetics

HHT is thought to affect 1:5000 people and is an autosomal dominant disease of which the majority is caused by mutations in the ENG (chromosome 9q34) or ACVRL1 gene (chromosome 12q13). Mutations in these genes account for up to 96% of HHT patients, leading to reduced levels of functional Endoglin and ALK1 protein.[33-36] A combined syndrome of HHT and Juvenile Polyposis is caused by mutations in the SMAD4 gene (chromosome 18q21).[37] All three genes play important roles in the transforming growth factor beta (TGF-β) superfamily signalling pathway which has a critical function in the development of the vascular system by regulating cell growth, differentiation, motility, tissue remodelling, wound repair and programmed cell death.

Phenotypes often vary greatly between affected individuals, even within families. This intra-familial variability may be explained by epigenetics, affecting the severity of symptoms. [38, 39] Genetic counselling can provide clarity on whether lifetime health monitoring is needed or not as HHT can be ruled out by genetic testing when a family mutation is known.

Impact of age on disease severity

Phenotypic variability is substantial within the HHT disease and penetrance of symptoms often varies. Symptoms of HHT are not present at birth but develop with age; especially telangiectases become more prevalent over the years.[15,40] Conversely, while the severity of gastrointestinal haemorrhage seems to increase with age, it is debated whether epistaxis does as well. Studies have shown that although the proportion of patients suffering from epistaxis increases with age, at an individual level, nosebleeds seem to vary in frequency and severity throughout their life.[12] Formation of cerebral vascular malformations is thought to be complete during childhood and PAVMs by the end of puberty, although AVMs can still increase in size under certain circumstances; for example in pregnancy.[15, 16, 24, 41] The fact that penetrance of symptoms is age dependant often impedes the ability to assign a definite diagnosis to children of parents with HHT. Consequently, the Curacao criteria can only be used in children to make the HHT diagnosis, but not exclude HHT. Theoretically it could be expected that this age-related deterioration leads to decreased life-expectancy. A small study has shown a decreased life-expectancy in HHT patients under the age of 60 years, but a normal life-expectancy when they pass that age.[11]

Lifestyle and other confounders of disease severity

Phenotypic variability between HHT molecular subtypes have been well documented, however explaining clinical variability between family members with the same mutation is more complex. Significant intra-familial variation in phenotype is thought to be the result of genetic modifiers. [38,39] When a genetic modifier is expressed it is able to alter the expression of another gene. In the case of HHT it seems to particularly influence the development of PAVMs. Another confounder may include the second hit model which assumes that a second mutation, or "hit", is needed to get functional proteins (Endoglin, ALK1 and SMAD4) below a crucial threshold, leading to the vascular malformations which make up the phenotype of HHT. The formation of telangiectases is often preceded by a form of trauma, including sunlight exposure, cold, hot or dry air or other climate related factors. Patients often report lifestyle choices to influence their epistaxis. Some patients find their epistaxis to be less severe when they make certain dietary choices or when they smoke.

Current treatments

Cerebral and pulmonary vascular malformations are treated by embolization when technically feasible, leading to a reduction of complication rates. [9,18,42] Treatment of HAVMs is only indicated when they lead to complications as high-output cardiac failure or portal hypertension. High-output cardiac failure is initially treated with conventional medical therapy, including salt and fluid restriction, diuretics, and beta-blockers. Bevacizumab has also proven to reduce high cardiac output as a result of HAVMs.[43] In severe cases a liver transplantation can provide a permanent solution.[26] Embolization of hepatic vascular malformations has been investigated but has shown to have a high mortality rate and is not performed in the Netherlands. [44]

The otorhinolaryngologist and gastroenterologist have an important role in treatment of epistaxis and gastro-intestinal haemorrhage. Treatment is mostly symptomatic and efficacy is variable. Management of epistaxis consists of topical, systemic, and surgical treatments. The frequency of epistaxis may be reduces by changing external factors as humidity and the use of nasal lubricants or topical agents. Systemic therapy can include hormone therapy, thalidomide and bevacizumab.[45-48] Hormone therapy, remarkably oestrogen and anti-oestrogen therapy, has shown to reduce epistaxis and gastrointestinal bleeding.[45,46]

Bevacizumab and thalidomide control neo-angiogenesis through different mechanisms but both have shown spectacular results. However, these systemic therapies, especially thalidomide, can lead to severe complications and should not be considered lightly. Surgical approaches to epistaxis include laser or Argon coagulation, septal dermoplasty (Saunders operation) and, in extremely severe cases, nasal closure (Young's procedure). Nasal and gastrointestinal bleeding often leads to iron-deficiency anaemia treated with iron replacement therapy or, in severe cases, with blood transfusion. This is often the reason physicians are not keen to prescribe anti-platelets and anti-coagulants to patients with HHT, even when indicated for co-morbidities, in fear of making nasal and gastrointestinal haemorrhage more severe. In order to stop or minimize gastrointestinal specific bleeding, therapeutic options includes endoscopic coagulation and surgical resection of bleeding sites.

Aim of this thesis

Currently the number of HHT centres of excellence worldwide is increasing, delivering specialized care to individuals affected by HHT and providing support and guidance to their families. Concentrating the care for this syndrome can not only improve the quality of care for patients, but can also lead to valuable opportunities providing a cohort of patients to include in clinical trials; (i) to obtain insight into factors that influence onset and severity of symptoms and (ii) when new treatments become available. This is vital to ensure adequate evidence for treatment efficacy in the case of a rare hereditary disease like HHT.

The aim of this thesis is to explore and identify more features of HHT patients. What characterises the HHT patient? For the development of more treatment modalities, unanswered questions about phenotype, complications patients suffer, their reaction to different therapies or external influences need to be solved. This thesis will address the question whether life expectancy of HHT patients is influenced, differentiating between the different disease causing mutations, and what issues influence the life expectancy – for example the cancer incidence in the HHT population and the association between HHT and PAH. It will also focus on the question how to screen children of affected parents for HHT and any pulmonary involvement to prevent any complications. In the final three chapters, different disease management strategies are discussed. What is the role of anti-platelets and anti-coagulants, and feasibility of their application to HHT patients, considering their

implication for epistaxis. What are the effects and what is the long term tolerance of Thalidomide and how diet influences epistaxis?

References

1. Legg W. A case of haemophilia complicated with multiple naevi. *Lancet*. 1876;2:856.

2. Babington BG. Hereditary epistaxis. *Lancet*. 1865:362-–363.

3. Sutton HG. Epistaxis as an indication of impaired nutrition, and of degeneration of the vascular system. *The medical mirror*. 1864:769-–81.

4. Rendu HJ. Épistaxis répétées chez un sujet porteur de petits angiomes cutanés et muqueux. *Gaz Hop*. :1322--3.

5. Osler W. On a family form of recurring epistaxis, associated with multiple telangiectases of the skin and mucous membranes. *Bull Johns Hopkins Hosp*. 1901(12):333–7.

6. Weber FP. Multiple hereditary developmental angiomata (telangiectases) of the skin and mucous membranes associated with recurring haemorrhages. *Lancet*. 1907:160–162.

7. Hanes FM. Multiple hereditary telangiectases causes hemorrhage (hereditary hemorrhagic telangiectasia). *Bull Johns Hopkins Hosp*. 1909(20):63-73.

8. McAllister KA, Grogg KM, Johnson DW, et al. Endoglin, a TGF-beta binding protein of endothelial cells, is the gene for hereditary haemorrhagic telangiectasia type 1. *Nat Genet*. 1994;8(4):345-351.

9. Faughnan ME, Palda VA, Garcia-Tsao G, et al. International guidelines for the diagnosis and management of hereditary haemorrhagic telangiectasia. *J Med Genet*. 2011;48(2):73-87.

10. Govani FS, Shovlin CL. Hereditary haemorrhagic telangiectasia: a clinical and scientific review. *Eur J Hum Genet*. 2009;17(7):860-871.

11. Kjeldsen AD, Vase P, Green A. Hereditary haemorrhagic telangiectasia: a population-based study of prevalence and mortality in Danish patients. *J Intern Med*. 1999;245(1):31-39.

12. Shovlin CL. Hereditary haemorrhagic telangiectasia: pathophysiology, diagnosis and treatment. *Blood Rev*. 2010;24(6):203-219.

13. Finnamore H, Le Couteur J, Hickson M, Busbridge M, Whelan K, Shovlin CL. Hemorrhage-adjusted iron requirements, hematinics and hepcidin define hereditary hemorrhagic telangiectasia as a model of hemorrhagic iron deficiency. *PLoS One*. 2013;8(10):e76516.

14. Kjeldsen AD, Kjeldsen J. Gastrointestinal bleeding in patients with hereditary hemorrhagic telangiectasia. *Am J Gastroenterol*. 2000;95(2):415-418.

15. Plauchu H, de Chadarevian JP, Bideau A, Robert JM. Age-related clinical profile of hereditary hemorrhagic telangiectasia in an epidemiologically recruited population. *Am J Med Genet*. 1989;32(3):291-297.

16. Krings T, Ozanne A, Chng SM, Alvarez H, Rodesch G, Lasjaunias PL. Neurovascular phenotypes in hereditary haemorrhagic telangiectasia patients according to age. Review of 50 consecutive patients aged 1 day-60 years. *Neuroradiology*. 2005;47(10):711-720.

17. Krings T, Kim H, Power S, et al. Neurovascular manifestations in hereditary hemorrhagic telangiectasia: imaging features and genotype-phenotype correlations. *AJNR Am J Neuroradiol*. 2015;36(5):863-870.

18. Shovlin CL, Jackson JE, Bamford KB, et al. Primary determinants of ischaemic stroke/brain abscess risks are independent of severity of pulmonary arteriovenous malformations in hereditary haemorrhagic telangiectasia. *Thorax*. 2008;63(3):259-266.

19. Kjeldsen AD, Torring PM, Nissen H, Andersen PE. Cerebral abscesses among Danish patients with hereditary haemorrhagic telangiectasia. *Acta Neurol Scand*. 2014;129(3):192-197.

20. Hewes RC, Auster M, White RI,Jr. Cerebral embolism--first manifestation of pulmonary arteriovenous malformation in patients with hereditary hemorrhagic telangiectasia. *Cardiovasc Intervent Radiol*. 1985;8(3):151-155.

21. Santhirapala V, Williams LC, Tighe HC, Jackson JE, Shovlin CL. Arterial oxygen content is precisely maintained by graded erythrocytotic responses in settings of high/normal serum iron levels, and predicts exercise capacity: an observational study of hypoxaemic patients with pulmonary arteriovenous malformations. *PLoS One*. 2014;9(3):e90777.

22. Shovlin CL. Circulatory contributors to the phenotype in hereditary hemorrhagic telangiectasia. *Front Genet*. 2015;6:101.

23. Ference BA, Shannon TM, White RI,Jr, Zawin M, Burdge CM. Life-threatening pulmonary hemorrhage with pulmonary arteriovenous malformations and hereditary hemorrhagic telangiectasia. *Chest*. 1994;106(5):1387-1390.

24. Shovlin CL, Sodhi V, McCarthy A, Lasjaunias P, Jackson JE, Sheppard MN. Estimates of maternal risks of pregnancy for women with hereditary haemorrhagic telangiectasia (Osler-Weber-Rendu syndrome): suggested approach for obstetric services. *BJOG*. 2008;115(9):1108-1115.

25. Buscarini E, Leandro G, Conte D, et al. Natural history and outcome of hepatic vascular malformations in a large cohort of patients with hereditary hemorrhagic teleangiectasia. *Dig Dis Sci*. 2011;56(7):2166-2178.

26. Lerut J, Orlando G, Adam R, et al. Liver transplantation for hereditary hemorrhagic telangiectasia: Report of the European liver transplant registry. *Ann Surg*. 2006;244(6):854-62; discussion 862-4.

27. Girerd B, Montani D, Coulet F, et al. Clinical outcomes of pulmonary arterial hypertension in patients carrying an ACVRL1 (ALK1) mutation. *Am J Respir Crit Care Med*. 2010;181(8):851-861.

28. Shovlin CL, Sulaiman NL, Govani FS, Jackson JE, Begbie ME. Elevated factor VIII in hereditary haemorrhagic telangiectasia (HHT): association with venous thromboembolism. *Thromb Haemost*. 2007;98(5):1031-1039.

29. Shovlin CL, Chamali B, Santhirapala V, et al. Ischaemic strokes in patients with pulmonary arteriovenous malformations and hereditary hemorrhagic telangiectasia: associations with iron deficiency and platelets. *PLoS One*. 2014;9(2):e88812.

30. Livesey JA, Manning RA, Meek JH, et al. Low serum iron levels are associated with elevated plasma levels of coagulation factor VIII and pulmonary emboli/deep venous thromboses in replicate cohorts of patients with hereditary haemorrhagic telangiectasia. *Thorax*. 2012;67(4):328-333.

31. Brosens LA, van Hattem A, Hylind LM, et al. Risk of colorectal cancer in juvenile polyposis. *Gut*. 2007;56(7):965-967.

32. Schwenter F, Faughnan ME, Gradinger AB, et al. Juvenile polyposis, hereditary hemorrhagic telangiectasia, and early onset colorectal cancer in patients with SMAD4 mutation. *J Gastroenterol*. 2012;47(7):795-804.

33. McDonald J, Wooderchak-Donahue W, VanSant Webb C, Whitehead K, Stevenson DA, Bayrak-Toydemir P. Hereditary hemorrhagic telangiectasia: genetics and molecular diagnostics in a new era. *Front Genet*. 2015;6:1.

34. McDonald MT, Papenberg KA, Ghosh S, et al. A disease locus for hereditary haemorrhagic telangiectasia maps to chromosome 9q33-34. *Nat Genet*. 1994;6(2):197-204.

35. Johnson DW, Berg JN, Baldwin MA, et al. Mutations in the activin receptor-like kinase 1 gene in hereditary haemorrhagic telangiectasia type 2. *Nat Genet*. 1996;13(2):189-195.

36. Shovlin CL, Hughes JM, Tuddenham EG, et al. A gene for hereditary haemorrhagic telangiectasia maps to chromosome 9q3. *Nat Genet*. 1994;6(2):205-209.

37. Gallione CJ, Repetto GM, Legius E, et al. A combined syndrome of juvenile polyposis and hereditary haemorrhagic telangiectasia associated with mutations in MADH4 (SMAD4). *Lancet*. 2004;363(9412):852-859.

38. Benzinou M, Clermont FF, Letteboer TG, et al. Mouse and human strategies identify PTPN14 as a modifier of angiogenesis and hereditary haemorrhagic telangiectasia. *Nat Commun*. 2012;3:616.

39. Kawasaki K, Freimuth J, Meyer DS, et al. Genetic variants of Adam17 differentially regulate TGFbeta signaling to modify vascular pathology in mice and humans. *Proc Natl Acad Sci U S A*. 2014;111(21):7723-7728.

40. Letteboer TG, Mager HJ, Snijder RJ, et al. Genotype-phenotype relationship for localization and age distribution of telangiectases in hereditary hemorrhagic telangiectasia. *Am J Med Genet A*. 2008;146A(21):2733-2739.

41. Shimoda Y, Osanai T, Nakayama N, et al. De novo arteriovenous malformation in a patient with hereditary hemorrhagic telangiectasia. *J Neurosurg Pediatr*. 2015:1-6.

42. Labeyrie PE, Courtheoux P, Babin E, Bergot E, Touze E, Pelage JP. Neurological involvement in hereditary hemorrhagic telangiectasia. *J Neuroradiol*. 2016;43(4):236-245.

43. Dupuis-Girod S, Ginon I, Saurin JC, et al. Bevacizumab in patients with hereditary hemorrhagic telangiectasia and severe hepatic vascular malformations and high cardiac output. *JAMA*. 2012;307(9):948-955.

44. Chavan A, Luthe L, Gebel M, et al. Complications and clinical outcome of hepatic artery embolisation in patients with hereditary haemorrhagic telangiectasia. *Eur Radiol*. 2013;23(4):951-957.

45. Yaniv E, Preis M, Shevro J, Nageris B, Hadar T. Anti-estrogen therapy for hereditary hemorrhagic telangiectasia - a long-term clinical trial. *Rhinology*. 2011;49(2):214-216.

46. Jameson JJ, Cave DR. Hormonal and antihormonal therapy for epistaxis in hereditary hemorrhagic telangiectasia. *Laryngoscope*. 2004;114(4):705-709.

47. Epperla N, Kapke JT, Karafin M, Friedman KD, Foy P. Effect of systemic bevacizumab in severe hereditary hemorrhagic telangiectasia associated with bleeding. *Am J Hematol*. 2016;91(6):E313-4.

48. Lebrin F, Srun S, Raymond K, et al. Thalidomide stimulates vessel maturation and reduces epistaxis in individuals with hereditary hemorrhagic telangiectasia. *Nat Med*. 2010;16(4):420-428.

Chapter two

Life expectancy of parents with Hereditary Haemorrhagic Telangiectasia

EM de Gussem, AE Hosman, CP Edwards, CJJ Westermann, RJ Snijder, ME Faughnan, JJ Mager

Orphanet J Rare Dis. 2016 Apr 22;11:46

ABSTRACT

Background: Hereditary Haemorrhagic Telangiectasia (HHT) is an autosomal dominant disease associated with epistaxis, arteriovenous malformations and telangiectasias. Disease complications may result in premature death.

Method: We investigated life-expectancies of parents of HHT patients compared with their non-HHT partners using self- or telephone-administered questionnaires sent to their children. Patients were extracted from the databases of 2 participating HHT Centres: the Toronto HHT Database (Toronto, Canada) and the St. Antonius Hospital HHT Database (Nieuwegein, The Netherlands).

Results: 225/407 (55%) of respondents were included creating HHT- (n=225) and control groups (n=225) of equal size. 213/225 (95%) of the HHT group had not been screened for organ involvement of the disease prior to death. The life expectancy in parents with HHT was slightly lower compared to parents without (median age at death 73.3 years in patients versus 76.6 years in controls, p0.018). Parents with *ACVRL* 1 mutations had normal life expectancies, whereas parents with *ENG* mutations died 7.1 years earlier than controls (p= 0.024). Women with *ENG* mutations lived a median of 9.3 years shorter than those without (p=0.04). Seven/123 (5%) of deaths were HHT related with a median age at death of 61.5 years (IQ range 54.4-67.7years).

Conclusion: Our study showed that the life expectancy of largely unscreened HHT patients was lower than people without HHT. Female patients with *ENG* mutations were most strikingly at risk of premature death from complications. These results emphasize the importance of referring patients with HHT for screening of organ involvement and timely intervention to prevent complications.

Introduction

Hereditary Haemorrhagic Telangiectasia (HHT) is an autosomal dominant disease with a prevalence of approximately 1/5000.[1] HHT is diagnosed genetically, or clinically according to the Curacao criteria.[2] Several disease causing mutations are known but most frequently patients are affected by mutations in the *ENG* (MIM 131195) gene (HHT-I) or the *activin A receptor type II-like 1 (ACVRL1*; MIM 601284) gene (HHT-II), coding for the proteins Endoglin and ALK-1 respectively. [3,4] The Curacao criteria are based on four clinical features: (i) the presence of spontaneous, recurrent epistaxis, (ii) mucocutaneous telangiectasia, (iii) visceral arteriovenous malformations (AVMs) and (iv) a first degree relative with HHT. Patients are diagnosed with HHT if they fulfil three out of four criteria. Two criteria results in a "possible HHT" diagnosis.

Telangiectasia can manifest on oral mucosa, nasal mucosa or on the face or fingers. Visceral AVMs mainly occur in the lungs (pulmonary AVM), brain (cerebral VM), gastro-intestinal tract and liver (hepatic VM).[5-7] In patients with (possible) HHT, screening for organ involvement is recommended to prevent complications. These include intrapleural or intrabronchial haemorrhage from pulmonary AVMs or – more often – paradoxical (sterile or septic) emboli which can lead to a stroke or brain abscess.[8-10] Cerebral vascular malformations (VMs) can lead to haemorrhagic stroke, and liver VMs can lead to right congestive heart failure, portal hypertension, biliary necrosis or portosystemic encephalopathy.[11] Frequent recurrent epistaxis and gastro-intestinal telangiectasia, which mainly occur in the stomach and duodenum, can lead to iron deficiency anaemia.[12]

To prevent complications and increase lifespan, patients should be referred to specialized HHT Centres for expert care and to screen for the presence of AVMs.[2] Previous studies in a small number of patients showed increased mortality in patients with HHT under 60 years of age.[1,13] To study the life expectancy of patients who did not receive treatment for their (asymptomatic) AVMs, we evaluated the age of death of parents of the current population referred to our clinics. The aim was to determine whether life expectancy of unscreened parents of our cohorts with HHT differed from that of their non-HHT partner.

Methods

Population

The participating hospitals of this two-centre study are St. Michael's Hospital in Toronto, Canada and St. Antonius Hospital in Nieuwegein, The Netherlands. Both hospitals are internationally recognized HHT Centres. Two thousand seven hundred and twenty-five patients screened for HHT were selected in June 2008 from the Toronto and St Antonius Hospital HHT Databases. The oldest generation known to the clinic with a confirmed genetic or clinical diagnosis of HHT, based on the Curacao criteria, was included. Respondents' admissions were excluded if a sibling had already returned a questionnaire on their parents or where HHT had been ruled out in both parents after screening in the clinic.

Study Protocol

The study protocol was approved by the research ethics boards of both participating hospitals (Medical Research Ethics Committees United). All selected patients received a letter with information on the study and a self-administered questionnaire. Patients were asked to return the questionnaire if both parents had deceased. If the HHT parent was still alive, patients were asked to forward the questionnaire to them with a request to complete it. If no answer was received within 4 weeks, patients were contacted by phone, by one of 2 well-trained interviewers (EG and CE), to answer the same questions.

The health status in terms of HHT of the parents of the participants was determined solely by the participants' response and was not objectified. However the participants used in this study had their diagnosis confirmed by the Curacao criteria or genetic testing and genealogies often show which side of the family has HHT. Considering the hereditary nature of HHT the questions on which parent was affected by HHT allowed deduction of which parent was not affected by HHT. Subsequently, parents could then be assigned as HHT-patient or control. In case it was unclear which parent had HHT, because of a de novo mutation or when the HHT parent showed very little symptoms or in case of unclear genealogy, the participant was excluded.

By using the parent not affected by HHT as control, we assume the socioeconomic status of one set of parents is comparable, making the control group and HHT-group equal in socioeconomic status. The questionnaire queried the mortal status of both parents and, if

applicable, the cause of death. Parents with HHT were divided in three groups: death definitely HHT related, possibly HHT related and not HHT related. Death secondary to pulmonary disease was categorized as possibly HHT related unless caused by pneumonia, emphysema, pulmonary embolism or tuberculosis; in these cases they were categorized as not HHT related deaths. Death secondary to an infection could be secondary to a paradoxical embolus leading to an abscess or sepsis and was categorized as possibly HHT related unless influenza or meningitis were the underlying causes; in these cases, they were categorized as not HHT related. Stroke was categorized as possibly HHT related. If death was reported secondary to cardiac disease it was categorized as possibly HHT related unless caused by a cardiac arrest, ruling it as not HHT related. To best interpret the results, the "possibly HHT related" group was then excluded from any further statistical analysis. The P-value was derived from cox-regression based on comparison of each of the two subgroups with controls.

Furthermore, the questionnaire queried HHT related symptoms in the HHT parent and asked whether this parent had received treatment for these symptoms or had been screened for HHT.

Statistical Analysis

Data was analysed using IBM SPSS statistics 22 and Microsoft Office Excel 2010. Survival data was analysed using Cox regression and Kaplan-Meijer curves. Given the inherent limitations of survey methodology, to assess whether survival of the control group was realistic, calculations from survival data reported for controls in the current survey were compared to the survival data of Dutch people in the national Dutch database (CBS statLine) and Canadian people in the national Canadian database (CANSIM).

Results

Of the 570 questionnaires sent out, 407 (71%) were completed, 9 (2%) were returned because of an incorrect address and 154 (27%) patients did not return the questionnaire or respond to phone calls. Of the respondents, 182 (45%) were excluded for the following reasons: incomplete response (24), parent without HHT was still alive (73), neither parent was diagnosed with HHT or had symptoms (the respondent was then assumed to have a *de*

novo mutation), parent had a low grade phenotype or one of the parents was not the genetic parent (52), or there were siblings among the respondents (to avoid dataset duplicates) (33).

In total 225 (55%) respondents were included, 85/225 were from St. Michael's Hospital and 140/225 were from St. Antonius Hospital, providing information on 550 parents; 225 HHT parents, and 225 controls. Of 157 (69.8%) HHT parents, the gene mutation causing HHT was known. In the control group, males had shorter lifespans (median age 74.0 years, IQ range 65.7- 80.9) than females (median age 80.1 years, IQ range 72.2- 86.8), with a hazard ratio of 1.684 (95% CI 1.284-2.208, p<0.001) (Table 1).

	HHT group				**Control group**			
	n°	**Median age (years)**	**IQ range 25% (years)**	**IQ range 75% (years)**	**n°**	**Median age (years)**	**IQ range 25% (years)**	**IQ range 75% (years)**
Total population	225	73.3	63.6	81.7	225	76.6	68.5	83.2
ENG	83	69.5	59.3	81.4				
ACVRL1	74	76.0	67.1	82.9				
Male	98	70.9	62.1	79.7	127	74.0	65.7	80.9
ENG	35	68.5	57.4	77.8				
ACVRL1	31	73.4	66.1	81.2				
Female	127	75.7	64.0	82.5	98	80.1	72.2	86.8
ENG	48	70.8	61.2	82.4				
ACVRL1	43	77.0	68.4	83.1				

Table 1. Comparing age at death (years) of parents with HHT (HHT group) and parents without HHT (control group), subdivided by sex and mutation.

This was as expected from the general Dutch and Canadian population (CBS statLine, CANSIM). The HHT group showed similar trends with respect to sex, but the difference was not statistically significant (hazard ratio 1.255, 95%CI 0.962-1.637, p=0.096).

Parents with HHT had a significantly shorter lifespan than parents without HHT. With a median age at death of 73.3 years (IQ range 63.3, 81.7) in the HHT group compared to a

median age at death in controls of 76.6 years (IQ range 68.5, 83.2), HHT parents died 3.3 years earlier than controls (HR 1.252, 95%CI 1.039-1.508, p=0.018) (Figure 1). Further analysis shows this difference could be attributed specifically to an *ENG* mutation. Parents with *ENG* mutations died significantly earlier than controls (HR 1.338, IQ range 1.038-1.723, p= 0.024) (Figure 2). More strikingly, females with an *ENG* mutation lived a median of 9.3 years shorter than females without the mutation (HR 1.442, 95% CI 1.017-2.043, p=0.04) (Figure 3). Males with an *ENG* mutation seemed to live 5.5 years shorter than non-HHT males, although this was not significant and may be because the data were underpowered (HR 1.361, IQ range 0.934-1.984, p=0.109). By contrast, the lifespan of parents with *ACVRL1* mutations was not significantly different from those without (HR 1.101, 95% CI 0.846-1.433, p= 0.476) (Figure 4).

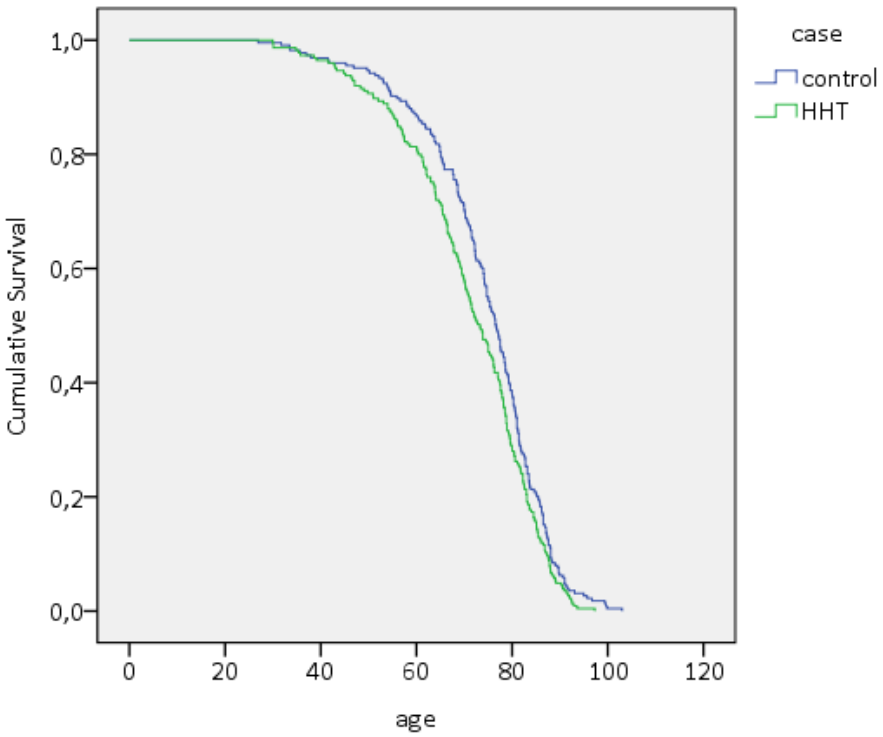


Figure 1. Kaplan-Meijer curve of HHT group versus control group. Log Rank: p=0.018

Parents with HHT who had died because of an HHT related complication had a lower median age at death compared to those who had died from no HHT related conditions (Table 2).

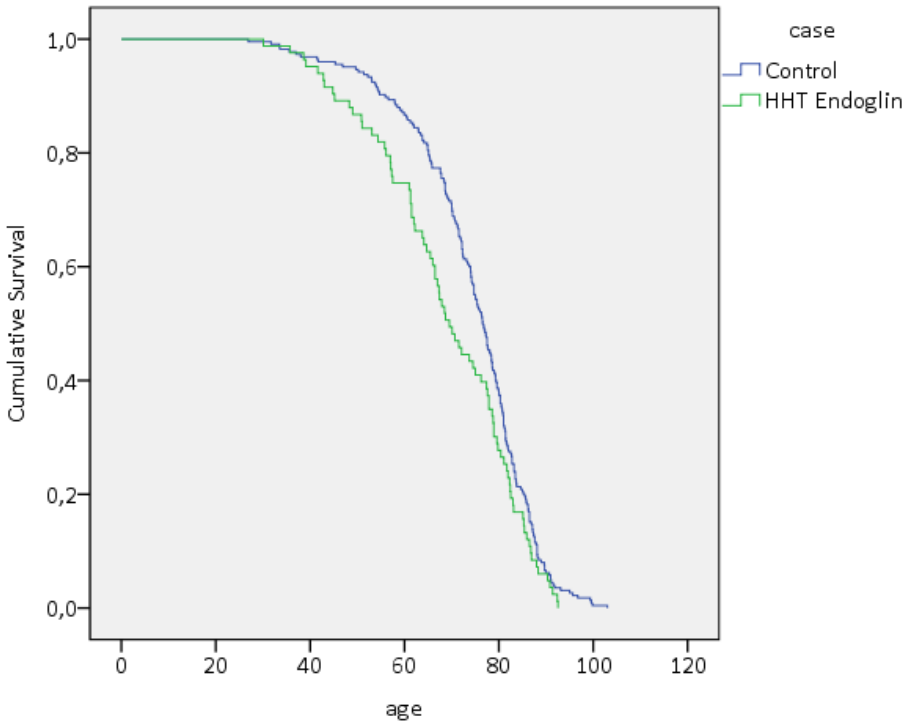


Figure 2. Kaplan-Meijer curve of unscreened Endoglin patients vs controls. Log Rank: p=0.024

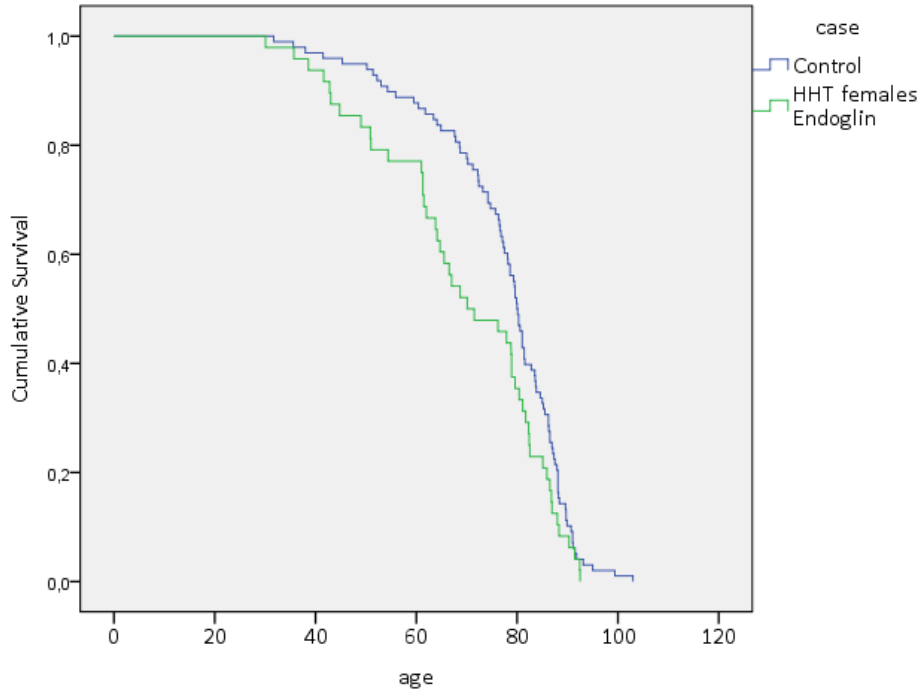


Figure 3. Kaplan-Meijer curve of unscreened female Endoglin patients vs female controls. Log Rank: p=0.038

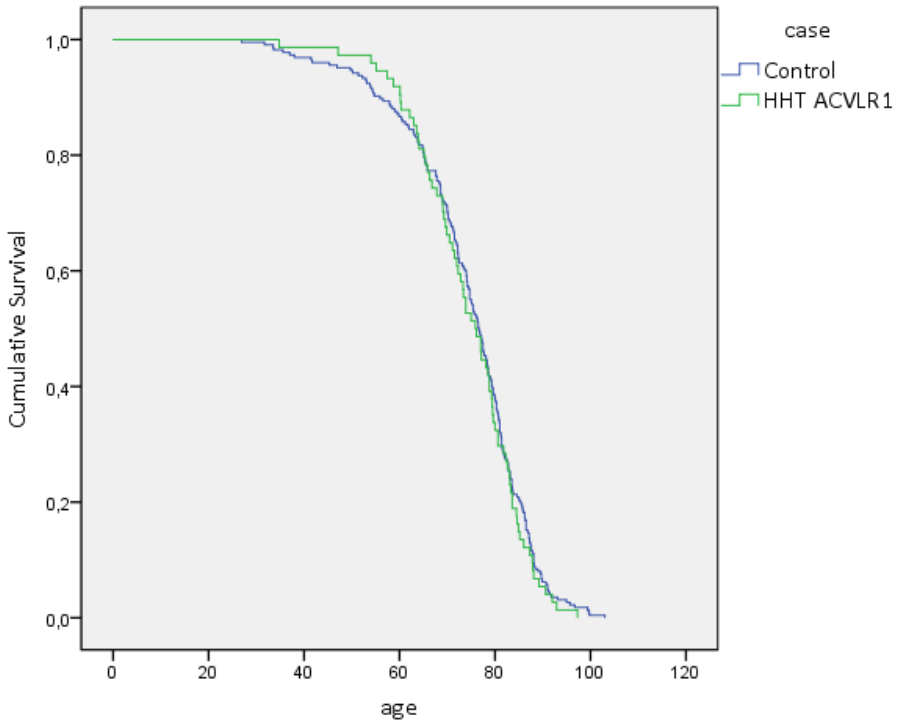


Figure 4. Kaplan-Meijer curve of unscreened ACVLR1 patients vs controls. Log Rank: p=0.476

Cause of death HHT related	**Yes**	**No**
Number (%)	6 (5%)	123 (95%)
Median age (IQ range)	61.5 (54.4-67.7)	75.0 (65.9-82.8)
Range	41.6-74.9	30.0-97.3
Causes of death (number)	Massive epistaxis (2) Pulmonary haemorrhage (2) Brain abscess (3)	Pulmonary disease (16) Cardiac disease (21) Infection (2) Abdominal aorta aneurysm (1) Malignancy (47) Bladder cyst (1) Dementia (2) Renal failure (2) Trauma (7) Unnatural (2) Old age (22)
P-value	0.002	0.343

Table 2. Causes of death of parents with HHT. P-value derived from cox-regression based on comparison with controls.

Discussion

This study illustrates that in an essentially unscreened and untreated population of HHT patients who were the parents of cohorts in our databases, the median life expectancy is significantly lower than that of their non-HHT partner. This is in alignment with a recent study performed on 675, largely unscreened, HHT patients showing a similar 3 year reduction in life expectancy, mainly related to neurological and haemorrhagic complications.[14] More detailed analysis of our data showed that the decrease in life expectancy in the otherwise unspecified HHT cohort is largely attributable to reduced life expectancy in women with a mutation in the *ENG* gene. Life expectancy of individuals with *ACVRL 1* mutations did not seem to be affected.

Reduced life expectancy is more pronounced in female with an *ENG* mutation, for reasons that are mainly unclear although we can hypothesize. Possible reasons might include the nature of the *ENG* phenotype, which is associated with a higher prevalence of pulmonary AVMs and cerebral VMs compared to the *ACVRL1* mutations. Cerebral VMs occur in approximately 8-16% of patients with *ENG* phenotype as opposed to 1-2% of HHT-II patients with *ACVRL1* mutation.[15-18] Pulmonary AVMs occur much more frequently in patients with *ENG* mutations (62%) compared to patients with *ACVRL1* mutations (10%) and complications may be life threatening.[19] Major pulmonary AVM related complications include life-threatening haemorrhage, as well as cerebral abscess (8%) and ischemic stroke (9 to 18%) due to paradoxical emboli, which can be fatal or leave patients severely physically impaired.[20,21] Fortunately, these complications can mostly be prevented through detection of pulmonary AVM by screening and treatment. [22]

Even though women with HHT have a higher risk of life threatening pulmonary AVM-related complications during pregnancy and possibly labour this did not appear to have a great impact on the study here: the overall life expectancy curves of women with *ENG* mutations only deviated in the 40-70 year range, and not during the fertile years (Figure 2). [23-26] However, this could be a result bias as women who died giving birth to their first child would not have had offspring to submit data for this study.

Seven parents whose death was definitely HHT related had a lower mean age at death compared to the two other groups. With present day standards of care, five of these deaths

secondary to pulmonary AVMs, i.e. pulmonary haemorrhage and brain abscess, could have been prevented by screening and treatment in advance.

There are limitations to the methodology used in this study. The population included is biased, since it only includes adult respondents. Patients with HHT who died during childhood and in their twenties have been largely missed, although these numbers are likely very low.[13, 27] Due to stringent inclusion criteria, parents without prominent symptoms of HHT were also excluded, as they might not have had HHT and their responding child might have had a spontaneous mutation. However, these parents could have had a less evident phenotype or died before becoming clearly symptomatic and therefore might have been incorrectly excluded. Furthermore, studies on male HHT patients specifically were underpowered. Underpowering of these analyses can be explained by a smaller number of male patients. This may be due to a female predominance resulting from a greater inclination of Dutch female patients to be referred to an HHT Centre for clinical evaluation.[16] Furthermore, we excluded 73 participants of which the parent without HHT was still alive. Of 53/73 (73%) participants, the deceased parent with HHT was male, this is consistent with a generally lower life expectancy for men compared to women. However, this led to a relatively smaller male HHT-group included in the study.

We studied life expectancy in a largely unscreened population in which only 5% of the participants had been examined in a specialized clinic. The goal of current screening is to prevent HHT related complications and improve life expectancy in HHT patients. Future studies will show if morbidity and mortality improve if patients are screened for visceral AVMs and treated when necessary.

To improve life expectancy in patients with HHT or suspected HHT, we strongly recommend referral to an HHT Centre of Excellence for appropriate screening and possible treatment of AVMs. International HHT guidelines recommend screening for pulmonary AVMs in all people with HHT and also recommend referral to expert centres for management of the various aspects of HHT. Taking the hereditary nature of HHT in account, asymptomatic family members should also be referred for screening since clinical manifestations like epistaxis and telangiectasia increase with age and might not be evident initially. Asymptomatic AVMs can be present in the young, potentially leading to life threatening complications.

Furthermore, increasing awareness of HHT is essential in order to improve patient care because despite greater awareness, HHT is still an under-diagnosed disease.[28, 29]

In conclusion, our study confirms the findings of recent literature that life expectancy in a largely unscreened population with HHT is worse than in their non-HHT partners, more specifically for patients with *ENG* mutations and especially women.[14] Because patients with *ACVRL1* mutations have a normal life expectancy, the reduction in life expectancy in patients with an *ENG* mutation is probably related to complications of untreated pulmonary AVMs and cerebral VMs. We propose that life expectancy in HHT can be normal if patients are screened and both pulmonary AVMs and cerebral VMs are properly treated in a timely way. To prevent complications of HHT, referral of patients with (suspected) HHT to an HHT Centre of Excellence for screening, and if necessary treatment, is highly recommended.

Acknowledgement

We would like to thank Pieter Zanen for aiding in the statistical analysis.

References

1. Kjeldsen AD, Vase P, Green A: Hereditary haemorrhagic telangiectasia: a population-based study of prevalence and mortality in Danish patients. J Intern Med 1999, 245(1):31-39.

2. Shovlin CL, Guttmacher AE, Buscarini E, Faughnan ME, Hyland RH, Westermann CJ, Kjeldsen AD, Plauchu H: Diagnostic criteria for hereditary hemorrhagic telangiectasia (Rendu-Osler-Weber syndrome). Am J Med Genet 2000, 91(1):66-67.

3. McDonald MT, Papenberg KA, Ghosh S, Glatfelter AA, Biesecker BB, Helmbold EA, Markel DS, Zolotor A, McKinnon WC, Vanderstoep JL: A disease locus for hereditary haemorrhagic telangiectasia maps to chromosome 9q33-34. Nat Genet 1994, 6(2):197-204.

4. Johnson DW, Berg JN, Baldwin MA, Gallione CJ, Marondel I, Yoon SJ, Stenzel TT, Speer M, Pericak-Vance MA, Diamond A, Guttmacher AE, Jackson CE, Attisano L, Kucherlapati R, Porteous ME, Marchuk DA: Mutations in the activin receptor-like kinase 1 gene in hereditary haemorrhagic telangiectasia type 2. Nat Genet 1996, 13(2):189-195.

5. Krings T, Kim H, Power S, Nelson J, Faughnan ME, Young WL, terBrugge KG, Brain Vascular Malformation Consortium HHT Investigator Group: Neurovascular manifestations in hereditary hemorrhagic telangiectasia: imaging features and genotype-phenotype correlations. AJNR Am J Neuroradiol 2015, 36(5):863-870.

6. Memeo M, Stabile Ianora AA, Scardapane A, Buonamico P, Sabba C, Angelelli G: Hepatic involvement in hereditary hemorrhagic telangiectasia: CT findings. Abdom Imaging 2004, 29(2):211-220.

7. Guttmacher AE, Marchuk DA, White RI,Jr: Hereditary hemorrhagic telangiectasia. N Engl J Med 1995, 333(14):918-924.

8. Woodall MN, McGettigan M, Figueroa R, Gossage JR, Alleyne CH,Jr: Cerebral vascular malformations in hereditary hemorrhagic telangiectasia. J Neurosurg 2014, 120(1):87-92.

9. Dupuis-Girod S, Giraud S, Decullier E, Lesca G, Cottin V, Faure F, Merrot O, Saurin JC, Cordier JF, Plauchu H: Hemorrhagic hereditary telangiectasia (Rendu-Osler disease) and infectious diseases: an underestimated association. Clin Infect Dis 2007, 44(6):841-845.

10. Mathis S, Dupuis-Girod S, Plauchu H, Giroud M, Barroso B, Ly KH, Ingrand P, Gilbert B, Godeneche G, Neau JP: Cerebral abscesses in hereditary haemorrhagic telangiectasia: a clinical and microbiological evaluation. Clin Neurol Neurosurg 2012, 114(3):235-240.

11. Garcia-Tsao G, Korzenik JR, Young L, Henderson KJ, Jain D, Byrd B, Pollak JS, White RI,Jr: Liver disease in patients with hereditary hemorrhagic telangiectasia. N Engl J Med 2000, 343(13):931-936.

12. Proctor DD, Henderson KJ, Dziura JD, Longacre AV, White RI,Jr: Enteroscopic evaluation of the gastrointestinal tract in symptomatic patients with hereditary hemorrhagic telangiectasia. J Clin Gastroenterol 2005, 39(2):115-119.

13. Sabba C, Pasculli G, Suppressa P, D'Ovidio F, Lenato GM, Resta F, Assennato G, Guanti G: Life expectancy in patients with hereditary haemorrhagic telangiectasia. QJM 2006, 99(5):327-334.

14. Donaldson JW, McKeever TM, Hall IP, Hubbard RB, Fogarty AW: Complications and mortality in hereditary hemorrhagic telangiectasia: A population-based study. Neurology 2015, 84(18):1886-1893.

15. Velthuis S, Vorselaars VM, van Gent MW, Westermann CJ, Snijder RJ, Mager JJ, Post MC: Role of transthoracic contrast echocardiography in the clinical diagnosis of hereditary hemorrhagic telangiectasia. Chest 2013, 144(6):1876-1882.

16. Letteboer TG, Mager JJ, Snijder RJ, Koeleman BP, Lindhout D, Ploos van Amstel JK, Westermann CJ: Genotype-phenotype relationship in hereditary haemorrhagic telangiectasia. J Med Genet 2006, 43(4):371-377.

17. Kjeldsen AD, Moller TR, Brusgaard K, Vase P, Andersen PE: Clinical symptoms according to genotype amongst patients with hereditary haemorrhagic telangiectasia. J Intern Med 2005, 258(4):349-355.

18. Bayrak-Toydemir P, McDonald J, Markewitz B, Lewin S, Miller F, Chou LS, Gedge F, Tang W, Coon H, Mao R: Genotype-phenotype correlation in hereditary hemorrhagic telangiectasia: mutations and manifestations. Am J Med Genet A 2006, 140(5):463-470.

19. van Gent MW, Post MC, Snijder RJ, Westermann CJ, Plokker HW, Mager JJ: Real prevalence of pulmonary right-to-left shunt according to genotype in patients with hereditary hemorrhagic telangiectasia: a transthoracic contrast echocardiography study. Chest 2010, 138(4):833-839.

20. Kjeldsen AD, Torring PM, Nissen H, Andersen PE: Cerebral abscesses among Danish patients with hereditary haemorrhagic telangiectasia. Acta Neurol Scand 2014, 129(3):192-197.

21. Shovlin CL, Jackson JE, Bamford KB, Jenkins IH, Benjamin AR, Ramadan H, Kulinskaya E: Primary determinants of ischaemic stroke/brain abscess risks are independent of severity of pulmonary arteriovenous malformations in hereditary haemorrhagic telangiectasia. Thorax 2008, 63(3):259-266.

22. Faughnan ME, Palda VA, Garcia-Tsao G, Geisthoff UW, McDonald J, Proctor DD, Spears J, Brown DH, Buscarini E, Chesnutt MS, Cottin V, Ganguly A, Gossage JR, Guttmacher AE, Hyland RH, Kennedy SJ, Korzenik J,

Mager JJ, Ozanne AP, Piccirillo JF, Picus D, Plauchu H, Porteous ME, Pyeritz RE, Ross DA, Sabba C, Swanson K, Terry P, Wallace MC, Westermann CJ, White RI, Young LH, Zarrabeitia R, HHT Foundation International - Guidelines Working Group: International guidelines for the diagnosis and management of hereditary haemorrhagic telangiectasia. J Med Genet 2011, 48(2):73-87.

23. de Gussem EM, Lausman AY, Beder AJ, Edwards CP, Blanker MH, Terbrugge KG, Mager JJ, Faughnan ME: Outcomes of pregnancy in women with hereditary hemorrhagic telangiectasia. Obstet Gynecol 2014, 123(3):514-520.

24. Shovlin CL, Sodhi V, McCarthy A, Lasjaunias P, Jackson JE, Sheppard MN: Estimates of maternal risks of pregnancy for women with hereditary haemorrhagic telangiectasia (Osler-Weber-Rendu syndrome): suggested approach for obstetric services. BJOG 2008, 115(9):1108-1115.

25. Yeomans ER, Gilstrap LC,3rd: Physiologic changes in pregnancy and their impact on critical care. Crit Care Med 2005, 33(10 Suppl):S256-8.

26. Lapinsky SE, Kruczynski K, Slutsky AS: Critical care in the pregnant patient. Am J Respir Crit Care Med 1995, 152(2):427-455.

27. Giordano P, Lenato GM, Suppressa P, Lastella P, Dicuonzo F, Chiumarulo L, Sangerardi M, Piccarreta P, Valerio R, Scardapane A, Marano G, Resta N, Quaranta N, Sabba C: Hereditary hemorrhagic telangiectasia: arteriovenous malformations in children. J Pediatr 2013, 163(1):179-86.e1-3.

28. Grosse SD, Boulet SL, Grant AM, Hulihan MM, Faughnan ME: The use of US health insurance data for surveillance of rare disorders: hereditary hemorrhagic telangiectasia. Genet Med 2014, 16(1):33-39.

29. Latino GA, Brown D, Glazier RH, Weyman JT, Faughnan ME: Targeting under-diagnosis in hereditary hemorrhagic telangiectasia: a model approach for rare diseases? Orphanet J Rare Dis 2014, 9:115-014-0115-7.

Chapter three

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

AE Hosman, HL Devlin, BM Silva, CL Shovlin

Orphanet J Rare Dis. 2013 Dec 20;8(1):195.

ABSTRACT

Background: Hereditary haemorrhagic telangiectasia (HHT) is inherited as an autosomal dominant trait, affects ~1 in 5,000, and causes multi-systemic vascular lesions and life-limiting complications. Life expectancy is surprisingly good, particularly for patients over 60ys. We hypothesised that individuals with HHT may be protected against life-limiting cancers.

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

Results: 1,307 participants completed the questionnaire including 1,007 HHT-subjects and 142 controls. The rigorous HHT diagnostic algorithm meant that 158 (12%) completed datasets were not assignable either to HHT or control status. For cancers predominantly recognised as primary cancers, the rates in the controls generally matched age-standardised rates for the general population. HHT subjects recruited through the survey had similar demographics to controls, although the HHT group reported a significantly greater smoking habit. Combining data of participants and uniquely-reported relatives resulted in an HHT-arm of 2,161 (58% female), and control-arm of 2,817 (52% female), with median ages of 66ys [IQR 53–77] and 77ys [IQR 65–82] respectively. In both crude and age-adjusted regression, lung cancers were significantly less frequent in the HHT arm than controls (age-adjusted odds ratio 0.48 [0.30, 0.70], p = 0.0012). Breast cancer prevalence was higher in HHT than controls (age-adjusted OR 1.52 [1.07, 2.14], p = 0.018). Overall, prostate and colorectal cancer rates were equivalent, but the pattern of colorectal cancer was modified, with a higher prevalence in younger HHT patients than controls.

Conclusions: These preliminary survey data suggest clinically significant differences in the rates of lung, breast and colorectal cancer in HHT patients compared to controls. For rare

diseases in which longitudinal studies take decades to recruit equivalent datasets, this type of methodology provides a good first-step method for data collection.

Introduction

Hereditary haemorrhagic telangiectasia (HHT, also known as Osler-Weber-Rendu syndrome) is inherited as an autosomal dominant trait, and affects approximately 1 in 5,000 people.[1-3] Affected individuals have multi-systemic vascular lesions that cause major morbidity and mortality.[4-6] Telangiectasia in the nasal mucosa and gastro-intestinal tract frequently haemorrhage leading to chronic iron deficiency anaemia and often transfusion-dependence.[1,3,7,8] Increasing age is associated with increasing severity and prevalence of telangiectasia, gastrointestinal bleeding, and comorbidities.[3,6,8] Pulmonary, cerebral, spinal and hepatic arteriovenous malformations (AVMs) affect high proportions of patients with HHT, and commonly cause complications including haemorrhagic, ischaemic and infective strokes; other major haemorrhage; and maternal death in pregnancy.[9-14] Hepatic AVMs may result in high output cardiac failure, and intractable complicated portal hypertension requiring liver transplantation.[15,16] Additional HHT-related pathologies include pulmonary arterial hypertension (PAH) when the prognosis appears worse than for patients with PAH due to *BMPR2* mutations, a higher risk of venous thromboemboli (enhanced in the setting of iron deficiency), and for patients with *SMAD4* mutations, colon cancer and other gastrointestinal cancers related to their juvenile polyposis.[17-21] Life-long monitoring and treatment is often needed. Patients often do not take secondary prophylaxis such as anti-platelets and anti-coagulants in view of the perceived risk of precipitating haemorrhage.[22]

It would be reasonably expected that patients with such severe potential disease complications, apparently increasing with age, should have higher mortality rates than the general population. Life expectancy data demonstrate a higher mortality rate in patients under 60 years of age, consistent with early mortality due to AVMs, especially cerebral AVM bleeds in childhood and young adults, and pregnancy-related deaths.[1,23] In one study, a retrospective analysis of Italian HHT patients' parents, increased mortality was demonstrated across all age groups.[23] However, in a 30 year prospective study in Denmark there was no evidence for an increase in mortality in HHT patients older than 60 years of age.[1] Although awaiting peer review, more recent data on North American and European cohorts, each of approximately 600 HHT patients or parents, also suggest surprisingly good survival rates. [24,25]

Amongst the explanations for the surprising life expectancy data could be that HHT-related mortality is offset by a reduction in deaths from more common diseases. Different rates of heart disease were proposed some years ago, though never formally published, and are the subject of a separate manuscript in preparation.[26] Based on personal and family histories from patients attending a specialised HHT service, we hypothesised that HHT patients may have less frequent life-limiting cancers.

Testing such a hypothesis in a rare disease population is not simple. To provide preliminary data in a human population even for the most common cancers such as breast, colorectal, lung and prostate cancer, carries major statistical and logistic difficulties. First, incidence rates (30–50 per 100,000 per annum for lung and colorectal cancers) are prohibitively small for realistic prospective studies in a rare disease population such as HHT. To generate sufficiently sized cohorts for any form of analysis requires pooling of cohorts from different geographical regions. This introduces variance through combining data from genetically unrelated populations, with differing risk factor exposures, and spanning time periods with varying incidence rates.[27,28] As a result, to have sufficient power to detect reductions in cancer rates requires population sizes of many thousands. Additionally, prior fatalities from life-limiting cancers mean that affected individuals may not survive to provide retrospective data at the point of clinic review or questionnaire: in the UK, 5 year survival following breast and prostate cancer is over 80%, but for colorectal cancer, just over 50%, and for lung cancer, less than 10%.[28] Animal models are therefore favoured, but while instructive in specific settings, such models cannot provide an integrated picture of the lifetime exposure risks for people in the setting of the repertoire of human genomic variation.

To design a study to test our hypothesis that cancer incidence may be reduced in HHT, and provide data to allow realistic power calculations to be performed for future studies, we developed an online questionnaire. This extended the techniques we used to capture fatal HHT cerebral haemorrhages, and maternal deaths in pregnancy, by allowing each individual to provide data on multiple family relatives.[14,29] This method presents a means of determining cancer rates at lower respondent/proband numbers than if only a single case per respondent was captured; inclusion of relevant questions regarding other family members allows identification of relatives that could have been reported on multiple occasions so allowing each to be captured only once. Questionnaire data are inevitably weakened by the self-reported nature, but comparison of subject and control groups

ascertained in comparable manners provides an opportunity to compare rates, even if these may not be formally assigned to classical incidence or prevalence rates that demand pre-defined populations.

Here we report a questionnaire-based study, which provides interesting suggestions that specific cancer types may differ between people affected with HHT and controls.

Methods

Study design

To capture cancer-histories in an unbiased manner, relevant questions were incorporated into a wider ethically-approved survey. Power calculations (detailed below) indicated that to distinguish incidence rates of the four most common cancer subtypes would require unrealistic response rates, so the study was designed to capture data on multiple relatives per respondent. The basic study design has been reported previously.[22,30] Briefly, in order to prevent participants altering their answers to conform to their guess of what the research hypothesis was, (hypothesis guessing), multiple questionnaires were incorporated into a single survey of questions regarding health and treatments for people with HHT and general population controls. As described elsewhere, the questionnaire was approved by the NRES Committee East Midlands-Derby 1 Research Ethics Committee; distributed by post, using the Imperial College London HHTIC London Clinical Service databases (2001 to present), during attendance at the HHT clinics, and advertised by the HHT Foundation International. [22,30,31]

Study design allowed participants the option of pausing whilst completing the questionnaire and continuing at a later time point, to optimise data collection and survey completion rates. Generic questions included in the analyses for this study were age, gender, and HHT-related questions which would permit independent assignment of the respondent's HHT-status based on the Curacao criteria, and allowed HHT-affected respondents to report which parent and grandparent had HHT.[32] Additional questions addressed personal cancer history, family cancer history, and prevalence of carcinogenic risk factors including smoking habits, diet, and industrial exposure to chemicals. These questions were not asked for the relatives due to the excessive number of questions this would have entailed, and the likelihood that no data would have been gathered as participants would have decided to stop the

questionnaire. Specific relatives' questions were therefore limited to age, gender, relationship, if HHT was known to be present, types of cancer present, age at first cancer, and if HHT affected management (if relevant) of cancer treatment (see Additional file 1 for exact wording). Free text options were provided allowing additional details to be reported.

Questions specifying particular cancers targeted the 20 most prevalent cancers in the western world with drop down boxes for 5 or 10 year age periods, and each of the specified cancers: skin (basal, squamous, melanoma, other, unsure), and non-skin cancers (brain, bladder, breast, cervical, colorectal, kidney, leukaemia, liver, lung, lymphoma, malignant melanoma, mesothelioma, mouth, myeloma, oesophagus, ovary, pancreas, prostate, stomach and uterus: see online Additional file 1).[27,28] All questions were standardised, although room was left for personal comment. Study methodology implied that it was not possible to ascertain whether primary or secondary cancers were being reported, but the methodology was identical for control and HHT groupings. In view of reported uncertainty regarding the types of skin cancer present, it was not the intention to analyse skin cancer data specifically: questions were included however, to ensure these cancers were captured by survey questions before non-skin cancers were reported.[27,28]

Power calculations

Recognising the varied pathogenic mechanisms involved in cancer subgroups, the primary study outcome was specific cancer types, namely the four most common non skin cancers in the UK: breast, colon, lung and prostate. Power calculations were performed assuming each respondent would report raw cancer data on seven unique individuals (grandparents, parents, siblings, and self); an average age of 55; equal gender distributions; and used incidence rate standard deviations of 9.0/100,000 (the maximum for the four cancers listed above).[27] Such calculations suggested that with 1,000 responses divided between HHT and non-HHT respondents, the study would have 80% power to detect a difference of 0.76/100,000 in incidence rates for lung (or colon) cancer. Since respondents and relatives would include men and women, fewer individuals would be captured for detection of gender-specific cancers. However, the two fold higher rates in the specific sex incidence rates for breast and prostate cancer rendered the calculations for colorectal cancer broadly comparable.[27]

Data for this study were downloaded on 30.6.2012, when 1,307 individuals had responded. Although the survey remained open (for increasingly limited question sets addressing outstanding medical questions) for a further 10 months, only 118 further individuals started the survey in this period.

Patient population

Ascertainment of HHT status:

All patients self-reported their HHT status, but it was critical to ensure that patients with HHT but unaware of their final diagnosis were not assigned control status, and conversely, that over-exuberant use of the HHT label was not allowed to result in inappropriate assignment of HHT. Therefore, questions were included to address the Curacao criteria which have been recently validated by a major molecular study.[32,33] The criteria are nosebleeds, mucocutaneous telangiectasia, visceral involvement (most commonly gastrointestinal telangiectasia, or AVMs at specified sites), and family history. Details of the precise question wording are provided in the online Additional file 1. To avoid bias, the telangiectasia question offered a variety of potential sites for "red spots" in tick box options, only two of which were to be considered as HHT telangiectasia (see Additional file 1). A positive family history was defined by a blood relative who had been diagnosed with HHT. Following data download, and prior to analyses of any cancer-related questions, all HHT-diagnostic questions were reviewed independently by two members of the HHT study team, to allow assignment of status as "HHT-subject," "control", or "unknown" based on the Curacao criteria, and schematic in Figure 1.[32] The senior author reviewed all assignments. Participants assigned to the "unknown" group were excluded from further data analysis.

In order to capture data on cancers with low incidence and high mortality rates, information on family members provided by the participants was used. Where the respondent had HHT, the questions on which parent and grandparent had HHT allowed deduction of which did not, assuming autosomal dominant inheritance as present in all reported HHT cases to date. Thus for HHT respondents, where it was known which side of the family HHT came from, HHT relatives could then be assigned as "HHT-subject," "control", or "unknown": specific care was taken to avoid under-diagnosis of HHT that was not yet manifest, thus controls were only selected from the side of the family without HHT. The status of "HHT-patient" or

"control" (from HHT unaffected family branches) allocated to family members was stringently assigned prior to analyses of any cancer-related questions. A subgroup of participants reported data during a period when software data collection did not record the age of their parents. For this subgroup, other age data (on themselves, their grandparents, their siblings and other deceased relatives) were complete. For these respondents, parental ages were estimated based on the mean age of mothers at first childbirth using published data for the years 1970, 1980, 1990, 2000, 2003 and 2009.[34] By cross referencing the names of the oldest patient known in the family to have HHT, and geographical location of the reported relatives, we were able to avoid double-counting relatives reported by multiple respondents. All data assignments were concluded blinded to other demographic and cancer data.

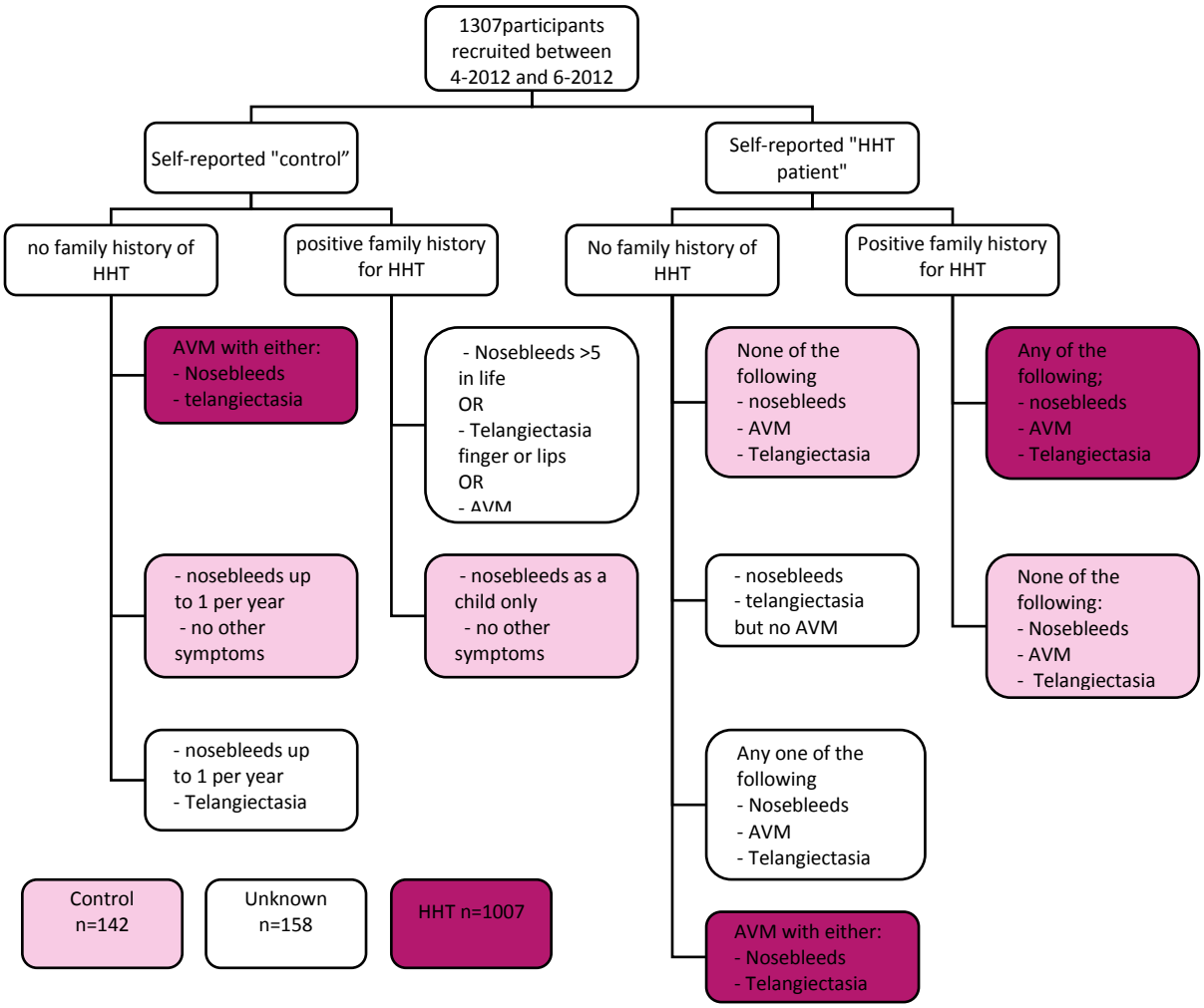


Figure 1. Stratification of diagnostic assignments. Flow chart indicating the application of the Curacao Criteria to Survey Respondents, stratified by whether they reported themselves as having HHT, and by the presence or absence of a family history.

Statistical methods

Basic demographic variables were calculated using STATA IC versions 11 and 12 (Statacorp, Texas), and Graph Pad Prism 5 (GraphPad Inc, US). An estimate of cancer rates per 100,000 people per year was calculated by adjusting for the specific population gender distribution and median age at the diagnosis of cancer. Recognising the inherent limitations of survey methodology, to assess if these estimates may be realistic, calculations from cancer data reported for controls in the current survey were compared to the 2008 age-standardised rates *(*ASRs) reported for the Developed World by Globocan.[27]

To address whether there may be a difference in rates between the HHT patients and controls captured in comparable methods using the current methodology, two way comparisons between HHT and control groups were performed using Mann Whitney, examining only survey respondents, only relatives, and combined data from all respondents and relatives. Each specified cancer type was used in turn as the dependent variable in logistic regression. Age-adjusted odds ratios for HHT status were calculated by performing logistic regression simultaneously examining the effect of age and HHT status on each specified cancer: p values for contribution from HHT status were calculated post estimation using the non-parametric Wald test which makes no assumption about independence of variables. To estimate age-standardised rates for graphical presentations, each individual's age was assigned to all of the 1–10 decades of life they had achieved, and cancers attributed to the decade in which they occurred. Thus almost all individuals provided more than one decade of life for analyses. Age adjusted rates were calculated for cancers where ages were specifically known, but inclusion of cancers where uncertain ages were spread equally across age groups did not materially alter the relationships.

Results

Survey population characteristics

At the time of data download, 1,307 participants had completed the questionnaire. Evaluation of HHT diagnostic criteria, as detailed in Figure 1, resulted in assignment of 1,007 with HHT, 158 unknowns (excluded from further analyses in this study), and 142 controls.

As demonstrated in Table 1, there was no difference in general demographics between HHT and control participants. Median ages were 55ys (range 18–90, interquartile range (IQR) ,46-

64) and 53ys (range 21–86, IQR 42–61) respectively; 65% of respondents were female (655/1012, [64.7%] HHT; 92/142 [64.8%] control); and there was also no difference in general demographics such as the international region of origin; diet as assessed crudely by vegetarian status/red meat intake; alcohol intake; or exposure to chemicals (Table 1). For smoking, similar percentages were current or former smokers (315/1007 [31%] HHT; 39/142 [27%] controls. Most were cigarette smokers, and most had stopped smoking by the time of the survey (Table 1). However, the smoking habit in terms of pack years smoked per smoker was significantly higher for HHT respondents than controls (Table 1). Crude cancer rates for the two populations are presented in online Additional file 2: Table S1.

Relatives and combined groupings

The survey also captured cancer data on 4,930 grandparents and parents. 1,154 were reported as HHT-affected. 2,675 relatives could be confidently assigned as controls as they were either relatives of control respondents, or from non-HHT branches of HHT families. The remaining relatives (n = 1,101) could not be assigned as they were in potentially HHT-affected branches of the families, and the diagnosis of HHT may not yet have manifest, [6-10] or they had been potentially reported by other survey respondents. Data from these relatives were therefore not analysed.

The respective median ages of survey respondents were 53ys [IQR 42–61] for controls and 55ys [IQR 46–64] for HHT subjects. Ages of reported relatives were higher at median 77ys [IQR 67–82] for controls; median 72ys [IQR 62–82] for HHT-affected relatives. Combining data of participants and relatives resulted in a control-arm of 2,817 (52% female, median age 77ys [IQR 65–82]), and HHT-arm of 2,166, (58% female, median age 66ys [IQR 53–77]).

Table 1 Demographics for HHT and control survey respondents

	Control				HHT				Total				Mann Whitney p value
	Total	Count	Mean	SD	Total	Count	Mean	SD	Total	Count	Mean	SD	
Gender (% female)	142	92	0.65	0.48	1007	654	0.65	0.48	1149	746	0.65	0.48	0.99
North America/Europe	142	124	0.87	0.33	1007	910	0.9	0.29	1149	1034	0.9	0.29	0.25
Australia/New Zealand	142	3	0.02	0.14	1007	42	0.04	0.2	1149	45	0.039	0.19	0.24
Asia	142	1	0.01	0.08	1007	4	0.004	0.06	1149	5	0.004	0.07	0.6
South America	142	0	0	0	1007	4	0.004	0.06	1149	4	0.004	0.06	0.45
Africa	142	0	0	0	1007	2	0.002	0.04	1149	2	0.0017	0.04	0.59
Current or former smoker	142	39	0.27	0.45	1007	315	0.31	0.46	1149	354	0.31	0.46	0.38
Current smoker	142	8	0.06	0.23	1007	67	0.07	0.25	1149	75	0.065	0.25	0.66
Former smoker	142	31	0.22	0.42	1007	248	0.25	0.43	1149	279	0.24	0.43	0.49
Passive smoker	142	1	0.01	0.08	1007	22	0.02	0.15	1149	22	0.019	0.14	0.24
Cigarettes	142	38	0.27	0.44	1007	308	0.31	0.46	1149	346	0.3	0.46	0.37
Number per week	38	38	0.31	0.23	308	308	0.34	0.33	346	346	38.1	21.4	0.4

Years smoked	38	38	15.8	13.5	308	308	17.3	11.5	346	346	17.1	13.4	0.37
Pack years per smoker	38	38	25.4	35.8	308	308	35.4	40	346	346	34	38.7	0.01
Cigars	142	3	0.02	0.14	1007	22	0.022	0.15	1149	25	0.022	0.15	0.96
Pipes	142	3	0.02	0.14	1007	18	0.018	0.13	1149	21	0.019	0.13	0.99
Other	142	1	0.01	0.84	1007	14	0.014	0.12	1149	15	0.013	0.11	0.5
Non vegetarian	130	123	0.95	0.23	969	929	0.96	0.2	1094	1047	0.96	0.21	0.51
Red meat 3x per week	130	75	0.58	0.5	950	542	0.57	0.5	1075	614	0.57	0.5	0.89
Industrial exposures	130	121	0.93	0.25	964	885	0.92	0.27	1090	1002	0.92	0.27	0.62
Alcohol	130	53	0.41	0.49	969	342	0.35	0.48	1099	395	0.36	0.48	0.22
Alcohol units per day	130	84	0.65	0.95	970	518	0.53	0.87	1095	602	0.55	0.88	0.17

Total: number of respondents reporting presences, absence or distribution of variable. Count: number of respondents with specified variable- demographics; originating in stated region; or describing stated intakes/use/exposures. SD, standard deviation.

Validation of survey methodology using control data

To validate the study methodology, the incidence of different cancers per 100,000 patients per year estimated cancer rate (per 100,000 patients per year) was calculated for the control group, and compared to ASRs for the Developed World from Globocan, recognising that Globocan ASRs were for primary cancers at the designated sites, whereas study methodology would include reports of metastatic cancers.[27] For the 18 most common non-skin cancers, Table 2 presents the crude data; adjustments for a population of average age 77ys, 52% female; and the ratios of the observed ASR/expected ASR. These ratios ranged from 0.43 to 2.3 (median 1.23). For the 15 "predominantly primary" cancers, the average ratio approximated to 1.0, compatible with robust study methodology. We concluded that while the data in the survey were not from a geographical or numerically-defined population, and while there were inevitably concerns about self-reported data, nevertheless, the survey data for controls were reflective of the cancer rates in the general population.

Of the 18 cancer types, three were at common sites of metastatic spread, namely lung, liver and brain. The ratio of ASRs for these cancer types (range 1.5-2.3, median 1.9) was significantly higher than for the other 15 cancer types (range 0.43-1.52, median 1.09, $p = 0.013$). Figure 2 illustrates the ASR ratios for the two subgroupings, plotted against the frequency of the particular cancer type. Since for the three "primary plus metastatic" sites, the cancers were reported more commonly than expected by primary ASRs, we concluded that the data were compatible with respondents reporting both primary and metastatic cancers for lung, liver and brain.

	Control survey population			Globocan ASR ^			Ratio
	Cases	Cases per 100,000	ASR per 100,000^	ASR (men)	ASR (women)	ASR if 52% female	Survey ASR/ Globocan ASR
Bladder	9	319	4.1	16.3	3.6	9.7	0.43
Brain	21	745	9.7	5.8	4.4	5.1	1.91
Breast	91	3230	42.0	0.0	66.4	34.5	1.22
Cervical	14	497	6.5	0.0	9.1	4.7	1.36
Colorectal	56	1988	25.8	37.7	24.3	30.7	0.84
Kidney	11	390	5.1	11.9	5.9	8.8	0.58
Leukaemia	17	603	7.8	9.1	5.9	7.4	1.05
Liver	27	958	12.4	8.2	2.7	5.3	2.33
Lung	106	3763	48.9	47.1	18.8	32.4	1.51
Lymphoma	19	674	8.8	12.5	9.0	10.7	0.82
Mouth	5	177	2.3	6.8	2.3	4.5	0.52
Myeloma	8	284	3.7	3.3	2.2	2.7	1.35
Oesophagus	9	319	4.1	6.5	1.3	3.8	1.09
Ovary	13	461	6.0	0.0	9.3	4.8	1.24
Pancreas	21	745	9.7	8.3	5.5	6.8	1.41
Prostate	56	1988	25.8	61.7	0.0	29.6	0.87
Stomach	39	1384	18.0	16.7	7.3	11.8	1.52
Uterus	20	710	9.2	0.0	13.0	6.8	1.36

Table 2. Calculation and comparison of age standardised rates (ASRs) for cancers in the control arm, compared to reported general population data. General population ASRs were Globocan ASRs for "More Developed Regions", 2008.[27] The study data represent cancer cases in 2817 control participants or relatives, with an average age of 77 ys, and 52% female. ^ Calculated assuming median age of 77 ys.

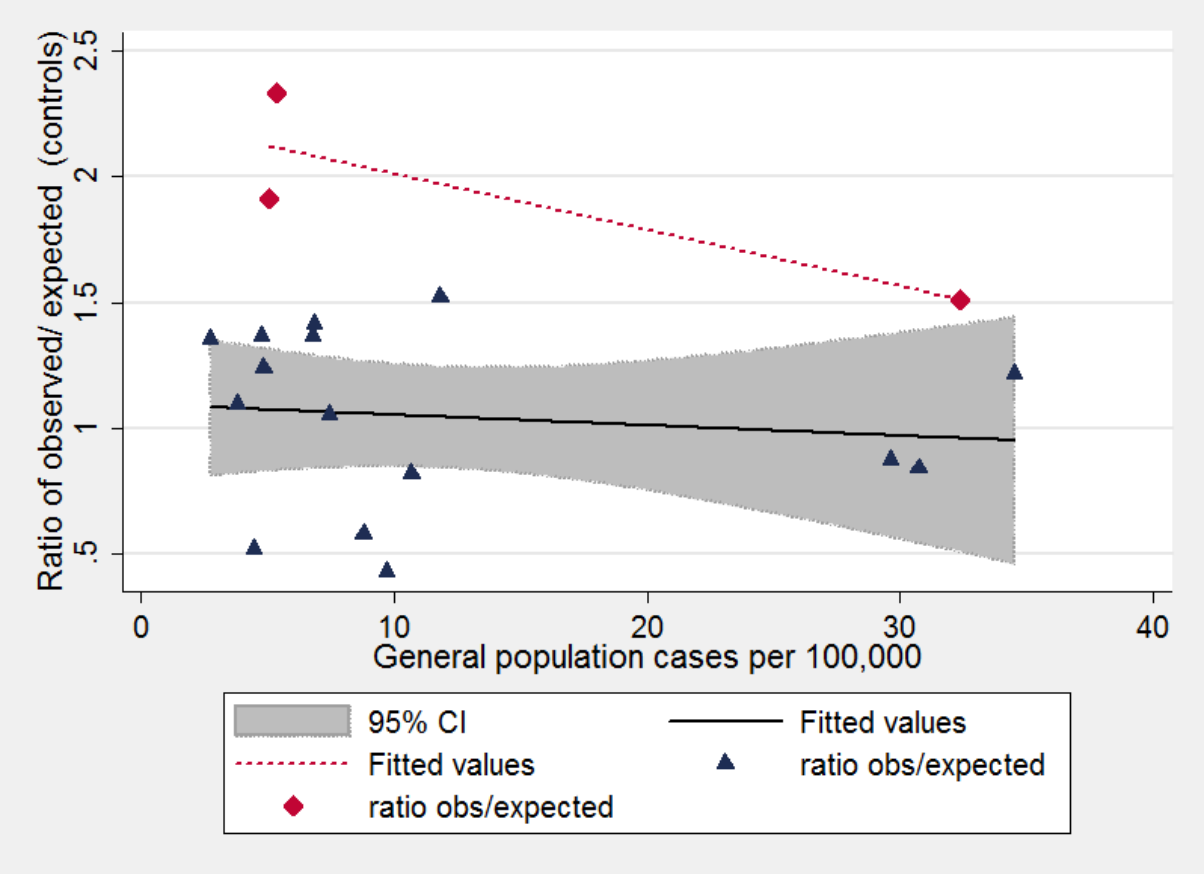


Figure 2 Validation data of cancer rates in controls. Comparison of age-standardized rates (ASRs) for survey control arm and Globocan ASRs for "More Developed Regions", 2008. The study data represent cancer cases in 2,817 control participants or relatives, with an average age of 77 ys, and 52% female, and plots the observed/expected ratios presented in Table 2, against the overall frequency of the specified cancer, since variance would be expected to be greater for less common cancers. Data are stratified by whether the cancers are predominantly primary only (navy symbols, black solid line with 95% confidence intervals); or primary and secondary sites (red symbols and red dotted line). This analysis and figure is courtesy of CLS.

Comparison of cancer rates in HHT patients and controls

Calculated cancer rates were then compared between the survey HHT and control groups. In crude analyses, fewer cancers were reported for HHT (398/2161, 18.4%) than controls (668/2817, 23.7%, p = 0.0012). As noted in Figure 3 and Table 3, in these crude figures, there appeared to be a lower frequency of solid tumours, and specifically of lung cancers in the HHT arm compared to controls. Recognising that primary and secondary lung and liver cancers carry high mortality, and that the HHT population comprised a greater proportion of respondents (introducing a bias as they needed to survive to the point of survey completion), cancer rates were examined in the "relatives only" subgroup, representing 1,154 HHT-affected relatives and 2,675 control relatives.[28] This revealed higher rates of

these life-limiting cancers than in the younger survey respondents, but again, the crude rates of lung and liver cancer were lower in the HHT group than in controls: Crude liver cancer rates for the relatives-only group (27 cases in controls, 10 in HHT) were 1,009 and 866 per 100,000. Crude lung cancer rates for the relatives-only group (101 cases in controls, 33 in HHT) were 3,775 and 2,860 per 100,000.

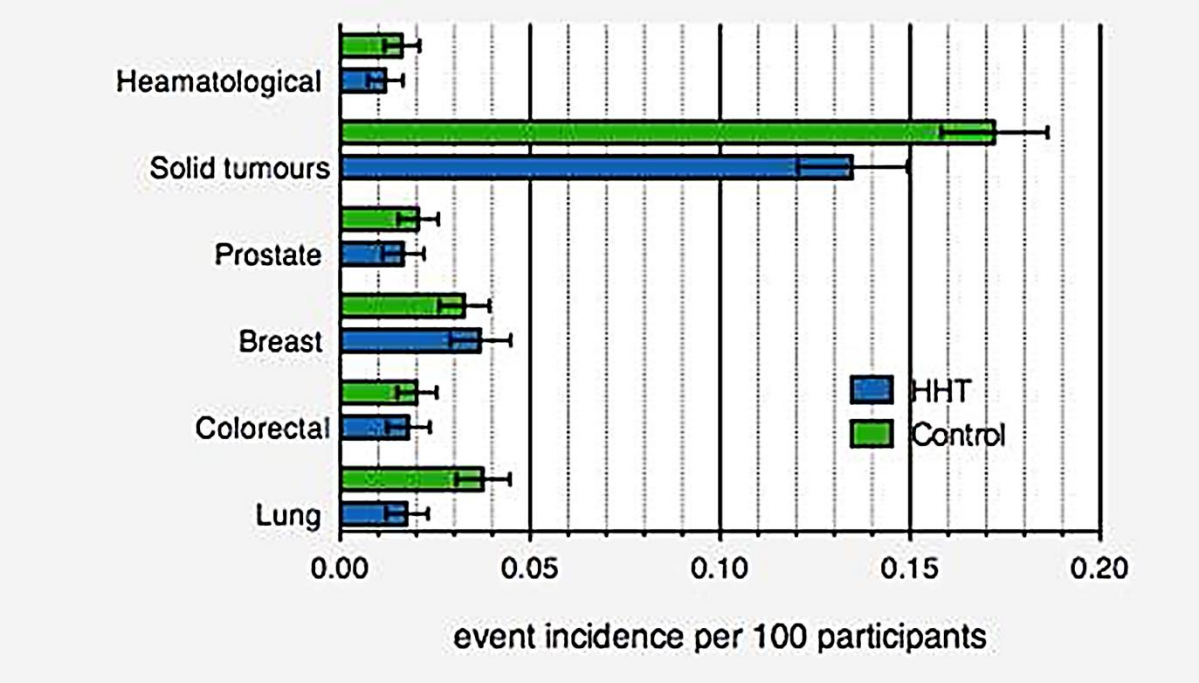


Figure 3. Reported numbers for haematological and solid cancers. Data are illustrated for the most common four cancers and haematological cancers, in 2,166 HHT patients and 2,817 controls (Error bars indicate 95% confidence intervals).

	Controls (n = 2817)†			HHT (n =2161) ‡			
	Cancer cases	**Cases per 100,000**	**SEM**	**Cancer cases**	**Cases per 100,000**	**SEM**	**p value**
Bladder	9	320	106	12	5090	153	0.3
Brain	21	746	162	11	463	146	0.21
Breast	91	3230	333	80	3702	406	0.38
Cervical	14	497	133	11	463	146	0.86
Colorectal	56	1988	263	37	1712	279	0.47
Kidney	11	391	118	11	463	146	0.7
Leukaemia	17	604	146	7	324	122	0.16
Liver	27	959	184	11	463	146	0.042
Lung	106	3763	359	38	1758	283	<0.0001
Lymphoma	19	675	154	16	740	185	0.79
Melanoma	67	2378	287	45	2082	307	0.48
Mesothelioma	1	36	36	0	0	0	0.38
Mouth	5	178	79	7	324	122	0.3
Myeloma	8	284	100	4	185	93	0.48
Oesophagus	9	320	106	4	185	93	0.35
Ovary	13	462	128	13	602	166	0.5
Pancreas	21	746	162	9	417	139	0.14
Prostate	56	1988	263	33	1527	264	0.22
Stomach	39	1384	220	14	648	173	0.012
Uterus	20	710	158	10	463	146	0.26
All cancers°	668	23713	800	398	22351	834	0.0012

Table 3. Crude incidence of the 20 most common non skin cancers in both study arms**.** † The control arm represented 142 survey respondents and 2,675 reported relatives. ‡ The HHT arm represented 1,007 survey respondents and 1,154 reported relatives. Note that for stomach and pancreatic cancer, the higher than expected control values (Table 2) suggest that these primary cancers may not have been appropriately assigned by respondents and relatives. °All cancers includes less common cancers. P value calculated by Mann Whitney.

Age-adjusted cancer rates

The individuals provided 36,887 separate decades of life for analyses: 15,053 in the HHT arm and 21,834 decades in the control arm. As expected, cancer rates were strongly age-related ($p < 0.0001$, all cancers). Age adjusted incidence rates were calculated for all cancers combined, and for the most common cancers. These data indicated that after age-adjustment, there was no significant difference in the overall rates of all cancers between HHT and controls (Table 4), but this masked different patterns amongst the four most common cancers: Following age-adjustment, there was no difference in prostate or colorectal cancer rates, but breast cancer was reported more frequently for HHT patients (age-adjusted OR 1.52 (1.07, 2.14), $p = 0.018$), and lung cancer significantly less frequently for HHT patients (age-adjusted OR 0.48 [0.30, 0.77], $p = 0.0023$).

	Crude odds ratio (95% CI)	**p value**	**Age adjusted odds ratio (95% CI)**	**p value**
All cancers	0.83 (0.72, 0.96)	0.012	1.04 (0.90, 1.21)	0.53
Prostate	1.11 (0.71, 1.76)	0.64	1.37 (0.87, 2.19)	0.18
Colorectal	1.04 (0.65, 1.65)	0.89	1.30 (0.81, 2.08)	0.28
Breast	1.18 (0.84, 1.65)	0.35	1.52 (1.07, 2.14)	0.018
Lung	0.38 (0.24, 0.60)	<0.0001	0.48 (0.30, 0.77)	0.0023

Table 4. Age adjusted odds ratios for the four most common cancers. Logistic regression was performed in all HHT patients and controls for all cancers, colorectal and lung cancers, in males only for prostate cancer, and in females only for breast cancer. The age adjusted p values for contribution of HHT status were calculated post estimation using Wald test.

The study had not been powered to detect differences in rates of liver cancer, but pooling with reported stomach cancer was considered logical, given stomach cancer was the most generic term available for abdominal cancer in these family reports, and was over-represented in the control group compared to Globocan.[28] Pooled data suggested HHT patients had fewer liver and stomach-designated abdominal cancers than controls (age-adjusted odd ratio 0.51 (0.25, 1.02), $p = 0.059$) (Table 5).

	Crude OR(95% CI)	p value	Age adjusted OR (95% CI)	p value
Liver	0.46 (0.18, 1.14)	0.095	0.59 (0.23, 1.49)	0.26
Stomach	0.43 (0.20, 0.95)	0.036	0.53 (0.24, 1.17)	0.12
Liver or stomach	0.51 (0.25, 1.03)	0.059	0.51 (0.25, 1.02)	0.059

Table 5. Age adjusted odds ratios for specified abdominal cancers. Logistic regression was performed in all HHT patients and controls. The age adjusted p values for contribution of HHT status were calculated post estimation using Wald test.

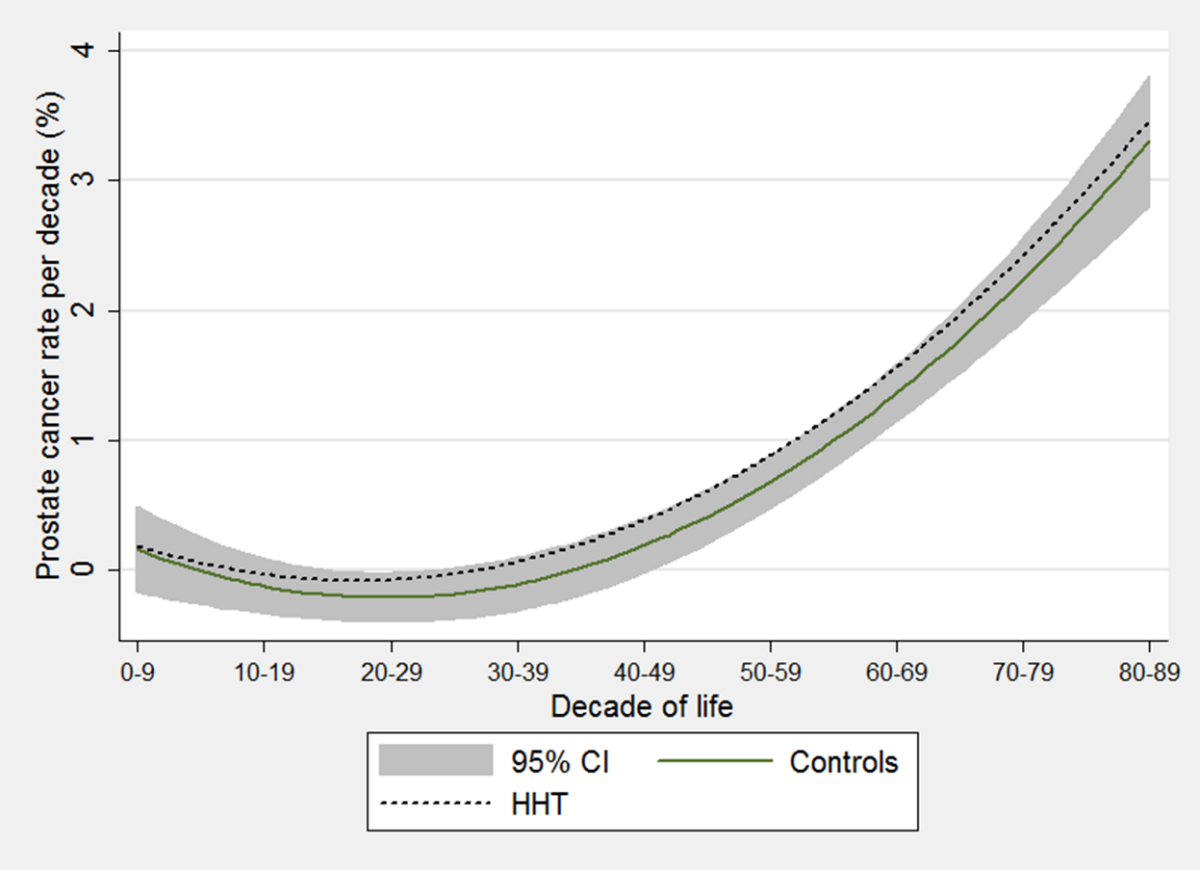


Figure 4. Age-specific prostate cancer rates. Quadratic regression plots for male-only HHT patients and controls. Shaded areas indicate 95% confidence intervals.

Patterns of age-related changes

To examine whether there were trends for differences between the HHT and control groups at specific periods of their lives, quadratic regression was used to present age-related changes graphically. As shown in Figure 4, for prostate cancer, there was an exponential rise in cancer with age in both controls and HHT patients. The best-fit quadratic regression line for HHT patients fitted within the 95% confidence intervals for the best-fit line in the control

population. These graphs represent the pattern that would be expected if there were no differences in prostate cancer rates in any age group, between HHT patients and controls.

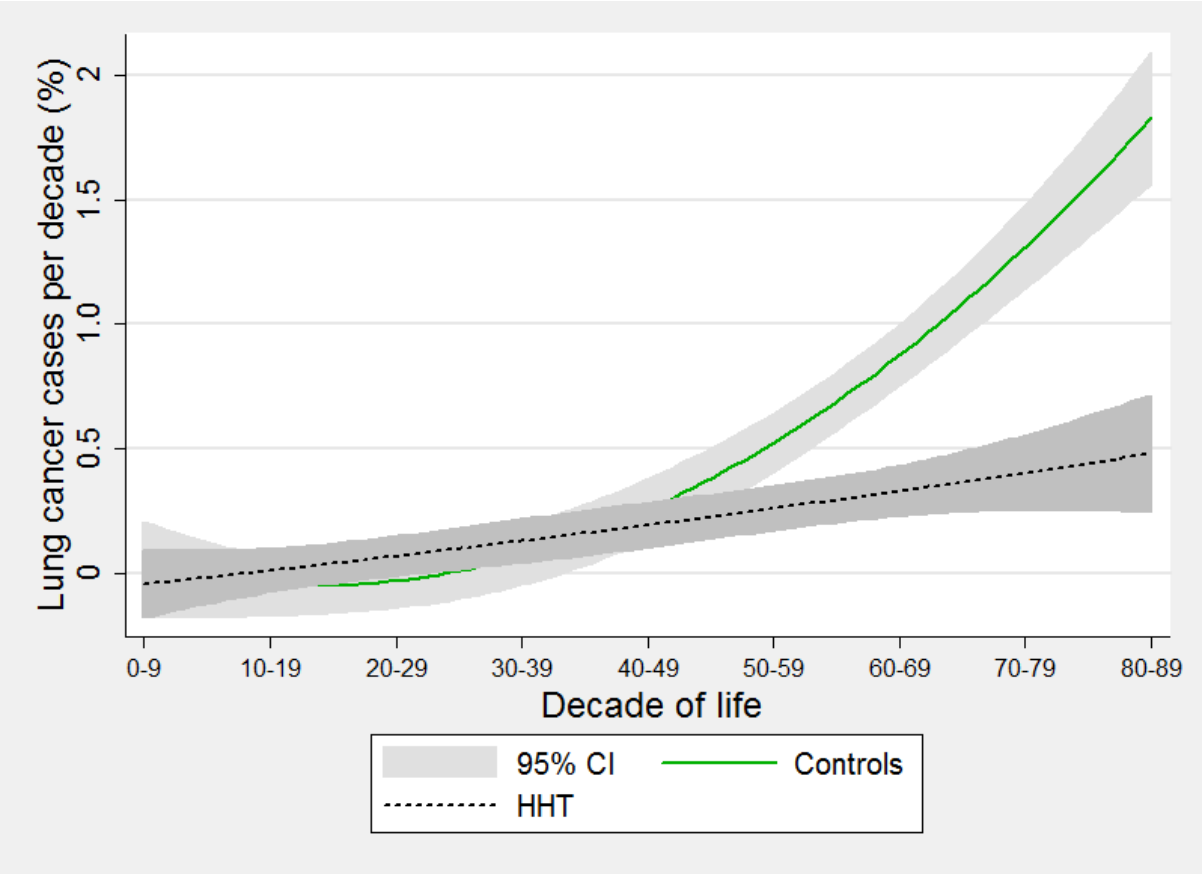


Figure 5. Age-specific lung cancer rates. Quadratic regression plots for all HHT patients and controls. Shaded areas indicate 95% confidence intervals. Note primary and secondary lung cancers are not distinguished.

For lung cancer, the control arm again demonstrated an exponential rise with age (Figure 5). In contrast, the best-fit line for lung cancer events in the HHT-arm was more linear, and less steep than the comparable curve for the controls. The 95% confidence intervals for the best-fit curves diverged after the 5th decade of life. These graphs represent the pattern that would be expected if lung cancer (primary or secondary) was less common in older HHT patients compared to equivalently aged members of the general population.

A similar trend was observed for liver cancer (Figure 6), although the study had not been powered to detect a difference in this less common cancer type. With the wider confidence limits, the 95% confidence intervals for the best-fit curves did not quite diverge. Again, these graphs represent the pattern that would be expected if liver cancer (primary or

secondary) was less common in older HHT patients compared to equivalently aged members of the general population.

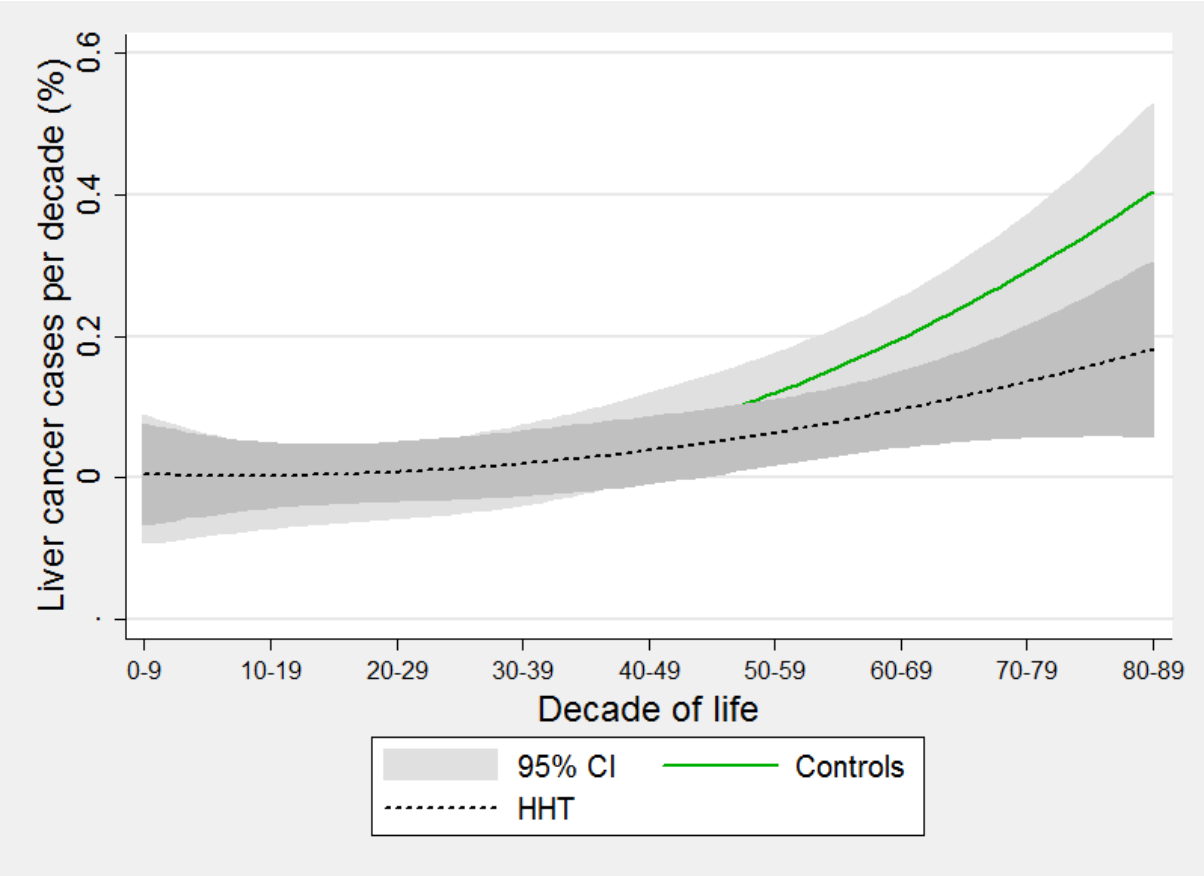


Figure 6. Age-specific liver cancer rates. Quadratic regression plots for all HHT patients and controls. Shaded areas indicate 95% confidence intervals. Note primary and secondary liver cancers are not distinguished.

For breast cancer, a different pattern was observed. For both controls and HHT patients there was a more linear increase in breast cancer cases with age (Figure 7). The curves diverged after 50 years of age but in this case, it was the HHT population who showed a greater increase of cancers with age. These graphs represent the pattern that would be expected if breast cancer was more common in older HHT patients compared to equivalently aged members of the general population.

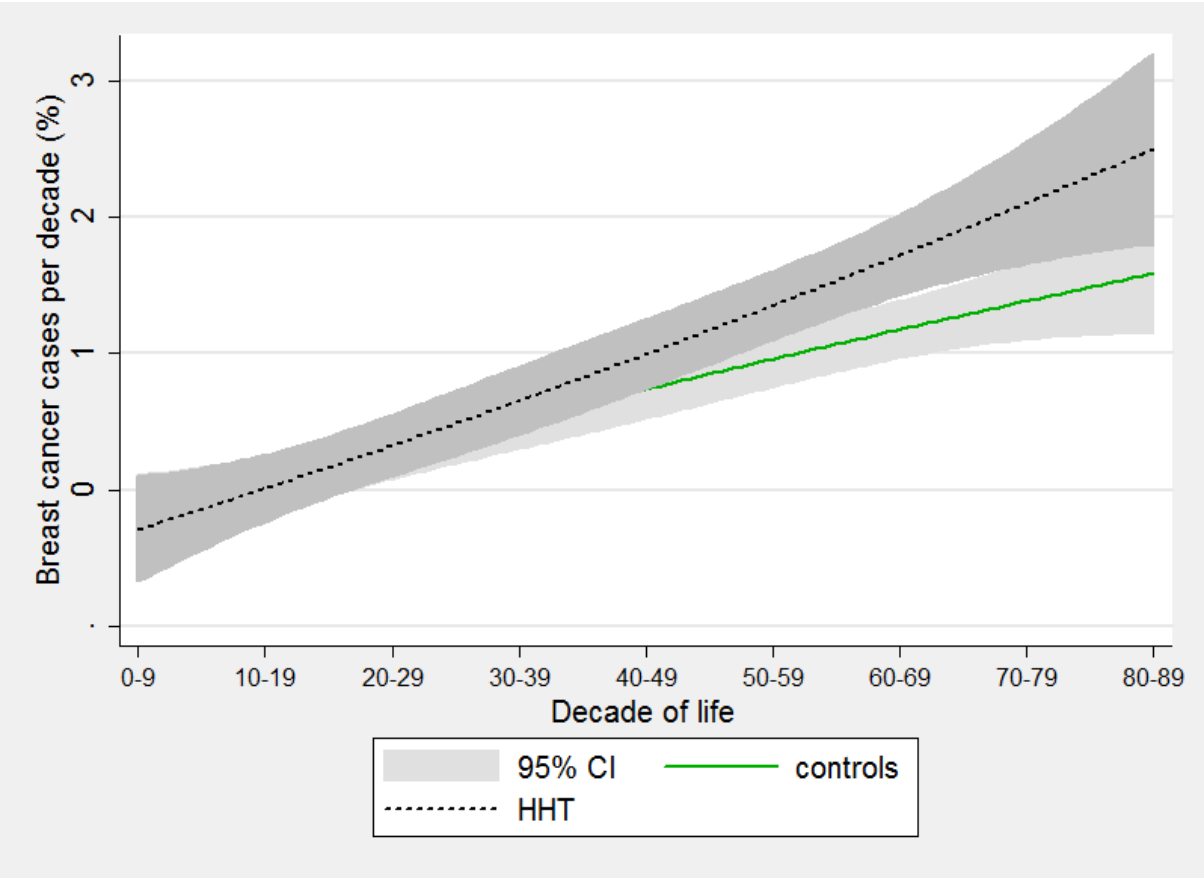


Figure 7. Age-specific breast cancer rates**.** Quadratic regression plots for female-only HHT patients and controls. Shaded areas indicate 95% confidence intervals.

It had been expected that rates of colorectal cancer would be higher in HHT because of the population subgroup with *SMAD4* mutations and juvenile polyposis. Crude and age-adjusted analyses had not revealed an overall difference in colorectal cancer rates between the control and HHT groups, but quadratic regression suggested a bimodal pattern (Figure 8). At younger ages, colorectal cancers were more common in HHT patients, but the rate of rise with age was less steep than for controls, and at older ages, the trend was for fewer cancers in HHT patients.

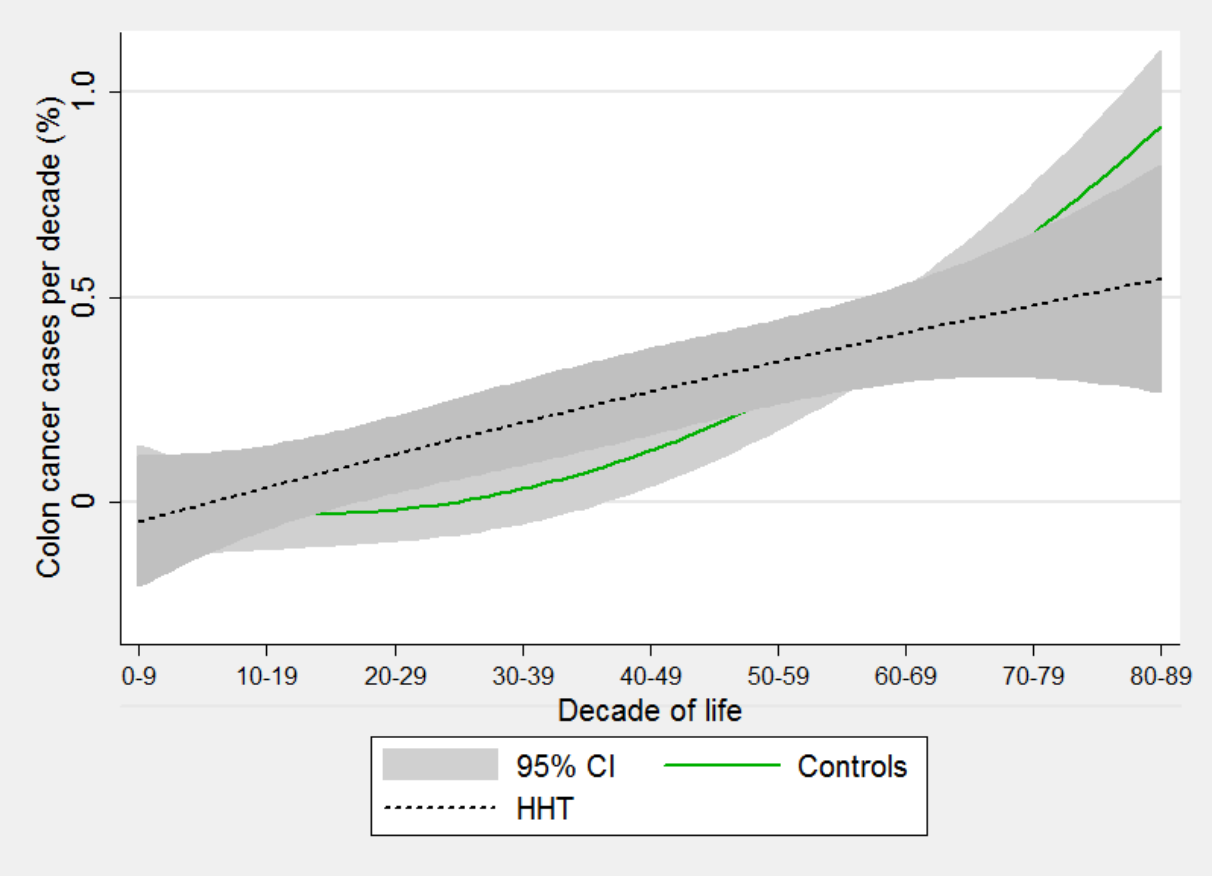


Figure 8. Age-specific colorectal cancer rates. Quadratic regression plots for all HHT patients and controls. Shaded areas indicate 95% confidence intervals.

Discussion

In this study, using a new tool to capture rates of uncommon conditions within a rare disease population, we demonstrated apparent differences in incidence of particular subtypes of cancer in HHT patients compared to controls. Lung and liver/abdominal cancers appeared to be less prevalent, and breast cancer rates higher in HHT patients. Overall, given the poorer survival from lung and liver cancer compared to breast cancer, the data could account for the surprisingly good life expectancy in older HHT patients.

The strengths of this study included the use of new methodology, designed as a family-based questionnaire powered to detect differences in rates of the four most common non-skin cancers between HHT patients and controls. The specific questionnaire was strengthened by the design, accessibility of the questions, standardized and objective inclusion criteria applied after data capture, and acquisition of data from a large number of subjects for a rare disease population. Design of the survey prevented “hypothesis guessing”

by participants by using questions on other common health issues that concealed the purpose of each section of the survey. Due to the familial nature of the condition, participants exhibited willingness to report detailed data on themselves and relatives, despite being unclear exactly why the questions were being asked. The large control group permitted validation of methodologies by comparing ASRs for specific cancers in the captured controls, to those reported for equivalent geographical populations.

Clearly there are limitations with this type of approach which relies on retrospective recollections with potential bias and honesty of data reporting. In addition, it may be limited by uncertainty on precise details of the HHT diagnosis. This was addressed by not merely using self-reported status, but also using a rigorous algorithm that meant that 12% of completed datasets were not assignable either to HHT or control status. While we cannot exclude that some individuals reporting they had AVMs at particular sites, or particular AVM treatments, were wrong, these were never used in isolation for the diagnosis of HHT (Figure 1). Absence of a molecular diagnosis in the majority of cases may be considered a limitation by scientists, but as clinicians recognize, only a proportion of HHT families can receive a molecular diagnostic confirmation. Conversely, given the current debate regarding the disease-causing status of many missense HHT mutations, incomplete descriptions of a change in one of the HHT genes were considerably more likely to be misreported than a clinical phenotype that was familiar to the patient.[35,36] The study was conducted on a predominantly western, English-speaking population aged between 18 and 90 years of age, though cross references were made to general population cancer data from equivalent countries. Detailed smoking and epidemiological habits of relatives were not available, although with the exception of smoking, the control and HHT respondent groups were similar in virtually all demographics analysed. We were particularly concerned with the potential bias of survival to study participation, because lung and liver cancers carry high mortality.[28] Had the reduced number of lung and liver cancers observed in HHT purely been due to survival bias (as participants needed to survive to the point of survey completion), more lung/liver cases should have been found in the HHT “relatives only” subgroup. Since lower rates of lung and liver cancer were reported for HHT relatives than control relatives, we concluded that even allowing for potential survival bias, the data suggested a genuine reduction in these cancers in HHT patients.

From laboratory and animal studies, there are opposing datasets suggesting HHT patients may be at higher or lower risk of cancer and metastases, reflecting the complexity of multistep cancer pathogenesis, and the importance of attempting to obtain data from patients, despite the methodological limitations compared to laboratory analyses. The majority of HHT patients have endoglin or ALK1 mutations, and are haploinsufficient, expressing approximately half normal endoglin or ALK1 in activated monocytes, human umbilical vein endothelial cells, and blood outgrowth endothelial cells.[35-43] Over-expression of both endoglin and ALK-1 is seen during tumour development and endothelial cell proliferation where new vessels are formed to support tumour growth.[44-53] Consequences of acute changes in endoglin and ALK1 expression are yet to be fully determined but include modulation of oncogenic genes such H-Ras; DNA repair enzymes, apoptosis, and resistance to chemotherapy.[45-47] For metastases, while there are data that acute use of anti-endoglin or anti-ALK1 antibodies attenuate endothelial sprouting and other early angiogenic processes, recent data suggest that long term deficiency may render endoglin deficient mice at enhanced risk of tumour metastatic spread, and that endoglin overexpression may be protective.[47-50] Conversely, there are data that cancer growth is reduced in endoglin $^{+/-}$ mice.[51,52] Importantly, both endoglin and ALK1 are emerging as successful targets for cancer therapies in the general population: The use of a soluble chimeric protein (ALK1-Fc), an inhibitor of ALK-1, has been shown to result in significant tumour-suppression both in vitro and in vivo.[53] Furthermore, Phase 1 and Phase 2 human trials have been performed with anti-endoglin antibodies with encouraging results.[54,55]

Our hypothesis based on clinical observations and the surprisingly good life expectancy data, was that cancer rates would be lower in HHT patients: This interpretation would be in-keeping with the data from the human trials.[53-55] The current study was powered to detect differences in lung cancer rates, and these emerged as significantly lower in HHT patients than controls ascertained using the same methodology. We cannot rule out a chance over-reporting of lung cancers only for the control arm, or that HHT patients who would have gone on to develop either primary lung cancer or lung metastases had already died from HHT or other causes, although in the latter case, as for lung cancer specific mortality above, we would have expected to see a higher rate in the relatives arm, but did not. The risk of primary lung cancer is strongly smoking associated, but it is difficult to attribute the lower rates of lung cancer to reduced smoking, as the data suggest smoking rates were if anything,

higher in HHT patients compared to controls. Data from our ongoing 2013 HHT Survey provide a plausible reason: of the first 137 smokers, two (1.5%) stated smoking seemed to start a nosebleed, but 13 (9.5%) stated smoking seemed to stop a nosebleed (Mann Whitney p = 0.0062).[56] We emphasize that the hazards of smoking mean smoking should not be viewed as a "therapeutic" option for HHT nosebleeds- smoking cessation is strongly recommended for HHT patients, as for the general population.

While the current study was underpowered to address liver and other abdominal cancer rates, these too appeared to be reduced. We therefore think it may be relevant that comparisons to age-standardized rates in the general population suggest a significant proportion of reported lung, liver and brain cancers were likely to be metastases from primary cancers elsewhere (Table 2, Figure 2). For lung cancer, we suggest it is possible that overall, HHT patients have natural protection against tumour development in terms of tumour initiation, growth, and/or metastases. Irrespective of the mechanism(s), given the dismal survival rates once lung cancer is present, reduced rates of lung cancer could account for the life expectancy paradox evident in the HHT population.[28]

In view of case reports and evidence that colorectal cancer risks are higher for patients with *SMAD4* mutations, we were surprised that the risk of colorectal cancer did not emerge more strongly for participants and/or relatives with HHT. The age-related changes would support an interpretation allowing for an enhanced risk in early life (most likely consequent on *SMAD4* and polyposis predispositions), but possible protection from other forms of colorectal cancer later in life.

Breast cancer was also expected to be higher in HHT patients: As for any discipline in which screening and treatment modalities include exposure to ionizing radiation, there are discussions about the degree to which health benefits may be offset by an increase in cancer rates.[57-61] In HHT, this is particularly true for brain, lung and breast tissues which lie within the radiation exposure fields for CT scans and angiography, essential to treat HHT cerebral and pulmonary AVMs respectively. Furthermore, endoglin, the protein mutated in HHT type 1, has been shown to suppress invasion and metastasis of breast cancer, with lower endoglin expression in the tumour compartment correlating with poorer clinical outcome.[54] Since HHT patients with endoglin mutations express approximately half normal endoglin, there would therefore be even more reason to predict that breast cancer rates should be higher in HHT patients.[37-43] However, only a modest increase was observed (age-

adjusted OR 1.52 (1.07, 2.14), p = 0.018). Whether this increase would be lessened by reduced radiation exposure is testable, but it is important to recognize that the lifetime risks of breast cancer (<1%) are substantially lower than the risks of strokes, brain abscess, and other complications, which are prevented by PAVM embolization.

Due to the divergent patterns particularly for lung and breast cancer, there were no evident trends comparing all solid cancers (Figure 9). This provides a cautionary note regarding pooling different disease states when faced with the demanding logistical or statistical requirements for studying comorbidities in patients with rare diseases. This could have been done in this study, for example powering the study to detect a difference in "all cancers", "all solid cancers", or "all haematological cancers". There were also no differences in the rates of pooled haematological cancers between HHT patients and controls (Figure 10). Instead of speculating on potential reasons, we prefer to emphasize that the study was underpowered to detect differences even when pooled, and that, as for solid cancers, pooling may have masked important differences between individual cancer types.

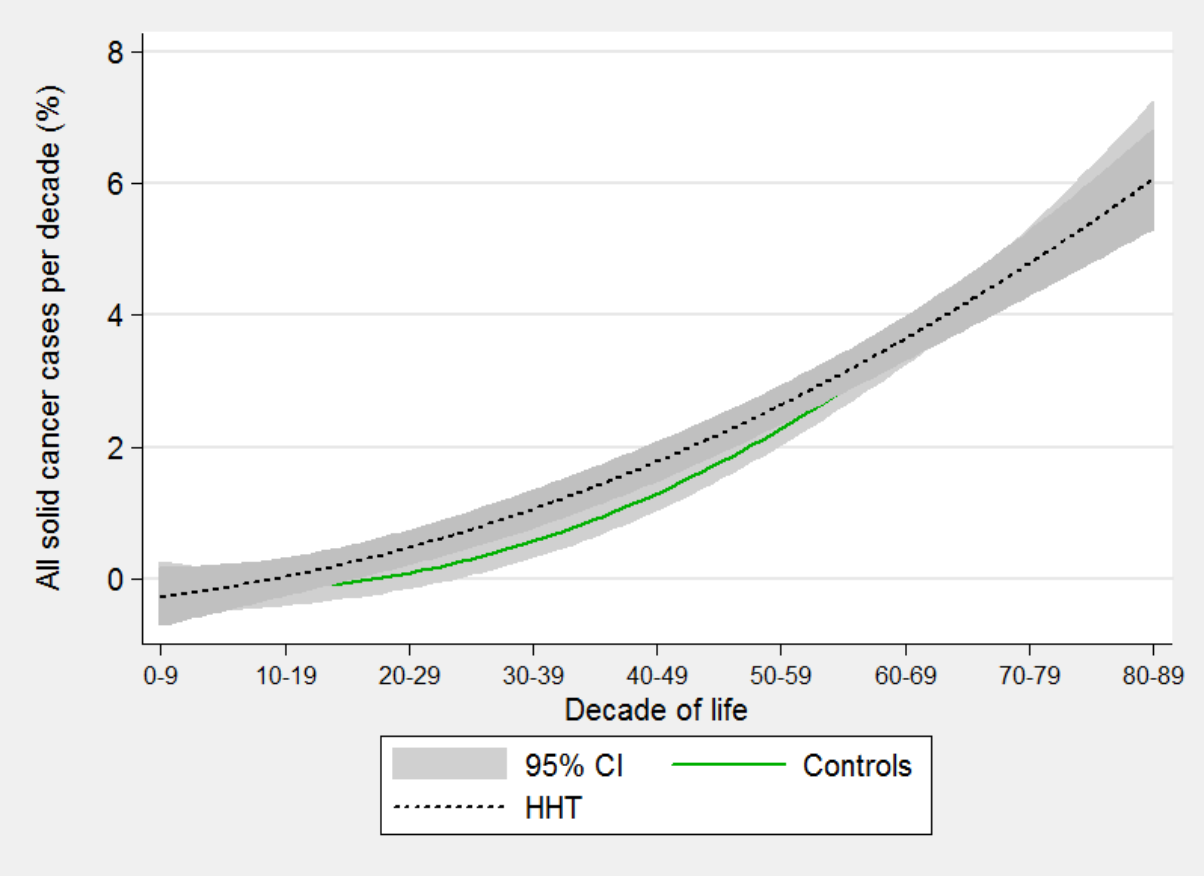


Figure 9. Age-specific rates of all solid, non-skin cancers**.** Quadratic regression plots for all HHT patients and controls. Data include brain, bladder, breast, cervical, colorectal, kidney, liver, lung, pancreas, prostate, stomach uterus, mouth and oesophageal cancers. Shaded areas indicate 95% confidence intervals.

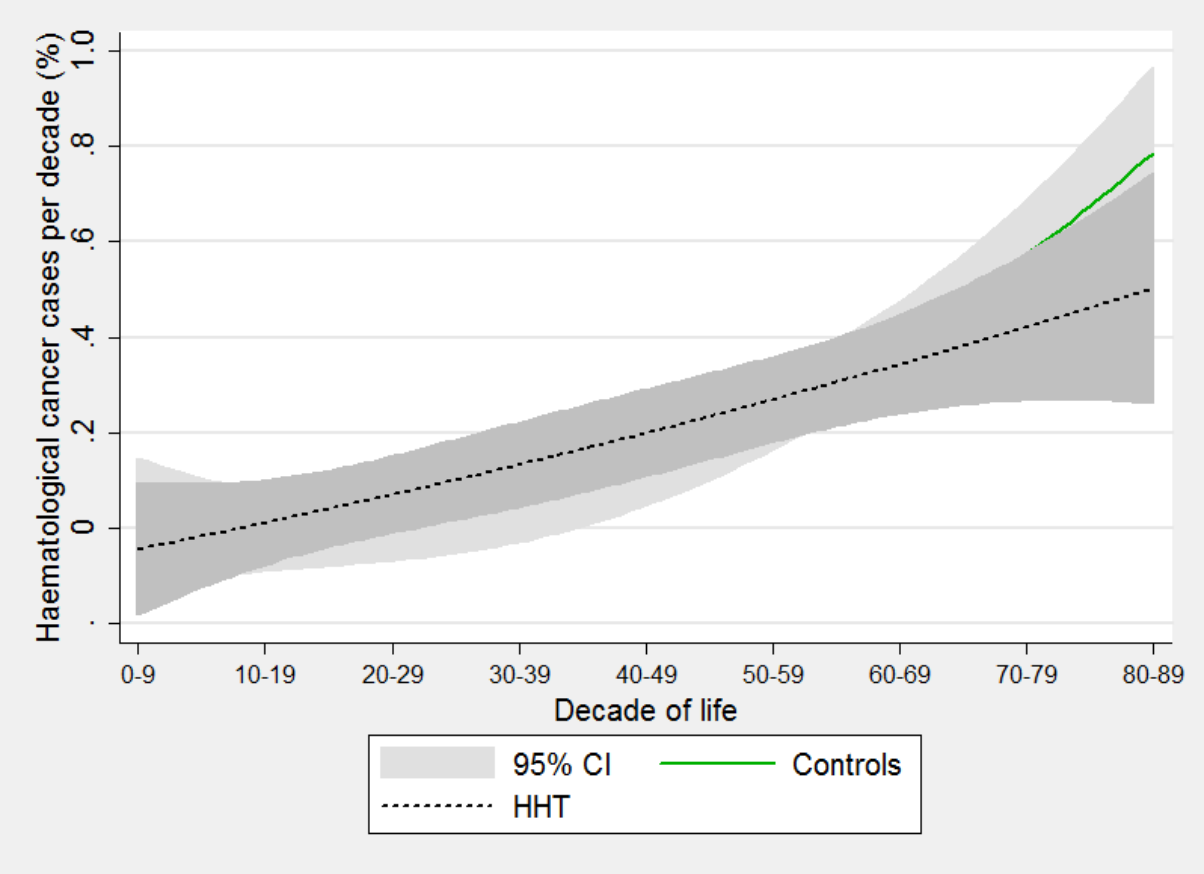


Figure 10. Age-specific rates of haematological cancers**.** Quadratic regression plots for all HHT patients and controls. Data include leukaemia, lymphomas and myeloma. Shaded areas indicate 95% confidence intervals.

Concluding remarks

Overall, for rare diseases in which longitudinal studies would take decades to recruit equivalent datasets prospectively, we suggest that this type of methodology is a good first-step method for data collection. Rapid high profile advertising in the specific populations (particularly via well-established patient support groups with links through email, Facebook and twitter to hundreds or thousands of affected/interested potential participants) renders prolonged data capture periods unnecessary. Such a tool provides the opportunity to address comorbidity risk reductions in rare disease populations, instead of risk increases which are easier to address statistically. Providing patients with rapid feedback from their participation in a somewhat arduous questionnaire is likely to increase their willingness to participate in further studies. This is important for rare disease populations where future research studies are likely to target the same patient groups. Additionally, if multiple research questions are addressed in the same survey, this reduces reporter bias, offers

opportunities for almost immediate delivery of results that matter to patients, yet could potentially be used to capture data of more interest to researchers than the participants themselves.[7,22,30]

For the HHT community, these study results are reassuring on multiple levels, and particularly in terms of absolute breast, brain and colorectal cancer rates given the inevitable speculation regarding potential risks based on available laboratory evidence. We suggest that the findings are also important to the scientific community, as they suggest that HHT patients may be protected from common cancers. Further studies are recommended to assess if factors that may be protecting the HHT population could also be harnessed for the benefit of the general population.

Author contributions

AEH assisted in design of survey cancer questions, participated in survey testing, recruitment and phenotypic assignments, performed the primary analyses, and drafted the manuscript. HLD participated in survey testing and recruitment, and performed phenotypic assignments. BMS participated in survey testing and recruitment. CLS conceived the study, performed the power calculations, generated the overall study questions and SurveyMonkey questionnaires, participated in phenotypic assignments, performed all population validations, population comparison and regression analyses, and wrote the final manuscript. All authors read and approved the final manuscript.

Acknowledgements

We would like to thank Cathleen Kinnear, Nicole Schaefer and Marianne Clancy of the HHT Foundation International for all their help in advertising and promoting the survey to their wide membership (www.hht.org). We are grateful to the worldwide HHT community and their family members for participating. AEH would like to thank the Erasmus scholarship, for financing the opportunity to perform this research project at Hammersmith Hospital. CLS also thanks Luis Calcado at SurveyMonkey Customer Support for technical support with survey formatting, James Moore for setting up the Imperial website entry page, and acknowledges support from the NIHR Biomedical Research Centre Funding Scheme (Imperial BRC), and donations from families and friends of HHT patients.

References

1. Kjeldsen AD, Vase P, Green A: Hereditary haemorrhagic telangiectasia: a population-based study of prevalence and mortality in Danish patients. J Intern Med 1999, 245:31–39.

2. Dakeishi M, Shioya T, Wada Y, Shindo T, Otaka K, Manabe M, Nozaki J, Inoue S, Koizumi A: Genetic epidemiology of hereditary hemorrhagic telangiectasia in a local community in the northern part of Japan. Hum Mut 2002, 19:140–148.

3. Plauchu H, de Chadarévian JP, Bideau A, Robert JM: Age-related clinical profile of hereditary hemorrhagic telangiectasia in an epidemiologically recruited population. Am J Med Genet 1989, 32(3):291–7.

4. Govani FS, Shovlin CL: Hereditary haemorrhagic telangiectasia: a clinical and scientific review. Eur J Hum Genet 2009, 17:860–71.

5. Faughnan ME, Palda VA, Garcia-Tsao G, Geisthoff UW, McDonald J, Proctor DD, Spears J, Brown DH, Buscarini E, Chesnutt MS, Cottin V, Ganguly A, Gossage JR, Guttmacher AE, Hyland RH, Kennedy SJ, Korzenik J, Mager JJ, Ozanne AP, Piccirillo JF, Picus D, Plauchu H, Porteous ME, Pyeritz RE, Ross DA, Sabba C, Swanson K, Terry P, Wallace MC, Westermann CJ, White RI, Young LH, Zarrabeitia R: International guidelines for the diagnosis and management of hereditary haemorrhagic telangiectasia. J Med Genet 2011, 48:73–87.

6. Shovlin CL: Hereditary hemorrhagic telangiectasia: pathophysiology, diagnosis and treatment. Blood Rev 2010, 24:203–19.

7. Finnamore H, Le Couteur J, Hickson M, Busbridge M, Whelan K, Shovlin CL: Hemorrhage-adjusted iron requirements, hematinics and hepcidin define hereditary hemorrhagic telangiectasia as a model of hemorrhagic iron deficiency. PLoS ONE 2013, 8(10):e76516.

8. Kjeldsen A, Kjeldsen J: Gastrointestinal bleeding in patients with hereditary hemorrhagic telangiectasia. Am J Gastroenterol 2000, 95:415–8.

9. Krings T, Ozanne A, Chng S, Alvarez H, Rodesch G, Lasjaunias P: Neurovascular phenotypes in hereditary haemorrhagic telangiectasia patients according to age. Review of 50 consecutive patients aged 1 day–60 years. Neuroradiology 2005, 47:711–20.

10. Shovlin CL, Jackson JE, Bamford KB, Jenkins IH, Benjamin AR, Ramadan H, Kulinskaya E: Primary determinants of ischemic stroke/ brain abscess risks are independent of severity of pulmonary arteriovenous malformations in hereditary hemorrhagic telangiectasia. Thorax 2008, 63:259–66.

11. Kjeldsen AD, Tørring PM, Nissen H, Andersen PE: Cerebral abscesses among Danish patients with hereditary haemorrhagic telangiectasia. Acta Neurol Scand. 2014 Mar;129(3):192-7.

12. Hewes RC, Auster M, White RI: Cerebral embolism- first manifestation of pulmonary arteriovenous malformation in patients with hereditary hemorrhagic telangiectasia. Cardiovasc Intervent Radiol 1985, 8:151–5.

13. Ference BA, Shannon TM, White RI Jr, Zawin M, Burdge CM: Life threatening pulmonary hemorrhage with pulmonary arteriovenous malformations and hereditary hemorrhagic telangiectasia. Chest 1994, 106:1387–1392.

14. Shovlin CL, Sodhi V, McCarthy A, Lasjaunias P, Jackson JE, Sheppard MN: Estimates of maternal risks of pregnancy for women with hereditary haemorrhagic telangiectasia suggested approach for obstetric services. BJOG 2008, 115:1108–15.

15. Buscarini E, Leandro G, Conte D, Danesino C, Daina E, Manfredi G, Lupinacci G, Brambilla G, Menozzi F, De Grazia F, Gazzaniga P, Inama G, Bonardi R, Blotta P, Forner P, Olivieri C, Perna A, Grosso M, Pongiglione G, Boccardi E, Pagella F, Rossi G, Zambelli A: Natural history and outcome of hepatic vascular malformations in a large c6363636363636363636363636363636363s with hereditary hemorrhagic telangiectasia. Dig Dis Sci 2011, 56(7):2166–78.

16. Lerut J, Orlando G, Adam R, Sabbà C, Pfitzmann R, Klempnauer J, Belghiti J, Pirenne J, Thevenot T, Hillert C, Brown CM, Gonze D, Karam V, Boillot O: European Liver Transplant Association. Liver transplantation for hereditary hemorrhagic telangiectasia: report of the European liver transplant registry. Ann Surg 2006, 244:854–62.

17. Girerd B, Montani D, Coulet F, Sztrymf B, Yaici A, Jaïs X, Tregouet D, Reis A, Drouin-Garraud V, Fraisse A, Sitbon O, O'Callaghan DS, Simonneau G, Soubrier F, Humbert M: Clinical outcomes of pulmonary arterial hypertension in patients carrying an ACVRL1 (ALK1) mutation. Am J Respir Crit Care Med 2010, 181:851–61.

18. Shovlin CL, Sulainam NL, Govani FS, Jackson JE, Begbie ME: Elevated Factor VIII in hereditary haemorrhagic telangiectasia (HHT): association with venous thromboembolism. Thromb Haemost 2007, 98:1031–9.

19. Livesey JA, Manning R, Meek JH, Jackson JE, Kulinskaya E, Laffan MA, Shovlin CL: Low serum iron levels are associated with elevated plasma levels of coagulation factor VIII and pulmonary emboli/deep venous thromboses in replicate cohorts of patients with hereditary hemorrhagic telangiectasia. Thorax 2012, 67:328–33.

20. Brosens LA, van Hattem A, Hylind LM, Iacobuzio-Donahue C, Romans KE, Axilbund J, Cruz-Correa M, Tersmette AC, Offerhaus GJ, Giardiello FM: Risk of colorectal cancer in juvenile polyposis. Gut 2007, 56:965–7.

21. Schwenter F, Faughnan ME, Gradinger AB, Berk T, Gryfe R, Pollett A, Cohen Z, Gallinger S, Durno C: Juvenile polyposis, hereditary hemorrhagic telangiectasia, and early onset colorectal cancer in patients with SMAD4 mutation. J Gastroenterol 2012, 47:795–804.

22. Devlin HL, Hosman AE, Shovlin CL: Antiplatelets and anticoagulants in hereditary hemorrhagic telangiectasia. New Engl J Med 2013, 368:876–8.

23. Sabbà C, Pasculli G, Suppressa P, D'Ovidio F, Lenato GM, Resta F, Assennato G, Guanti G: Life expectancy in patients with hereditary haemorrhagic telangiectasia. QJM 2006, 99:327–34.

24. de Gussem EM, Edwards CP, Westermann CJJ, Faughnan ME, Mager JJ: Life expectancy of parents with hereditary hemorrhagic telangiectasia. Hematol Meet Rep 2009, 3(Suppl 4):11–2.

25. Goodwin J, Nisenbaum R, Edwards C, Faughnan ME: Survival in hereditary hemorrhagic telangiectasia. Hematol Meet Rep 2009, 3(Suppl 4):12.

26. Guttmacher AE, McKinnon WC, Taylor LA, Berg J, Porteous MEM, Korsenik J, Fayad PB, Shovlin C, Burdge CM, Jacobson B, Van Cutsem E, Westermann CJJ, Kjeldsen A, Vase P, White RI, McDonald J, Marchuk DA: The natural history of hereditary haemorrhagic telangiectasia: data from a patient questionnaire. Curaçao: Conference Proceedings for HHT Foundation Int., Scientific Meeting; 1997.

27. GLOBOCAN: Estimated cancer Incidence, Mortality, Prevalence and Disability-adjusted life years (DALYs) Worldwide in 2008. ; 2008. http://globocan.iarc.fr.

28. Cancer Research UK: CancerStats: Cancer Statistics for the UK. http://www.cancerresearchuk.org/cancer-info/cancerstats/.

29. Easey AJ, Wallace GM, Hughes JM, Jackson JE, Taylor WJ, Shovlin CL: Should asymptomatic patients with hereditary haemorrhagic telangiectasia (HHT) be screened for cerebral vascular malformations? Data from 22,061 years of HHT patient life. J Neurol Neurosurg Psych 2003, 74:743–8.

30. Silva BM, Hosman AE, Devlin HL, Shovlin CL: A questionnaire-based study suggests lifestyle and dietary factors influencing nosebleed severity in hereditary hemorrhagic telangiectasia (HHT). Laryngoscope 2013, 123:1092–9.

31. HHT Foundation International. www.hht.org.

32. Shovlin CL, Guttmacher AE, Buscarini E, Faughan M, Hyland R, Westermann CJJ, Plauchu H: Diagnostic criteria for hereditary hemorrhagic telangiectasia (Rendu-Osler-Weber syndrome). Am J Med Genet 2000, 91:66–7.

33. van Gent MW, Velthuis S, Post MC, Snijder RJ, Westermann CJ, Letteboer TG, Mager JJ: Hereditary hemorrhagic telangiectasia: how accurate are the clinical criteria? Am J Med Genet A 2013, 161:461–6.

34. World Fertility Data, United Nations Population Division.
http://www.un.org/esa/population/publications/WFD%202008/WP_WFD_2008/Data.html.

35. Govani FS, Giess A, Mollet IM, Begbie ME, Jones M, Game L, Shovlin CL: Directional next-generation RNA sequencing and examination of premature termination codon mutations in endoglin/hereditary haemorrhagic telangiectasia. Mol Syndromol 2013, 4:184–196.

36. McDonald J, Gedge F, Burdette A, Carlisle J, Bukjiok C, et al: Multiple sequence variants in hereditary hemorrhagic telangiectasia cases: illustration of complexity in molecular diagnostic interpretation. J Mol Diagnostics 2009, 11(6):569–575.

37. Pece N, Vera S, Cymerman U, White R, Wrana J, Letarte M: Mutant endoglin in Hereditary Hemorrhagic Telangiectasia type I is transiently expressed intracellularly and is not a dominant negative. J Clin Invest 1997, 100:2568–2579.

38. Pece-Barbara N, Cymerman U, Vera S, Marchuk D, Letarte M: Expression analysis of four endoglin missense mutations suggests haploinsufficiency is the predominant mechanism for Hereditary Hemorrhagic Telangiectasia type I. Hum Mol Genet 1999, 8:2171–2181.

39. Pece-Barbara N, Vera S, Kathirkamathamby K, Liebner S, Di Guglielmo GM, Dejana E, Wrana JL, Letarte M: Endoglin null endothelial cells proliferate faster and are more responsive to transforming growth factor beta1 with higher affinity receptors and an activated Alk1 pathway. J Biol Chem 2005, 280:27800–27808.

40. Bourdeau A, Cymerman U, Paquet ME, Meschino W, McKinnon WC, Guttmacher AE, Becker L, Letarte M: Endoglin expression is reduced in normal vessels but still detectable in arteriovenous malformations of patients with hereditary hemorrhagic telangiectasia type 1. Am J Pathol 2000, 156:911–923.

41. Cymerman U, Vera S, Pece-Barbara N, Bourdeau A, White RI Jr, Dunn J, Letarte M: Identification of Hereditary Hemorrhagic Telangiectasia type I in newborns by protein expression and mutation analysis of endoglin. Pediatr Res 2000, 47:24–35.

42. Paquet ME, Pece-Barbara N, Vera S, Cymerman U, Karabegovic A, Shovlin C, Letarte M: Analysis of several endoglin mutants reveals no endogenous mature or secreted protein capable of interfering with normal endoglin function. Hum Mol Genet 2001, 10:1347–1357.

43. Fernandez-L A, Sanz-Rodriguez F, Zarrabeitia R, Perez-Molino A, Morales C, Restrepo CM, Ramirez JR, Coto E, Lenato GM, Bernabeu C, Botella LM: Mutation study of Spanish patients with hereditary hemorrhagic telangiectasia and expression analysis of Endoglin and ALK1. Hum Mutat 2006, 27:295.

44. Seki T, Yun J, Oh SP: Arterial endothelium-specific activin receptor- like kinase 1 expression suggests its role in arterialization and vascular remodeling. Circ Res 2003, 93:682–9.

45. Santibanez JF, Pérez-Gómez E, Fernandez-L A, Garrido-Martin EM, Carnero A, Malumbres M, Vary CP, Quintanilla M, Bernabéu C: The TGF-beta co-receptor endoglin modulates the expression and transforming potential of H-Ras. Carcinogenesis 2010, 31:2145–54.

46. Ziebarth AJ, Nowsheen S, Steg AD, Shah MM, Katre AA, Dobbin ZC, Han HD, Lopez-Berestein G, Sood AK, Conner M, Yang ES, Landen CN: Endoglin (CD105) contributes to platinum resistance and is a target for tumor-specific therapy in epithelial ovarian cancer. Clin Cancer Res 2013, 19:170–82.

47. Uneda S, Toi H, Tsujie T, Tsujie M, Harada N, Tsai H, Seon BK: Anti-endoglin monoclonal antibodies are effective for suppressing metastasis and the primary tumors by targeting tumor vasculature. Int J Cancer 2009, 125:1446–53.

48. van Meeteren LA, Thorikay M, Bergqvist S, Pardali E, Stampino CG, Hu-Lowe D, Goumans MJ, ten Dijke P: Anti-human activin receptor-like kinase 1 (ALK1) antibody attenuates bone morphogenetic protein 9 (BMP9)-induced ALK1 signaling and interferes with endothelial cell sprouting. J Biol Chem 2012, 287:18551–61.

49. Anderberg C, Cunha SI, Zhai Z, Cortez E, Pardali E, Johnson JR, Franco M, Páez-Ribes M, Cordiner R, Fuxe J, Johansson BR, Goumans MJ, Casanovas O, ten Dijke P, Arthur HM, Pietras K: Deficiency for endoglin in tumor vasculature weakens the endothelial barrier to metastatic dissemination. J Exp Med 2013, 210:563–79.

50. Henry LA, Johnson DA, Sarrió D, Lee S, Quinlan PR, Crook T, Thompson AM, Reis-Filho JS, Isacke CM: Endoglin expression in breast tumor cells suppresses invasion and metastasis and correlates with improved clinical outcome. Oncogene 2011, 30:1046–58.

51. Quintanilla M, Ramirez JR, Pérez-Gómez E, Romero D, Velasco B, Letarte M, López-Novoa JM, Bernabéu C: Expression of the TGF-beta coreceptor endoglin in epidermal keratinocytes and its dual role in multistage mouse skin carcinogenesis. Oncogene 2003, 22:5976–5985.

52. Jerkic M, Rodríguez-Barbero A, Prieto M, Toporsian M, Pericacho M, Rivas-Elena JV, Obreo J, Wang A, Pérez-Barriocanal F, Arévalo M, Bernabéu C, Letarte M, López-Novoa JM: Reduced angiogenic responses in adult Endoglin heterozygous mice. Cardiovasc Res 2006, 69:845–54.

53. Mitchell D, Pobre EG, Mulivor AW, Grinberg AV, Castonguay R, Monnell TE, Solban N, Ucran JA, Pearsall RS, Underwood KW, Seehra J, Kumar R: ALK1-Fc Inhibits Multiple Mediators of Angiogenesis and Suppresses Tumor Growth. Mol Cancer Ther 2010, 9:379–388.

54. Rosen LS, Hurwitz HI, Wong MK, Goldman J, Mendelson DS, Figg WD, Spencer S, Adams BJ, Alvarez D, Seon BK, Theuer CP, Leigh BR, Gordon MS: A phase I first-in-human study of TRC105 (Anti-Endoglin Antibody) in patients with advanced cancer. Clin Cancer Res 2012, 18:4820–9.

55. Bernabeu C, Shovlin CL: TRC-105, an anti-endoglin chimeric mAb for the potential treatment of cancer. Thomson Reuters Pharma. https://www.thomson-pharma.com/portal/page/portal/Reports/ drug_id=17606.

56. Elphick A, Shovlin CL: Relationships between epistaxis, migraines, and triggers in hereditary hemorrhagic telangiectasia. Laryngoscope 2013. 10.1002/lary.24526.

57. Berrington De González A, Darby S: Risk of cancer from diagnostic X-rays: estimates for the UK and 14 other countries. Lancet 2004, 363:345–51.

58. Chodick G, Ronckers C, Shalev V, Ron E: Excess lifetime cancer mortality risk attributable to radiation exposure from computed tomography examinations in children. Isr Med Assoc J 2007, 9:584–7.

59. Rice H, Frush D, Farmer D, Waldhausen J: APSA Education Committee Review of radiation risks from computed tomography: essentials for the pediatric surgeon. J Pediatr Surg 2007, 42:603–7.

60. Thierry-Chef I, Simon SL, Land CE, Miller DL: Radiation dose to the brain and subsequent risk of developing brain tumors in pediatric patients undergoing interventional neuroradiology procedures. Radiat Res 2008, 170:553–65.

61. Ma S, Kong B, Liu B, Liu X: Biological effects of low-dose radiation from computed tomography scanning. Int J Radiat Biol 2013, 89:326–33.

Chapter four

The correlation between pulmonary arterial hypertension and different types of hereditary haemorrhagic telangiectasia: a review

AE Hosman, VMM Vorselaars, CJJ Westermann, RJ Snijder, JJ Mager, MJ Goumans, MC Post

Submitted

Abstract

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

Pulmonary arterial hypertension

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

PAH is defined by an increased mPAP (of ≥ 25 mmHg at rest), pulmonary capillary wedge pressure ≤15 mmHg and pulmonary vascular resistance of > 3 Wood units, for all of which the gold standard of measurement is a right heart catheterization (RHC), although right ventricular pressure measurement on an echocardiogram can also give an estimation of the severity of the disease.[1]

Clinical features are the result of decrease in cardiac output due to right heart failure and include progressive dyspnea, decreased exercise tolerance and fatigue.

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

Hereditary Haemorrhagic Telangiectasia

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

cause HHT type 1 which is characterized by a higher prevalence of pulmonary and cerebral AVMs, mucocutaneous telangiectasia and epistaxis compared to *ACVRL1* mutations, or HHT type 2. The second has a higher prevalence of hepatic AVMs.

Patients with HHT, especially women with an ENG mutation, who have not been screened and treated pre-emptively have a slightly lower life expectancy than family members without HHT and severe epistaxis can result in a decreased quality of life.[6] Complications from pulmonary AVMs mainly include hypoxemia and paradoxical (sterile or septic) emboli, although many patients remain asymptomatic. Hepatic shunting can lead to portal hypertension, biliary necrosis and high output cardiac failure due at least two-to three fold elevation of cardiac output.[7,8] Complications of cerebral AVMs are rare (approximately 0.5% per year) but its consequence can be devastating.[9]

Clinical signs are not only variable in subtype but also variable in severity between family members with identical mutations.[10] Etiological factors and genetic modifiers are thought to explain this clinical variability.[11,12]

Molecular Mechanism

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

arteriovenous malformations explaining the abnormal morphogenesis of vasculature in HHT.[14,21]

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

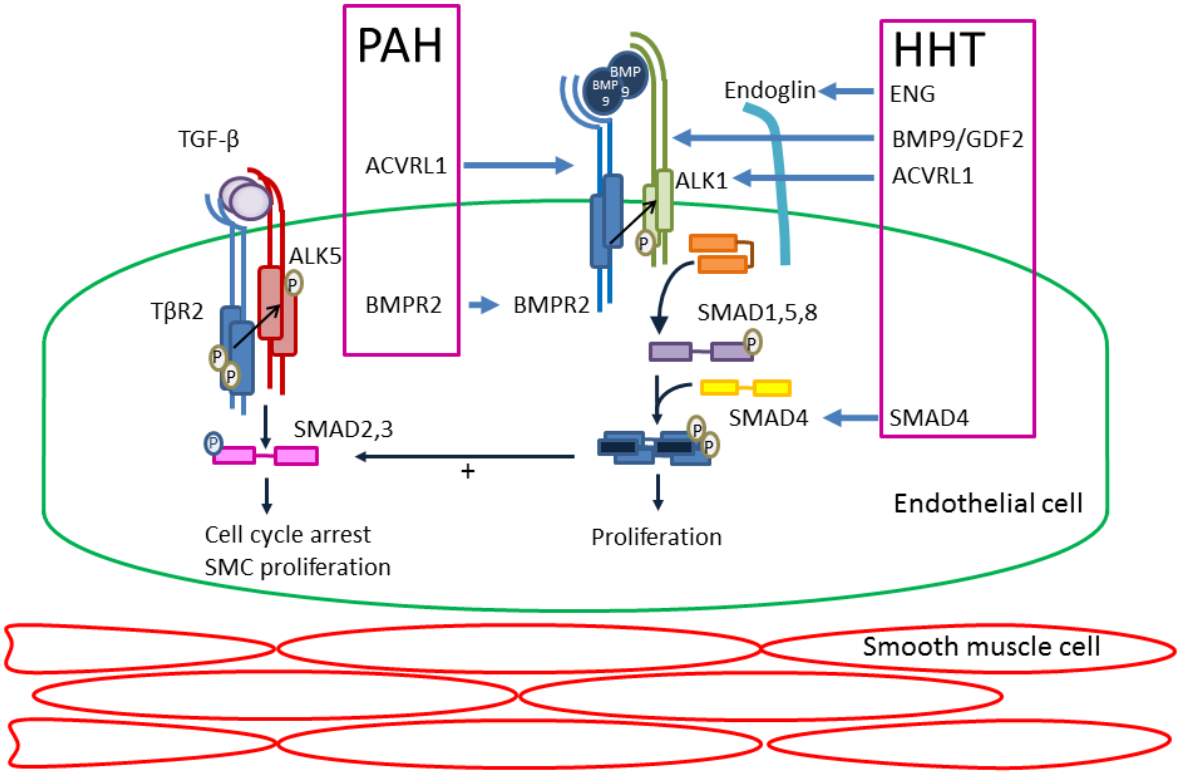


Figure 1. Schematic diagram illustrating the TGF-B pathway and the genes and proteins involved in PAH and HHT. Illustrated are two pathways of ALK5/SMAD2-3 and ALK1/SMAD1-5.

PAH and HHT

Heritable PAH is a rare but severe complication of HHT. *ACVRL1* mutations have been recognized to lead to this combined syndrome for several years. Thirty-nine patients with PAH and *ACVRL1* mutations have been described in literature.[29-39] Many different *ACVRL1* mutations have been described in HPAH patients, but there seems to be a predominance of mutations in exon 10 (http://www/hhtmutation.org).[34] Knowledge of PAH in the field of HHT is especially important since this combination usually leads to a worse outcome than PAH alone.[34] Twenty-two of the patients described in these case reports were diagnosed under the age of 18 (56%). Compared to *BMPR2* mutation carriers and non-carriers (idiopathic PAH), *ACVRL1* mutation carriers are diagnosed at a younger age and have a worse prognosis despite similar therapy and better hemodynamics at time of diagnosis.[34] This suggest that the disease progresses more rapidly with severe consequences. Even though it is rare for HHT to be complicated by PAH, physicians should be aware of the combination and perform an echocardiogram when clinical signs indicate so, especially in patients with *ACVRL1* mutations.

Conversely, clinical signs of HHT in patients with HPAH based on *ACVRL1* mutations might not always be apparent initially.

Ten patients with HPAH and HHT resulting of ENG mutations have been described in literature but this association is still disputed.[32,39-42] No data exist about the prognosis of patients with PAH and *ENG* mutations.

Although more patients with HHT and pulmonary hypertension (PH) have been described in various reports, this often involves PH due to left sided heart disease or high output PH due to a left to right shunt in the presence of arteriovenous malformations in the liver.[35,43,44]

Furthermore, both PAH and HHT show an impaired inflammatory response and inflammation often leads to disease progression.[45,46] This information may be interesting for future therapy, although this is yet to be explored. Furthermore, in both diseases a difference is seen in men and women. Epidemiologic data shows a female predominance in many types of PAH and life-expectancy of females with HHT caused by an ENG mutation seems to be impacted greatly.[6,47] Although it is thought female hormones play an important role in both diseases, the exact mechanisms are not yet fully understood.[48-50]

Management of HPAH in HHT

Although literature is limited, treatment with the typical therapies used for HPAH is recommended. This includes a combination of different PAH specific medication (endothelin receptor antagonists (ERA), phosphodiesterase inhibitors (PD5I), prostacyclins and soluble guanylate cyclase stimulators) and supporting therapy (e.g. diuretics and oxygen) [1]. Two case reports describe successful treatment with the ERA bosentan in PAH and HHT, which improves exercise capacity, laboratory findings and hemodynamic parameters.[51,52] Recently the first case of a patient successfully treated with sildenafil (PD5I) was documented.[53]

Although vasoreactivity testing is recommended in all patients with HPAH, there was no reaction on pulmonary vasodilators in a study with 23 *ACVRL1* patients. Treatment with calcium channel blockers seems therefore not indicated.[1,34]

It is important to realize that embolization of pulmonary AVMs could potentially increase the pulmonary arterial pressure, although to which extent this might contribute to the progression of PAH is not yet known.[54-57] Furthermore, the risk of sudden rupture of pulmonary AVMs may be increased in PAH patients.[58]

Pulmonary Hypertension as complication of HHT

This review discusses the role of PAH and HHT particularly, but it is important to note that other types of PH, associated with HHT, can occur by several different mechanisms. Most often, post capillary PH develops as a final result of the hyperkinetic state associated with liver AVMs. Especially in HHT, anemia due to epistaxis and gastro-intestinal bleeding may trigger this cascade due to increased cardiac output. Pre-capillary PH may be the result of chronic thromboembolic PH (CTEPH) since HHT patients may encounter an increased thrombotic risk.[59]

Conclusion

The combination of PAH and HHT is rare but may have severe consequences. Both diseases can be the result of genetic affecting the TGF-β signaling pathway, essential for angiogenesis. Clinical signs may not specific but early diagnosis is important for appropriate

treatment. Therefore awareness of this disease combination is important for all clinicians working with HHT or PAH patients.

Acknowledgments

All authors would like to thank the SWORO foundation and ZonMw (Topzorg project).

References

1. Galie N, Humbert M, Vachiery JL, Gibbs S, Lang I, Torbicki A, Simonneau G, Peacock A, Vonk Noordegraaf A, Beghetti M, Ghofrani A, Gomez Sanchez MA, Hansmann G, Klepetko W, Lancellotti P, Matucci M, McDonagh T, Pierard LA, Trindade PT, Zompatori M, Hoeper M, Aboyans V, Vaz Carneiro A, Achenbach S, Agewall S, Allanore Y, Asteggiano R, Paolo Badano L, Albert Barbera J, Bouvaist H, Bueno H, Byrne RA, Carerj S, Castro G, Erol C, Falk V, Funck-Brentano C, Gorenflo M, Granton J, Iung B, Kiely DG, Kirchhof P, Kjellstrom B, Landmesser U, Lekakis J, Lionis C, Lip GY, Orfanos SE, Park MH, Piepoli MF, Ponikowski P, Revel MP, Rigau D, Rosenkranz S, Voller H, Luis Zamorano J. 2015 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension: The Joint Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS): Endorsed by: Association for European Paediatric and Congenital Cardiology (AEPC), International Society for Heart and Lung Transplantation (ISHLT). *Eur Heart J* 2016; 37: 67-119.

2. Tillet E, Bailly S. Emerging roles of BMP9 and BMP10 in hereditary hemorrhagic telangiectasia. *Front Genet* 2015; 5: 456.

3. Wang G, Fan R, Ji R, Zou W, Penny DJ, Varghese NP, Fan Y. Novel homozygous BMP9 nonsense mutation causes pulmonary arterial hypertension: a case report. *BMC Pulm Med* 2016; 16: 17-016-0183-7.

4. Faughnan ME, Palda VA, Garcia-Tsao G, Geisthoff UW, McDonald J, Proctor DD, Spears J, Brown DH, Buscarini E, Chesnutt MS, Cottin V, Ganguly A, Gossage JR, Guttmacher AE, Hyland RH, Kennedy SJ, Korzenik J, Mager JJ, Ozanne AP, Piccirillo JF, Picus D, Plauchu H, Porteous ME, Pyeritz RE, Ross DA, Sabba C, Swanson K, Terry P, Wallace MC, Westermann CJ, White RI, Young LH, Zarrabeitia R, HHT Foundation International - Guidelines Working Group. International guidelines for the diagnosis and management of hereditary haemorrhagic telangiectasia. *J Med Genet* 2011; 48: 73-87.

5. McDonald J, Wooderchak-Donahue W, VanSant Webb C, Whitehead K, Stevenson DA, Bayrak-Toydemir P. Hereditary hemorrhagic telangiectasia: genetics and molecular diagnostics in a new era. *Front Genet* 2015; 6: 1.

6. de Gussem EM, Edwards CP, Hosman AE, Westermann CJ, Snijder RJ, Faughnan ME, Mager JJ. Life expectancy of parents with Hereditary Haemorrhagic Telangiectasia. *Orphanet J Rare Dis* 2016; 11: 46-016-0427-x.

7. Buscarini E, Plauchu H, Garcia Tsao G, White RI,Jr, Sabba C, Miller F, Saurin JC, Pelage JP, Lesca G, Marion MJ, Perna A, Faughnan ME. Liver involvement in hereditary hemorrhagic telangiectasia: consensus recommendations. *Liver Int* 2006; 26: 1040-1046.

8. Vorselaars VM, Velthuis S, Snijder RJ, Vos JA, Mager JJ, Post MC. Pulmonary hypertension in hereditary haemorrhagic telangiectasia. *World J Cardiol* 2015; 7: 230-237.

9. Willemse RB, Mager JJ, Westermann CJ, Overtoom TT, Mauser H, Wolbers JG. Bleeding risk of cerebrovascular malformations in hereditary hemorrhagic telangiectasia. *J Neurosurg* 2000; 92: 779-784.

10. Letteboer TG, Mager JJ, Snijder RJ, Koeleman BP, Lindhout D, Ploos van Amstel JK, Westermann CJ. Genotype-phenotype relationship in hereditary haemorrhagic telangiectasia. *J Med Genet* 2006; 43: 371-377.

11. Benzinou M, Clermont FF, Letteboer TG, Kim JH, Espejel S, Harradine KA, Arbelaez J, Luu MT, Roy R, Quigley D, Higgins MN, Zaid M, Aouizerat BE, van Amstel JK, Giraud S, Dupuis-Girod S, Lesca G, Plauchu H, Hughes CC, Westermann CJ, Akhurst RJ. Mouse and human strategies identify PTPN14 as a modifier of angiogenesis and hereditary haemorrhagic telangiectasia. *Nat Commun* 2012; 3: 616.

12. Kawasaki K, Freimuth J, Meyer DS, Lee MM, Tochimoto-Okamoto A, Benzinou M, Clermont FF, Wu G, Roy R, Letteboer TG, Ploos van Amstel JK, Giraud S, Dupuis-Girod S, Lesca G, Westermann CJ, Coffey RJ,Jr, Akhurst RJ. Genetic variants of Adam17 differentially regulate TGFbeta signaling to modify vascular pathology in mice and humans. *Proc Natl Acad Sci U S A* 2014; 111: 7723-7728.

13. Shi Y, Massague J. Mechanisms of TGF-beta signaling from cell membrane to the nucleus. *Cell* 2003; 113: 685-700.

14. Goumans MJ, Lebrin F, Valdimarsdottir G. Controlling the angiogenic switch: a balance between two distinct TGF-b receptor signaling pathways. *Trends Cardiovasc Med* 2003; 13: 301-307.

15. Gore B, Izikki M, Mercier O, Dewachter L, Fadel E, Humbert M, Dartevelle P, Simonneau G, Naeije R, Lebrin F, Eddahibi S. Key role of the endothelial TGF-beta/ALK1/endoglin signaling pathway in humans and rodents pulmonary hypertension. *PLoS One* 2014; 9: e100310.

16. Goumans MJ, Valdimarsdottir G, Itoh S, Lebrin F, Larsson J, Mummery C, Karlsson S, ten Dijke P. Activin receptor-like kinase (ALK)1 is an antagonistic mediator of lateral TGFbeta/ALK5 signaling. *Mol Cell* 2003; 12: 817-828.

17. Goumans MJ, Valdimarsdottir G, Itoh S, Rosendahl A, Sideras P, ten Dijke P. Balancing the activation state of the endothelium via two distinct TGF-beta type I receptors. *EMBO J* 2002; 21: 1743-1753.

18. Lamouille S, Mallet C, Feige JJ, Bailly S. Activin receptor-like kinase 1 is implicated in the maturation phase of angiogenesis. *Blood* 2002; 100: 4495-4501.

19. Ota T, Fujii M, Sugizaki T, Ishii M, Miyazawa K, Aburatani H, Miyazono K. Targets of transcriptional regulation by two distinct type I receptors for transforming growth factor-beta in human umbilical vein endothelial cells. *J Cell Physiol* 2002; 193: 299-318.

20. Li C, Hampson IN, Hampson L, Kumar P, Bernabeu C, Kumar S. CD105 antagonizes the inhibitory signaling of transforming growth factor beta1 on human vascular endothelial cells. *FASEB J* 2000; 14: 55-64.

21. Tual-Chalot S, Mahmoud M, Allinson KR, Redgrave RE, Zhai Z, Oh SP, Fruttiger M, Arthur HM. Endothelial depletion of Acvrl1 in mice leads to arteriovenous malformations associated with reduced endoglin expression. *PLoS One* 2014; 9: e98646.

22. Morrell NW, Adnot S, Archer SL, Dupuis J, Jones PL, MacLean MR, McMurtry IF, Stenmark KR, Thistlethwaite PA, Weissmann N, Yuan JX, Weir EK. Cellular and molecular basis of pulmonary arterial hypertension. *J Am Coll Cardiol* 2009; 54: S20-31.

23. van der Bruggen CE, Happe CM, Dorfmuller P, Trip P, Spruijt OA, Rol N, Hoevenaars FP, Houweling AC, Girerd B, Marcus JT, Mercier O, Humbert M, Handoko ML, van der Velden J, Vonk Noordegraaf A, Bogaard HJ, Goumans MJ, de Man FS. Bone Morphogenetic Protein Receptor Type 2 Mutation in Pulmonary Arterial Hypertension: A View on the Right Ventricle. *Circulation* 2016; 133: 1747-1760.

24. Huang Z, Wang D, Ihida-Stansbury K, Jones PL, Martin JF. Defective pulmonary vascular remodeling in Smad8 mutant mice. *Hum Mol Genet* 2009; 18: 2791-2801.

25. David L, Mallet C, Mazerbourg S, Feige JJ, Bailly S. Identification of BMP9 and BMP10 as functional activators of the orphan activin receptor-like kinase 1 (ALK1) in endothelial cells. *Blood* 2007; 109: 1953-1961.

26. Wooderchak-Donahue WL, McDonald J, O'Fallon B, Upton PD, Li W, Roman BL, Young S, Plant P, Fulop GT, Langa C, Morrell NW, Botella LM, Bernabeu C, Stevenson DA, Runo JR, Bayrak-Toydemir P. BMP9 mutations cause a vascular-anomaly syndrome with phenotypic overlap with hereditary hemorrhagic telangiectasia. *Am J Hum Genet* 2013; 93: 530-537.

27. Long L, Ormiston ML, Yang X, Southwood M, Graf S, Machado RD, Mueller M, Kinzel B, Yung LM, Wilkinson JM, Moore SD, Drake KM, Aldred MA, Yu PB, Upton PD, Morrell NW. Selective enhancement of endothelial BMPR-II with BMP9 reverses pulmonary arterial hypertension. *Nat Med* 2015; 21: 777-785.

28. Morrell NW, Bloch DB, ten Dijke P, Goumans MJ, Hata A, Smith J, Yu PB, Bloch KD. Targeting BMP signalling in cardiovascular disease and anaemia. *Nat Rev Cardiol* 2016; 13: 106-120.

29. Trembath RC, Thomson JR, Machado RD, Morgan NV, Atkinson C, Winship I, Simonneau G, Galie N, Loyd JE, Humbert M, Nichols WC, Morrell NW, Berg J, Manes A, McGaughran J, Pauciulo M, Wheeler L. Clinical and molecular genetic features of pulmonary hypertension in patients with hereditary hemorrhagic telangiectasia. *N Engl J Med* 2001; 345: 325-334.

30. Harrison RE, Flanagan JA, Sankelo M, Abdalla SA, Rowell J, Machado RD, Elliott CG, Robbins IM, Olschewski H, McLaughlin V, Gruenig E, Kermeen F, Halme M, Raisanen-Sokolowski A, Laitinen T, Morrell NW, Trembath RC. Molecular and functional analysis identifies ALK-1 as the predominant cause of pulmonary hypertension related to hereditary haemorrhagic telangiectasia. *J Med Genet* 2003; 40: 865-871.

31. Abdalla SA, Gallione CJ, Barst RJ, Horn EM, Knowles JA, Marchuk DA, Letarte M, Morse JH. Primary pulmonary hypertension in families with hereditary haemorrhagic telangiectasia. *Eur Respir J* 2004; 23: 373-377.

32. Harrison RE, Berger R, Haworth SG, Tulloh R, Mache CJ, Morrell NW, Aldred MA, Trembath RC. Transforming growth factor-beta receptor mutations and pulmonary arterial hypertension in childhood. *Circulation* 2005; 111: 435-441.

33. Smoot LB, Obler D, McElhinney DB, Boardman K, Wu BL, Lip V, Mullen MP. Clinical features of pulmonary arterial hypertension in young people with an ALK1 mutation and hereditary haemorrhagic telangiectasia. *Arch Dis Child* 2009; 94: 506-511.

34. Girerd B, Montani D, Coulet F, Sztrymf B, Yaici A, Jais X, Tregouet D, Reis A, Drouin-Garraud V, Fraisse A, Sitbon O, O'Callaghan DS, Simonneau G, Soubrier F, Humbert M. Clinical outcomes of pulmonary arterial hypertension in patients carrying an ACVRL1 (ALK1) mutation. *Am J Respir Crit Care Med* 2010; 181: 851-861.

35. Lyle MA, Fenstad ER, McGoon MD, Frantz RP, Krowka MJ, Kane GC, Swanson KL. Pulmonary Hypertension in the setting of Hereditary Hemorrhagic Telangiectasia. *Chest* 2015; 149: 362-371.

36. Montani D, Price LC, Girerd B, Chinet T, Lacombe P, Simonneau G, Humbert M. Fatal rupture of pulmonary arteriovenous malformation in hereditary haemorrhagic telangiectasis and severe PAH. *Eur Respir Rev* 2009; 18: 42-46.

37. Chida A, Shintani M, Yagi H, Fujiwara M, Kojima Y, Sato H, Imamura S, Yokozawa M, Onodera N, Horigome H, Kobayashi T, Hatai Y, Nakayama T, Fukushima H, Nishiyama M, Doi S, Ono Y, Yasukouchi S, Ichida F, Fujimoto K, Ohtsuki S, Teshima H, Kawano T, Nomura Y, Gu H, Ishiwata T, Furutani Y, Inai K, Saji T, Matsuoka R, Nonoyama S, Nakanishi T. Outcomes of childhood pulmonary arterial hypertension in BMPR2 and ALK1 mutation carriers. *Am J Cardiol* 2012; 110: 586-593.

38. Fujiwara M, Yagi H, Matsuoka R, Akimoto K, Furutani M, Imamura S, Uehara R, Nakayama T, Takao A, Nakazawa M, Saji T. Implications of mutations of activin receptor-like kinase 1 gene (ALK1) in addition to bone morphogenetic protein receptor II gene (BMPR2) in children with pulmonary arterial hypertension. *Circ J* 2008; 72: 127-133.

39. Machado RD, Southgate L, Eichstaedt CA, Aldred MA, Austin ED, Best DH, Chung WK, Benjamin N, Elliott CG, Eyries M, Fischer C, Graf S, Hinderhofer K, Humbert M, Keiles SB, Loyd JE, Morrell NW, Newman JH, Soubrier F, Trembath RC, Viales RR, Grunig E. Pulmonary Arterial Hypertension: A Current Perspective on Established and Emerging Molecular Genetic Defects. *Hum Mutat* 2015; 36: 1113-1127.

40. Chaouat A, Coulet F, Favre C, Simonneau G, Weitzenblum E, Soubrier F, Humbert M. Endoglin germline mutation in a patient with hereditary haemorrhagic telangiectasia and dexfenfluramine associated pulmonary arterial hypertension. *Thorax* 2004; 59: 446-448.

41. Chen YJ, Yang QH, Liu D, Liu QQ, Eyries M, Wen L, Wu WH, Jiang X, Yuan P, Zhang R, Soubrier F, Jing ZC. Clinical and genetic characteristics of Chinese patients with hereditary haemorrhagic telangiectasia-associated pulmonary hypertension. *Eur J Clin Invest* 2013; 43: 1016-1024.

42. Girerd B, Montani D, Jais X, Eyries M, Yaici A, Sztrymf B, Savale L, Parent F, Coulet F, Godinas L, Lau EM, Tamura Y, Sitbon O, Soubrier F, Simonneau G, Humbert M. Genetic counselling in a national referral centre for pulmonary hypertension. *Eur Respir J* 2016; 47: 541-552.

43. Olivieri C, Lanzarini L, Pagella F, Semino L, Corno S, Valacca C, Plauchu H, Lesca G, Barthelet M, Buscarini E, Danesino C. Echocardiographic screening discloses increased values of pulmonary artery systolic pressure in 9 of 68 unselected patients affected with hereditary hemorrhagic telangiectasia. *Genet Med* 2006; 8: 183-190.

44. Sopena B, Perez-Rodriguez MT, Portela D, Rivera A, Freire M, Martinez-Vazquez C. High prevalence of pulmonary hypertension in patients with hereditary hemorrhagic telangiectasia. *Eur J Intern Med* 2013; 24: e30-4.

45. Stenmark KR, Meyrick B, Galie N, Mooi WJ, McMurtry IF. Animal models of pulmonary arterial hypertension: the hope for etiological discovery and pharmacological cure. *Am J Physiol Lung Cell Mol Physiol* 2009; 297: L1013-32.

46. Li C, Guo B, Ding S, Rius C, Langa C, Kumar P, Bernabeu C, Kumar S. TNF alpha down-regulates CD105 expression in vascular endothelial cells: a comparative study with TGF beta 1. *Anticancer Res* 2003; 23: 1189-1196.

47. Benza RL, Gomberg-Maitland M, Miller DP, Frost A, Frantz RP, Foreman AJ, Badesch DB, McGoon MD. The REVEAL Registry risk score calculator in patients newly diagnosed with pulmonary arterial hypertension. *Chest* 2012; 141: 354-362.

48. Pugh ME, Hemnes AR. Pulmonary hypertension in women. *Expert Rev Cardiovasc Ther* 2010; 8: 1549-1558.

49. Yaniv E, Preis M, Shevro J, Nageris B, Hadar T. Anti-estrogen therapy for hereditary hemorrhagic telangiectasia - a long-term clinical trial. *Rhinology* 2011; 49: 214-216.

50. Albinana V, Bernabeu-Herrero ME, Zarrabeitia R, Bernabeu C, Botella LM. Estrogen therapy for hereditary haemorrhagic telangiectasia (HHT): Effects of raloxifene, on Endoglin and ALK1 expression in endothelial cells. *Thromb Haemost* 2010; 103: 525-534.

51. Bonderman D, Nowotny R, Skoro-Sajer N, Adlbrecht C, Lang IM. Bosentan therapy for pulmonary arterial hypertension associated with hereditary haemorrhagic telangiectasia. *Eur J Clin Invest* 2006; 36 Suppl 3: 71-72.

52. Chang SA, Jang SY, Ki CS, Kang IS, Kim DK. Successful bosentan therapy for pulmonary arterial hypertension associated with hereditary hemorrhagic telangiectasia. *Heart Vessels* 2011; 26: 231-234.

53. Miyake R, Fujino T, Abe K, Hosokawa K, Ohtani K, Morisaki H, Yamada O, Higo T, Ide T. Pulmonary arterial hypertension associated with hereditary hemorrhagic telangiectasia successfully treated with sildenafil. *Int J Cardiol* 2016; 214: 275-276.

54. Shovlin CL, Tighe HC, Davies RJ, Gibbs JS, Jackson JE. Embolisation of pulmonary arteriovenous malformations: no consistent effect on pulmonary artery pressure. *Eur Respir J* 2008; 32: 162-169.

55. Vorselaars VM, Velthuis S, Mager JJ, Snijder RJ, Bos WJ, Vos JA, van Strijen MJ, Post MC. Direct haemodynamic effects of pulmonary arteriovenous malformation embolisation. *Neth Heart J* 2014; 22: 328-333.

56. Cottin V, Plauchu H, Bayle JY, Barthelet M, Revel D, Cordier JF. Pulmonary arteriovenous malformations in patients with hereditary hemorrhagic telangiectasia. *Am J Respir Crit Care Med* 2004; 169: 994-1000.

57. Remy-Jardin M, Dumont P, Brillet PY, Dupuis P, Duhamel A, Remy J. Pulmonary arteriovenous malformations treated with embolotherapy: helical CT evaluation of long-term effectiveness after 2-21-year follow-up. *Radiology* 2006; 239: 576-585.

58. Montani D, Price LC, Girerd B, Chinet T, Lacombe P, Simonneau G, Humbert M. Fatal rupture of pulmonary arteriovenous malformation in hereditary haemorrhagic telangiectasis and severe PAH. *Eur Respir Rev* 2009; 18: 42-46.

59. Shovlin CL, Sulaiman NL, Govani FS, Jackson JE, Begbie ME. Elevated factor VIII in hereditary haemorrhagic telangiectasia (HHT): association with venous thromboembolism. *Thromb Haemost* 2007; 98: 1031-1039.

Chapter five

Screening Children for Pulmonary Arteriovenous Malformations: evaluation of 18 years of experience

AE Hosman, EM de Gussem, WAF Balemans, A Gauthier, CJJ Westermann, RJ Snijder, MC Post, JJ Mager

Submitted

Abstract

Background: Hereditary Hemorrhagic Telangiectasia (HHT) is an autosomal dominant disease with multi-systemic vascular dysplasia. Early diagnosis through screening is important to prevent serious complications. How best to screen children of affected parents for pulmonary arteriovenous malformations (PAVMs) is often subject to debate. Transthoracic contrast echocardiogram (TTCE) is considered optimal in screening for PAVMs in adults. Guidelines for the screening of children are not specific, reflecting the lack of scientific evidence on the best method to use.

Objective: Aims of this study are (i) to evaluate our current screening method, consisting of history, physical examination, pulse oximetry and chest radiography and (ii) to assess whether postponing more invasive screening for PAVMs until adulthood is safe.

Methods: This is a prospective observational cohort study using a patient database.

Results: Over a period of 18 years (mean follow-up 9.21 years, SD 4.72 years), 436 children from HHT families were screened consecutively. 175/436 (40%) children had a diagnosis of HHT. PAVMs were detected in 39/175 (22%) children, 33/39 requiring treatment by embolotherapy. None of the screened children suffered any PAVM-associated complications with this screening method.

Conclusion This study shows that a conservative screening method during childhood is sufficient to detect large PAVMs and protect children with HHT for PAVM-related complications. Postponing TTCE until adulthood to detect any smaller PAVMs does not appear to be associated with major risk.

Introduction

Hereditary Hemorrhagic Telangiectasia (HHT), also known as Rendu-Osler-Weber syndrome, is an autosomal dominant inherited disease affecting approximately 1:5000 people.[1-3]

Most cases of HHT result from a pathogenic DNA sequence variant in the ENG, ACVLR1 or SMAD4 gene leading to multi-systemic vascular dysplasia.[4-7] Affected individuals often suffer from spontaneous epistaxis and gastrointestinal bleeding due to mucosal telangiectasia, frequently leading to iron deficiency anemia.[8] Pulmonary- (PAVM) and cerebral-arteriovenous malformations (CAVM) and hepatic vascular malformations (HVM) can cause profound morbidity and mortality.[9,10] Major PAVM-related complications include ischemic cerebral events and cerebral abscesses due to (septic or sterile) paradoxical emboli, hemoptysis and haemothorax.

A clinical diagnosis is based on the Curacao criteria which include recurrent spontaneous epistaxis, telangiectasia, visceral arteriovenous malformations and a first-degree family member with confirmed HHT.[11] The prevalence of vascular malformations depends on the type of mutation: ENG mutations are associated with a higher prevalence of PAVMs (62%) compared to ACVRL1 mutations (10%) and more CAVMs – 8-16% compared to 1-2% respectively.[10,12-15] Penetrance of symptoms varies greatly not only between disease causing mutations but also within families and especially with age. Affected children often do not show symptoms until after puberty which makes a clinical diagnosis difficult.[16] Consequently, unless genetic testing is performed, all children of parents with HHT are suspect for HHT. The HHT Guidelines recommend all children of parents affected by HHT should be screened regardless of age. However, no consensus is reached on which methodology is best to use stating that “the choice of screening tests should be decided on a case by case basis”.[17] This reflects the lack of scientific evidence on the relationship between the outcome of different early screening methods in children with their ultimate diagnosis and the detection of high-risk vascular abnormalities as adults. Potential benefits of early detection of serious malformations have to be weighed against the hazards and ethical concerns of screening for an often initially asymptomatic disease. Especially since HHT is an autosomal dominant disease and as such, half of the offspring of HHT patients will not have HHT. Literature clearly indicates that transthoracic contrast echocardiogram (TTCE) is optimal for detecting PAVMs in adults and probably also in the pediatric HHT population,

as a small study has shown.[18,19] However, TTCE requires intravenous access which can be stressful for children and raise ethical questions on the benefit. Any TTCE showing a shunt greater than a grade 1 will need to be followed up by chest CT scans, even when PAVMs are too small to be embolized.[10, 12, 17, 19]

In this study we review our approach (detailed history, physical examination, pulse oximetry and chest radiography) to screen for PAVMs in children. This screening method only detects large PAVMs, which will be treated if technically feasible. However, small PAVMs will be missed and will not be detected until full screening is performed at adulthood with a TTCE. The question is whether small asymptomatic PAVMs, that are not detected using this screening method during childhood, pose a relevant risk or can be left untreated until more sensitive screening can be carried out in adulthood.

The purpose of this study is to determine whether this conservative approach during childhood is sufficient and adequate to prevent PAVM related complications prior to adulthood. Our hypothesis is that a conservative mode of screening for PAVMs during childhood, in which small PAVMs may be missed, has no relevant consequences on morbidity or mortality.

Patients and Methods

This study is a partly retrospective (data before the year 2004) and prospective (data after the year 2004) observational cohort study determining the safety of a conservative screening method for PAVMs. Included were children between the ages of 0 and 18 years first screened for HHT between 1998 and 2015 in the St Antonius Hospital (Nieuwegein, The Netherlands), the Dutch National HHT Centre of Excellence. Here, children are generally screened every 5 years from 3 years of age onward using (i) history (to detect epistaxis or hypoxemia related symptoms like exercise intolerance, poor growth, headaches, etc), (ii) physical examination, capillary microscopy and nasal examination by an otorhinolaryngologist (to detect telangiectases, cyanosis, dyspnea or clubbing), (iii) pulse oximetry (to detect hypoxemia as a result of a potential pulmonary shunt) and (iv) a chest radiography (to screen for visible PAVMs).[20,21] Further investigation and imaging are performed when abnormalities are found: suspect history, saturation with pulse oximetry

<96%, or a density suspect for a PAVM on the chest radiography. When HHT is diagnosed or cannot be excluded, the patient is advised to use prophylactic antibiotics in case of bacteremic procedures, as recommended by the International HHT Guidelines since smaller PAVMs cannot be excluded.[17]

The HHT diagnosis is based on either the Curacao criteria and/or genetic testing.[11] Patients were categorized as (i) confirmed HHT diagnosis, (ii) possible HHT or (iii) no HHT. A diagnosis was considered confirmed when a child met three or more Curacao criteria or a positive genetic test. The HHT diagnosis was considered possible when a child met two Curacao criteria. Children were viewed not to have HHT if they met none or one Curacao criterion, or if genetic testing was negative for the confirmed family mutation.

All patients who reached adulthood where contacted to be re-screened as adults using TTCE following the 2009 HHT guidelines.[17] Patients who had reached adulthood but who had not been re-screened as adults were contacted by telephone and asked if they had suffered any HHT related complications including, brain abscess, cerebral hemorrhage, cerebral ischemic events , hemoptysis or hemothorax.

Patient follow-up years are defined as the number of years from moment of screening to the date of data collection.

The study was approved by the St Antonius Hospital Research Ethical Board.

Data were collected on October 5th, 2015 from the St Antonius Hospital HHT Database.

Results

In total 436 children were screened for HHT in the Dutch National HHT Centre between 1998 and 2015. Currently, 175 patients have a definite HHT diagnosis accounting for 1612 patient follow-up years (table 1). This includes 33 cases in whom the initial diagnosis was later changed from “no HHT” or “possible HHT” to “definite HHT” when symptoms became more prominent or when DNA testing revealed a HHT mutation. In 172/175 (98%) HHT patients the family mutation was known (table 1). In 11 cases the HHT mutation was confirmed in the child prior to initial screening. A possible HHT diagnosis was made in 125 patients. One hundred and thirty-six children did not have HHT based on genetic testing (42%) or based on the Curacao criteria (58%) (table 1). However, the latter might still

contain HHT patients as Curacao criteria are not sensitive enough to exclude the diagnosis at a young age. The 436 children had a mean age of 9 years at the time of screening. The HHT patients had a mean follow-up of 9.2 years (SD 4.7 years) and a mean screening incidence rate of 2.03 (SD 1.8).

	HHT	**Possible HHT**	**no HHT**
Total (number)	175	125	136
Mean age 1st screening (years)	9.6	9.8	9.8
SD (years)	4.3	4.3	4.4
Range (years)	1-17	3-17	2-17
Diagnosis based on			
Genetics, n (%)	47 (27%)		33 (24%)
Curacao criteria, n (%)	83 (47%)	125 (100%)	79 (58%)
Both, n (%)	45 (26%)		24 (18%)
Family mutation			
Endoglin, n (%)	122 (69%)	64 (51%)	78 (57%)
ACVRL1, n (%)	40 (23%)	42 (34%)	54 (40%)
SMAD4, n (%)	10 (6%)	2 (2%)	1 (1%)
Type unknown, n (%)	3 (2%)	17 (14%)	3 (2%)
Clinical evaluation			
Chest radiography , n (%)	168 (96%)	123 (98%)	130 (96%)
Chest radiography normal, n (%)	134 (77%)	117 (94%)	129 (95%)
Chest radiography suspect for PAVM, n (%)	22 (13%)	6 (5%)	1 (1%)
Chest CT, n (%)	50 (35%)	6 (5%)	1 (1%)
PAVM, n (%)	39 (25%)	0 (0%)	0 (0%)

Figure 1. patient characteristics: Complications after screening

Chest radiography was performed in 168/175 definite HHT patients (96%) and followed by a chest CT in 50/168 patients (30%) because of abnormalities evident on chest radiography (14/50, 28%), abnormal physical examination including pulse oximetry (28/50, 56%) (table 1) or both (8/50, 16%). In 39/50 (78%) patients who underwent a chest CT, a PAVM was

detected. Thirty-three/39 (85%) children with PAVMs underwent embolization; 29 of them before the age of 18 years. The 6/39 (9%) children who did not undergo embolization had PAVMs too small for embolization and are still being monitored. In total, 72 embolization sessions were performed in the 29 children. Nineteen children (66%) underwent multiple embolization sessions as a result of persistent perfusion or reperfusion of previously embolized PAVMs.

Eighty-eight/175 (50%) HHT patients had reached adulthood at the time of data analysis of whom 57/88 (65%) had been re-screened as an adult using TTCE, and chest CT scan when indicated. Of the 31 adults who had not yet been re-screened as adults, 23 could be contacted by telephone and none of them had suffered complications. The remaining 8 patients were considered lost to follow-up. (figure 1) Of the patients with possible HHT, 45 had reached adulthood, 9 were rescreened and 27 were contacted by phone; none of the 36/45 had suffered complications. Nine possible HHT patients were lost to follow-up.

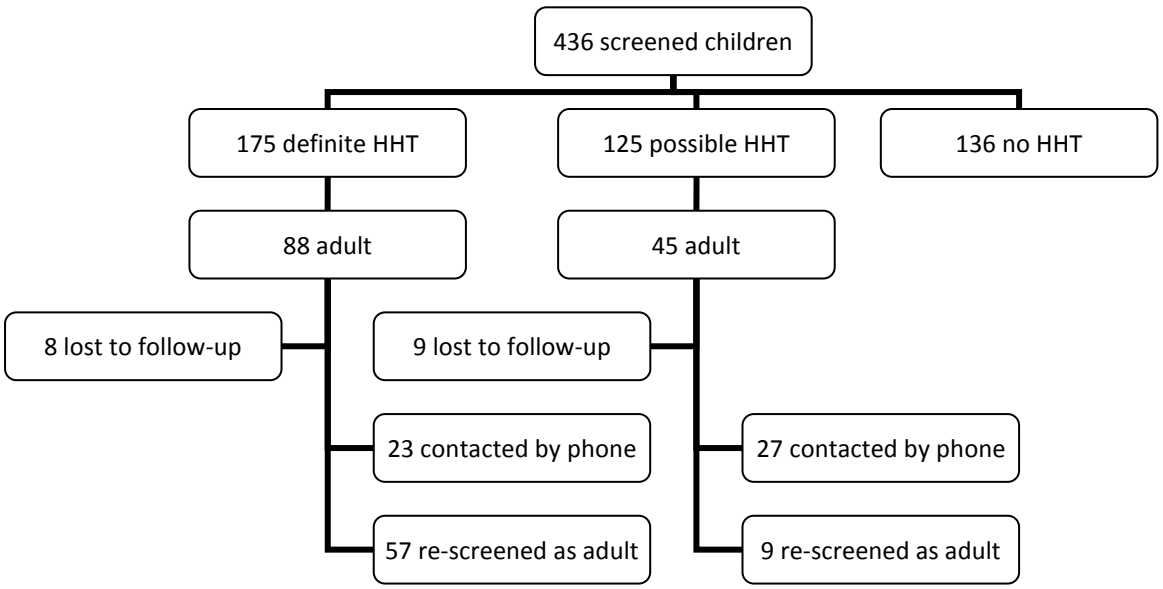


Figure 1. CONSORT flow diagram

In 6/57 (11%) definite HHT patients in whom no PAVM was detected through screening as a child, a PAVM was detected when they were rescreened as adults using a TTCE. They all underwent a chest CT based on a positive TTCE. In 1/57 (1.8%) patient, embolization was technically feasible. In the other 5/57 (8.8%) patients embolization was not technically

feasible initially and patients were monitored regularly. Two/57 (3.5%) were subsequently embolized several years later when the size of their PAVMs increased.

None of the 419 children in the follow-up cohort suffered a brain abscess, ischemic stroke or hemoptysis as a result of PAVMs during follow-up.

One patient, first screened at the age of 4 and diagnosed with HHT but with no pulmonary or neurologic symptoms, normal oxygen saturations and normal chest radiography, presented with consecutive transient symptoms of paresthesia of the left hand followed by paresthesia of the tongue at the age of 15 years. Initially she was suspected of suffering a thromboembolic cerebral event; however further evaluation revealed it was migraine accompagnée presumed to be associated with the PAVM found on the chest CT scan.[22,23] She had a history of migraines, which ceased after embolization of the PAVM.

Discussion

Historically the St Antonius hospital has systematically screened children for pulmonary involvement since 1998 using a patients history, physical examination, pulse oximetry and chest radiography. From 2004 onward, all adults screened for HHT undergo a TTCE to detect any pulmonary right to left shunts because of its high specificity, low complication risk and radiation free technique.[24] However, at that point in time no complications in children due to missed PAVMs had been recorded and the St Antonius hospital HHT team decided to continue this method of screening without TTCE and record all data on complications prospectively. The goal of this study was to evaluate the safety of this conservative approach in children of HHT families for the presence of pulmonary arteriovenous malformations, not to determine the sensitivity and specificity of our screening method.

PAVM-related complications include cerebral abscess (8%) and ischemic stroke (9 to 18%) due to paradoxical (septic or sterile) emboli.[10,25-27] There is consensus that these potentially life threatening complications make screening essential for the well-being of patients and treatment is warranted even when patients are asymptomatic.[28,29]

TTCE is proven to be more sensitive and specific than a chest radiography and oximetry and will also reveal small shunts which are often undetectable on high resolution chest CTs. A study on 92 pediatric HHT patients has shown that using TTCE as primary screening tool would allow more than 40% of the pediatric patients screened for PAVMs to be spared the radiation dose of chest CT.[19] Consequently, in 60% of that pediatric cohort the TTCE is followed by a chest CT scan to evaluate the cause of the shunt and feasibility of embolization. Eventually, in 14% of children embolotherapy was considered to be indicated.[19] This is in range of the embolization rate found in our cohort where 33/175 (19%) of children with HHT underwent embolization, but far less patients in our cohort were subjected to a chest CT, e.g. 30%.

When a PAVM does not qualify for embolization, follow-up using a chest CT is advised every 1 to 5 years.[17] The level of radiation exposure during a high resolution chest CT is 3,6mSv compared to 0,04mSv of a chest radiography and risk of radiation-induced cancer may be increased by as much as 24% (relative risk) in patients undergoing CT scanning in childhood. [30-33]

On the other hand, using chest radiography and oximetry small PAVMs will be overlooked. Consequently, because PAVMs cannot yet be ruled out antibiotic prophylaxis will be advised to patients who do not have any PAVMs and did not need antibiotic prophylaxis in hindsight. Furthermore, PAVM related complications may have important effects on neurologic development at a young age and devastating case-reports have been published.[34] However, it seems small PAVMs might not pose as much as a threat in children as in adults. In a study of 44 pediatric patients, respiratory and neurological complications occurred only in patients with large PAVMs.[35] Even more interesting, preliminary data of 164 pediatric patients shows PAVM related serious adverse events occurred only in patients with large PAVMs and a baseline oxygen saturation lower than 85%. [36] Those data coincide with the follow-up results of the cohort in this study where none of the screened HHT patients suffered any complications.

Although embolization of large PAVMs in adults reduces the risk of ischemic stroke and septic emboli, the long-term outcome of embolization of PAVMs in children with HHT is still far from clear and might not be as good as previously thought.

Recently, reperfusion rates of 70% have been reported for embolized PAVMs in young patients compared to 8-49% in adults.[37,38] Crawford et al. showed, in a retrospective study with 9.4 years of mean follow-up of 20 embolizations of PAVMs in 12 patients, 83% of reperfusion of PAVMs was due to collateral reperfusion.[39] They conclude that persistence of PAVMs after embolotherapy in children with HHT may be higher than previously suggested in studies in adults.

The strength of this study is that this is the first study on screening of children in this context with a sample size this large. Furthermore, we assume there is little selection bias considering the St Antonius hospital is the only HHT referral center in the Netherlands and children are referred to our center from other hospitals limiting the chance of children screened for HHT elsewhere. Limitations of this study are that only half of the HHT cohort had reached adulthood and only a third had been rescreened as adults using TTCE. Secondly, antibiotic prophylaxis is advised in all children where a right to left shunt cannot be ruled out which could impact the complication rate.

Conclusion

This study provides a good indication of the safety of our conservative method of screening for PAVMs, performed in a large cohort, over a long period of time. We recognize and accept that smaller PAVMs will be missed, but consider these small PAVMs at that stage as irrelevant. In 18 years of experience, with a mean follow-up of more than 9 years and more than 1600 patient years, none of our patients have suffered complications from these undetected PAVMs. We support continuing the screening of all children of HHT parents for relevant PAVMs but suggest that using less invasive and less stressful screening of children for pulmonary involvement, based on physical examination, pulse oximetry and a chest radiograph, instead of TTCE, is sufficient to prevent serious complications. Taking all the current evidence into consideration we argue that screening with TTCE can be postponed until adult age.

Acknowledgement

All authors would like to thank the SWORO foundation and ZonMW for funding.

References

1 Bideau A, Plauchu H, Brunet G, Robert J. Epidemiological investigation of Rendu-Osler disease in France: its geographical distribution and prevalence. *Popul*. 1989;44(1):3-22.

2 Westermann CJ, Rosina AF, De Vries V, de Coteau PA. The prevalence and manifestations of hereditary hemorrhagic telangiectasia in the Afro-Caribbean population of the Netherlands Antilles: a family screening. *Am J Med Genet A*. 2003;116A(4):324-328.

3 Kjeldsen AD, Vase P, Green A. Hereditary haemorrhagic telangiectasia: a population-based study of prevalence and mortality in Danish patients. *J Intern Med*. 1999;245(1):31-39.

4 McDonald MT, Papenberg KA, Ghosh S et al. A disease locus for hereditary haemorrhagic telangiectasia maps to chromosome 9q33-34. *Nat Genet*. 1994;6(2):197-204.

5 Johnson DW, Berg JN, Baldwin MA et al. Mutations in the activin receptor-like kinase 1 gene in hereditary haemorrhagic telangiectasia type 2. *Nat Genet*. 1996;13(2):189-195.

6 Shovlin CL, Hughes JM, Tuddenham EG et al. A gene for hereditary haemorrhagic telangiectasia maps to chromosome 9q3. *Nat Genet*. 1994;6(2):205-209.

7 Gallione CJ, Repetto GM, Legius E et al. A combined syndrome of juvenile polyposis and hereditary haemorrhagic telangiectasia associated with mutations in MADH4 (SMAD4). *Lancet*. 2004;363(9412):852-859.

8 Kjeldsen AD, Kjeldsen J. Gastrointestinal bleeding in patients with hereditary hemorrhagic telangiectasia. *Am J Gastroenterol*. 2000;95(2):415-418.

9 Shovlin CL. Hereditary haemorrhagic telangiectasia: pathophysiology, diagnosis and treatment. *Blood Rev*. 2010;24(6):203-219.

10 Velthuis S, Vorselaars VM, van Gent MW et al. Role of transthoracic contrast echocardiography in the clinical diagnosis of hereditary hemorrhagic telangiectasia. *Chest*. 2013;144(6):1876-1882.

11 Shovlin CL, Guttmacher AE, Buscarini E et al. Diagnostic criteria for hereditary hemorrhagic telangiectasia (Rendu-Osler-Weber syndrome). *Am J Med Genet*. 2000;91(1):66-67.

12 van Gent MW, Post MC, Snijder RJ, Westermann CJ, Plokker HW, Mager JJ. Real prevalence of pulmonary right-to-left shunt according to genotype in patients with hereditary hemorrhagic telangiectasia: a transthoracic contrast echocardiography study. *Chest*. 2010;138(4):833-839.

13 Bayrak-Toydemir P, McDonald J, Markewitz B et al. Genotype-phenotype correlation in hereditary hemorrhagic telangiectasia: mutations and manifestations. *Am J Med Genet A*. 2006;140(5):463-470.

14 Kjeldsen AD, Moller TR, Brusgaard K, Vase P, Andersen PE. Clinical symptoms according to genotype amongst patients with hereditary haemorrhagic telangiectasia. *J Intern Med*. 2005;258(4):349-355.

15 Letteboer TG, Mager JJ, Snijder RJ et al. Genotype-phenotype relationship in hereditary haemorrhagic telangiectasia. *J Med Genet*. 2006;43(4):371-377.

16 Plauchu H, de Chadarevian JP, Bideau A, Robert JM. Age-related clinical profile of hereditary hemorrhagic telangiectasia in an epidemiologically recruited population. *Am J Med Genet*. 1989;32(3):291-297.

17 Faughnan ME, Palda VA, Garcia-Tsao G et al. International guidelines for the diagnosis and management of hereditary haemorrhagic telangiectasia. *J Med Genet*. 2011;48(2):73-87.

18 Cottin V, Plauchu H, Bayle JY, Barthelet M, Revel D, Cordier JF. Pulmonary arteriovenous malformations in patients with hereditary hemorrhagic telangiectasia. *Am J Respir Crit Care Med*. 2004;169(9):994-1000.

19 Karam C, Sellier J, Mansencal N et al. Reliability of contrast echocardiography to rule out pulmonary arteriovenous malformations and avoid CT irradiation in pediatric patients with hereditary hemorrhagic telangiectasia. *Echocardiography*. 2015;32(1):42-48.

20 Mager JJ, Westermann CJ. Value of capillary microscopy in the diagnosis of hereditary hemorrhagic telangiectasia. *Arch Dermatol*. 2000;136(6):732-734.

21 Pasculli G, Quaranta D, Lenato GM et al. Capillaroscopy of the dorsal skin of the hands in hereditary hemorrhagic telangiectasia. *QJM*. 2005;98(10):757-763.

22 van Gent MW, Mager JJ, Snijder RJ et al. Relation between migraine and size of echocardiographic intrapulmonary right-to-left shunt. *Am J Cardiol*. 2011;107(9):1399-1404.

23 Koppen H, Palm-Meinders IH, Mess WH et al. Systemic right-to-left shunts, ischemic brain lesions, and persistent migraine activity. *Neurology*. 2016;86(18):1668-1675.

24 Nanthakumar K, Graham AT, Robinson TI et al. Contrast echocardiography for detection of pulmonary arteriovenous malformations. *Am Heart J*. 2001;141(2):243-246.

25 Woodall MN, McGettigan M, Figueroa R, Gossage JR, Alleyne CH,Jr. Cerebral vascular malformations in hereditary hemorrhagic telangiectasia. *J Neurosurg*. 2014;120(1):87-92.

26 Dupuis-Girod S, Giraud S, Decullier E et al. Hemorrhagic hereditary telangiectasia (Rendu-Osler disease) and infectious diseases: an underestimated association. *Clin Infect Dis*. 2007;44(6):841-845.

27 Mathis S, Dupuis-Girod S, Plauchu H et al. Cerebral abscesses in hereditary haemorrhagic telangiectasia: a clinical and microbiological evaluation. *Clin Neurol Neurosurg*. 2012;114(3):235-240.

28 Kjeldsen AD, Torring PM, Nissen H, Andersen PE. Cerebral abscesses among Danish patients with hereditary haemorrhagic telangiectasia. *Acta Neurol Scand*. 2014;129(3):192-197.

29 Shovlin CL, Jackson JE, Bamford KB et al. Primary determinants of ischaemic stroke/brain abscess risks are independent of severity of pulmonary arteriovenous malformations in hereditary haemorrhagic telangiectasia. *Thorax*. 2008;63(3):259-266.

30 Rijksinstituut voor Volksgezondheid en Milieu. Stralingsbelasting. . 2015;

31 Rijksinstituut voor Volksgezondheid en Milieu. Gemiddelde effectieve dosis per type CT-onderzoek. . 2013;

32 Rijksinstituut voor Volksgezondheid en Milieu. Gemiddelde effectieve dosis voor verschillende typen röntgenverrichtingen. . 2011;

33 Mathews JD, Forsythe AV, Brady Z et al. Cancer risk in 680,000 people exposed to computed tomography scans in childhood or adolescence: data linkage study of 11 million Australians. *BMJ*. 2013;346:f2360.

34 Shovlin CL. Circulatory contributors to the phenotype in hereditary hemorrhagic telangiectasia. *Front Genet*. 2015;6:101.

35 Giordano P, Lenato GM, Suppressa P et al. Hereditary hemorrhagic telangiectasia: arteriovenous malformations in children. *J Pediatr*. 2013;163(1):179-86.e1-3.

36 Ibrahim SM, Henderson KJ, Rashid S, Hussein SM, White Jr. RI, Pollak JS. Correlation of oxygen saturation and serious adverse events in pediatric patients with documented or suspected pulmonary arteriovenous malformation. [abstract]. *Angiogenesis*. 2015;18:537.

37 Shimohira M, Kawai T, Hashizume T et al. Reperfusion Rates of Pulmonary Arteriovenous Malformations after Coil Embolization: Evaluation with Time-Resolved MR Angiography or Pulmonary Angiography. *J Vasc Interv Radiol*. 2015;26(6):856-864.e1.

38 Mager JJ, Overtoom TT, Blauw H, Lammers JW, Westermann CJ. Embolotherapy of pulmonary arteriovenous malformations: long-term results in 112 patients. *J Vasc Interv Radiol*. 2004;15(5):451-456.

39 Crawford H, MD, McDonald, Jamie, MS,LGC, Hardman, Rulon L, MD, PhD, O'Hara R, MD. Long-term embolotherapy treatment outcomes of pulmonary arteriovenous malformation in children with hereditary hemorrhagic telangiectasia. *Angiogenesis.* 2015;18(4):538.

Chapter six

Antiplatelet and anticoagulant Agents in Hereditary Haemorrhagic Telangiectasia

HL Devlin, AE Hosman, CL Shovlin

New England Journal of Medicine. 2013 Feb 28;368(9):876-8

Hereditary haemorrhagic telangiectasia (HHT) leads to haemorrhage, particularly nosebleeds (epistaxis), because of vascular telangiectasia and arteriovenous malformations.[1] Thrombotic conditions, including venous thromboemboli and ischemic stroke, are relatively common in patients with HHT.[2,3] These emergencies are managed by clinicians who may not be very familiar with HHT. Current guidance on the use of antiplatelet and anticoagulant agents in HHT is based on anecdotal evidence and expert opinion.[4]

To evaluate the effects of antiplatelet and anticoagulant agents on haemorrhage in HHT, relevant questions were included as a subsection of a broad international survey (www.imperial.ac.uk/medicine/HHTsurvey2012) that was approved by the National Research Ethics Service East Midlands–Derby 1 Committee, United Kingdom.

The unbiased survey methods are reported elsewhere and described in the Supplementary Appendix, available with the full text of this letter at NEJM.org.[5] A total of 1302 persons responded to the online survey, including 973 with a diagnosis of HHT that was supported by sufficient diagnostic features in the survey. The ages of the respondents ranged from 14 to 89 years (median, 53.1), and 655 of 973 were female (67.3%).

Of the 973 respondents with HHT, 700 (71.9%) stated they had never used antiplatelet or anticoagulant agents. Of these 700 respondents, 381 (54.4%) reported that they were advised by a doctor not to use these agents because; they had HHT (in 334 patients [87.7%]), they already had bleeding (in 191 patients [50.1%]), or they had an arteriovenous malformation (in 75 patients [19.7%]). Five respondents stated that treatment for myocardial infarction had been withheld from a relative because of a diagnosis of HHT in the relative, and this had contributed to the relative's death.

A total of 273 participants with HHT (28.1%) reported the use of antiplatelet or anticoagulant therapy (Table 1). As expected, high proportions of participants reported worsening of nosebleeds (Table 1) that sometimes led to hospitalization, discontinuation of therapy, or both. Five respondents stated that treatment for myocardial infarction had led to haemorrhages that worsened the outcome in relatives with HHT who had later died.

It was surprising, however, that 153 of 379 patients with HHT who received antiplatelet or anticoagulant therapy (40.4%) reported no change in their nosebleeds. Nine of 379 patients (2.4%) reported an improvement in their nosebleeds (Table 1). Lower-dose aspirin (75 mg), heparin, and other anticoagulant agents appeared to aggravate nosebleeds less than higher-dose aspirin (300 mg) or warfarin (Table 1). In addition, more haemorrhagic events other than nosebleeds were reported by patients who received anticoagulant agents than by patients who received antiplatelet agents (19.5% vs. 8.8%) (P=0.003 by Fisher's exact test). Nevertheless, no change or an improvement in nosebleeds was reported by 43 of 93 patients who received heparin (46%) and by 21 of 55 patients who received warfarin (38%).

These data indicate unexplained and wide variation in the side effect profiles with respect to haemorrhage associated with the use of anticoagulant and antiplatelet therapies in patients with HHT. Lower-dose agents, particularly antiplatelet agents, did not appear to be associated with haemorrhage in high proportions of patients. These findings provide support for the use of antiplatelet or anticoagulant agents, with caution, in patients with HHT if there is a very strong indication for their use.

Table 1. Reported Effects of Antiplatelet and Anticoagulant Agents in 273 Patients with Hereditary Haemorrhagic Telangiectasia.

Agent	Nosebleeds the same		Nosebleeds Worse		Other haemorrhages		No haemorrhage	
	No. of patients / total no.	% (95% CI)	No. of patients / total no.	% (95% CI)	No. of patients / total no.	% (95% CI)	No. of patients / total no.	% (95% CI)
Antiplatelet agents								
Aspirin								
300mg	28/80	35.0 [24.3, 45.7]	52/80	65.0 [54.3, 75.7]	4/80	22.2 [0.1, 10.1]	74/80	94.9 [89.9, 99.9]
75mg	58/127	45.6 [36.9, 54.5]	67/127	52.8 [44.0, 61.6]	14/127	11.1 [5.6, 16.7]	112/127	88.9 [83.3, 94.5]
Other antiplatelet agents †	10/22	45.5 [22.9, 68.1]	dec-22	54.6 [32.0, 77.1]	2/22	9.1 [-4.0, 22.1]	20/22	90.9 [779, 104.0]
Anticoagulant agent								
Heparin								
Subcutaneous	22/52	42.3 [28.4, 56.2]	28/52	53.9 [39.8, 67.9]	11/52	21.2 [9.7, 32.6]	41/52	78.9 [67.4, 90.3]
Intravenous	16/41	39.0 [23.4, 54.6]	22/41	53.7 [37.7, 69.6]	8/41	19.5 [6.9, 32.2]	33/41	80.5 [67.8, 93.2]
Warfarin	19/55	34.6 [21.6, 47.5]	34/55	61.8 [48.6, 75.1]	10/55	18.5 [7.8, 29.2]	44/55	81.5 [70.8, 92.2]
Dabigatran.	0/2		2/2	1.0 [1.0,1.0]	0/0	0	2/2	1 [1.0,1.0]

All antiplatelet therapies	96/229	41.6 [35.2, 48.0]	131/229	56.7 [50.3, 63.2]	20/229	8.9 [5.1, 12.6]	206/229	91.2 [87.4, 94.9])
All anticoagulant therapies	57/150	38.5 [30.6, 46.5]	86/150	58.1 [50.1, 66.2]	29/150	19.5 [13.0, 25.9]	120/150	80.5 [74.1, 87.0]
All agents	153/379	40.4 [35.4, 45.3]	217/379	57.3 [52.3, 62.3]	49/379	13.1 [9.6, 16.5]	326/279	86.9 [83.5, 90.4]

* Some patients received more than one agent.

† Other antiplatelet agents were abciximab, clopidogrel, dipyridamole, prasugrel, ticlodipine and tirofiban. Among patients who received both aspirin and clopidogrel, the number of patients who reported that nosebleeds were worse with aspirin than with clopidogrel was equal to the number of patients who reported that nosebleeds were worse with clopidogrel than with aspirin. Nine patients noted an improvement in nosebleeds associated with the following: aspirin at a dose of 75mg (two patients), subcutaneous heparin (two patients), intravenous heparin (three patients), or warfarin (two patients).

References

1. Shovlin CL. Hereditary haemorrhagic telangiectasia: Pathophysiology, diagnosis and treatment. Blood Rev 2010;24:203-219.

2. Livesey JA, Manning RA, Meek JH, et al. Low serum iron levels are associated with elevated plasma levels of coagulation factor VIII and pulmonary emboli/deep venous thromboses in replicate cohorts of patients with hereditary haemorrhagic telangiectasia. Thorax 2012;67:328-333.

3. Shovlin CL, Jackson JE, Bamford KB, et al. Primary determinants of ischaemic stroke/brain abscess risks are independent of severity of pulmonary arteriovenous malformations in hereditary haemorrhagic telangiectasia. Thorax 2008;63:259-266.

4. Faughnan ME, Palda VA, Garcia-Tsao G, et al. International guidelines for the diagnosis and management of hereditary haemorrhagic telangiectasia. J Med Genet 2011;48:73-87.

5. Silva BM, Hosman AE, Devlin HL, Shovlin CL. Lifestyle and dietary influences on nosebleed severity in hereditary hemorrhagic telangiectasia. Laryngoscope, 2013 May; 123(5):1092-9.

Chapter seven

Follow up of Thalidomide treatment in patients with Hereditary Haemorrhagic Telangiectasia

AE Hosman, CJJ Westermann', R Snijder, F Disch, CL Mummery, JJ Mager

Rhinology. 2015 Dec;53(4):340-4

ABSTRACT

Background: Patients with a hereditary vascular disorder called Rendu-Osler-Weber syndrome (Hereditary Haemorrhagic Telangiectasia, HHT) haemorrhage easily due to weak-walled vessels. Haemorrhage in lungs or brain can be fatal but patients suffer most from chronic and prolonged nosebleeds (epistaxis), the frequency and intensity of which increases with age. Several years ago, it was discovered serendipitously that the drug Thalidomide had beneficial effects on the disease symptoms in several of a small group of HHT patients: epistaxis and the incidence of anaemia were reduced and patients required fewer blood transfusions. In addition, they reported a better quality of life. However, Thalidomide has significant negative side effects, including neuropathy and fatigue.

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

Results: Only a minority of patients had continued taking the drug despite its beneficial effects on their symptoms and that the side effects were the primary reason to stop.

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

Introduction

Thalidomide, a drug also known as Softenon, is by many associated with its notorious side-effects on foetal development in humans in the 1960s. Babies born to mothers taking Thalidomide during early pregnancy for nausea often failed to develop limbs and had multiple other severe birth defects. Thalidomide was discredited but has more recently been reintroduced and is now successfully used in the treatment of multiple myeloma and other afflictions.[1] It is now also being investigated and used in the treatment of symptoms associated with Hereditary Haemorrhagic Telangiectasia (HHT, also known as Rendu-Osler-Weber syndrome). This is an autosomal dominant disease is characterised by multi-systemic vascular lesions, afflicting about 1 in 5000 people.[2-4] It is caused by mutations in receptors of the transforming growth factor ß family, most notably endoglin and activin receptor like kinase type 1 (ALK1), which are expressed on endothelial cells of blood vessels.[5] Symptoms include telangiectasia, blood vessel abnormalities typically evident around lips, oral mucosa and fingertips. Gastrointestinal and nasal bleeding often lead to chronic iron deficiency, anaemia, and complications due to liver-, lung- and brain arteriovenous malformations.[6] For patients, anaemia due to gastrointestinal bleeding and social limitations due to nasal bleeding have the most effect on the quality of daily life. Animal and patient studies have shown Thalidomide has a positive effect on HHT related symptoms.[7,8,13-18] However, Thalidomide is also known to coincide with severe side-effects including neuropathy, severe skin reactions, angina and dyspnoea, oedema, drowsiness, general malaise and tremor. It has also been reported to cause deep vein thrombosis in one patient using Thalidomide to control nosebleeds secondary to HHT. The mechanism of action of Thalidomide in patients with HHT has been extensively discussed in Lebrin et al.[7] In short, Thalidomide promotes vessel maturation and enhances the coverage of smooth muscle cells around vessels, making vessels more stable and lowering nosebleed frequency in HHT subjects. The cohort of patients included in the first study was relatively small and the patients served as their own (historical) controls.[7] These patients and others who were prescribed Thalidomide later, were, however, never systematically followed up to evaluate the long term outcome, severity of side effects and drug compliance. The study here was designed for this purpose with view to considering a larger scale, double blinded trial.

Methods

Patient cohort

The St. Antonius Hospital is the only HHT-centre in the Netherlands and most patients in the Netherlands are known to the hospital or are treated by the group of specialists authoring this paper. In general, HHT is diagnosed clinically using the Curacao criteria in which patients are scored for evidence of nosebleeds, mucocutaneous telangiectasia, visceral involvement (most commonly gastrointestinal telangiectasia, or AVMs at specified sites), and a family history of HHT.[5] Severely affected patients are often referred to the St Antonius Hospital for treatment of nosebleeds if the frequency of blood transfusions, degree of anaemia is excessive. The patients who have also often had nasal cauterization without prolonged success are those that have been offered Thalidomide prior to the final option of the Saunder's operation. This means that the patients included in this study are a subset of all patients in the Netherlands but are among the most severely affected subset. In 2014 there were 1283 HHT patients on record in the St. Antonius Hospital, of whom less than 1% were treated with Thalidomide. All patients who received Thalidomide between 2009 and 2014 made up the cohort for this study as no patients in the Netherlands are treated with Thalidomide elsewhere than the St Antonius Hospital. In order to capture information on patient experience with Thalidomide, a paper questionnaire was used to collect data. The data was returned anonymously and was analysed using Excel and Graph Pad Prism 5 (GraphPad Inc, US). The questionnaire was composed of general health questions, symptoms of HHT before taking Thalidomide, after 3 months and currently or just before ceasing Thalidomide use and personal experience. In an attempt to assess the severity of symptoms objectively, HHT patients provided information on the number of nosebleeds per week, the mean duration of each nosebleed and the number of blood transfusions needed due to severe anaemia.

The questionnaire was a retrospective collection of data but 4 patients had already been included in the initial study (short term follow up) published in Nature Medicine (2010).[7] In that study, patients kept a detailed diary of nosebleeds (frequency and severity) in the three months before and after treatment. This is routine procedure for this subgroup of patients that are particularly severely affected. These patients included in the present study are therefore used to monitoring their own symptom severity. Furthermore, even

though most of the data is subjective, the number of blood transfusions received in a specific period is objective.

Ethical approval

The questionnaire was approved for use in this study by the Medical Ethical Committee of the St Antonius Hospital, Nieuwegein, The Netherlands.

Results

Nineteen patients met the criteria from records to be included. However, 4 patients had died from non-HHT related causes, 1 patient declined to participate and 2 did not respond, leaving 12 participants. The cohort was made up out of 6 males and 6 females with a median age of 69.5 years [IQR 60.0-80.5]. Subjects had been treated daily with 50 to 100 mg of Thalidomide orally. The most common types of treatment patients had undergone previously are reported: 11 of 12 patients used iron tablets for iron deficiency anaemia, 8 had undergone argon laser treatment, 7 had used Sofradex, 6 had been subjected to cauterisation, 4 had undergone embolization, and 3 had nasal epithelium transplantation (Saunder's operation). Thalidomide was used for a median time of 7 months [IQR 3.0-23.3]. Reported influence on frequency and severity of nosebleeds is shown in table 1. The number of blood transfusions each patient underwent in the study period is also given in table 1. This is an objective measure of disease severity.

In the questionnaire, differences in symptoms during Thalidomide use could be scored by the patients as: much worse, worse, no difference, better and much better (Figure 1). Three patients rated their nosebleeds "better" during Thalidomide use, and 7 patients thought their nosebleeds were "much better". Five patients rated oral bleedings or bleeding located in the facial area as much better. Remaining patients did not notice any difference at these sites. One patient reported having had a liver haemorrhage even though this is an exceptionally rare complication. However, according to our patient records, none of the participating patients has ever had a liver haemorrhage raising doubt on whether this patient understood the question.

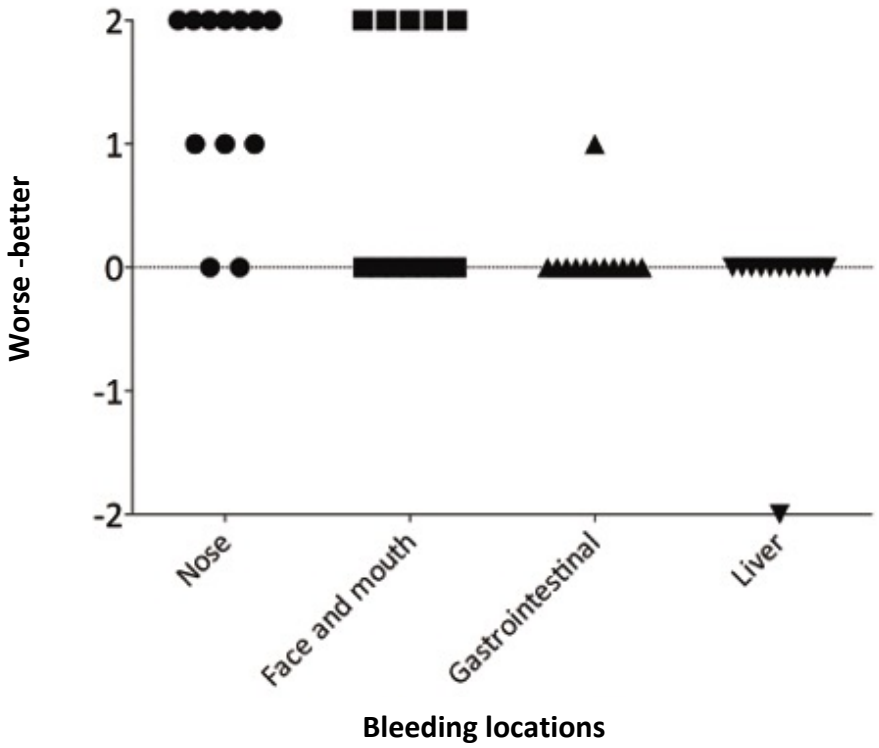


Figure 1. Difference in bleeding in nose, facial and oral area, gastrointestinal and liver haemorrhage using Thalidomide scored -2 “much worse”, -1 “worse”, 0 “ no difference”, 1 “ better”, 2 “much better”.

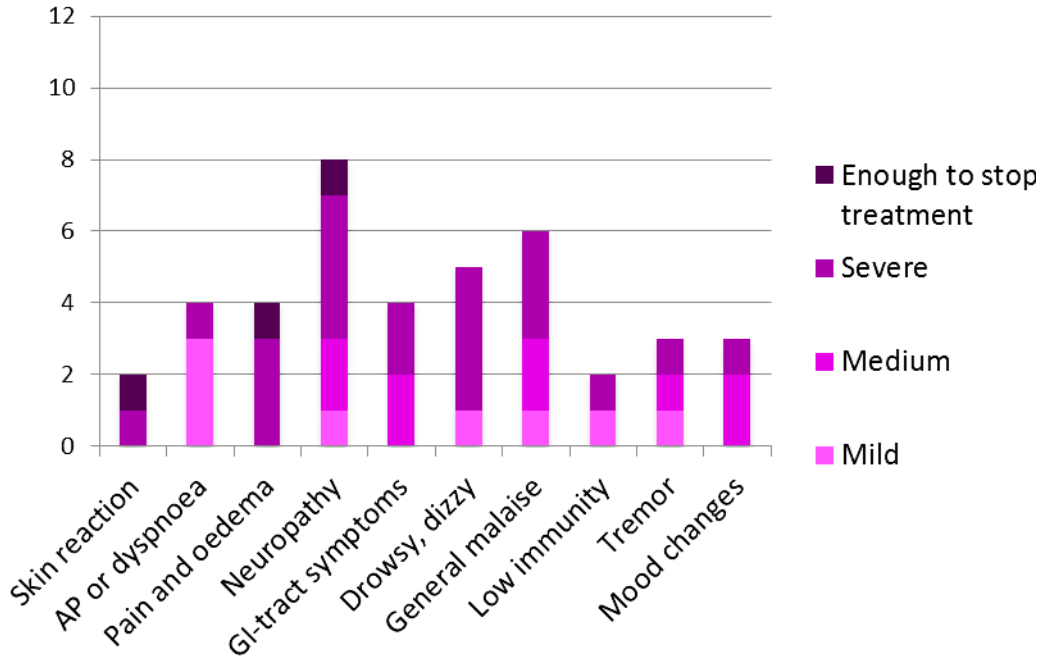


Figure 2. Side- effects experienced by patients. Side-effects are shown on the x-axis, the number of patients on y-axis. Degree of severity is color-coded.

Four patients were still using Thalidomide after respectively 3, 27, 30 and 50 months. One patient stopped because of the lack of effect on their symptoms and the remaining 7 patients discontinued use because of side-effects (see Figure 2).

When asked if the advantages of Thalidomide weighed up against the disadvantages, 5 participants report they agreement, 3 returned neutral answers and 4 disagreed. Nine out of 12 patients would recommend trying Thalidomide to other HHT patients.

No.	Before Thalidomide use				After 3 months of Thalidomide				Current situation of just before stopping			
	Severity	Nosebleeds /week	Duration	Transfusion	Severity	Nosebleeds /week	Duration	Transfusion	Severity	Nosebleeds /week	Duration	Transfusion
1	***				**							
2	***	15	30		**	7	20	0	*			
3	****		30-60	1	**	1	15-30	0	**	0	0	0
4	****	29	0	3	**	2	5	0	*	2	5	0
5	****	50	15-90	12	**	13	15	4	*	2	5	4
6	***	210	1-5	2	**	10	1-5	2		2	1-5	0
7	****	45	5-45	10	***	20	10-15	6				0
8	****	4	3-60	2	**	2	10	0	*	1	1-2	0
9	****	1	10-60		***		50					
10	****	7		0	**	5		0				
11	***	7										
12	****	40	5-30	0	**	15	5	0	**	15	5	0
Mean		40.8	25	3.75		7.75	18	1.33		5.67	3.8	0.57

Table 1. Severity of epistaxis.

Severity score: * mild, ** medium, *** severe, **** extreme. N/w: number of epistaxis per week. Duration of epistaxis is measured in minutes. “Transfusions” indicates the number of blood transfusions received in that period.

Discussion

The majority of HHT patients included here had tried an array of therapies for reducing nosebleeds by the time they were considered for Thalidomide treatment. These included compression techniques, bilateral embolization, surgical arterial ligature, laser therapy, sclerosing agents, electrocautery, septodermoplasty, adjuvant medical treatments including oestrogens, progesterone and antifibrinolytic agents. Most relieve symptoms only temporarily so that there is a significant unmet medical need for a more stable, less invasive alternatives.[8,9]

Several reports have described beneficial effects of Thalidomide on some of the symptoms of HHT: nosebleeds were reduced in some cases and as a direct or indirect result secondary symptoms, caused by blood loss, were also reduced.[7] Similar results were described by Balduini et al in a preliminary study of 11 patients.[18] Reduced nosebleed frequency was observed in 5 within a few weeks of initiating treatment. In addition, *in vivo* studies in mutant mice heterozygous for the Endoglin gene and in HHT patients as well *in vitro* studies, have indicated a mechanism through which Thalidomide could act in promoting vessel stability: up regulation of the expression of platelet derived growth factor-β (PDGF-β) by endothelial cells results in enhanced recruitment of pericytes expressing PDGF receptors to the vessel wall. The pericytes subsequently differentiate to smooth muscle cells as a result of this interaction with endothelial cells and improved vessel maturation occurs. In addition, Thalidomide increases the number of smooth muscle cells in mice and in the nasal epithelium of HHT patients.[7] Furthermore, anti-inflammatory and immunosuppressive properties of Thalidomide have been hypothesized as preventing *de novo* pulmonary arteriovenous malformation development.[10] This would bode well for optimism in the use of Thalidomide to treat HHT and, indeed, the vast majority of participants in this study reported an improvement in their symptoms. However, side-effects seemed to be a limiting factor for use in practice. Neuropathy was reported in 8 out of 12 patients of whom more than half reported the neuropathy to be severe. Although this study group in total is very limited in size, severe side effects have been reported in patients with multiple myeloma treated with Lenalidomide by Katodritou et al.[11] Adverse events were reported in 68.9 % of patients (myelosuppression in 49.4 %) and 12.7 % of patients needed hospitalization. Peripheral neuropathy was reported in 2.5

% of patients and deep vein thrombosis in 5.7 %. Almost 39% of patients discontinued the treatment completely due to side-effects. Overall, 34 cases in ten studies of HHT patients treated with Thalidomide and Bevacizumab have been reported. Follow-up varies from 1 to 60 months (mean 13.5 months).[6,12-20] Although the majority of cases reported Thalidomide as being a successful treatment with reduced blood transfusions and epistaxis, adverse reactions were often underreported or not mentioned. One patient developed deep vein thrombosis, although it is questionable whether the Thalidomide was directly responsible.[16] Although no other major adverse events are reported, some patients discontinued the treatment without further documentation on motives.

Overall, Thalidomide appears to be beneficial for treating HHT even though it is effectively one of many Thalidomide side effects ("off target use"). Yet the severity of the side–effects would likely be a disincentive to begin such a trial where the chance that patients would continue using the drug long term are somewhat limited. Nevertheless, in particularly severe cases where other treatments have been exhausted, there may be justification for incidental use. Whilst most insurance companies or health systems would not cover the cost of a drug under these circumstances, the costs of Thalidomide are low and for the patients in this study, the costs were not prohibitive enough to opt out of the study.

Acknowledgements

The authors are grateful to the patients at the Netherlands HHT Expertise Centre for their willingness to participate in this study. In addition, we thank Lucia Mijs, Daniëlle Barendregt and Margré Kooman of the CGS Prins Maurits School in Middelharnis for their initiation and enthusiastic participation in this project and their contribution to the questionnaire and its analysis. HHT studies at the St Antonius Hospital and LUMC are supported by the SWORO Foundation for HHT patients

References

1. Eriksson T, Björkman S, Höglund P: Clinical pharmacology of thalidomide. Eur J Clin Pharmacol. 2001 Aug;57(5):365-76.

2. Kjeldsen AD, Vase P, Green A: Hereditary haemorrhagic telangiectasia: population-based study of prevalence and mortality in Danish patients. J Intern Med 1999, 245:31–39

3. Dakeishi M, Shioya T, Wada Y et al: Genetic epidemiology of hereditary hemorrhagic telangiectasia in a local community in the northern part of Japan. Hum Mut 2002, 19:140–148.

4. Plauchu H, de Chadarévian JP, Bideau A, Robert JM: Age-related clinical profile of hereditary hemorrhagic telangiectasia in an epidemiologically recruited population. Am J Med Genet 1989, 32(3):291–7.

5. Shovlin CL. Hereditary haemorrhagic telangiectasia: pathophysiology, diagnosis and treatment. Blood Rev. Nov;24(6):203-19. doi: 10.1016/j.blre.2010.07.001. Epub 2010 Sep 25. Review.

6. Govani FS, Shovlin CL: Hereditary haemorrhagic telangiectasia: a clinical and scientific review. Eur J Hum Genet 2009, 17:860–71

7. Lebrin F, Srun S, Raymond K et al: Thalidomide stimulates vessel maturation and reduces epistaxis in individuals with hereditary hemorrhagic telangiectasia. Nat Med. 2010 Apr;16(4):420-8.

8. Franchini M, Frattini F, Crestani S, Bonfanti C.: Novel treatments for epistaxis in hereditary hemorrhagic telangiectasia: a systematic review of the clinical experience with thalidomide.. J Thromb Thrombolysis. 2013 Oct;36(3):355-7. doi: 10.1007/s11239-012-0840-5. Review.

9. Silva BM, Hosman AE, Devlin HL, Shovlin CL: Lifestyle and dietary influences on nosebleed severity in hereditary hemorrhagic telangiectasia. Laryngoscope. 2013 May;123(5):1092-9. doi: 10.1002/lary.23893. Epub 2013 Feb 12.

10. Lacout A, Marcy PY, El Hajjam M, Lacombe P.: Pulmonary arteriovenous malformations etiologies in HHT patients and potential utility of thalidomide. Med Hypotheses. 2013 May;80(5):587-8

11. Katodritou E, Vadikolia C, Lalagianni C et al: "Real-world" data on the efficacy and safety of lenalidomide and dexamethasone in patients with relapsed/refractory multiple myeloma who were treated according to the standard clinical practice: a study of the Greek Myeloma Study Group. Ann Hematol. 2014 Jan;93(1):129-39. 17.

12. Kursting R: Using Thalidomide in a patient with epithelioid leiomyosarcoma and Osler-Weber-Rendu disease. Oncology (Wilston park) 16: 21-24. 2002

13. Kersemeakers MAH, Westermann CJJ, Mager JJ, De Weerdt O, Disch FMJ, Snijder RJ: Thalidomide for the treatment of epistaxis in hereditary hemorrhagic telangiectasia. Hematol Meet Rep 1: 22-23. 2007

14. Buscarini E, Manfredi G, Gazzaniga P et al: on behalf of HHT-NET Thalidomide for the treatment of chronic severe bleeding in hereditary hemorrhagic telangiectasia. Hematol Meet Rep 3: 20-21. 2009

15. Gossage JR, Chamberlain SM, Sridhar S, Kumar A: An interim report of thalidomide treatment of recurrent angioectasia related gastrointestinal bleeding. Hematol Meet Rep 2009; 3:21

16. Penaloza A, Vekemans MC, Lambert C, Hermans C: Deep vein thrombosis induced by thalidomide to control epistaxis secondary to hereditary hemorrhagic telangiectasia. Blood Coagul Fibrinolysis 22: 616-618. 2011

17. Alam MA, Sami S, Babu S: Successful treatment of bleeding gastro-intestinal angiodysplasia in hereditary haemorrhagic telangiectasia with thalidomide BMJ Case Rep. 2011; 10:1136

18. Balduini CL, Pallega F, Bellistri F et al: Efficacy of thalidomide in treatment of severe recurrent epistaxis in hereditary hemorrhagic telangiectasia (HHT): ongoing results of a prospective study. Blood Transfus 10(4): s36. 2012

19. Chavan A, Schumann-Binarsch S, Luthe L et al: Systemic therapy with bevacizumab in patients with hereditary hemorrhagic telangiectasia (HHT). Vasa 42:106-10 mar 2013

20. Chen CH, Hsu HH, Hu RH et al: Long-term therapy with thalidomide in hereditary hemorrhagic telangiectasia: case report and literature review. J Clin Pharmacol 52: 1436-1440. Sept 2012

Chapter eight

Lifestyle and Dietary Influences on Nosebleed Severity in Hereditary Haemorrhagic Telangiectasia

BM Silva, AE Hosman, HL Devlin, CL Shovlin

Laryngoscope, 2013 May; 123(5):1092-9

ABSTRACT

Objectives/Hypothesis: To identify factors influencing the severity of epistaxis in hereditary haemorrhagic telangiectasia (HHT).

Study Design: Participants with and without HHT were recruited from a specialist service and online following advertisement by the HHT Foundation International. Both groups were asked to complete a nonbiased questionnaire.

Methods: The reported effects of specific treatments or lifestyle factors on epistaxis were assigned positive values if beneficial, negative values if detrimental, or zero if ‘‘no difference’’ and were summed to enable statistical analysis.

Results: Epistaxis affected 649 of 666 (97%) participants with HHT and was significantly more frequent than in control participants. Specialist invasive treatments were reported as beneficial, laser therapy more frequently than cauterization. Medical treatments commonly used for HHT epistaxis (female hormones, antioestrogens, tranexamic acid, aminocaproic acid, nasal creams, and bevacizumab) also had significantly positive (beneficial) scores. Lifestyle and dietary factors were generally detrimental, but room humidification, nasal lubrication, and saline treatments were all reported as beneficial (95% confidence intervals greater than zero). Multiple food items were volunteered as being detrimental to epistaxis. The most frequently reported items were alcohol (n = 45; 6.8% of participants) and spices (n = 26, 3.9% of participants). Remaining foods reported to exacerbate epistaxis were also found to be high in salicylates (including red wine, spices, chocolate, coffee, and certain fruits), natural antiplatelet activity (garlic, ginger, ginseng, ginkgo biloba, and vitamin E15), or omega-3 acids (oily fish, salmon).

Conclusions: This study supports existing treatments and suggests lifestyle and dietary manoeuvres that may also improve nosebleeds in HHT.

Introduction

Hereditary haemorrhagic telangiectasia (HHT), also known as Osler-Rendu-Weber syndrome, is an autosomal dominant disorder that leads to the development of abnormal vessels.[1] The most common clinical feature is epistaxis (nosebleeds), which often occurs on a daily basis.[2] Other disease manifestations include gastrointestinal telangiectasia and arteriovenous malformations, particularly in the pulmonary, hepatic, and cerebral circulations. Careful epidemiologic studies reveal that HHT affects approximately 1 in 5,000 individuals.[3,4] HHT is caused by mutations in the endoglin, ALK1/ACVRL1, or SMAD4 genes.[5] The mutated genes encode proteins that mediate transforming growth factor-beta superfamily signalling in vascular endothelial cells. Disruption of signalling pathways in HHT pathogenicity results in remodelled vasculature, with dilation of venules and concomitant arteriovenous communications.[6,7] The current perspective of pathogenesis is that HHT vessels result from aberrant responses to injury-induced angiogenic stimuli, when the mutated genes in HHT appear to result in the inability of a blood vessel to mature appropriately.[1,6–8] More than 90% of HHT patients experience spontaneous recurrent epistaxis secondary to telangiectasia of the nasal mucosa.[8] Both severity and frequency of HHT-related epistaxis vary widely.[9] Frequency may range from a few bleeds yearly to several each day, and severity may range from a few drops to unmanageable flows with acute hemodynamic disturbances. Chronic iron-deficiency anaemia and transfusion dependence are common.[1,9] Increased frequency and duration of epistaxis in those with HHT are associated with a decreased quality of life.[10,11]

Otorhinolaryngologic management of HHT epistaxis includes cauterization, laser photocoagulation, septal dermoplasty, and Young's procedure (nostril closure).[12,13] Ligation and embolization treatments have also been used.[9] Medical treatments using both oestrogen and antioestrogen therapy are now supported by small randomized, controlled trials.[14,15] There is increasing interest in the use of anti-angiogenesis therapies, such as bevacizumab (Avastin) and thalidomide.[6,16,17] Therapeutic manipulation of coagulation and fibrinolytic pathways and antioxidant therapies are also employed to try to limit blood loss in HHT.[18-21] Many patients require more than one modality, with more invasive surgical treatments generally reserved for patients remaining transfusion dependent or severely symptomatic despite standard therapy.[22] Anecdotal reports from patients in our clinic

suggested several lifestyle factors and conservative treatments that may have provocative or beneficial effects on nosebleeds in HHT. Only a proportion had been examined in prior formal publications.[9,23] The goal of this study was to use a nonbiased patient survey to identify factors associated with changes in epistaxis severity in HHT.

Materials and methods

Study Design

To capture patient experiences of treatment and lifestyle factors affecting nosebleeds in an unbiased manner, we incorporated relevant questions into a wider ongoing survey regarding health and treatments for people with HHT and general population controls. The full study addressed general and HHT demographics, factors influencing HHT nosebleed severity, and prevalence and behaviour of common medical conditions in the participant and relatives. The study received a favourable ethics opinion by NRES Committee East Midlands-Derby 1 Research Ethics Committee before online launch. For the current study, relevant data were downloaded on May 10, 2012. Participants were asked ‘‘Have you had any of these […] treatments for HHT nosebleeds?’’ and were then asked to score the effects of treatments as ‘‘much better,’’ ‘‘a bit better,’’ ‘‘no different really,’’ ‘‘a bit worse,’’ or ‘‘much worse.’’ Additional options of ‘‘sometimes better—but not always’’ and ‘‘sometimes worse—but not always’’ were provided for invasive treatments (cauterization, laser treatment, septal dermoplasty, Young’s procedure [nostril closure], arterial ligation, and embolization). They had the opportunity to tick more than box per treatment if it didn’t always have the same result for medical treatments. Participants were also asked to score the effects of specific lifestyle changes as ‘‘much better,’’ ‘‘a bit better,’’ ‘‘no different really,’’ ‘‘a bit worse,’’ or ‘‘much worse.’’ Finally, they were given the opportunity to provide information on any other medical treatment or lifestyle or dietary item that seemed to lead to a change in their nosebleeds and then were asked to detail whether that was a beneficial or detrimental change.

Patient Population

Potential participants were recruited through the Imperial College London HHTIC London Clinical Service databases (2001 to present) by post, during attendance at the HHT clinics, and by advertisement by the HHT Foundation International. Answers from individuals who filled in paper-format questionnaires were transcribed into the online survey by the study team. Non-HHT participants were also recruited and provided comparison data for nosebleed frequency in the HHT and general populations.

Statistical Methods

Question responses ‘‘much better,’’ ‘‘a bit better’’/’’better,’’ ‘‘no different,’’ ‘‘a bit worse’’/‘‘worse,’’ ‘‘much worse,’’ ‘‘sometimes better—but not always,’’ and ‘‘sometimes worse—but not always’’ were converted into numeric format (+2, +1, 0, -1, -2, +0.5, and -0.5, respectively) to generate ‘‘response means.’’ Interventions volunteered by participants were scored as +1 (‘‘better’’) or -1 (‘‘worse’’) and summed to generate a ‘‘score’’. ‘‘Scores’’ could not be compared to ‘‘response means’’ because of the absence of the formal ‘‘no difference’’ response category (occasional individuals volunteered a ‘‘no difference’’ effect using free text). GraphPad Prism version 5.00 (GraphPad Software, San Diego, CA) was used to calculate descriptive statistics and to compare variables quantified in the same manner using the Fisher exact test (proportions) or Mann Whitney U test (two variables).

Results

Population Characteristics

In total, 771 responses were received at the time of data download from 666 individuals with HHT and 105 controls. The average age of HHT and non-HHT participants was 54 years (range, 21–87) and 53 years (range, 21–86), respectively. Females accounted for 436 of 663 (66%) HHT participants and 70 of 105 (67%) non-HHT participants. The majority of participants were Caucasian and were living in the United States, United Kingdom, Europe, Canada, or Australia. The proportion of HHT participants reporting epistaxis (649/666, 97%) was significantly higher than the proportion of non-HHT patients (70/105, 67%), $P < .0001$. The frequency of nosebleeds experienced by the HHT participants was also higher (Fig. 1A).

Treatment Modalities in HHT-Related Epistaxis

A total of 326 participants reported the use of specialist invasive treatments for HHT epistaxis (Fig. 1B). Of the 170 requiring emergency packing, 164 (96%) had used either (or more commonly both) cauterization or laser therapy. More invasive treatments were almost always used in patients who had also received cauterization and/or laser therapy. Approximately half of those treated by cauterization (138/267, 52%) or laser photocoagulation (109/221, 49%), and smaller proportions receiving other modalities, provided a response report (Fig. 2). The graded responses to each specified treatment (spanning beneficial, no difference, and detrimental options) were scored to generate a response mean. Of those patients reporting an effect, most reported a benefit, and all invasive treatment modalities demonstrated positive responses. For the two major outpatient procedures, laser therapy was more frequently reported as beneficial than cauterization (Mann Whitney U, $P < .0001$).

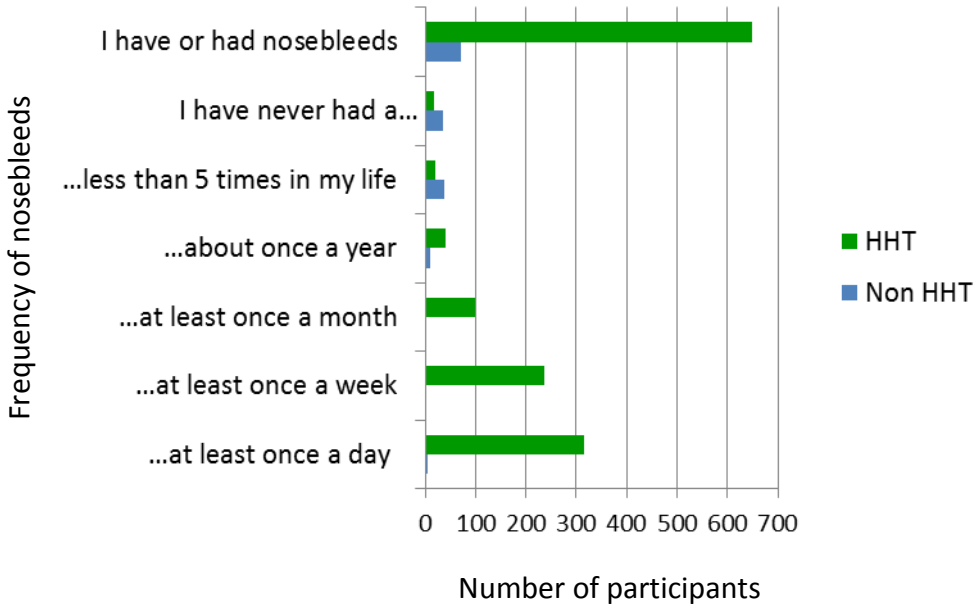


Figure 1A. Nosebleed frequency and treatments. Comparison of frequency of nosebleeds reported by individuals with and without hereditary haemorrhagic telangiectasia (HHT).

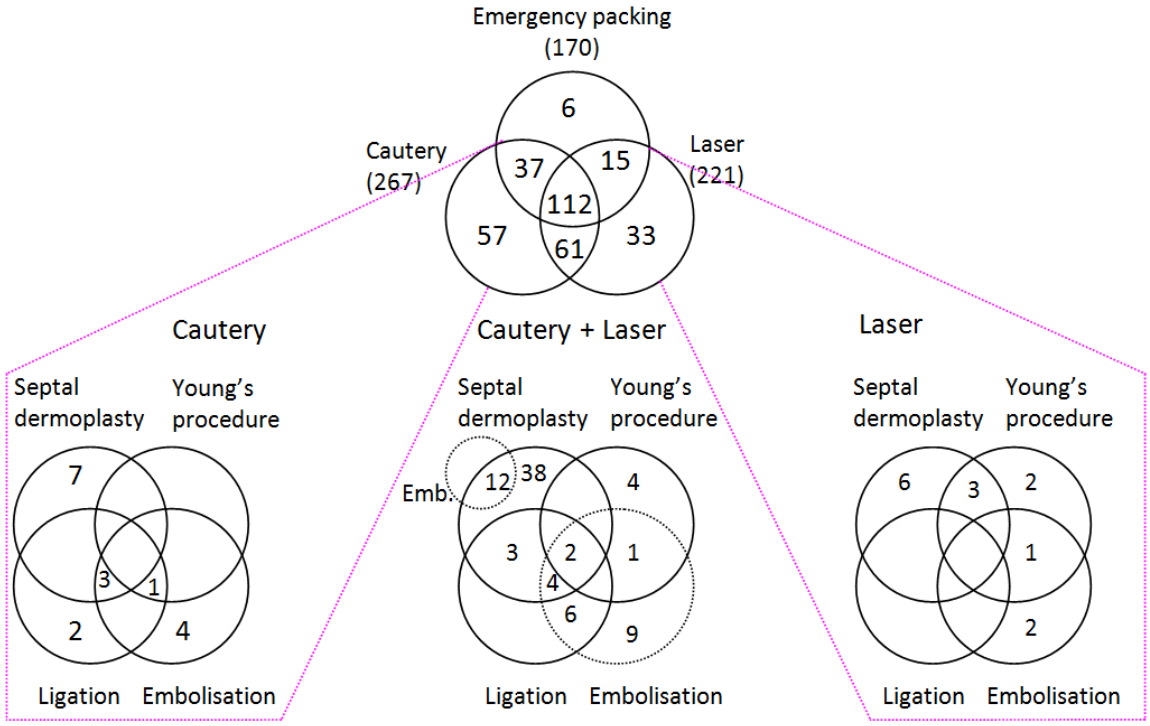


Figure 1 B Treatment modalities used by HHT patients: The upper Venn diagram indicates the use of emergency and common outpatient modalities; the lower shows the use of specialized treatments, stratified according to whether respondents also had cauterization (left panel), cauterization and laser treatment (middle panel), or laser treatment (right panel). Four participants reported treatment by specialized treatments (septal dermoplasty, embolization) without laser and/or cautery: they are indicated on the upper panel.

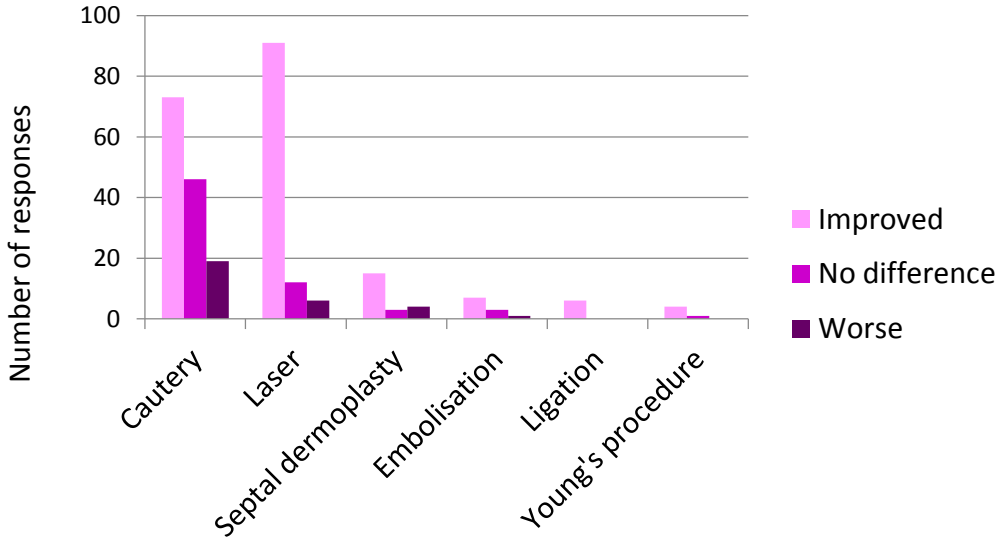


Figure 2A. Reported effect of specialized treatments on nosebleed severity. Graphic indication for each treatment showing the number of reports where the treatment was reported as improving nosebleeds, making no difference, or worsening nosebleeds.

	Total receiving treatment	Total (%) reporting response	Response mean	95% confidence intervals
Cautery	267	138 (52%)	0.42	0.26-0.58
Laser	221	109 (49%)	0.98	0.82-1.13
Septal dermoplasty	84	22 (26%)	0.80	0.27-1.32
Embolisation	19	5 (26%)	0.10	-0.01-2.21
Ligation	19	6 (31%)	1.42	0.72-2.11
Young's procedure	47	11 (23%)	0.68	0.08-1.29

Figure 2 B Indication of proportion of respondents and response means (with 95% confidence intervals) for each treatment modality.

Medical treatments for HHT epistaxis fell into two categories (Fig. 3). Female hormones (which could have been used for any indication including oral contraception and hormone replacement) and antioestrogens (tamoxifen/ raloxifene) were specified in the questionnaire; the graded responses (spanning beneficial, no difference, and detrimental options) were scored to generate a response mean. High proportions of individuals receiving these agents provided a response report: 275 of 330 (83%) of those receiving female hormones and 30 of 33 (91%) receiving tamoxifen/raloxifene. Both were generally reported as beneficial, although the response mean for tamoxifen/raloxifene was significantly higher than that for female hormones (Mann Whitney U, P = .0037). The other four groups volunteered by study participants (aminocaproic acid, bevacizumab, nasal antibiotic ointments, and tranexamic acid) were also more frequently reported as having a beneficial effect on epistaxis.

For these volunteered agents, participant responses (beneficial or detrimental) were summed to generate positive (beneficial) ‘‘scores’’.

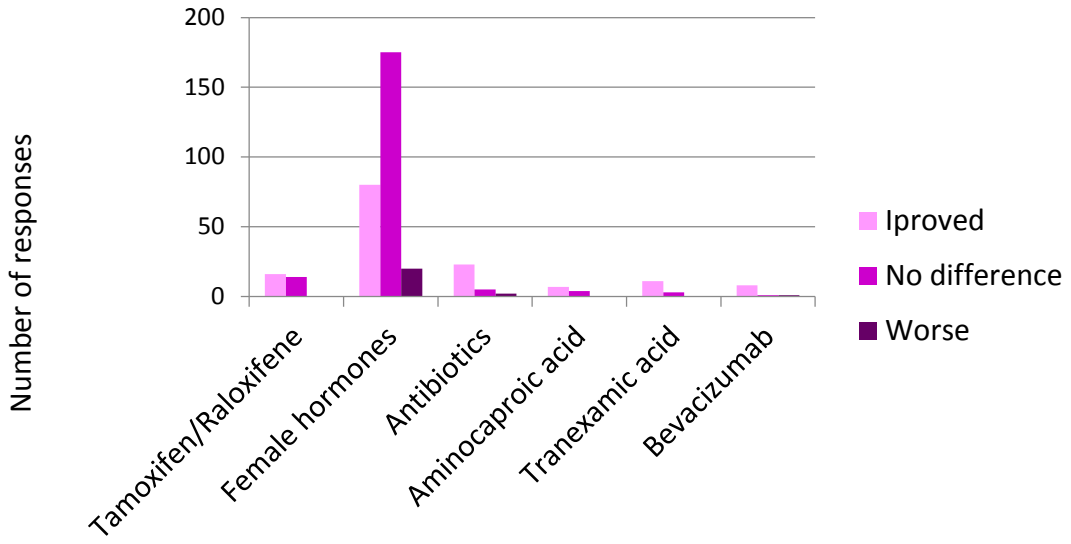


Figure 3 A Reported effect of recognized medical treatments for hereditary haemorrhagic telangiectasia nosebleeds. Graphic indication for each treatment showing the number of reports where the treatment was reported as improving nosebleeds, making no difference, or worsening nosebleeds.

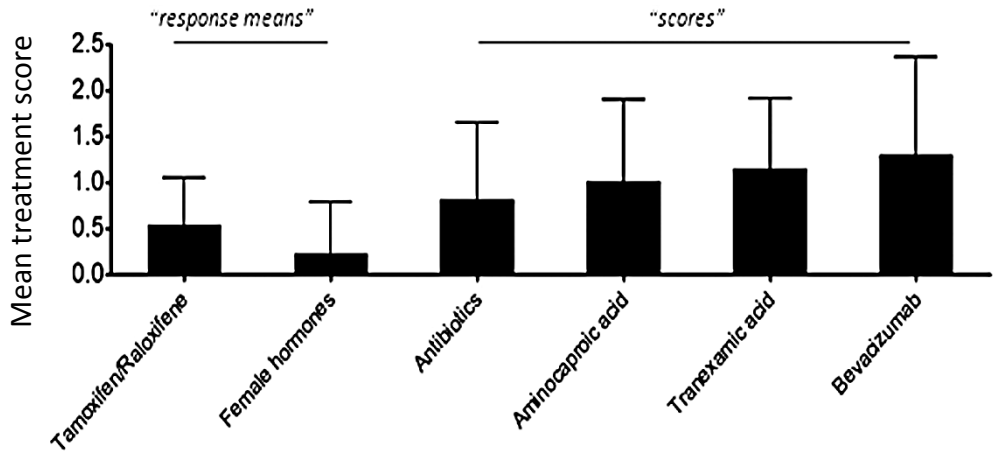


Figure 3 B The mean treatment effect reported (with 95% confidence intervals) for each treatment. Note that treatment effects cannot be compared between the two different categories (response means or scores).

	Total receiving treatment	Total (%) reporting response	Mean treatment effect reported	95% Confidence intervals
Tamoxifen/Raloxifene*	33	30 (91%)	0.53	0.34, 0.72
Female hormones*	330	275 (83%)	0.22	0.15, 0.29
Antibiotics^	?	30	0.80	0.48, 1.12
Aminocaproic acid^	?	11	1.00	0.40, 1.60
Tranexamic acid^	?	14	1.14	0.70, 1.59
Bevacizumab^	?	10	1.30	0.54, 2.06

Figure 3C. Indication of proportion of respondents and mean treatment effect reported for each treatment modality (*response means; ^scores). Note that the total number of respondents using nasal antibiotics, aminocaproic acid, tranexamic acid, and bevacizumab were not captured, as these treatment effects were volunteered by the participants. The likely absence of individuals for whom effects on epistaxis would have been reported as ''no difference'' means that these scores cannot be compared to the response means for oestrogens or antioestrogens.

Other Medical and Lifestyle Influences

Other agents used by HHT patients also fell into questionnaire-specified and participant-volunteered categories (Fig. 4). Steroid hormones (excluding topical agents or inhalers) and iron tablets were specified in the questionnaire: each was as commonly reported with beneficial as detrimental effects. The other agents were volunteered by study participants. Nasal humidification regimens were all reported as having beneficial effects on nosebleeds (95% confidence intervals [95% CI] for scores greater than zero). Surprisingly, the Chinese herb Yunnan Baiyao was frequently reported as being beneficial (score, 1.5 [95% CI, 1.06–1.94]), as were nasal decongestants (score, 1.31 [95% CI, 0.17–2.45]). As expected, HHT participants using antiplatelet agents, anticoagulants, and nonsteroidal anti-inflammatory drugs were more likely to report a detrimental effect. Similarly, the use of omega-3 acid supplements and allergy treatments were reported as detrimental (scores -1.2, [95% CI, -0.64 to -1.76] and -0.6 [95% CI, -0.21 to -1.41], respectively).

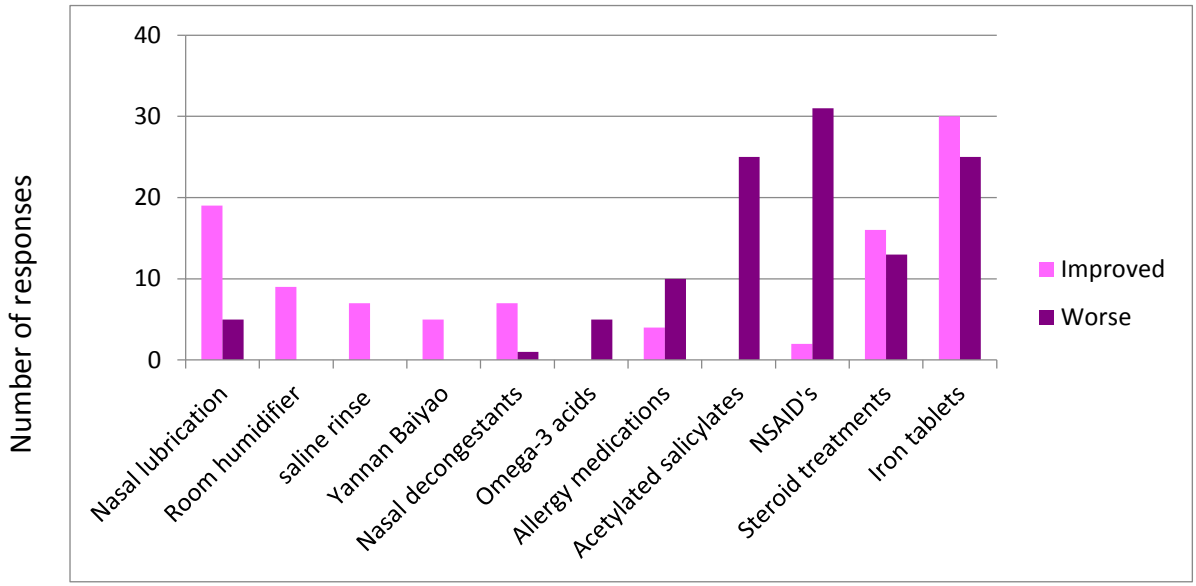


Figure 4A. Reported effect of other medical treatments on hereditary haemorrhagic telangiectasia nosebleeds. Graphic indication for each treatment showing the number of reports where the treatment was reported as improving or worsening nosebleeds. Epistaxis responses to steroid therapy and iron tablets were specifically requested: the ''no difference'' responses (77.4% [96/124] of steroid users; 88% [403/457] of iron tablet users) are not illustrated for clarity.

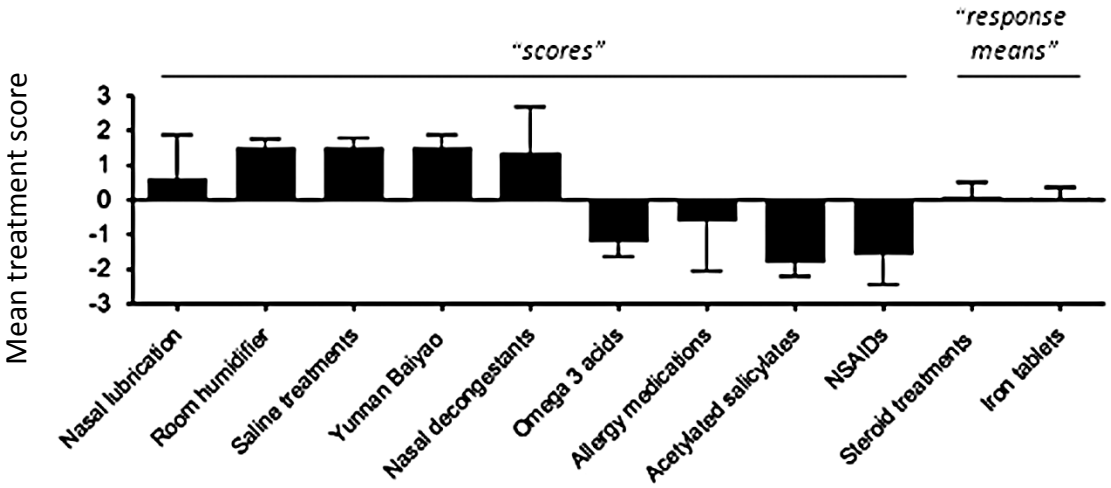


Figure 4B. The mean treatment effect reported (with 95% confidence intervals) for each treatment. Note that response means cannot be compared to scores.

	Number of reports	Mean effect size	95% Confidence interval
Nasal lubrication^	31	0.61	0.15, 1.08
Room humidifier^	9	1.50	1.31, 1.69
saline rinse^	7	1.50	1.23, 1.78
Yannan Baiyao^	8	1.50	1.06, 1.94
Nasal decongestants^	5	1.31	0.17, 2.45
Omega-3 acids^	5	-1.20	-1.76, -0.64
Allergy medications^	15	-0.60	-1.41, -0.21
Acetylated salicylates^	24	-1.79	-1.97, 1.62
Non steroid anti-inflammatory drugs^	33	-1.56	-1.88, -1.24
Steroid treatments*	124	0.03	-0.05, -0.12
Iron tablets*	456	0.015	-0.016, 0.047

Figure 4C. Indication of number of respondents and mean treatment effect reported for each treatment modality (^scores; *response means). NSAIDs ¼ nonsteroidal anti-inflammatory drugs.

The effects of specific lifestyle variables requested (different foods/drinks, season/temperature, mood/stress/ anxiety, lifestyle activities/sports, and nasal knock/ injury) were all reported as detrimental (Fig. 5). When participants volunteered details (Fig. 5), the majority of reports (all negative) cited extremes and changes in temperature (n = 133), low humidity (n = 80), both temperature and low humidity (n = 23), or springtime allergies (n = 31). For activities and sports, reports were generally negative, although two participants reported beneficial effects from aerobic sports. The most unexpected data were the 25 different food items reported (Fig. 6): all except soy were reported only as having detrimental effects on epistaxis. The most frequently reported items were alcohol (n = 45, 6.8% of participants) and spices (n = 26, 3.9% of participants). We noted that most of the foods reported to exacerbate epistaxis were high in salicylates (including spices, chocolate, coffee, and certain fruits), other natural compounds reported to have antiplatelet activities (red wine, garlic, ginger, ginseng, ginkgo biloba, and vitamin E), or in omega-3 acids (oily fish, salmon).[24-31]

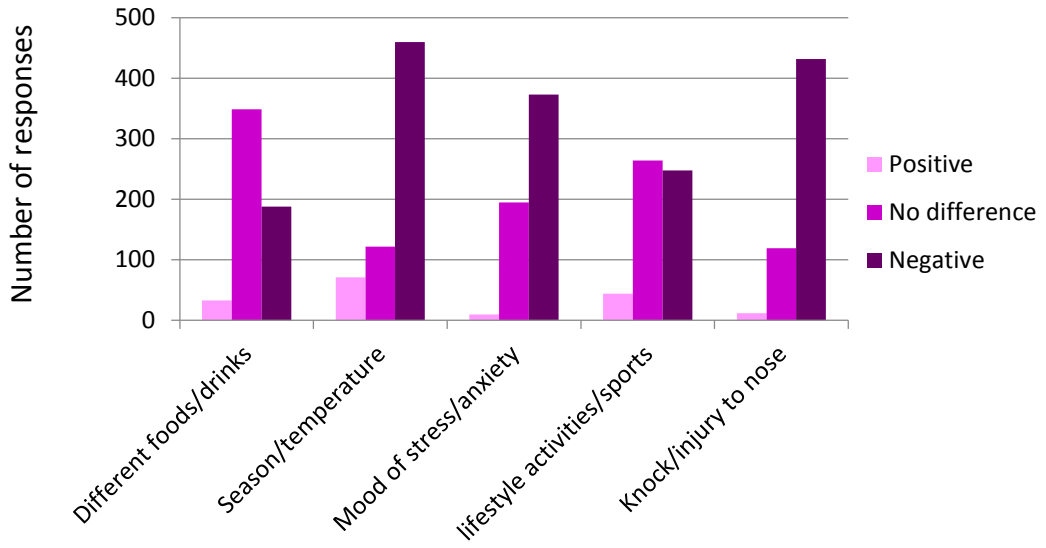


Figure 5A. Reported effect of lifestyle factors on hereditary haemorrhagic telangiectasia nosebleeds. Graphic indication of the effects of each change reported as improving nosebleeds, making no difference, or worsening nosebleeds.

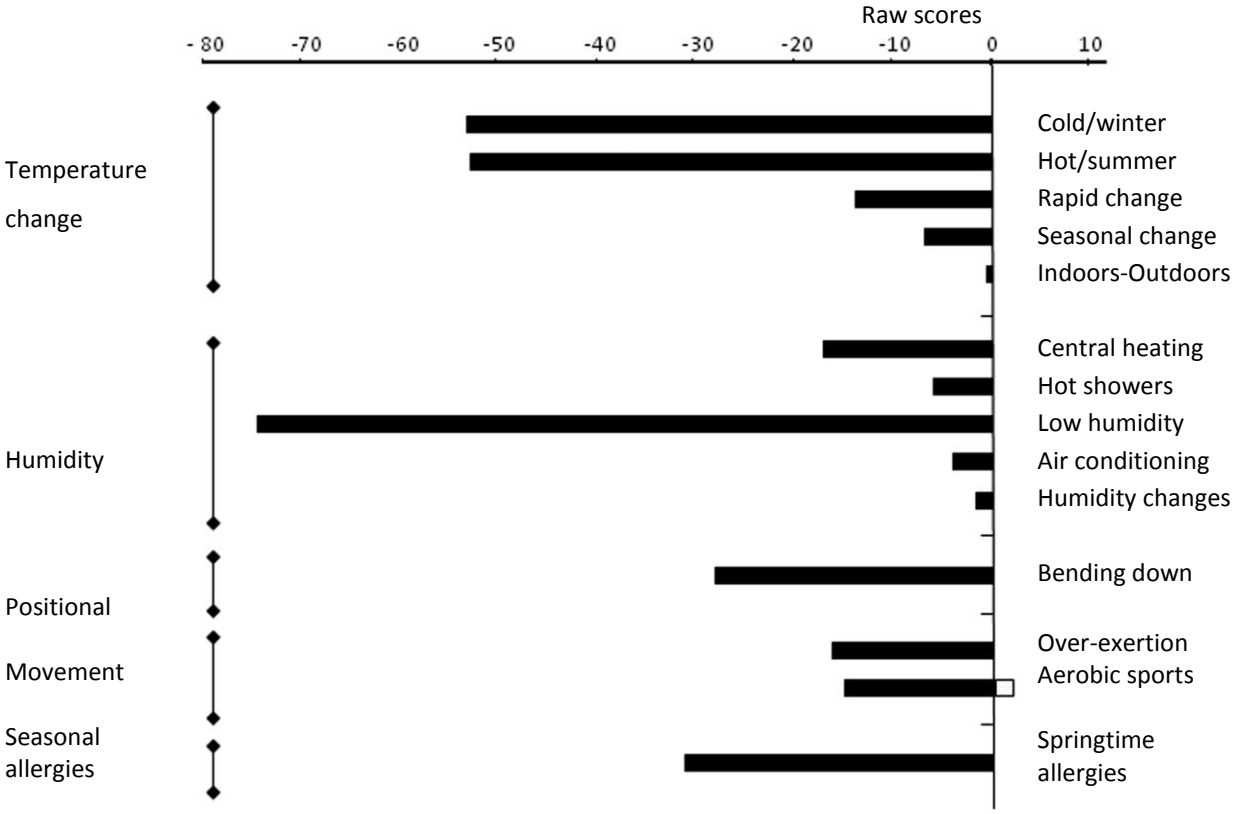


Figure 5B. The number of reports of specific environmental changes, grouped according to type (black bars ¼ negative/detrimental, white bar ¼ positive/beneficial).

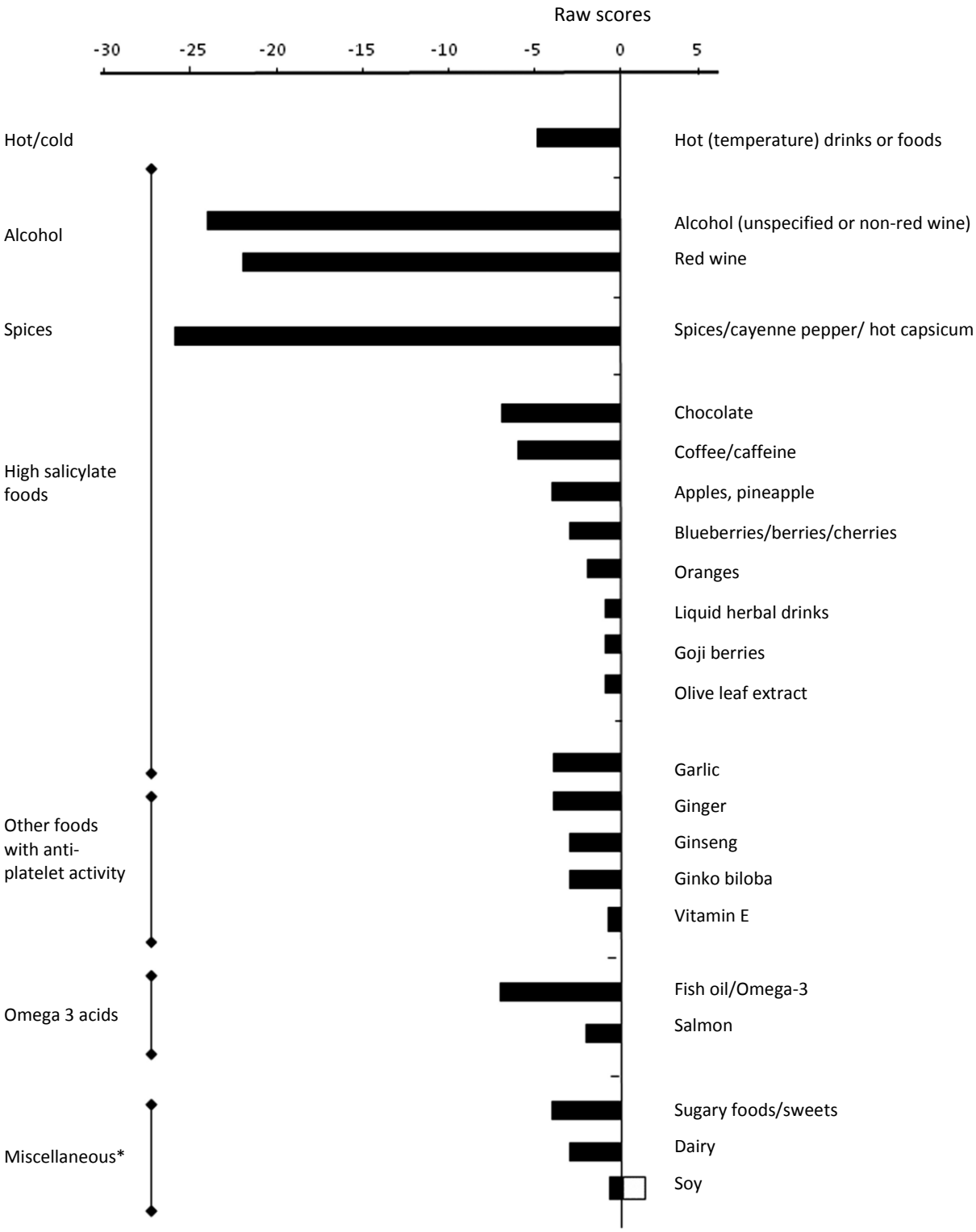


Figure 6. Reported effect of dietary factors on hereditary haemorrhagic telangiectasia nosebleeds. The number of reports for specific dietary items, grouped according to type (black bars ¼ negative/detrimental, white bar ¼ positive/ beneficial). *There were additional single deleterious reports for vitamin C, cheese, carbonated drinks, red meat, soy, and bananas, although two individuals volunteered a beneficial effect with soy.

Discussion

Management of HHT epistaxis is difficult, and because it is a rare disease, individual practitioners rarely treat large number of patients outside of HHT centres. In this study using a nonbiased questionnaire and international HHT patients, we highlight the need for multiple different treatment modalities, which were generally reported as beneficial. More importantly, the data provide potential options for lifestyle modifications. The strengths of the study were the use of a nonbiased questionnaire amenable to quantification. Limitations were that only a proportion of participants reported the effect of specific variables on their epistaxis: 83% to 91% for oestrogen and antioestrogen therapies, approximately 50% for laser and cauterization, low for more invasive modalities, and not ascertainable for other treatments and lifestyle factors. The intention of dedicated treatments was known to the patients, and the reported beneficial responses may therefore have included placebo responses. However, the goal of the study was not to evaluate the beneficial effects of specific treatments but to identify other factors modifying nosebleed severity. Overall, the findings of treatment benefits strengthened our confidence in the subsequent reports associated with environmental and lifestyle factors: Salicylate content and antiplatelet activities of dietary food items are not generally appreciated, and we suspect, but cannot prove, that this was not known to the participants reporting effects on their nosebleeds. The use of a subjective questionnaire-based study meant that the effects attributable to one particular treatment or variable could not be isolated from potentially independent and/or interactive effects of other therapeutic, lifestyle, pathogenic, or genetic variables. The responses were not reported consistently by all patients, and the reasons for this variability are not yet understood. That said, the data do add to the limited published literature. Use of specific otorhinolaryngologic treatments in HHT is guided by expert opinion and the findings of case series. For example, recent international guidance recommended the use of laser therapy over cauterization.[9] Our study findings provide direct evidence to suggest that the use of laser treatment may be preferable to cauterization in HHT. Medical therapies, particularly hormonal therapies that are supported by randomized control trials in HHT, were also reported as beneficial.[14,15] To date no formal comparisons have been made between oestrogens and antioestrogens in the treatment of HHT-related epistaxis. The current study begins to suggest that the use of antioestrogen treatment may be favourable

to hormone treatment, although the lower hormonal score may in part reflect the variety of oestrogen/ progesterone dosing regimens used by participants. Beneficial effects were also reported for aminocaproic acid, tranexamic acid, antibiotic ointments, and bevacizumab. No data were volunteered by participants for thalidomide or antioxidants. Trauma to the nose and high anxiety levels were reported to worsen nosebleeds, along with almost all patient-reported variables relating to environmental factors. These findings support both wide anecdotal consensus and the findings of a single questionnaire-based study (n = 49), which reported changes in temperature, low humidity, sneezing, bending over, strenuous activity, and alcohol as aggravating factors for nosebleeds in HHT.[23] These factors are likely to influence epistaxis through drying of the nasal mucosa by fluctuations in temperature and humidity, external trauma to telangiectasia, and increased perfusion pressure of the vessels whilst bending over. Many of these factors also lead to epistaxis in the general population, owing to the superficial and easily traumatized multidirectional arterial anastomotic system of the nose.[32,33] We believe the most important data from this study relate to potential forms of self-management in this chronic condition. Consensus opinion has suggested the use of humidification and regular lubrication of the nostrils, based on anecdotal reports and the likelihood that crusting and drying of the nasal mucosa will exacerbate damage of the nasal telangiectasia, precipitating haemorrhage. [8,9] For example, saline irrigation of the nostrils has been postulated as a useful method of preventing deeper crusting when tolerated by the patient.[34] In our study, the positive (beneficial) scores for room humidification (1.50 [95% CI, 1.31–1.69]), saline treatments (1.50 [95% CI, 1.23–1.78]), and nasal lubrication (0.61 [95% CI, 0.15–1.08]) provide evidence to encourage use. For individuals with nasal congestion, the beneficial reports for nasal decongestants (score, 1.31 [95% CI, 0.17–2.45]) are intriguing and likely reflect reduced nasal irritation and damage to telangiectasia secondary to reduced blowing of the nose. Eight participants reported using Yunnan Baiyao, a traditional Chinese herbal medicine widely used in the treatment of haemorrhages and wounds and shown in several randomized, controlled trials to reduce intraoperative bleeding due to its haemostatic properties.[35–38] Similarly, two participants reported beneficial effects from soy, which reduced bleeding from mechanically injured vessels in thrombocytopenic dogs and was associated with decreased risk of subarachnoid haemorrhage in a Japanese population.[39-40] For these agents, further study of efficacy, tolerability, and mechanisms that may be involved in alleviating HHT epistaxis is indicated in

a wider group of patients. As for other potentially pro-thrombotic agents, examination of side-effect profiles is important, particularly with the recent demonstration of the heightened risk of venous thromboemboli in iron-deficient HHT patients.[41] Nosebleeds are more frequent and prolonged for individuals in the general population using antiplatelet and anticoagulant therapies, and the current study suggests this may be the case for HHT patients.[40,42] There were also deleterious reports for food items reported to have similar activities. The two most commonly reported groups of agents to exacerbate HHT epistaxis were alcohol (especially red wine) and spices. Total salicylates and salicylic acid are high in a wide variety of spices, with antiplatelet activity of curcumin, a food spice from turmeric, reported by numerous groups.[24-26, 43–45] Curcumin is also recognized to inhibit vascular smooth muscle cell functions relevant to responses to vascular injury, the process currently considered to precipitate HHT haemorrhage.[46] There is already substantial literature regarding the mechanisms by which red wine appears to have a beneficial effect protecting against cardiovascular disease in the apparent ‘‘French paradox.’’[47] We can also speculate that the detrimental effects of alcohol in the current study may be attributable not only to vasodilatation but also to the reported association between alcohol and reduced platelet aggregation.[48] Chocolate was the next most commonly reported item to exacerbate HHT epistaxis (Fig. 6). Multiple studies have emphasized different antiplatelet effects of cocoa, its flavonol constituents, and specifically dark chocolate.[49–56] Similarly, antiplatelet activity for omega-3 is recognized, with reduced microvascular thromboses demonstrated in a porcine model, although it is not clear whether this usually translates to clinically relevant haemorrhagic risk.[57-62] In the current study, items containing fish oil and omega-3 were reported as worsening epistaxis by 15 HHT patients. In addition, most fruits, especially berry fruits and dried fruits, many vegetables, tea, and honey contain salicylate, although functional evidence of antiplatelet activities has generally not been reported to date.[24–26] Antiplatelet activity is also recognized for a wide variety of additional food and dietary items: Pribitkin et al. and Ciocon et al. emphasized the importance of garlic, ginger, ginseng, and Ginkgo biloba (the 4 Gs), and in the current study each was reported by multiple different HHT participants as exacerbating their nosebleeds (Fig. 6).[28,29]

Conclusion

This study suggests that HHT patients with troublesome epistaxis may be advised to try simple risk-free strategies including avoidance or monitored intake of high-salicylate foods and other dietary items reported as deleterious in this study. Acute aggravation of HHT epistaxis could serve as an activity biomarker for proposed dietary manoeuvres to protect against atherosclerosis and vascular diseases.

Acknowledgments

The authors are grateful to the East Midlands-Derby 1 Research Ethics Committee and Imperial AHSC Joint Research Compliance Office for efficient ethical reviews. Dr. Shovlin also thanks Luis Calcado at SurveyMonkey Customer Support for technical support with survey formatting; James Moore for setting up the Imperial website entry page; and Cathleen Kinnear, Nicole Schaefer, and Marianne Clancy of the HHT Foundation International for advertising the study.

References

1. Shovlin CL. Hereditary haemorrhagic telangiectasia: pathophysiology, diagnosis and treatment. Blood Rev 2010;24:203–219.

2. AAssar OS, Friedman CM, White RI Jr. The natural history of epistaxis in hereditary hemorrhagic telangiectasia. Laryngoscope 1991;101:977–980.

3. Kjeldsen AD, Vase P, Green A. Hereditary haemorrhagic telangiectasia: apopulation-based study of prevalence and mortality in Danish patients. J Intern Med 1999;245:31–39.

4. Dakeishi M, Shioya T, Wada Y, et al. Genetic epidemiology of hereditary hemorrhagic telangiectasia in a local community in the northern part of Japan. Hum Mutat 2002;19:140–148.

5. Abdalla SA, Letarte M. Hereditary haemorrhagic telangiectasia: current views on genetics and mechanisms of disease. J Med Genet 2006;43:97–110.

6. Park S, Wankhede M, Lee Y, et al. Real-time imaging of de novo arteriovenous malformation in a mouse model of hereditary hemorrhagic telangiectasia. J Clin Invest 2009;119:3487–3496.

7. Lebrin F, Srun S, Raymond K, et al. Thalidomide stimulates vessel maturation and reduces epistaxis in individuals with hereditary hemorrhagic telangiectasia. Nat Med 2010;16:420–428.

8. McDonald J, Bayrak-Toydemir P, Pyeritz RE. Hereditary hemorrhagic telangiectasia: an overview of diagnosis, management, and pathogenesis. Genet Med 2011;13:607–616.

9. Faughnan M, Palda V, Garcia-Tsao G, et al. International guidelines for the diagnosis and management of hereditary hemorrhagic telangiectasia. J Med Genet 2011;48:73–87.

10. Ingrand I, Ingrand P, Gilbert-Dussardier B, et al. Altered quality of life in Rendu-Osler-Weber disease related to recurrent epistaxis. Rhinology 2011;49:155–162.

11. Loaec M, Moriniere S, Hitier M, Ferrant O, Plauchu H, Babin E. Psychosocial quality of life in hereditary haemorrhagic telangiectasia patients. Rhinology 2011;49:164–167.

12. Mahoney EJ, Shapshay SM. Nd-YAG laser photocoagulation for epistaxis associated with hereditary hemorrhagic telangiectasia. Laryngoscope 2005;115:373–375.

13. Harvey RJ, Kanagalingam J, Lund VJ. The impact of septodermoplasty and potassium-titanyl-phosphate (KTP) laser therapy in the treatment of hereditary hemorrhagic telangiectasia-related epistaxis. Am J Rhinol 2008;22:182–187.

14. Van Cutsem E, Rutgeerts P, Vantrappen G. Treatment of bleeding gastrointestinal vascular malformations with oestrogen-progesterone. Lancet 1990;335:953–955.

15. Yaniv E, Preis M, Hadar T, Shvero J, Haddad M. Antiestrogen therapy for hereditary hemorrhagic telangiectasia: a double-blind placebo-controlled clinical trial. Laryngoscope 2009;119:284–288.

16. Karnezis TT, Davidson TM. Efficacy of intranasal Bevacizumab (Avastin) treatment in patients with hereditary hemorrhagic telangiectasia-associated epistaxis. Laryngoscope 2011;121:636–638.

17. Brinkerhoff BT, Choong NW, Treisman JS, Poetker DM. Intravenous and topical intranasal bevacizumab (Avastin) in hereditary hemorrhagic telangiectasia. Am J Otolaryngol 2012;33:349–351.

18. Sabba C, Gallitelli M, Palasciano G. Efficacy of unusually high doses of tranexamic acid for the treatment of epistaxis in hereditary hemorrhagic telangiectasia. N Engl J Med 2001;345:926.

19. Saba HI, Morelli GA, Logrono LA. Treatment of bleeding in hereditary hemorrhagic telangiectasia with aminocaproic acid. N Engl J Med 1994; 330:1789–1790.

20. Morales-Angulo C, Perez del Molino A, Zarrabeitia R, Fernandez A, Sanz- Rodriguez F, Botella LM. Treatment of epistaxes in hereditary haemorrhagic telangiectasia (Rendu-Osler-Weber disease) with tranexamic acid. Acta Otorrinolaringol Esp 2007;58:129–132.

21. de Gussem E, Snijder R, Disch F, Zanen P, Westermann C, Mager J. The effect of N-acetylcysteine on epistaxis and quality of life in patients with HHT: a pilot study. Rhinology 2009;47:85–88.

22. Lund V, Howard D. A treatment algorithm for the management of epistaxis in hereditary haemorrhagic telangiectasia. Am J Rhinol 1999;13: 319–322.

23. Folz BJ, Tennie J, Lippert BM, Werner JA. Natural history and control of epistaxis in a group of German patients with Rendu-Osler-Weber disease. Rhinology 2005;43:40–46.

24. Swain AR, Dutton SP, Truswell AS. Salicylates in foods. Am Diet Assoc 1985;85:950–960.

25. Swain AR. The role of natural salicylates in food intolerance. (PhD Thesis, University of Sydney). 1988. Available at: http://www.sswahs.nsw.gov.au/ rpa/allergy/research/students/1988/AnneSwainPhDThesis.pdf.

26. Paterson JR, Srivasatava R, Baxter GJ, Graham AB, Lawrence JR. Salicylic acid content of spices and its implications. J Agric Food Chem 2006;54:2891–2896.

27. Stanger MJ, Thompson LA, Young AJ, Lieberman HR. Anticoagulant activity of select dietary supplements. Nutr Rev 2012;70:107–117.

28. Pribitkin ED, Boger D. Herbal therapy: What every facial plastic surgeon must know. Arch Facial Plastic Surg 2001;3:127–132.

29. Ciocon JO, Ciocon DG, Galindo DJ. Dietary supplements in primary care: Botanicals can affect surgical outcomes and follow-up. Geriatrics 2004; 58:20–24.

30. Spolarich AE, Andrews L. An examination of the bleeding complications associated with herbal supplements, antiplatelet and anticoagulant medications. J Dent Hyg 2007;81:67.

31. Violi F, Pignatelli P, Basili S. Nutrition, supplements, and vitamins in platelet function and bleeding. Circulation 2010;121:1033–1044.

32. Pope LER, Hobbs CGL. Epistaxis: an update on current management. Postgrad Med J 2005;81:309–314.

33. Saban Y, Amodeo CA, Bouaziz D, Polselli R. Nasal arterial vasculature: medical and surgical applications. Arch Facial Plast Surg 2012, doi: 10.1001/archfacial.2012.202. [Epub ahead of print].

34. Sautter NB, Smith TL. Hereditary hemorrhagic telangiectasia-related epistaxis: innovations in understanding and management. Int Forum Allergy Rhinol 2012;2:422–431.

35. Pan SF, Sun Y, Li F, et al. Effects of Yunnan Baiyao on peri-operative bleeding of patients undergoing cervical open-door laminoplasty: a multi-center randomised double-blind placebo-control trial. Zhonghua Yi Xue Za Zhi 2006;86:1888–1890.

36. Li NC, Pan BN, Wang HJ, et al. The effect of Yunnan Baiyao on reduction of intra-operative bleeding of the patients undergoing transurethral resection of prostate. Zhonghua Yi Xue Za Zhi 2007;87:1017–1020.

37. Tang ZL, Wang X, Yi B, Li ZL, Liang C, Wang XX. Prophylactic administration of Yunnan Baiyao capsule reduces intra-operative blood loss in orthognathic surgery. Zhonghua Kou Qiang Yi Xue Za Zhi 2008;43: 542–545.

38. Tang ZL, Wang X, Yi B, Li ZL, Liang C, Wang XX. Effects of the preoperative administration of Yunnan Baiyao capsules on intraoperative blood loss in bimaxillary orthognathic surgery: a prospective, randomised, double-blind, placebo-controlled study. Int J Oral Maxillofac Surg 2009; 38:261–266.

39. Djerassi I, Farber S, Roy AR, Yoshimura H. Effects of soybean phosphatides on the hemorrhagic tendency due to thrombocytopenia. J Clin Invest 1962;41:770–778.

40. Okamoto K, Horisawa R. Soy products and risk of an aneurysmal rupture subarachnoid hemorrhage in Japan. Eur J Cardiovasc Prev Rehabil 2006;13:284–287.

41. Livesey JA, Manning R, Meek JH, et al. Low serum iron levels are associated with elevated plasma levels of coagulation factor VIII and pulmonary emboli/deep venous thromboses in replicate cohorts of patients with hereditary haemorrhagic telangiectasia. Thorax 2012;67:328–333.
42. Tay HL, Evans JM, McMahon AD, MacDonald TM. Aspirin, nonsteroidal anti-inflammatory drugs, and epistaxis. A regional record linkage case control study. Ann Otol Rhinol Laryngol 1998;107:671–674.

43. Srivastava KC, Bordia A, Verma SK. Curcumin, a major component of food spice turmeric (Curcuma longa) inhibits aggregation and alters eicosanoid metabolism in human blood platelets. Prostaglandins Leukot Essent Fatty Acids 1995;52:223–227.

44. Shah BH, Nawaz Z, Pertani SA, et al. Inhibitory effect of curcumin, a food spice from turmeric, on platelet-activating factor- and arachidonic acidmediated platelet aggregation through inhibition of thromboxane formation and Ca2þ signaling. Biochem Pharmacol 1999;58:1167–1172.

45. Mayanglambam A, Dangelmaier CA, Thomas D, Damodar Reddy C, Daniel JL, Kunapuli SP. Curcumin inhibits GPVI-mediated platelet activation by interfering with the kinase activity of Syk and the subsequent activation of PLCgamma2. Platelets 2010;21:211–220.

46. Yang X, Thomas DP, Zhang X, et al. Curcumin inhibits platelet-derived growth factor-stimulated vascular smooth muscle cell function and injury-induced neointima formation. Arterioscler Thromb Vasc Biol 2006;26:85–90.

47. Li H, Forstermann U. Red wine and cardiovascular health. Circ Res 2012; 111:959–961.

48. Mukamal KJ, Massaro JM, Ault KA, et al. Alcohol consumption and platelet activation and aggregation among women and men: the Framingham Offspring Study. Alcohol Clin Exp Res 2005;29:1906–1912.

49. Rein D, Paglieroni TG, Wun T, et al. Cocoa inhibits platelet activation and function. Am J Clin Nutr 2000;72:30–35.

50. Pearson DA, Paglieroni TG, Rein D, et al. The effects of flavanol-rich cocoa and aspirin on ex vivo platelet function. Thromb Res 2002;106: 191–197.

51. Murphy KJ, Chronopoulos AK, Singh I, et al. Dietary flavanols and procyanidin oligomers from cocoa (Theobroma cacao) inhibit platelet function. Am J Clin Nutr 2003;77:1466–1473.

52. Heptinstall S, May J, Fox S, Kwik-Uribe C, Zhao L. Cocoa flavanols and platelet and leukocyte function: recent in vitro and ex vivo studies in healthy adults. J Cardiovasc Pharmacol 2006;47:S197–S205.

53. Innes AJ, Kennedy G, McLaren M, Bancroft AJ, Belch JJ. Dark chocolate inhibits platelet aggregation in healthy volunteers. Platelets 2003;14: 325–327.

54. Hermann F, Spieker LE, Ruschitzka F, et al. Dark chocolate improves endothelial and platelet function. Heart 2006;92:119–120.

55. Flammer AJ, Hermann F, Sudano I, et al. Dark chocolate improves coronary vasomotion and reduces platelet reactivity. Circulation 2007;116: 2376–2382.

56. Hamed MS, Gambert S, Bliden KP, et al. Dark chocolate effect on platelet activity, C-reactive protein and lipid profile: a pilot study. South Med J 2008;101:1203–1208.

57. Dyerberg J, Bang HO. Haemostatic function and platelet polyunsaturated fatty acids in Eskimos. Lancet 1979;2:433–435.

58. Moertl D, Berger R, Hammer A, et al. Dose-dependent decrease of platelet activation and tissue factor by omega-3 polyunsaturated fatty acids in patients with advanced chronic heart failure. Thromb Haemost 2011; 106:457–465.

59. Cohen MG, Rossi JS, Garbarino J, et al. Insights into the inhibition of platelet activation by omega-3 polyunsaturated fatty acids: beyond aspirin and clopidogrel. Thromb Res 2011;128:335–340.

60. Thorwest M, Balling E, Kristensen SD, et al. Dietary fish oil reduces microvascular thrombosis in a porcine experimental model. Thromb Res 2000;99:203–208.

61. Harris WS. Expert opinion: omega-3 fatty acids and bleeding-cause for concern? Am J Cardiol 2007;99:44C–46C.

62. Phang M, Lazarus S, Wood LG, Garg M. Diet and thrombosis risk: nutrients for prevention

Chapter nine

Summary and general discussion

This thesis addresses some of the open clinical questions on the epidemiology, pathology, diagnostics and therapy of Hereditary Haemorrhagic Telangiectasia.

Chapter 2 reports the life expectancy of HHT patients most of whom were the unscreened and untreated parents of individuals with confirmed HHT. The study showed that the life expectancy in parents with HHT was slightly lower compared to parents without. This is likely the result of complications associated with HHT. Further analysis showed that parents with ACVRL 1 mutations had normal life expectancies, whereas parents with Endoglin mutations died 7.1 years earlier than controls. Female patients with Endoglin mutations were most strikingly at risk of premature death from complications. These results emphasize the importance of referring patients with HHT for screening to establish any vital organ involvement and timely intervention to prevent complications. Interestingly enough, life-expectancy seemed to normalize in all HHT groups after the age of 70. This phenomenon could be explained by results described in chapter 3.

Chapter 3 analyses cancer rates in an international HHT cohort. Questionnaire derived data of 2,161 HHT patients and 2,817 control individuals were included. In both crude and age-adjusted regression, lung cancers were significantly less frequent in the HHT arm than controls. By contrast, breast cancer prevalence was higher in HHT than in controls. Overall, prostate and colorectal cancer rates were equivalent, but the pattern of colorectal cancer differed, with a higher prevalence in younger HHT patients than controls. Lung cancer was associated with a high mortality rate and the decreased prevalence in the HHT group could explain the near normal life expectancy of HHT patients in later decades of life.

Chapter 4 describes the relationship between pulmonary arterial hypertension (PAH) and HHT. Hereditary PAH is a rare but severe complication of HHT. ACVRL1 mutations have been recognized as leading to this combined syndrome for several years. Understanding PAH in the context of HHT is especially important since this combination usually leads to a worse outcome than PAH alone. Twenty-two of the patients described in these case reports were diagnosed under the age of 18 (56%). Compared to BMPRII mutation carriers and non-carriers (idiopathic PAH), ACVRL1 mutation carriers are diagnosed at a younger age and have a worse prognosis despite similar therapy and better haemodynamics at time of diagnosis.

This suggests that the disease progresses more rapidly with severe consequences. Even though it is rare for HHT to be complicated by PAH, physicians should be aware that HHT and PAH do present in combination and that in these cases they perform an echocardiogram when clinical signs so indicate, especially in patients with ACVRL1 mutations.

Chapter 5 addresses the question how to screen children of patients with HHT for possible pulmonary arteriovenous malformations. The conservative screening methodology, which includes a full history, physical examination, pulse oximetry and chest radiography, used in the St Antonius Hospital for the past 18 years was evaluated in terms of the actual incidence of HHT-related complications or major incidents. Four hundred and thirty-six children from HHT families were screened using this method. 175/436 (40%) children had a definite diagnosis of HHT, based on either the Curacao criteria or a DNA-test. Pulmonary arteriovenous malformations (PAVMs) were detected in 44/175 (25%) children, 40/44 requiring treatment by embolotherapy. None of the children screened had suffered any PAVM-associated complications at any point of their follow-up, so it appears that this screening method is fit for purpose in identifying risk of this complication. Although small PAVMs would likely have been missed using this method, the study showed that detecting large PAVMs is sufficient to prevent children with HHT developing PAVM-related complications. Postponing invasive screening to detect any smaller PAVMs, which includes high radiation doses that can cause malignancies, until adulthood does not appear to be associated with major risk.

Chapter 6 discusses the use of antiplatelet and anticoagulant therapy in HHT patients. Many patients with HHT are not treated with antiplatelet and anticoagulants when indicated because of concern that it might result in (increased) haemorrhage. This study showed unexplained and wide variation in the side effect profiles with respect to haemorrhage associated with the use of anticoagulant and antiplatelet therapies in patients with HHT. However, data also show that 40% of HHT patients using antiplatelet and anticoagulant therapy do not experience any change in either incidence or severity of their epistaxis. These findings provide cautious support for the use of antiplatelet or anticoagulant agents in cases where there is a very strong indication for their use.

Chapter 7 evaluates the results of a long-term follow-up of 12 patients treated with Thalidomide to reduce epistaxis. Only a minority of patients had continued taking the drug after its initial prescription despite its beneficial effects on their symptoms. Side effects that included fatigue and constipation as well as rather more severe neuropathy in the lower limbs, were the primary reason to stop. Thus despite symptom reduction in the case of Thalidomide, alternative treatments with fewer side-effects are clearly still necessary for treating epistaxis in HHT patients and a large-scale clinical trial with Thalidomide for treatment of epistaxis is not justified at the present time, although incidental use in the most severely affected patients can be considered.

Chapter 8 identifies factors influencing the severity of epistaxis in HHT patients. 97% of the HHT patients included in the study were affected by epistaxis. Specialist invasive- and medical treatments commonly used for HHT-related epistaxis had significant benefit. Many lifestyle and dietary factors could be identified by patients that were generally detrimental and a few appeared to have benefit. Atmospheric room humidification, nasal lubrication, and saline treatments for example were all reported as beneficial. Alcohol, spices and foods high in salicylates, other natural antiplatelet activity or omega-3 acids were identified as being detrimental. These results support existing guidelines and suggested that lifestyle and dietary advice may also improve nosebleed incidence and severity in HHT.

The aim of this thesis was to further characterise HHT patients; who they are, how they are affected by HHT and how they react to different therapies or external factors. These are all clinical issues that need to be answered to further develop treatment modalities, to give evidence based advice, to improve life-expectancy and to better quality of life of HHT patients.

Co-morbidities and protective features

The outcome of this small series of studies indicated that HHT symptoms and severity can be modified by a number of co-morbidity factors. Examples included PAH, and antiplatelet or anticoagulant therapies for treating cardiovascular disease. Likewise, HHT appeared to affect the incidence of other conditions, both positively and negatively, for example in the case of some of the most common types of cancer. HHT appeared to be protective for some

kinds of cancer but enhance others. Whether common mechanisms underlie this and the effects are directly linked to the mutations themselves or are indirect and mediated by typical HHT lifestyle, treatment or clinical parameters is unclear. HHT is caused by defective signalling to vascular cells by TGFβ superfamily members, but TGFβ controls many other vital functions in the body, most notably the immune and inflammatory system. The close link between HHT symptoms and their aggravation by inflammation is widely acknowledged clinically but there have been relatively few studies on systemic (e.g. autoimmune) versus local inflammation in patients and its influence on the manifestation of disease. As more families are identified, there may be sufficient numbers of patients to include so that an adequately powered study can be carried out that addresses more co-morbidity indications. Without sufficient power, as is often the case with rare diseases, incorrect conclusions may be drawn. For example, a similar cancer study to the one discussed in chapter 3 was underpowered and results led to very different conclusions showing no association between cancer incidence and HHT.[1] This conclusion stresses the point that appropriately powered studies are essential in order to provide informative data to guide patient management and focus future research.[2] It is not only important to recognise the inhomogeneity of the population in terms of HHT and wider genotypes, but also to adjust for important confounding variables such as smoking and iatrogenic radiation exposure.[3] Furthermore, using a family-based methodology gives control for some of the plethora of cancer susceptibility alleles and shared environmental exposures segregating within families.[2]

Screening

The life expectancy calculated in chapter 2 is based on a population largely unscreened and untreated HHT patients. More research will be needed to determine the life-expectancy of the current generation that has been screened and often treated pre-emptively. A regular critical evaluation of screening and treatment methodology and continuous improvement of care is vital for patients. In chapter 5 the methodology on how to screen children for PAVMs is discussed. This is a topic often discussed and disputed by HHT professionals. Although the ultimate goal of protecting children for complications of HHT is clear, the best way to achieve this best is still controversial. Generating more long-term data on this subject will increase liability of outcomes. Linking HHT databases with larger national and international biobanks may be one way to address this issue.

The ethical concerns of a diagnosis in children with variable degrees of long term health risk are complex and with constantly evolving imaging and screening modalities, continuous evaluation of different screening methods is crucial for a balanced discussion and development of evidence based methods for the future.

Furthermore, currently all patients needing follow-up after embolization of PAVMs undergo a thoracic computer tomography scan (CT scan). Although radiation exposure using CT scans has greatly decreased in recent years, the cumulative dose of radiation can be high, especially in patients with multiple PAVMs who undergo regular follow-up. Other methods to follow-up embolized PAVMs, like magnetic resonance imaging and transthoracic contrast-echocardiograms, may prove to be a good and radiation free alternative. Although experience with these methods is increasing, large amounts of data, accumulated over long periods of time, are needed to evaluate whether these methods are sufficiently sensitive to replace thoracic CT scans as a long-term follow-up method for embolized PAVMs.

Treatment

Understanding of both HHT and other diseases with similar molecular pathologies might lead to new treatment options. As discussed in chapter 4, hereditary forms of PAH have a very similar genetic and molecular background to HHT. Recent studies have shown possible novel treatment for PAH and perhaps HHT. A haplo-insufficiency of ALK1, Endoglin, BMPR2 or BMP9 results in reduced levels of active receptors and can lead to both diseases.[4, 5] Studies have shown that when mice, affected by PAH due to a BMPR2 deficiency, are treated with excessive BMP9, the symptoms of PAH are reversed within 4 weeks.[6] Given the similarity between the molecular mechanism of PAH and HHT, the option of treatment with BMP9 in HHT patients could be explored in the future.

Conversely, insight in the pathophysiology of HHT might be useful in the treatment of solid tumours in the general population. In chapter 3 we observe a significantly reduced prevalence of lung cancer in the HHT population. Currently, both anti-endoglin and anti-ALK1 therapies are being studied for the treatment of solid tumours of which first results are encouraging.[7-9] Interestingly, the patients treated with anti-endoglin and anti-ALK1 antibodies develop a similar phenotype to HHT affected patients (telangiectasia and epistaxis).

Lastly, and for patients perhaps the most pressing matter, is future research into treatment and prevention of epistaxis and anaemia. Although pulmonary and cerebral arteriovenous malformations lead to significant morbidity and mortality, epistaxis and anaemia greatly impact quality of life of HHT patients.[10] Both systemic treatment with Thalidomide and Bevacizumab have been explored but side effects can be problematic as shown in chapter 7. Local treatment would be a preferred approach, but so far local treatment with Bevacizumab has been unsuccessful. [11, 12]

In conclusion, ongoing advances in medical and scientific understanding of this hereditary disease help to continuously improve patient care. Future challenges lie in several areas including genetics and their relation to the widely variable HHT phenotype, optimisation of screening, diagnostics and treatment, and improving life expectancy and quality of life of the current and future generations affected by HHT.

References

1 Duarte CW, Black AW, Lucas FL, Vary CP. Cancer incidence in patients with hereditary hemorrhagic telangiectasia. J Cancer Res Clin Oncol. 2016;

2 Hosman AE, Shovlin CL; Cancer and hereditary haemorrhagic telangiectasia. J Cancer Res Clin Oncol. 2016 Nov 11. [Epub ahead of print]

3 Hanneman K, Faughnan ME, Prabhudesai V. Cumulative Radiation Dose in Patients With Hereditary Hemorrhagic Telangiectasia and Pulmonary Arteriovenous Malformations. Canadian Association of Radiologists Journal. 2014;65(2):135-140.

4 Wooderchak-Donahue WL, McDonald J, O'Fallon B et al. BMP9 mutations cause a vascular-anomaly syndrome with phenotypic overlap with hereditary hemorrhagic telangiectasia. Am J Hum Genet. 2013;93(3):530-537.

5 Wang G, Fan R, Ji R et al. Novel homozygous BMP9 nonsense mutation causes pulmonary arterial hypertension: a case report. BMC Pulm Med. 2016;16:17-016-0183-7.

6 Long L, Ormiston ML, Yang X et al. Selective enhancement of endothelial BMPR-II with BMP9 reverses pulmonary arterial hypertension. Nat Med. 2015;21(7):777-785.

7 Doi T, Lee KH, Kim TM et al. A phase I study of the human anti-activin receptor-like kinase 1 antibody PF-03446962 in Asian patients with advanced solid tumors. Cancer Med. 2016;5(7):1454-1463.

8 Uneda S, Toi H, Tsujie T et al. Anti-endoglin monoclonal antibodies are effective for suppressing metastasis and the primary tumors by targeting tumor vasculature. Int J Cancer. 2009;125(6):1446-1453.

9 Rosen LS, Hurwitz HI, Wong MK et al. A phase I first-in-human study of TRC105 (Anti-Endoglin Antibody) in patients with advanced cancer. Clin Cancer Res. 2012;18(17):4820-4829.

10 Geirdal AO, Dheyauldeen S, Bachmann-Harildstad G, Heimdal K. Quality of life in patients with hereditary hemorrhagic telangiectasia in Norway: a population based study. Am J Med Genet A. 2012;158A(6):1269-1278.

11 Dupuis-Girod S, Ambrun A, Decullier E et al. Effect of Bevacizumab Nasal Spray on Epistaxis Duration in Hereditary Hemorrhagic Telangectasia: A Randomized Clinical Trial. JAMA. 2016;316(9):934-942.

12 Whitehead KJ, Sautter NB, McWilliams JP et al. Effect of Topical Intranasal Therapy on Epistaxis Frequency in Patients With Hereditary Hemorrhagic Telangiectasia: A Randomized Clinical Trial. JAMA. 2016;316(9):943-951.

Nederlandse Samenvatting

Hereditaire Hemorragische Teleangiëctastieën, ook wel bekend als de Ziekte van Rendu-Osler-Weber, is een erfelijke, autosomaal dominante aandoening welke wordt gekenmerkt door afwijkende vaatvorming wat resulteert in spontane, recidiverende bloedneuzen (epistaxis) en kleine- en grote vaatafwijkingen, respectievelijk teleangiëctastieën en arterioveneuze malformaties (AVMs) genoemd. In 90-95% van de patiënten kan een mutatie worden gevonden in het *ENG, ACVRL1* of *SMAD4* gen. De AVMs welke typisch worden gezien bij deze aandoening komen voornamelijk in de longen, lever en hersenen voor. Hoewel ze vaak asymptomatisch zijn, kunnen ze tot belangrijke complicaties leiden. De meest gevreesde complicaties worden veroorzaakt door paradoxale septische of steriele embolieën welke door pulmonale AVMs kunnen passeren naar de systemische circulatie en herseninfarcten of hersenabcessen veroorzaken. Door deze pulmonale AVMs tijdig te behandelen kunnen deze complicaties worden voorkómen. Een andere complicatie is ijzergebreksanemie als gevolg van bloedneuzen en gastro-intestinale bloedingen. Deze kunnen danig ernstig zijn dat ziekenhuisopname en bloedtransfusies nodig zijn. De mate waarin een individu lijdt aan symptomen hangt af van multipele factoren waaronder de gen mutatie, genetic modifiers en omgevingsfactoren.

In **hoofdstuk 2** wordt de levensverwachtingen van de ouders van HHT patiënten onderzocht. Door de hereditaire eigenschap van HHT kon de ouder aangedaan door HHT vergeleken worden met de ouder niet aangedaan door HHT. De levensverwachting bij de (onbehandelde) ouders met HHT was iets lager in vergelijking met ouders zonder HHT (gemiddelde leeftijd bij overlijden 73,3 jaar bij patiënten versus 76,6 jaar bij de controle groep). Ouders met *ACVRL1* mutaties hadden een normale levensverwachting, terwijl ouders met *ENG* mutaties 7,1 jaar eerder stierven dan mensen in de controle groep. Vrouwelijke patiënten met *ENG* mutaties hadden het meest risico op vroeg overleiden. Deze resultaten benadrukken het belang van verwijzen patiënten met HHT voor de screening van orgaan betrokkenheid en tijdig ingrijpen om complicaties te voorkomen.

In **hoofdstuk 3** wordt gekeken naar het vóórkomen van kanker in HHT patiënten en mensen niet aangedaan door HHT. Er werd een database opgezet aan de hand van vragenlijsten van 1307 deelnemers, waaronder 1007 HHT-patiënten en 142 controles. Door ook informatie over familieleden te includeren werd een cohort 2161 HHT aangedane mensen (58%

vrouwen), en 2817 controles (52% vrouwen) gecreëerd. Resultaten tonen een beduidend lagere prevalentie van longkanker in de HHT groep dan in de controle groep. De borstkanker prevalentie was hoger bij HHT dan controles. Over het algemeen was het vóórkomen van prostaat- en darmkanker gelijk in beide groepen, maar de trend van het vóórkomen van colorectaal kanker was afwijkend met een hogere prevalentie bij jongere patiënten in de HHT groep dan de controlegroep. Deze resultaten suggereren klinisch significante verschillen in het vóórkomen van de long-, borst- en colorectaal kanker in HHT patiënten in vergelijking met mensen zonder HHT.

Hoofdstuk 4 geeft een overzicht van de relatie tussen pulmonale arteriële hypertensie en HHT; met name het type veroorzaakt door *ACVRL 1* mutaties. Erfelijke pulmonale arteriële hypertensie (HPAH) is een zeldzame, maar ernstige complicatie van HHT. Beide ziekten kunnen het gevolg zijn van genetische mutaties in *ACVLR1* en *ENG* genen welke coderen voor eiwitten die betrokken zijn bij de transforming growfactor-beta (TGF-β) superfamilie, een signalerings-pathway die essentieel is voor angiogenese. Veranderingen binnen deze pathway kunnen leiden tot zowel de proliferatieve vasculopathie van HPAH en arterioveneuze vaatafwijkingen gezien in HHT. Klinische symptomen van de ziekte combinatie zijn niet specifiek maar vroege diagnose is belangrijk voor de juiste behandeling.

Hoofdstuk 5 evalueert de methode waarop kinderen worden gescreend in het St Antonius ziekenhuis voor de ziekte van Rendu-Osler-Weber en daarmee geassocieerde pulmonale arterioveneuze malformaties (PAVMs). Hier zijn 436 kinderen gescreend in de afgelopen 18 jaar middels het afnemen van anamnese, lichamelijk onderzoek, zuurstof saturatie meting en een röntgen foto van de thorax. Hoewel bij deze methode waarschijnlijk kleine PAVMs worden gemist lijkt dit klinisch geen consequenties te hebben. Bovendien betekent dit minder stralingsbelasting voor kinderen dan wanneer ze met regelmaat een CT scan moeten ondergaan. Data laat zien dat geen enkel kind een complicatie door heeft gemaakt ten gevolge van kleine, gemiste PAVMs, welke op volwassen leeftijd pas zullen worden gevonden. Deze bevindingen ondersteunen het gebruik van deze screeningsmethode.

In **hoofdstuk 6** wordt de het effect van plaatjesaggregatieremmers of anticoagulantia op epistaxis bestudeerd. Hoewel HHT bekend staat om zijn associatie met bloedingen komen er

ook relatief vaak trombo-embolische en ischemische complicaties voor. Normaliter wordt dit behandeld middels plaatjesaggregatieremmers of anticoagulantia, echter zijn veel clinici huiverig deze voor te schrijven vanwege de hemorragische kenmerken van HHT. Om de effecten van plaatjesaggregatieremmers en antistollingsmiddelen op bloedingen in HHT te evalueren, werden relevante vragen opgenomen als onderdeel van een breed internationaal onderzoek.
Van de 973 respondenten met HHT, verklaarden er 700 (71,9%) dat zij had nooit plaatjesaggregatieremmers of anticoagulantia hadden gebruikt. Het was echter verrassend dat 153 van de 379 patiënten die wel plaatjesaggregatieremmers of anticoagulantia (40,4%) hadden gekregen, geen verandering in hun neusbloedingen bemerkten. Lagere dosering van middelen, in het bijzonder plaatjesaggregatieremmers, bleek niet geassocieerd te worden met bloedingen in de hoge aantallen patiënten. Deze bevindingen ondersteunen voorzichtig gebruik van plaatjesaggregatieremmers of anticoagulantia bij patiënten met HHT als er een sterke indicatie is.

In **hoofdstuk 7** wordt het lang termijn gebruik van Thalidomide geëvalueerd. Thalidomide, ook bekend als Softenon, wordt sporadisch gebruikt als één van de laatste redmiddelen in zeer ernstige vormen van HHT. Het verminderd epistaxis en voorkomt daardoor anemie waardoor patiënten minder bloedtransfusiebehoeftig worden. Thalidomide heeft echter belangrijke negatieve bijwerkingen, waaronder neuropathie en vermoeidheid.
Twaalf patiënten werden geïncludeerd waarvan slechts een klein deel thalidomide was blijven gebruiken. Ondanks de gunstige effecten op hun symptomen waren bijwerkingen de belangrijkste reden om te stoppen. Dit doet ons concluderen dat alternatieve therapieën nog steeds nodig zijn voor de behandeling van epistaxis in HHT patiënten.

In **hoofdstuk 8** worden factoren geïdentificeerd die mogelijk invloed hebben op de ernst van epistaxis. Zeshonderdnegenenveertig van de 666 (97%) individuen met HHT had klachten van spontane, recidiverende epistaxis. Specialistische invasieve behandelingen hadden een gunstig effect op epistaxis; lasertherapie vaker dan cauterisatie. Medicamenteuze behandelingen welke vaak werden gerapporteerd in de behandeling voor epistaxis hadden ook significant gunstige scores. Meerdere etenswaren werden opgegeven als ongunstig zijnde voor epistaxis. De meest frequent gemelde items waren alcohol en specerijen.

Voeding hoog in salicylaten, natuurlijke plaatjesaggregatieremmers, of omega-3 zuren werden als schadelijk gerapporteerd. Deze data ondersteunt de bestaande behandelingen en toont dat leefstijl en voedingspatroon epistaxis bij HHT patiënten kan beïnvloeden.

Lijst van publicaties

Devlin HL, Hosman AE, Shovlin CL; Antiplatelet and anticoagulant agents in hereditary hemorrgagic telangiectasia; New England Journal of Medicine, 2013 Febuary; 368(9):876-8

Silva BM, Hosman AE, Devlin HL, Shovlin CL; Lifestyle and dietary influences on nosebleed severity in hereditary hemorrhagic telangiectasia; Laryngoscope, 2013 May; 123(5):1092-9

Hosman AE, Devlin HL, Silva BM, Shovlin CL; Specific cancer rates may differ in patients with hereditary haemorrhagic telangiectasia compared to controls; Orphanet J Rare Dis. 2013 Dec 20;8(1):195.

Lim KH, Thomas G, van Beers EJ, Hosman AE, Mourits MP, van Noesel CJ, Kater AP, Reinartz SM; Acute sinusitis and blindness as the first presentation of chronic lymphocytic leukaemia. Neth J Med. 2014 Dec;72(10):548-50.

Hosman AE, Westermann CJ, Snijder R, Disch F, Mummery CL, Mager JJ; Follow-up of Thalidomide treatment in patients with Hereditary Haemorrhagic Telangiectasia; Rhinology. 2015 Dec;53(4):340-4.

de Gussem EM, Hosman AE, Edwards CP, Westermann CJ, Snijder RJ, Faughnan ME, Mager JJ; Life expextancy of parents with Hereditary Haemorrhagic Telangiectasia; Orphanet J Rare Dis. 2016 Apr 22;11:46.

Hosman AE, Gussem de EM, Balemans WAF, Gauthier A, Westermann CJJ, Snijder RJ, Post MC, Mager JJ; Screening Children for Pulmonary Arteriovenous Malformations: evaluation of 18 years' experience; submitted

Hosman AE, Vorselaars VMM, Westermann CJJ, Snijder RJ, Mager JJ, Goumans MJTH, Post MC; Pulmonary arterial hypertension and hereditary hemorrhagic telangiectasia; submitted

Hosman AE, Shovlin CL; Cancer and hereditary hemorrhagic telangiectasia; J Cancer Res Clin Oncol. 2017 Feb;143(2):369-370

Dankwoord

Aan het eind gekomen van dit proefschrift blik ik met trots en enige opluchting terug; die strakke deadlines zijn gehaald! En daarvoor wil ik iedereen enorm bedanken die daar een aandeel in heeft gehad, want het was absoluut teamwork.

Met name dank aan mijn promotoren, professor Grutters en professor Goumans, dat jullie mij hebben geaccepteerd als PhD.
Geachte professor Grutters, beste Jan, bedankt voor de geruststellingen, de begeleiding tijdens mijn flits-bezoek als PhD-student en dat ik mee mocht met al die extreem sportieve ILD uitjes.
Geachte professor Goumans, beste Marie-José, bedankt voor al je geduld tijdens het uitleggen die complexe moleculaire pathways en dat je me een kijkje liet nemen in de wereld van de basale wetenschap. Ik kijk enorm uit naar onze toekomstige samenwerking om onderzoek in kliniek en basale wetenschap te combineren.

Geachte dr. Mager, beste Hans-Jurgen, bedankt voor deze kans. Bedankt dat je mij als kersverse dokter de kans hebt gegeven te werken en te leren in zo een bijzondere omgeving. De klinische en wetenschappelijke ervaring zijn van onschatbare waarde en hebben bij als persoon gevormd.
Geachte dr. Snijder en dr. Post, beste Repke en Marco, dank voor jullie begeleiding, kennis, ervaring, enthousiasme voor het vak en dank voor jullie realistische perspectief wanneer, meer dan eens, mijn iet wat naïeve enthousiasme de overhand nam.

Een speciaal woord van dank aan ZonMw (middels het Topzorg project) en SWORO voor de financiering van onze projecten. Dank aan de Hart en Vaat groep en onze patiënten voor de participatie in onze onderzoeken en dank aan Ingrid en Wilma voor de fijne alledaagse samenwerking. Veerle, enorm bedankt dat we zo vaak als ROW-PhDers even konden sparren. Aan de ILD-onderzoeksgroep; bedankt dat jullie mij hebben geadopteerd als enige niet-ILDer in de kamer, ik heb genoten!

Dear dr. Shovlin, dear Claire, thank you for inspiring me with your enthusiasm for good patient care, teaching me proper science and especially for making me like science. You have

kick-started my career in a way I can never thank you enough for. You have been a fantastic teacher and role model to me.
Hannah, thank you for the mental- and emotional support, the tea and the Hobnobs'. It's been great working with you!

I would like to thank my grandparents for their unconditional love and support. I will forever treasure the many weekends visiting you during my stay in London for my first scientific projects.

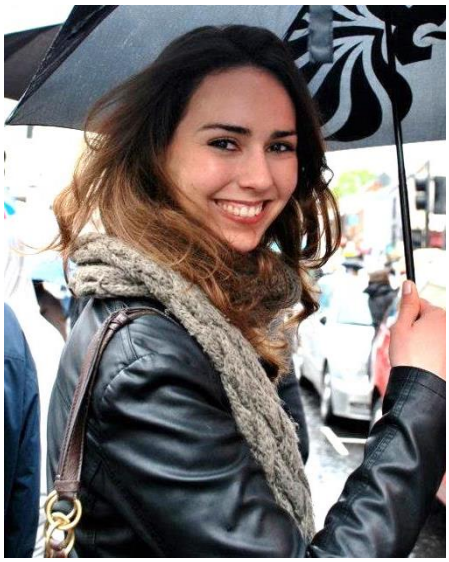
Dank aan al mijn vrienden voor de ontspanning buiten werktijden en vandaag in het speciaal mijn paranimfen Nika en Michelle.

Alle lof en dank aan mijn zusje, Kelly, voor haar creativiteit en fantastische hulp in de vormgeving en de kaft van dit boekje. Waanzinnig bedankt lieve ylleK! Dank aan mijn broertje, Tim, voor de technical support, de spelletjes en de gezelligheid.

Lieve pap en mam, natuurlijk past mijn dank aan jullie nooit op een paar regels. Toch doe ik graag een poging. Bedankt voor alles; voor alle inspiratie, adviezen, levenslessen, klushulp, liefde en support in grote en kleine dingen. Bedankt dat jullie mij alle kansen bieden om alles uit mijzelf te halen.

Liefste Elmar, ik ben blij dat jij er voor mij bent, ook als je niet weet waar ik het in hemelsnaam over heb of als ik er met mijn gestuntel weer een zooitje van maak. Ik hou van jou!

Curriculum Vitae



Anna Ellen Hosman was born in Amsterdam on October 11th, 1989. After she finished high school in 2007 at “het nieuwe lyceum” in Bilthoven she followed a year of courses in biology, neurophysiology and human pathology at Harvard University Extension School (Boston, Massachusetts). In 2008 she was accepted to study medicine at the University of Amsterdam where she received her medical degree in 2015. During her studies she did her academic internship in 2012 at Hammersmith Hospital (London) under the supervision of dr. Claire Shovlin researching different aspects of hereditary hemorrhagic telangiectasia. The fascination for this condition led to a cooperation with the Antonius Hospital and the Leiden University Medical Center studying the long term effects of Thalidomide in HHT patients in 2014. In April 2015 she started a PhD under the supervision of dr. Johannes-Jurgen Mager and Dr. Repke Snijder, while also working as a clinical physician of pulmonary medicine at the St Antonius Hospital.