INTRANASAL BEVACIZUMAB IN TREATING HEREDITARY HEMORRHAGIC TELANGIECTASIA ASSOCIATED EPISTAXIS

Long-term effectiveness and a novel correlation of Pentraxin 3 with epistaxis severity

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ABBREVIATIONS

ACVRL1	Activin receptor-like kinase 1
AVM	Arteriovenous malformation
BMP	Bone morphogenetic protein
CL	Cantril's self-anchoring ladder
CNS	Central nervous system
CVM	Cerebral vascular malformation
ESS	Epistaxis severity score
FGF	Fibroblast growth factor
HADS	Hospital anxiety and depression scale
Hgb	Hemoglobin
HHT	Hereditary hemorrhagic telangiectasia
IFT	Epistaxis intensity, frequency and need for blood transfusion score
JPHT	Juvenile polyposis and HHT
KTP	Potassium titanyl phosphate
MRI	Magnetic resonance imaging
OUH	Oslo University Hospital
OPG	Orthopantomogram
PAVM	Pulmonary arteriovenous malformation
PROMs	Patient reported outcome measures
PTX3	Pentraxin 3
QoL	Quality of life
REK	Regional ethics committee
RISBI	Repeated intranasal submucosal injections

RTM	Regression to the mean
SD	Standard deviation
SF-36	Short form 36
SPVM	Spinal vascular malformation
TGFβ	Transforming growth factor β
VEGF	Vascular endothelial growth factor
VEGFR	Vascular endothelial growth factor receptor
VAS	Visual analogue score

LIST OF INCLUDED PAPERS

I. **Steineger J**, Osnes T, Heimdal K, Dheyauldeen, S

Long-term experience with intranasal bevacizumab therapy

Laryngoscope. 2018 Oct;128(10):2237-2244

II. **Steineger J**, Geirdal AØ, Osnes T, Heimdal K, Dheyauldeen S

Intranasal bevacizumab injections improve quality of life in HHT patients

Laryngoscope. 2019 Jul; Epub ahead of print.

III. **Steineger J**, Ueland T, Aukrust P, Michelsen A, Osnes T, Heimdal K, Dheyauldeen, S

Pentraxin 3 level is elevated in hereditary hemorrhagic telangiectasia and reflects the severity of disease-associated epistaxis

Laryngoscope. 2019 Jan;129(1):E44-E49.

THE THESIS AT A GLANCE

Hereditary hemorrhagic telangiectasia (HHT) is a rare, genetic disorder that causes abnormal blood vessels in mucous membranes, skin and internal organs. HHT often leads to episodes of nosebleeds (epistaxis) which can be very frequent and bothersome, with substantial negative impact on the quality of life (QoL) in affected individuals. At present, there is no cure, and the treatment is supportive. Because the severity of epistaxis varies greatly from patient to patient, the treatment needs to be individually customized. Numerous treatment options are available, with associated side effects and limitations. Thus, the development of new treatment methods is constantly indicated.

The development of blood vessels (angiogenesis) is abnormal in HHT, and this gives rise to the clinical features observed in the disorder. Bevacizumab is a relatively new and intriguing therapeutic option in HHT, and works by inhibiting angiogenesis. It works by binding the pro-angiogenic molecule Vascular Endothelial Growth Factor (VEGF). When bevacizumab is injected in the nose it can improve the epistaxis severity in most of the HHT patients, and the side effects are believed to be minimal. Yet, previous studies regarding this treatment had short observation periods and a low number of included patients. In addition, based on case reports and our own experience, we suspected that some patients would have a gradual loss of treatment response over time. Accordingly, we aimed to study the effect of intranasal bevacizumab injections over a long-term period of several years with respect to epistaxis severity and quality of life, in a cohort of 33 patients included from 2011 in a prospective, uncontrolled case series study. Additionally, any adverse outcomes would be reported.

During the observation period, it became clear that the treatment response was very individual. Most of the patients had a very good response; some gradually lost the effect, while a minority was resistant from the start. Thus, it became important for us

to investigate if it was possible to predict which patients would benefit from bevacizumab injections. Consequently, we investigated if VEGF and other related angiogenic factors in the serum of HHT patients correlated with the epistaxis severity. The same factors were also compared to healthy controls. Measuring the epistaxis severity in HHT is important to evaluate the treatment effectiveness. However, the present scoring systems are based on subjective evaluations of the patients and additional objective methods are warranted.

1. INTRODUCTION

Hereditary hemorrhagic telangiectasia (HHT) is a rare, autosomal dominant inherited disease that leads to formation of abnormal blood vessels, and typically presents with frequent epistaxis. Multiple, tiny dilated blood vessels in the skin and mucous membranes are typical features in affected individuals. These lesions are called telangiectasias and resemble superficially located red dots.

In addition, abnormalities in larger vessels occur, producing direct communication between arteries and veins. These abnormal vessels are known as arteriovenous malformations (AVMs) and are seen in internal organs, such as lungs, liver and the central nervous system (CNS). Because of their fragile structure, the telangiectasias in the nose and gut mucosa are prone to bursting, usually causing recurrent epistaxis or less commonly gastrointestinal (GI) bleeding. Thus, affected patients may suffer from iron deficiency anemia and diminished quality of life (QoL). There is no cure at present, and although numerous treatment options are available to control and prevent bleeding, there is an unmet clinical need for additional therapies. This thesis includes papers that focus on a new treatment method in treating HHT associated epistaxis. Additionally, the novel finding of a biomarker that may have implications for clinical practice and contribute to the understanding of the disease mechanism in HHT is discussed. Based on our findings, we have renewed the treatment algorithm for HHT associated epistaxis at Oslo University Hospital (OUH), Rikshospitalet.

Historic background

The disease was first described as a "Severe, recurrent epistaxis and a malformation of the vascular system" by Sutton[1] (1864). Babington[2] (1865) was the first to recognize this as an inherited epistaxis syndrome. Later on, Rendu[3] (1896) reported the widespread nature of the cutaneous and mucosal telangiectasias. Then, Osler[4] (1904) clearly differentiated the disorder from hemophilia and further established its

hereditary character. Following this, Weber[5] (1907) was first to describe a case series of patients with the condition. Hanes[6] (1909) was the first physician to use the term "hereditary hemorrhagic telangiectasia" in a paper. The disease is also known as "Rendu-Osler-Weber disease" or sometimes simply as "Morbus Osler". Almost a century later (1994) the ENG mutation at chromosome 9 causing HHT type 1 was identified.[7] The other common genetic mutation in ACVRL1 in chromosome 12 causing HHT type 2 was described shortly thereafter (1996).[8] The clinical diagnostic Curacao criteria were published in 2000[9] and the evidence-based international guidelines were first developed in 2006 and published in 2011.[10]

Epidemiology

About 1 in 5000-8000 individuals suffer from HHT worldwide[11,12], with regional discrepancies.[13] Moreover, isolated communities may show a skewed geographical distribution.[14,15] For example, in Northern Norway there is a community with a disproportionate high number of HHT patients due to a "founder mutation".[14]

Because HHT is an autosomal dominant disease, the expected male to female ratio is anticipated to be 1:1. Despite this, most studies show a higher female to male ratio.[16,17] This is probably due to a recruitment bias where females are more oriented towards self-care than males.

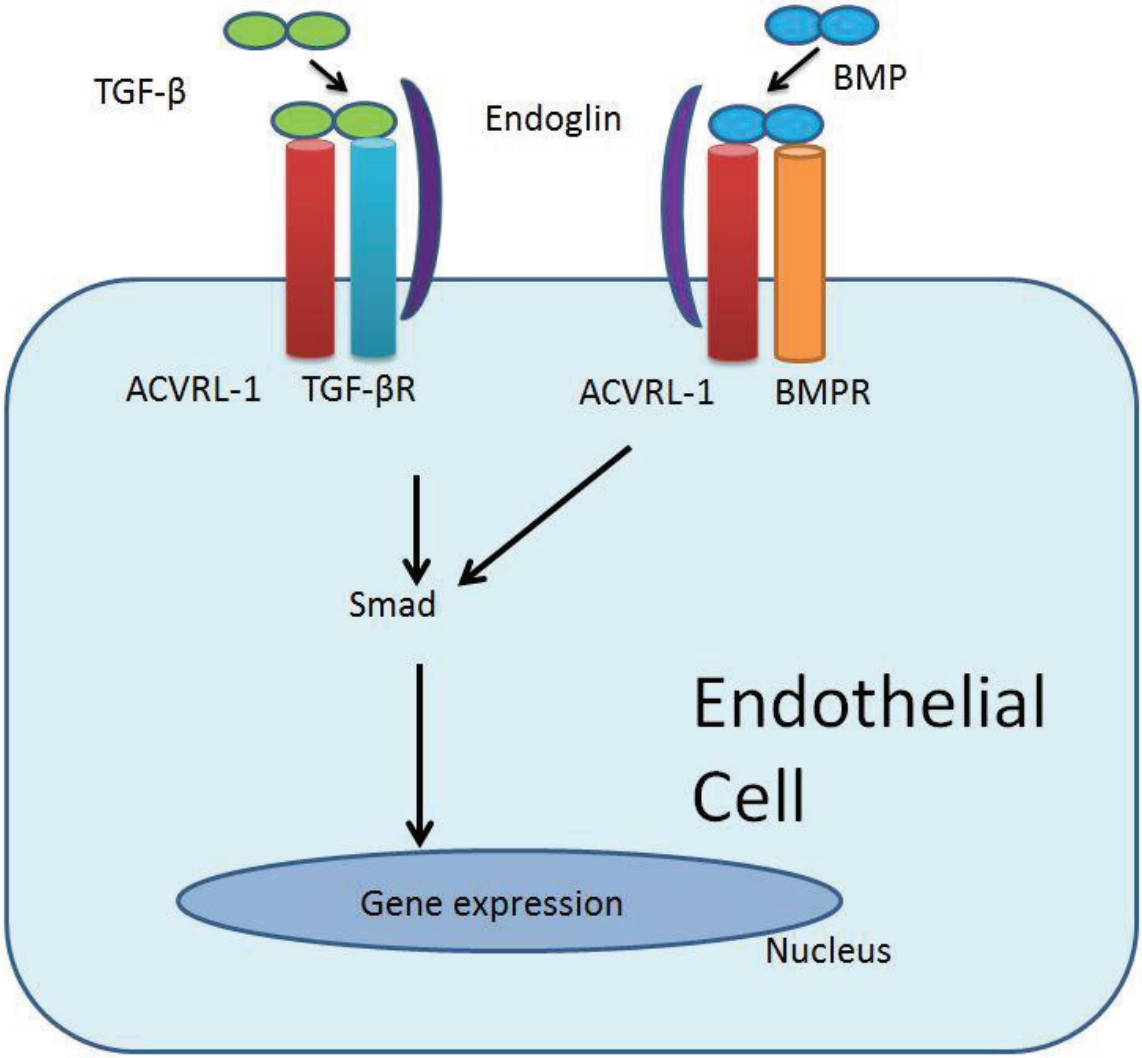
Genetics and pathogenesis

HHT is inherited in an autosomal dominant pattern, with an age dependent penetrance, and many patients have only minor symptoms until later in life.[18] The disorder is not clinically evident at birth, but rather gradually evolves into the recognizable phenotype as the telangiectasias develop.[19,20] Most cases of HHT are due to pathogenic variants in ENG encoding for endoglin in chromosome 9[7], or in ACVRL1 in chromosome 12 encoding for activing receptor-like kinase 1.[8] The transmembrane proteins ACVRL1 and endoglin activates SMAD4, which influence transcription in the nucleus. Mutation in SMAD4 leads to an often more severe form of HHT with juvenile GI-tract

polyposis (JPHT), but is much rarer than the former two.[7,8,21] All three variants result in haploinsufficiency (loss of gene product required to maintain normal function), again leading to abnormal signaling by the transforming growth factor-beta/bone morphogenetic protein (TGFβ/BMP) pathway in the vascular endothelial cells. This pathway is responsible for cell differentiation, angiogenesis, homeostasis and migration (Fig. 1). The abnormal signaling in the TGFβ/BMP pathway leads to a persistent angiogenesis and the development of the characteristic vascular lesions (telangiectasias and AVMs) in HHT.

The initial morphologic change in the pathogenesis of HHT appears to be focal dilatation of post-capillary venules. These venules gradually increase in size and eventually become twisted and connect to capillary arterioles through capillary segments. Finally, these segments disappear, leading to direct arteriovenous communication. In telangiectasias, the venules show excessive layers of smooth muscle cells without any elastic fibers or an incomplete layer of smooth muscle cells. Similar to telangiectasias, AVMs lack intervening capillaries and consist of direct connections between arteries and veins, but are much larger than telangiectasias.[22,23]

Figure 1: The signaling pathway involved in the pathogenesis of HHT



ACVRL-1: Activing receptor-like kinase-1; BMP: Bone morphogenetic protein; TGFβ: Transforming growth factorβ; BMPR: Bone morphogenetic protein receptor; TGFβR: Transforming growth factorβ receptor.

The type of gene mutation influences the HHT phenotype, but a significant variance is also observed between family members who carry the same mutation. Mutation at ENG causes HHT1[7], and mutation at ACVRL1 causes HHT2[8], and together they constitute approximately 85% of patients with the disorder.[24] Owing to the extreme allelic heterogeneity of the disorder, over a thousand ENG and ACVRL1 variants to date are described to lead to HHT-like phenotypes.[25] HHT1 has a higher prevalence of pulmonary AVMs compared to HHT2, while HHT2 is reported to have a later onset and a lower penetrance.[7,26] On the other hand, patients with HHT2 may have higher risk of liver manifestations and GI bleeding.[27] The more rare mutation in SMAD4 is

responsible for 1-2% of the cases[28] and is associated with juvenile polyposis[29]. This syndrome is known as JPHT (juvenile polyposis and HHT) and is a precancerous condition. About 15% of HHT patients have an unidentified genetic cause.[21]

A variable disease pattern in families with identical gene mutations is observed in HHT. This suggests that the phenotype is affected by not only the gene mutation, but also environmental or additional genetic factors.[30,31]

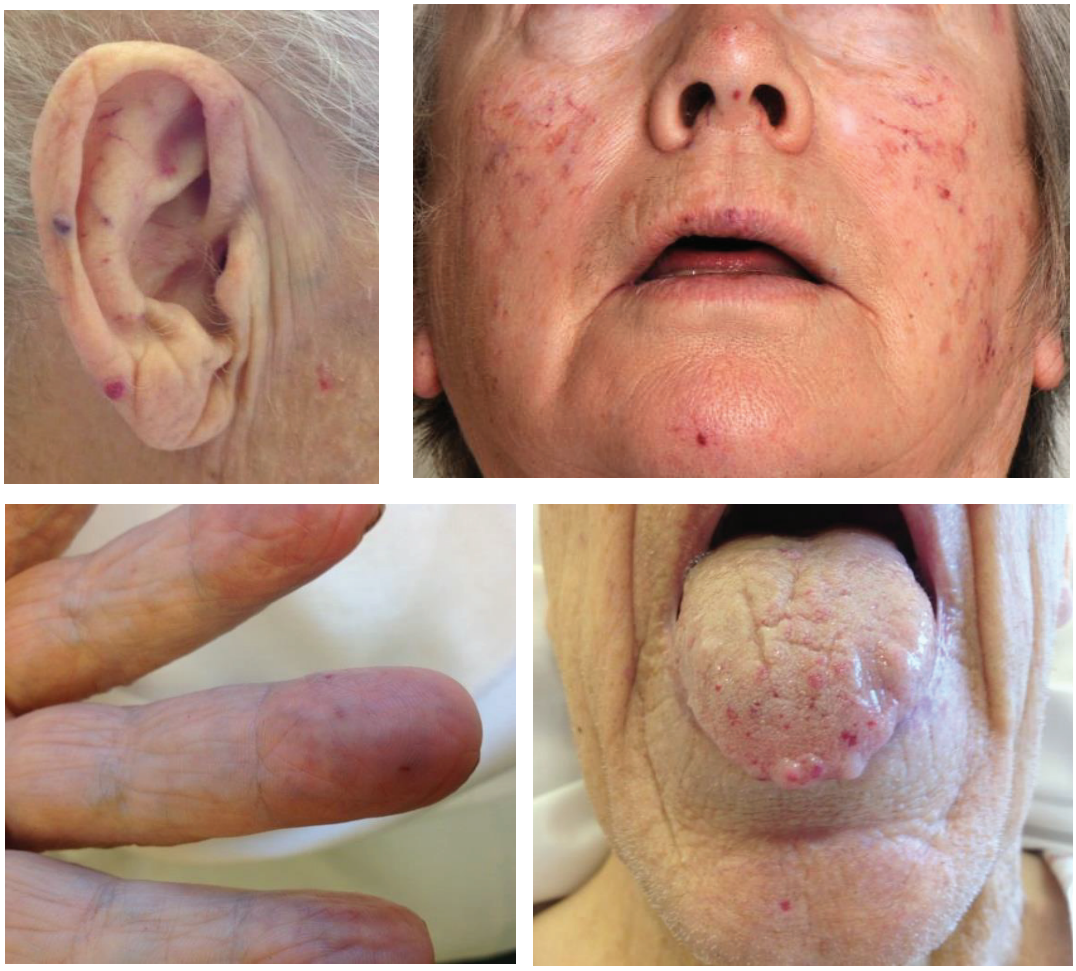
The "second hit" hypothesis in HHT suggests stimuli such as wounding, high blood velocity[32], angiogenesis or inflammation must be present in addition to haploinsufficiency, for the vascular lesions observed in the disorder to develop.

Of note, HHT patients have raised levels of Vascular Endothelial Growth Factor (VEGF) in serum.[33-35] VEGF is a key pro-angiogenic factor, involved in both physiological and pathological de novo formation of new blood vessels. This insight is recently applied in clinical practice, by the use of angiogenic inhibitors in the treatment of HHT.

Microvascular disease traits

The microvascular traits of HHT are due to development of pathological dilated vessels, termed telangiectasias, in the nose, skin and GI-tract. They arise when dilated and elongated venules communicate directly with dilated arterioles, associated with the absence of the normal intervening capillary bed. They resemble red spots up to a few millimeters in size, and may easily burst and bleed (Figure 2).

Figure 2: multiple telangiectasias of the skin and mucous membranes in HHT patients



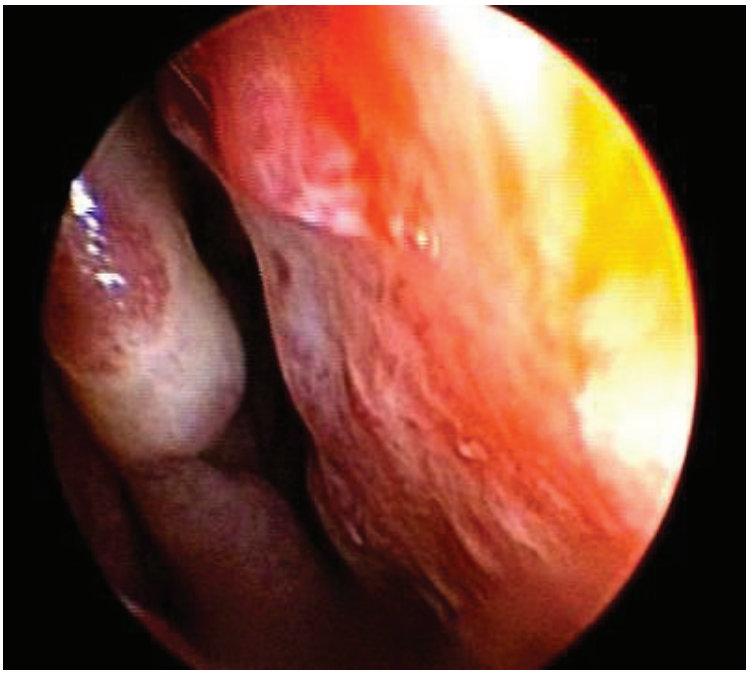
Epistaxis

Recurrent, spontaneous and easily provoked epistaxis from the telangiectasias in the nasal mucosa (Figure 3) is the most prevalent symptom in HHT.[16] It affects about 90% of all patients with the disorder, and has the greatest negative impact on QoL[36,37] overall. Further, epistaxis is usually the first sign of disease.[17] About 95% of patients with the disorder suffer from epistaxis before the age of 50, and the majority within the first two decades in life.[17,38]

Epistaxis in HHT is recurrent and varies from mild and self-limiting, to profuse, transfusion-dependent or rarely even life threatening episodes.

HHT associated epistaxis is discussed in more detail in Chapter 2.

Figure 3: Telangiectasias of the nasal mucosa (Right nasal cavity)



Telangiectasias

In addition to the nasal mucosa, telangiectasias tend to appear in oral mucosal membranes, as well as the skin, in up to 90% of patients with HHT. Other predilection sites include the tongue, lips, fingertips, hands and ears. Cutaneous telangiectasias rarely bleed and are mostly a cosmetic concern.[39] The patients commonly develop more cutaneous telangiectasias as they age.[17]

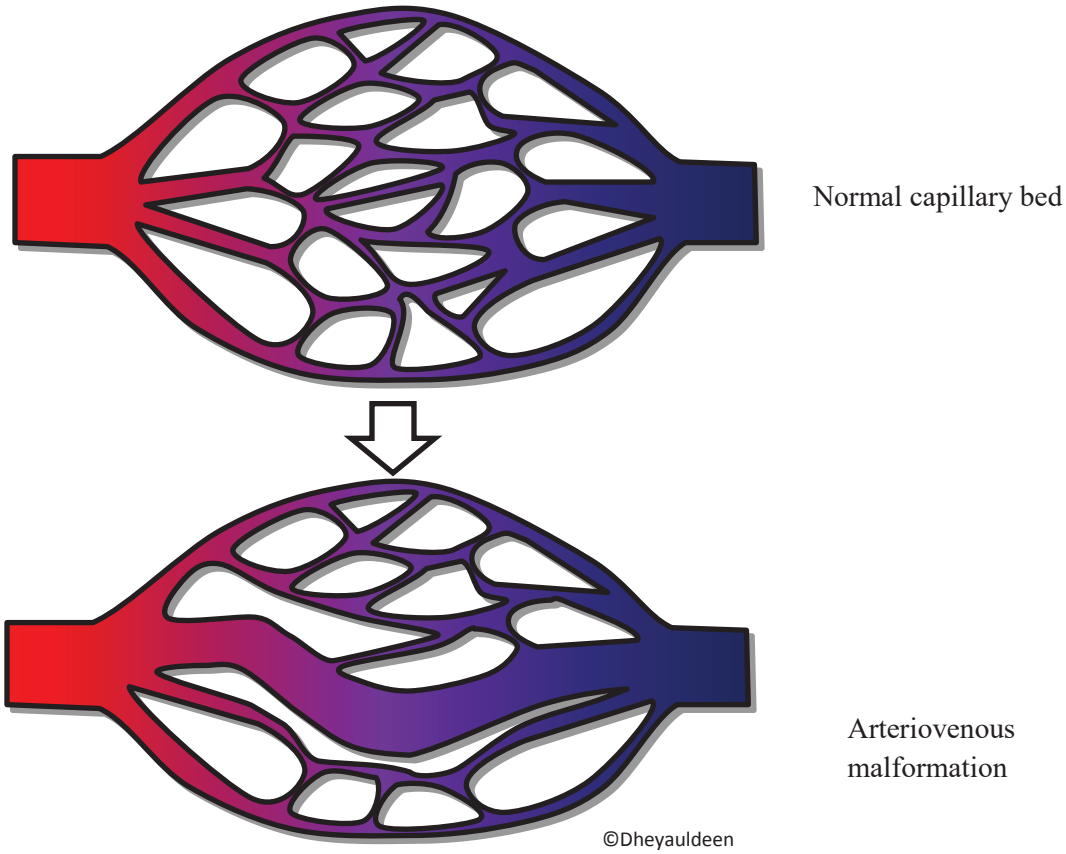
GI-tract

GI telangiectasias are frequent in HHT patients, and occur primarily in the stomach and upper small intestines. One study described 64% gastric and 91% small intestine telangiectasias among 23 HHT patients.[40] However, it should be emphasized that GI telangiectasias are most often asymptomatic. GI bleeding is seen in 13-30% of HHT patients (opposed to 3% in the general population), and onset is usually from age 40.[41,42] These numbers may be underestimated however, as patients and doctors are likely to attribute the anemia with epistaxis rather than GI-hemorrhage.

Macrovascular disease traits

The macrovascular lesions observed in HHT develop by the same pathogenic mechanism as telangiectasias, by formation of AVMs. Yet, whereas telangiectasias are numerous and comprehensively dispersed, AVMs are much larger in size and observed predominantly in the liver, lung and CNS (Figure 4).

Figure 4: Development of an arteriovenous malformation



Liver

In a study of 584 patients diagnosed with HHT, hepatic AVMs were reported in 40.6% of HHT2 and 7.6% of HHT1 patients.[43] However, as HHT patients are not routinely screened for liver involvement, the incidence was estimated to be higher. The majority of HHT patients with hepatic AVMs are non-symptomatic. In one study, CT screening showed liver involvement in 79% of 24 studied HHT patients, whereas only 26% had symptoms of hepatic disease.[44] The most common symptoms of hepatic AVM is high output heart failure due to portal hypertension, biliary disease and portal hypertension.[45]

Lung

The prevalence of pulmonary AVMs (PAVMs) depends on HHT genotype. They are identified in up to 58% of HHT1 and 18% of HHT2 patients.[46,47] They tend to become apparent after puberty, but may also occur in children.[48] PAVMs occur predominantly in the lower lobes and may be multiple. They act as shunts between the systemic and pulmonary circulation, due to the loss of intervening capillaries. These "right to left" shunts interrupt the oxygenation of the pulmonary arterial blood passing through, and may lead to hypoxemia. In addition, the shunts may cause paradoxical embolic stroke, and infrequently brain abscess.[49] Hemorrhagic complications are rare, but can arise from spontaneous PAVM rupture, and this may lead to massive hemoptysis or hemothorax. Approximately 20% of HHT patients without evidence of PAVM on CT display evidence of right to left shunting on contrast echocardiography.[47] Moreover, 20% of unselected HHT patients show evidence of increased pulmonary artery pressure.[50] This is usually not due to primary pulmonary hypertension, but rather secondary to hepatic AVMs. [51]

Central nervous system

HHT patients are estimated to display cerebral vascular malformations (CVMs) in 11-23% of cases[52,53], including cerebral arteriovenous malformations; micro AVMs; arteriovenous fistula and telangiectasias.[54] These CVMs are most often multiple[55], and can be present in all HHT phenotypes, without specific genotype predominance.[54]

CVMs are usually non-symptomatic, but may rarely lead to seizures, cerebral ischemia or intracranial bleeding.[56] A retrospective study estimated a 0.5% annual risk of bleeding from CVMs in HHT.[57]

Spinal vascular malformations (SVMs) in HHT are usually fistulas and occur far more infrequently than CVMs. SVMs most often present with acute severe neurological decline, and surgery, embolization or a combination of both is the preferred treatment[58].

Diagnosis

The diagnosis of HHT is clinical and based on the recognition of the classical features that characterize the disorder. The consensus clinical diagnostic criteria (known as the Curaçao criteria) were published in 2000[9] (Table 1).

Table 1: The diagnostic Curaçao criteria

Feature	**Description**
Nosebleeds	Spontaneous and recurrent
Telangiectasias	Multiple, at characteristic sites (nasal mucosa, oral cavity, lips and fingers)
Internal organ manifestations	Gastrointestinal telangiectasias (with or without bleeding) Pulmonary AVM Liver AVM CNS AVM
Family history	A first-degree relative with a positive diagnosis according to these criteria.

A diagnosis of HHT is definite if three or four criteria are fulfilled, possible or suspected in the presence of two criteria, and unlikely if one or no criterion is met.

Genetic testing is available, and increasingly more used as cost decrease. Yet, genetic tests are only positive in approximately 85 % of patients with HHT[59], so a negative test

does not rule out the diagnosis. Genetic testing is especially useful in relatives of HHT patients who do not meet the diagnostic criteria (often children and young adults). At OUH, all patients with clinical suspicion of HHT are gene tested.

Screening

According to the international guidelines for HHT published in 2011[10], screening is recommended for PAVMs and CVMs. Transthoracic contrast echocardiography is the initial recommended method for PAVM, followed by an unenhanced thoracic CT scan if positive contrast echocardiography. It is recommended to repeat the screening for PAVM every 5 years. In Norway, the screening for PAVM is initiated from age 16. Children under the age of 16 years are only scanned for PAVMs if they have symptoms, to avoid unnecessary radiation exposure. PAVMs may bleed, leading to fatal complications in later pregnancy.[60] Therefore, women with HHT who plan to become pregnant should undergo evaluation for PAVMs before pregnancy.

Screening for CVMs in HHT is performed by cerebral MRI, but remains controversial in asymptomatic patients. In 2014, the ARUBA trial (A Randomized trial of Unruptured Brain Arteriovenous Malformation) showed increased risk of death or stroke after intervention for asymptomatic CVMs in the general population.[61] CVMs in HHT may have even lower risk of hemorrhage compared to CVMs in the general population.[62] Thus, many experts now recommend against screening for CVMs in asymptomatic HHT patients.

Screening for liver AVMs is not generally recommended. An exception is made for patients with one or two Curaçao criteria, where genetic tests are inconclusive or unavailable. Investigations for liver involvement are also indicated in HHT patients who have symptoms of hepatic disease.[10] In these cases, a Doppler ultrasound is the appropriate diagnostic method.

HHT patients with anemia and hemoglobin (Hgb) levels disproportionate to epistaxis severity should be referred to gastro-duodenoscopy to assess the possibility of GI-hemorrhage. Finally, all patients with the SMAD4 gene mutation have increased risk

of GI cancer, and should undergo GI screening with endoscopy and capsule surveillance starting from age 15-18 years and 25 years, respectively.[10]

Treatment

Curative treatment for HHT is not available, since gene therapy does not yet exists. The treatment for HHT associated manifestations includes AVM-specific and epistaxis specific options. Discussing the various strategies for AVM-specific interventions is beyond the scope of this thesis. Most of the various treatment options for HHT associated epistaxis are summarized in Table 2.

Prognosis

HHT has previously been associated with a reduced life expectancy, approaching a decrease in 7 years in one study.[63,64] Gender and gene mutation are also prognostic factors, where women with the ENG mutation is at most risk with a median decrease in life expectancy of 9.3 years.[65] The increase in premature death is due to acute complications. On the other hand, if optimal management of the disorder is performed (e.g. screening and treatment for PAVMs, treatment for iron deficiency anemia etc.), the reduction in life expectancy may improve to the level of the general population[66]. Moreover, a recent epidemiological study suggests that HHT patients are affected to a lesser degree of cancer than the background population[66].

2. BACKGROUND

Epistaxis in HHT

Recurrent epistaxis, caused by bleeding from telangiectasias in the nasal mucosa, is the most common and usually the earliest symptom in HHT.

Individuals with HHT display a great variation in epistaxis, across geography[13,14] but also within the same families.[67]

Epistaxis is the most annoying symptom for HHT patients, with the greatest negative impact on QoL.[36,37] HHT-associated epistaxis is typically spontaneous, easily provoked and recurrent, and often leads to functional and social distress. It may also

cause secondary health problems such as iron deficiency anemia, malaise and dyspnea.[16]

The epistaxis specific scoring systems in HHT

To offer the appropriate treatment for HHT patients, it is important to evaluate the degree of epistaxis severity. In addition, a correct assessment of the epistaxis severity is essential as an outcome measure in HHT therapeutic research. Consequently, different groups have developed numerous epistaxis specific scoring systems for use in HHT during the past decades. For many years, there were no commonly accepted systems, which made it difficult to compare results between various research groups. In 2008, Al-Deen et al proposed the IFT, which measure the epistaxis intensity, frequency and need for blood transfusion in HHT.[68] Shortly thereafter, Hoag et al. published the Epistaxis Severity Score[69] (ESS), through a Cure HHT grant. The ESS also encompass the duration of epistaxis episode, in addition to the parameters measured with IFT.

Treatment options for HHT associated epistaxis

At present, there is no cure for HHT, since gene therapy is not yet available. Hence, the treatment strategy can be summarized into four groups:

1. Compensation therapy: compensate for hemorrhage with blood transfusions and oral or intravenous iron supplements.

2. Obliteration or surgical therapy of affected vessels: e.g. surgical resection of cerebral AVM, replacing the nasal mucosa by skin grafts (septodermoplasty) and obliteration of telangiectasias by laser photocoagulation.

3. Bleeding prevention therapy: by using antifibronolytic agents (such as tranexamic acid) or hormonal therapy (e.g. Tamoxifen).

4. Anti-angiogenic therapy: correcting the underlying dysregulated angiogenesis with bevacizumab, thalidomide or similar agents.

Table 2 summarizes the most commonly used treatments to control HHT associated epistaxis.

Table 2: treatment options for HHT associated epistaxis

Compensation

- Red packed cells transfusions
- Iron supplements

Cautery

- Electro
- Chemo
- Cryo

Laser

- Argon
- KTP
- Nd:Yag
- Diode
- Pulsed- dye

Hormonal

- Topical
  - Ostriol
- Systemic
  - Tamoxifen (antiestrogenic)
  - Ostradiol
  - Medoxyprogesterone

Antifibrinolytic

- Tranexamic acid
- Ethamsylate

Surgical

- Modified Septodermoplasty
- Closure of the nose (Young's procedure)

Anti – VEGF

- Bevacizumab
- Thalidomide

Selective arterial embolization

Sclerotherapy

Radiotherapy

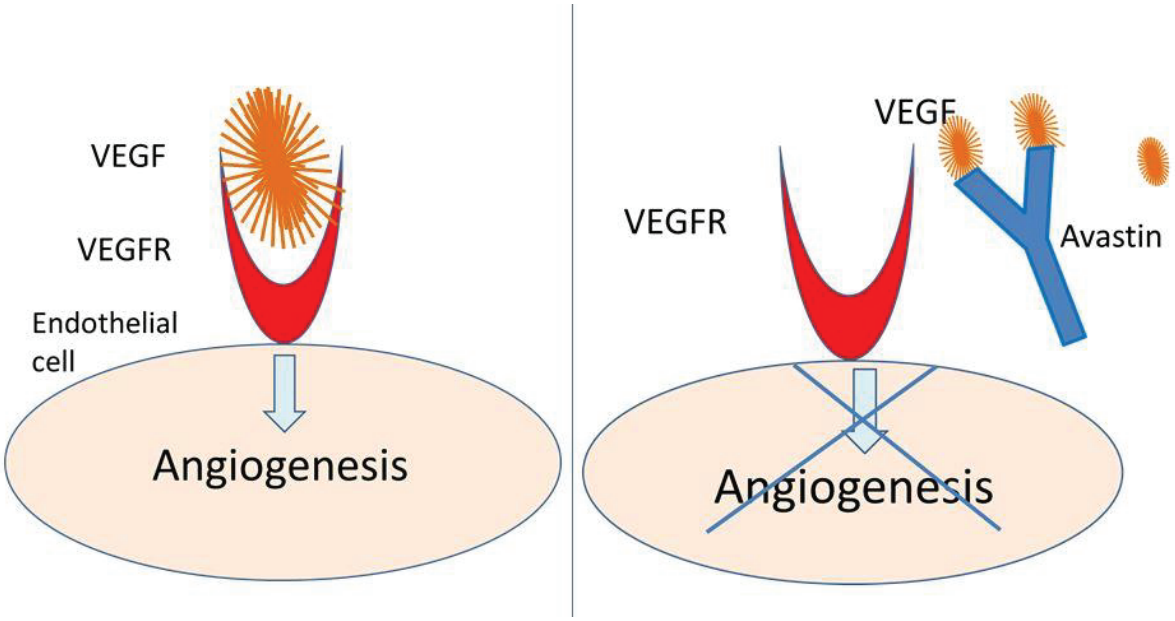
KTP:Potassium titanyl phosphate; VEGF: Vascular endothelial growth factor

Most of the methods for treating HHT related epistaxis are expensive, associated with possible side effects or contraindications. Many HHT patients respond successfully to one method for a certain period before the treatment becomes less effective and the need for an alternative method arise. Thus, there is a need for continuous development of new and improved treatment options. In the last decade, the improved understanding of HHT pathogenesis identified VEGF as a possible target to treat the disorder.

Bevacizumab

Bevacizumab (Avastin®, Genentech, Roche, South San Francisco, California, USA) is a recombinant humanized antibody, designed to bind and inhibit the pro-angiogenic molecule VEGF. The mechanism of action on a molecular level is shown in Fig. 5.

Figure 5: The proposed action of Bevacizumab



VEGF: Vascular endothelial growth factor; VEGFR: Vascular endothelial growth factor receptor

Bevacizumab is effective in treating colorectal and other cancers in combination with chemotherapy, where it improves survival.[70] By binding to VEGF extracellularly, bevacizumab inhibits the pathological angiogenesis that occurs during malignant tumor growth. The oncologic dose of bevacizumab is 5-15 mg/kg intravenously every two to four weeks. At oncologic doses, bevacizumab is associated with several side effects. The most serious side effects include hemorrhage (GI or lung), GI perforation and thromboembolisms. Other side effects include hypertension, epistaxis, nasal septum perforation, cytopenia, proteinuria, and impaired wound healing.[70,71] In recent years, some reports have documented osteonecrosis of the jaw following bevacizumab treatment, either alone or in combination with other drugs, like steroids or bisphosphonate.[72] All the reported cases occurred in patients who received bevacizumab in oncologic doses intravenously. Osteonecrosis *outside* the jaw has also been reported in the literature as a side effect of bevacizumab administered in oncologic doses.[73,74]

Bevacizumab in HHT

Interestingly, bevacizumab was discovered as a therapeutic agent in HHT accidentally. In 2006, a patient with HHT received bevacizumab in combination with pemetrexed for treatment of a malignant mesothelioma. The physicians noted a profound improvement in the severity of epistaxis and transfusion requirements in the patient.[75] Later on, HHT patients have been treated with bevacizumab to improve epistaxis, GI bleeding and liver AVMs. For HHT-associated epistaxis, bevacizumab has been administered both systemically and locally (intranasally), with variable doses and intervals. Nevertheless, the role of intranasal bevacizumab in HHT is still uncertain. Recently, two literature reviews came to differing conclusions in this regard.[76,77] Moreover, long term-studies that examine the response to the treatment and potential associated adverse effects are absent. Also of importance, long-term intranasal bevacizumab treatment has not been studied with respect to impact on quality of life.

Angiogenic factors in HHT

The abnormal function of the regulatory proteins in the TGF-β superfamily has traditionally been viewed as responsible for the disease manifestations observed in HHT. The pathogenesis is complex and not fully understood but it is recognized that the characteristic vascular lesions of the disorder occur due to a dysregulated angiogenesis. In line with this, Cirulli et al[33] and later Sadick et al[34] described elevated levels of the pro-angiogenic molecule VEGF in the serum of HHT patients. The disease is profoundly heterogeneous, as evident in family members with the same mutation who present with different phenotypes[67]. This implies the involvement of other mediators than members of the TGF-β family and VEGF in the pathogenesis.

3. AIMS

The following issues are addressed in the thesis:

1. Is long-term treatment with intranasal injections of bevacizumab effective and safe for HHT patients? (Paper 1).

2. Can repeated intranasal injections of bevacizumab improve the health related quality of life in patients with HHT associated epistaxis? (Paper 2).

3. Can VEGF levels in serum predict the severity of epistaxis in HHT? (Paper 3).

4. Can other inflammatory or angiogenic molecules predict the severity of epistaxis in HHT? (Paper 3).

Paper 1 and Paper 2

The purpose of paper 1 was to describe the long term outcomes of repeated intranasal submucosal bevacizumab injections (RISBI) on HHT associated epistaxis. The effect was measured with the epistaxis specific scoring systems ESS and IFT, in addition to Hgb levels. Furthermore, any adverse effects or events were recorded.

The aim of paper 2 was to further determine the impact of RISBI on HHT associated epistaxis with respect to health related QoL and psychological distress. The patients included in paper 1 were invited to complete QoL questionnaires before and after a period of RISBI.

Paper 3

The premise for paper 3 was based on two main points.

First, prior research has emphasized a raised level of VEGF in the serum of HHT patients, providing a rationale for treatment with the VEGF-inhibitor bevacizumab.[33,34] Nevertheless, other molecules closely related to VEGF, affecting angiogenesis and endothelial inflammation have not been examined. A discovery of increased levels of

other mediators may clarify pathogenic mechanisms in the disorder, and even open up new therapeutic opportunities.

Secondly, the observed variable sensitivity to RISBI makes it interesting to examine the possible correlation between serum level of VEGF and related angiogenic factors with the clinical presentation of the disease. Disease specific variables were epistaxis severity, measured with ESS and IFT, and the presence of internal organ manifestations seen in HHT. In addition, we evaluated the angiogenic factors in blood sampled from HHT patients attending the clinic and compared them to healthy controls. Biomarkers for HHT can help in the following aspects:

1. Diagnostic aspects: Biomarkers can provide supplementary evaluation of epistaxis severity. The current evaluation tools are limited to patient reported outcome measures (PROMs), epistaxis specific scoring systems (e.g. ESS and IFT) and Hgb levels. PROMs, measured by SF-36 may not be sensitive enough to detect small, but clinically relevant changes in the study population. For example, the SF-36 is reported to not correlate significantly with the frequency of epistaxis measured by ESS[78], and ESS and IFT also have limitations. Additionally, the Hgb levels alone are inadequate for epistaxis evaluation, since factors such as GI bleeding, dehydration, iron supplements and blood transfusions may influence the level of Hgb.

2. Prognostic aspects: Biomarkers can predict outcomes and evaluate responses to treatment. The identification of serum or plasma markers that can predict the risk of future episodes of epistaxis in HHT is needed. Prognostic biomarkers can have value in clinical decision making when selecting the appropriate treatment for HHT patients.

4. MATERIALS AND METHODS

OUH, Rikshospitalet is responsible for treating most of the patients with HHT in Norway. The majority of these patients require repeated treatment to decrease epistaxis severity. The ENT department in OUH offers several treatment options for HHT associated epistaxis. These include different types of laser photocoagulation, argon plasma coagulation, septodermoplasty, and medical therapy. In 2011, intranasal submucosal bevacizumab injections were implemented as a treatment alternative for HHT associated epistaxis at our department.

Intranasal submucosal bevacizumab injections

The injection technique used is based on the vascular anatomy of the nose.[79]

Preparation

The procedure is performed either in local with sedation or general anesthesia. The patients receive 1 to 2 grams of paracetamol and 5 mg oxycodone orally 30 to 60 minutes preoperatively. Some patients also receive 5 to 7.5 mg midazolam. The nose is gently packed with gauze soaked in a solution of topical tetracaine (16 mg/mL) and adrenalin (0.2 mg/mL) for 30 to 60 minutes. Repeated doses of fentanyl (50 µg/mL) are administered intravenously during the procedure.

Bevacizumab dose

The first eight injections were performed with a total dose of 100 mg bevacizumab submucosally at four specific anatomical sites. Subsequently, the dosage of bevacizumab was increased to 200 mg based on a written recommendation we received from a senior authority (Dr. T. Davidson at the Nasal Disorder Clinic in San Diego).

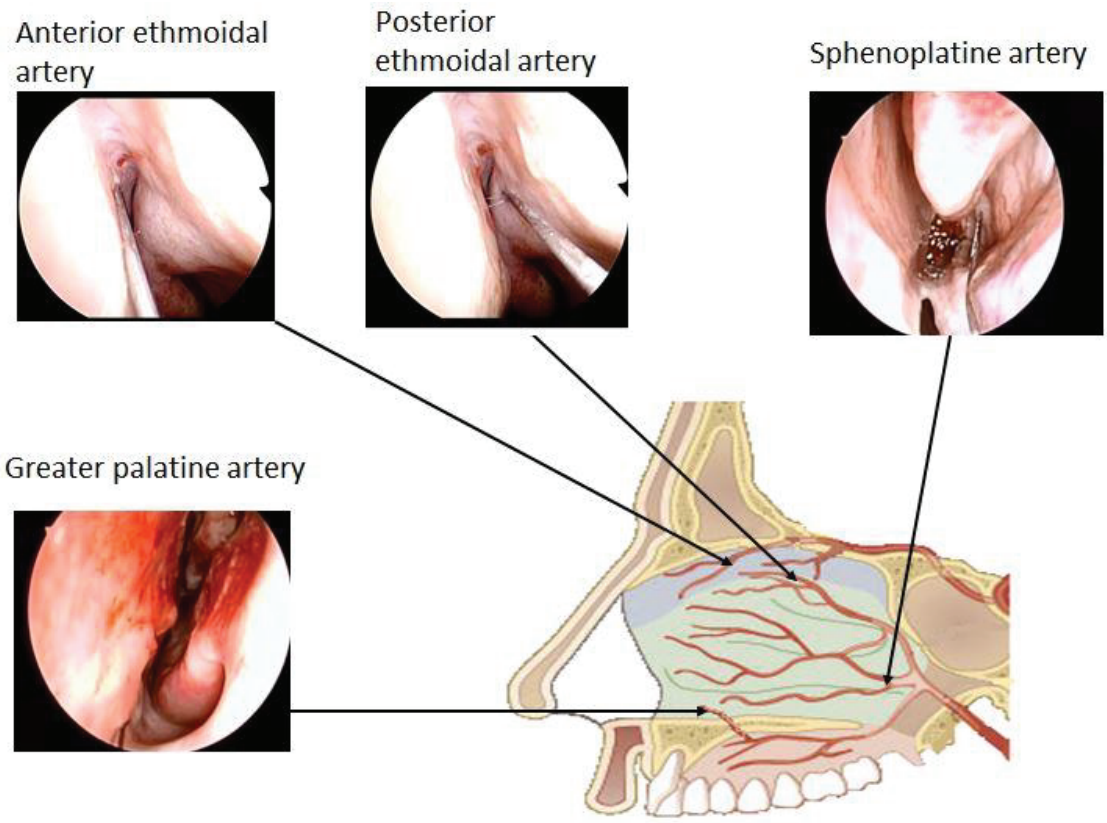
Injection technique and procedure

Each of the following areas was injected with 1 mL 25 mg/ml bevacizumab on each side: 1) the sphenopalatine area, 2) upper part of bony septum, 3) upper part of the lateral nasal wall, and 4) the anterior floor of the nose. These four areas correspond to

the points of entry of the main arteries responsible for the blood supply to the nasal mucosa, which are the sphenopalatine artery, the anterior ethmoid artery, posterior ethmoid artery, and greater palatine artery (Figure 5).

A long 23 gauge needle was used, and the procedure was assisted with a 0 or 30 degree rigid endoscope. Additional tetracaine/adrenalin packing or Surgicel was used to treat any bleeding during the procedure. Patients who reported discomfort when undergoing the procedure were given successive treatments under general anesthesia.

Figure 6: The injection sites of bevacizumab



Paper 1

All patients scheduled for RISBI between June 2011 and August 2013 were included in the study. The indication for RISBI was lack of long-lasting improvement in epistaxis severity from other treatment methods. These options included pulsed dye laser, diode laser, argon plasma coagulation and septodermoplasty. All the included patients were previously treated with pulsed dye and/or diode laser. Approximately

half of the patients had been treated with argon plasma, when laser was ineffective. Septodermoplasty was performed on two of the included patients previously, without satisfactory long-term control on epistaxis.

All of the included patients were followed up until; a) discontinuation of RISBI due to a gradual loss of effect; b) discontinuation of RISBI due to an adverse event; or c) end of the observational period in April 2017.

The follow up included evaluation with ESS and IFT scoring (Appendix 4 and 5). This evaluation was performed prior to initiation of RISBI, and repeated 6-8 weeks after each injection up to and including the third treatment. Finally, an endpoint evaluation was performed in April 2017, for the patients who still received RISBI. In addition, Hgb levels were measured immediately before, and 4 to 8 weeks after each injection throughout the entire study period.

The patients completed ESS and IFT surveys before each treatment session at the clinic. Afterwards, they were provided with ESS and IFT questionnaires to take home, fill out and send back to the clinic 6-8 weeks later. The Hgb samples were collected at the clinic before injections, and at the family doctor's office or local hospital 4-8 weeks post-treatment.

The injection intervals were based on the individual response to the treatment. After the first injection, all patients were scheduled to a follow up appointment 6 months later. After this, further appointments were made on a case-by case basis depending on the treatment response. The patients were told to contact the ENT department at an earlier time than the scheduled appointment if the epistaxis severity increased. This was based on the subjective evaluation by the patient regarding epistaxis intensity, duration and frequency. Thus, additional treatment was offered when the effect of the previous injection began to decline. Similarly, the patient had the opportunity to postpone the scheduled treatment if he/she still had a good response to the previous injection.

Paper 2

The patients we treated with RISBI from June 2011 to August 2013 were consecutively invited to a QoL and psychological distress study. Thus, the patients were included from the same cohort as in paper 1.

Measures

The included patients answered QoL and Hospital anxiety and depression scale (HADS) questionnaires before the first treatment and 6-8 weeks after the last treatment (Table 3). At the same time, the severity of epistaxis was assessed by the ESS and the IFT.

Quality of Life

For a comprehensive evaluation, three different levels of QoL were measured. The levels were overall-, health-related-, and disease specific QoL. We used Cantril's Self-Anchoring Ladder (CL) to measure overall QoL, Short Form 36 (SF-36) to measure health-related QoL, and a symptom specific-QoL question to measure the disease specific QoL.

1. CL was used to assess the subjective overall life satisfaction. This is a self-administered questionnaire with one question; "How is your life?" The response alternatives are 0 -10 (0 = worst QoL, 10 = best QoL).

2. Short Form-36 (SF-36) measures eight dimensions: physical functioning (PF), role limitation due to physical health problems (RP), bodily pain (BP), general health (GH), vitality (VT), social functioning (SF), role limitations due to emotional problems (RE), and mental health (MH)[80]. From these dimensions two component scales are constructed; the Physical component scale (PCS) and the Mental component scale (MCS). The MCS is generated by the VT, SF, RE, and MH, while the PF, RP, BP, and GH constitutes the PCS. According to standard (SF-36) scoring, all scores were transformed into a 0 (worst) to 100 (best) scales. Score <40% was regarded as poor QoL. The Norwegian validated version 12 of SF-36 was used[81].

3. The disease-specific quality of life was assessed using the following question: “To which level does the HHT impact your Quality of life?” The answer alternatives are from 1 which corresponds to ‘‘no impact on QoL’’ and to 10 which corresponds to ‘‘the worst possible QoL’’.

A cut-off score was chosen at 6 or lower (1–6 score corresponds to no or small impact on QoL). Higher score of SF-36 and CL reflect better QoL, while higher disease specific score reflects that the disease has more impact on the QoL.

Hospital Anxiety and Depression Scale

The Hospital Anxiety and Depression Scale (HADS) scale[82,83] was used to measure the level of distress (Appendix 2). This self-report questionnaire consists of 14 items, and is subdivided into the two scales: anxiety (HADS-A) and depression (HADS-D). Both HADS-A and HADS-D have seven items, and the answer alternatives range from 0 (not present) to 3 (maximally present). This gives a sum score from 0 to 21 on each scale. Higher scores reflect more severe symptoms. A score of 8 or greater is recognized as clinically relevant anxiety or depression.

Table 3: The measurements used to assess HR-QoL and Psychological distress

Measure	Questionnaire used	Remarks
Overall QoL	Cantril's ladder	One question
HR-QoL	SF-36	36 questions, 8 dimensions
DS-QoL	Disease specific question	One question
Psychological distress	HADS (A+D)	Anxiety: 7 questions Depression: 7 questions

Paper 3

Blood samples were collected from consecutive HHT patients attending the ENT department at OUH for HHT associated epistaxis during the period from February 2012 to August 2013. The grade of epistaxis was evaluated using the ESS and IFT at the day of sampling. Some patients attended the clinic more than once during this period (usually with 4-6 months intervals). Consequently, blood samples were collected and the severity of epistaxis was scored more than once in these patients.

The first sample from each patient was used as a baseline sample in studying the correlation of angiogenic molecule levels with the presence of internal organ manifestations. Blood samples were also obtained from 16 healthy control persons.

All the molecules were measured in duplicate with enzyme immunoassays using antibodies. Coefficients of variation were <10% for all assays.

Appendix 1 shows the measured molecules in the study, all related to endothelial cell activation.

Statistical measures and methods

In Paper 1 and 2, the T-test was used to compare the difference between pre- and post-treatment measurements. In addition, in paper 2 regression analyses was performed to examine any associations between demographic variables (gender and age) and QoL scores.

In paper 3, the non-parametric Mann-Whitney test was used to calculate significance of difference between the patients and the controls, since a normal distribution of data could not be assumed. Multivariable linear regression on transformed levels was used to adjust for differences in age between patients and controls. Additionally, the Spearman's rank correlation was performed to determine any correlation between the epistaxis severity and the levels of angiogenic factors.

SPSS version 24 was used for all the statistical calculations.

5. RESULTS

The effectiveness and safety of RISBI

From June 2011 to August 2013, 33 HHT patients (17 females) were included and scheduled for RISBI. The observational period continued to April 2017. The total number of treatments with intranasal bevacizumab injection was 210. The mean age, gender and gene mutation of the included patients are shown in Table 4.

Table 4: Patient characteristics

	All patients	Non-responders†	Responders
Total number	33	4	29
Gender			
Female	17	0	17
Male	16	4	12
Age	57.2 ± 11.2 (35-82)	47 ± 2.9 (43-50)	58.7 ± 11.2 (35-82)
Gene mutation			
ENG	11	1	10
ACVRL1	17	3	14
Gene unidentified	2	0	2
Not tested	3	0	3

† No response in ESS and IFT 6-8 weeks after the initial injection with bevacizumab

ACVRL1 = activin-receptor-like kinase 1; ENG = endoglin; ESS = epistaxis severity score; IFT = intensity, frequency and the need of blood transfusion score

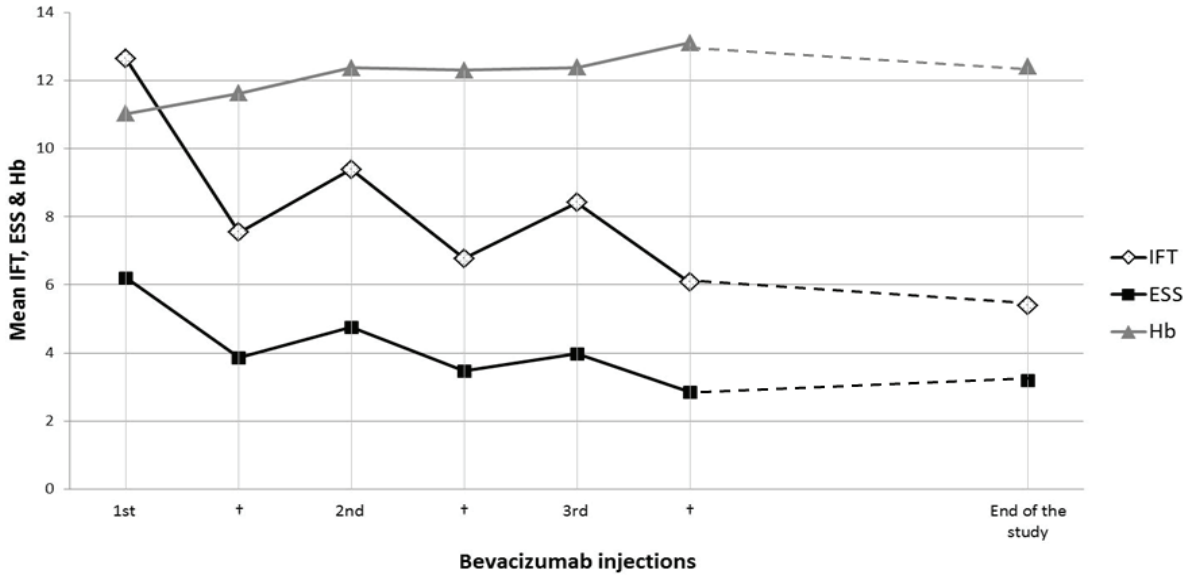
The effectiveness of RISBI

A positive response to the injection was defined as any reduction in the ESS and IFT measured 6-8 weeks after the procedure, without a specific cut-off value.

Twenty-nine patients (87.8%) showed a positive response after the first intranasal bevacizumab injection. These patients were scheduled for RISBI and prospectively followed-up.

The mean treatment and observation period was 38.8 months ± 21.8 (range 2-66 months). The mean IFT, ESS and Hgb measured before and 4-8 weeks after each treatment and at the end of the study are shown in Figure 7.

Figure 7: The mean IFT, ESS and Hgb before and 4-8 weeks after each treatment, and at the end of the study



† 4-8 weeks after injection

The duration of the epistaxis episode, represented by the second component in the ESS, significantly improved. Similarly, the epistaxis frequency reflected by the first component of ESS and the "F" value in the IFT significantly improved, as did the intensity of the epistaxis represented by the "I" value in the IFT scoring.

Conversely, four patients (all males) showed no response in ESS and IFT after the initial therapy. The treatment was repeated in two of these patients but without achieving a beneficial effect. The other two patients declined further injections. These four patients were referred to as "*non-responders"*. Three of these carried the *ACVRL1* and one carried the *ENG* mutation.

In 11 of the patients who responded initially to RISBI, the effect of the injection became gradually shorter. In these patients, RISBI was discontinued before the end of the study when the effect lasted less than 8 weeks. The mean duration from the start of RISBI to the discontinuation in this group was 28.4 ± 23.2 months (range 2-55 months).

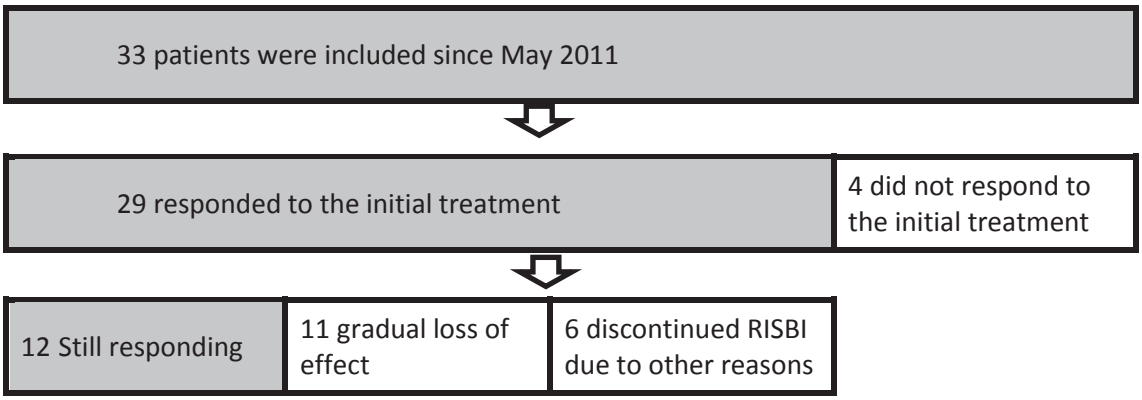
In one patient, RISBI was discontinued after 39 months because of a serious systemic adverse event in the form of osteonecrosis in the knees.[84] Additionally, one patient had a very good response after only one treatment, but RISBI was discontinued since the patient moved abroad and was therefore excluded from further calculations.

Two patients showed a favorable response after the first treatment session but were unable to attend further scheduled injections due to comorbidities. Another two patients died before the end of the study due to non-HHT associated disease. Both of them had responded positively to RISBI.

Twelve (36.3%) of the patients showed positive responses to RISBI until the end of the study. The mean duration from the first to the last injection among these patients was 54 ± 10.4 months (33-66 months).

The long-term response in all the included 33 patients is summarized in figure 8.

Figure 8: The long-term response to RISBI in the included patients



The interval between the injections

The repetition intervals of the injections were individually customized for each patient. Each patient was offered new treatment as soon as the effect of the previous treatment began to diminish. This was based on the subjective evaluation of the patient, in terms of intensity and frequency of epistaxis and the duration of the epistaxis episodes. All patients who initially responded to the injection needed repeated injections in order to maintain long-term treatment effectiveness.

The mean duration between injections was 5.1 ± 2.0 months SD (range 7 weeks - 11 months).

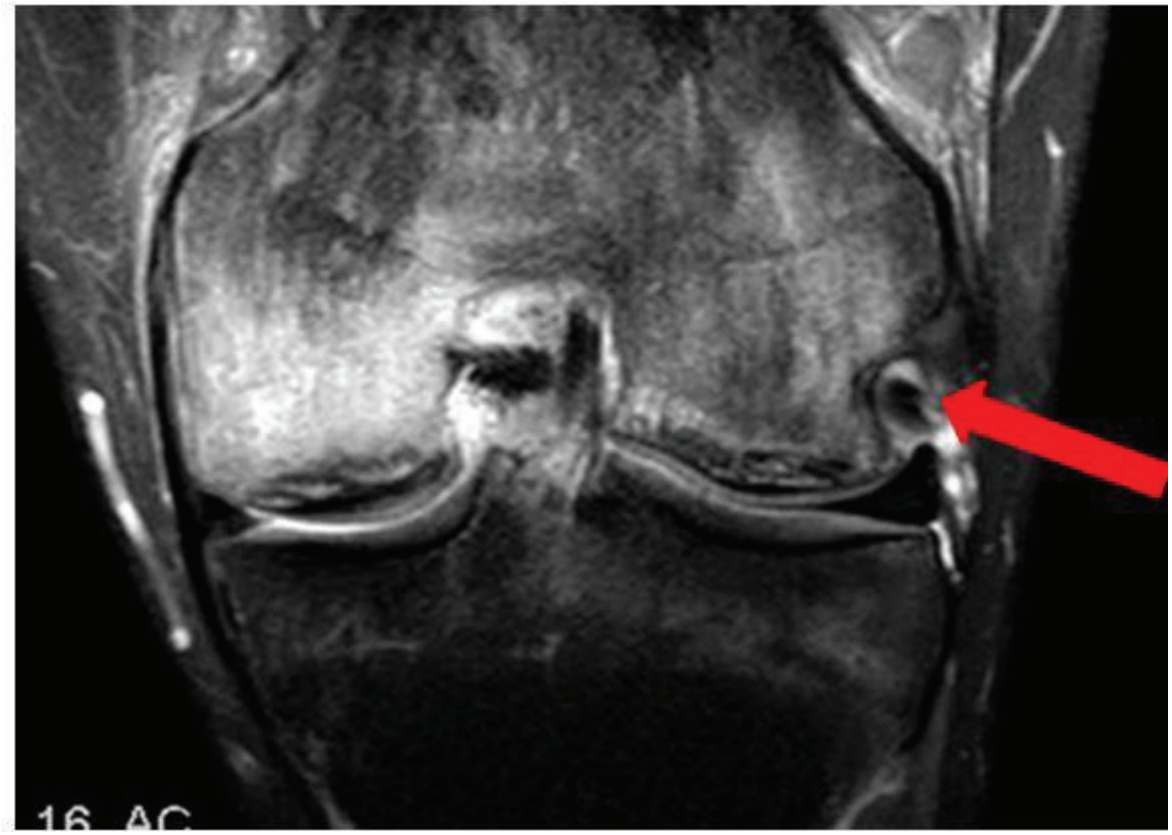
Safety of RISBI

When reporting the safety outcome of a medical treatment, it is recommended to distinguish between adverse effects and adverse event. According to the American Food and Drug Administration (FDA) an adverse effect (or adverse drug reaction) is an apparently harmful or unpleasant reaction, resulting from an intervention related to the use of a medicinal product. An adverse event, on the other hand, can be defined as an adverse outcome during or after the use of a drug or other intervention, but not necessarily caused by it.[85]

In our study, there were no local adverse outcomes, including septal perforation, during the 5.5 years long observation period. Yet, one serious systemic adverse event

was detected. One patient developed gradually increasing severe bilateral pain in the knees to such an extent that he was almost unable to walk. After approximately 6 months, he required either a wheelchair or crutches to get around. Magnetic resonance imaging (MRI) revealed osteoporosis, bone marrow edema and osteonecrosis affecting both knees (Figure 9), in addition to an insufficiency fracture of the right femoral neck.

Figure 9: MRI (proton density fat suppressed sequence) of the left knee in a HHT patient after treatment with RISBI



Note the bone marrow edema in both condyles in addition to an osteonecrosis developing in the lateral femoral condyle (arrow).

Bevacizumab was suspected as the causative agent, because other known risk factors for osteonecrosis were absent. The patient had received in total 8 doses of 200 mg bevacizumab injected intranasally, with an average interval between treatments of 5.6 months. Bevacizumab therapy was discontinued, and treatment with intravenous bisphosphonate (Aclasta®) was initiated. The patient gradually underwent symptomatic remission, without the need for surgery. Follow up MRI one year after

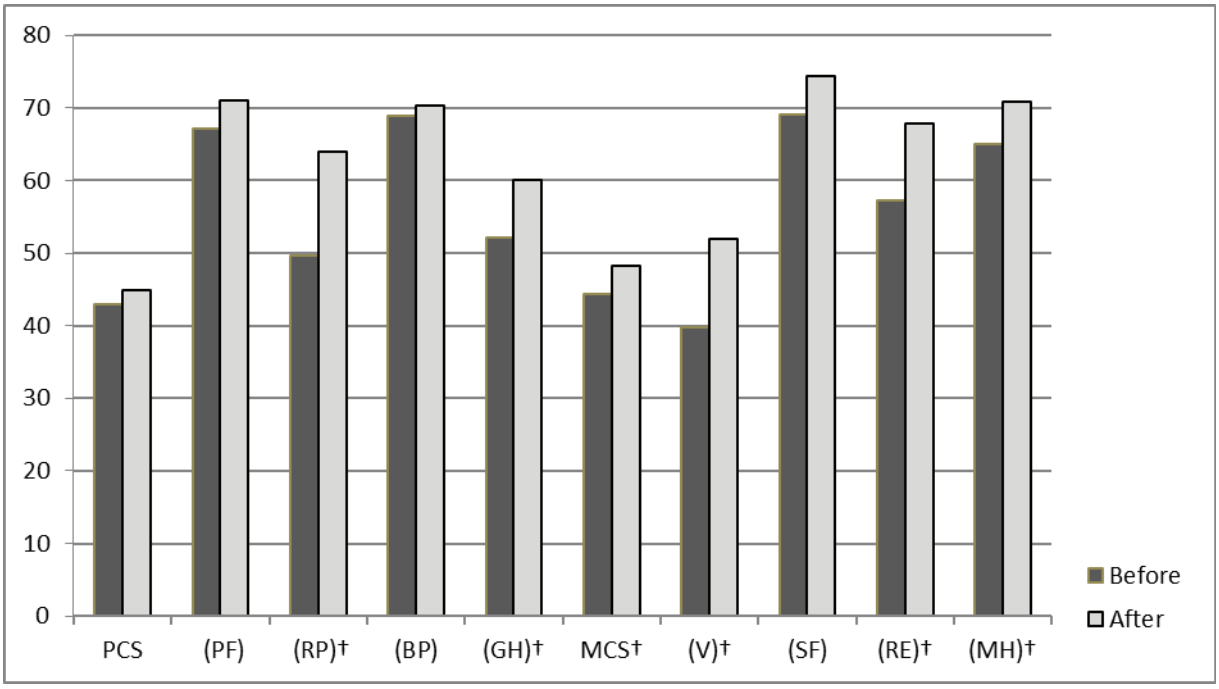
the onset of symptoms showed signs of improvement with regression of bone marrow lesions in the left knee; this was about nine months after the last submucosal injection of bevacizumab.

The effect of RISBI on health related quality of life

As described, thirty-three HHT patients were treated with RISBI during the inclusion period. These patients were consecutively invited to complete questionnaires related to health-related QoL (SF-36) and psychological distress (HADS). Ten patients did not respond, or declined the invitation. Thus, the sample consisted of 23 patients (a response rate of 70%), 14 females and 9 males. It was not possible to perform attrition analysis.

As shown in Figure 10, all QoL dimensions of SF-36 showed improvement at the end of the observation period.

Figure 10: QoL and mental health of the 23 HHT patients before and after RISBI



†Statistically significant result

BP = Bodily Pain; GH = General Health; HHT = hereditary hemorrhagic telangiectasia; HR-QoL = health-related quality of life; MCS = Mental Component Scale; PCS = Physical Component Scale; PF = Physical Functioning; RISBI = repeated intranasal submucosal bevacizumab injections; RP = Role Physical; SF= Social Functioning; SF-36 = Short Form 36; V= Vitality

In the Mental component scale, all dimensions were strongly positively affected, with the exception of Social functioning which showed minor improvement. In the Physical component scale, the "Role physical" and "General health" dimensions demonstrated a significant improvement, while the other dimensions showed a positive trend only. Additionally, the overall QoL measured with Cantril's ladder significantly improved ($p<0.05$) with a medium to strong effect size: 0.62. The Disease specific- QoL showed slight improvement, but was not statistically significant.

Both the scales for anxiety and depression (HADS-A and HADS-D) were significantly improved ($p=0.05$ and 0.02, respectively) with a medium to strong effect size after treatment (0.53 and 0.61, respectively).

The ESS showed a significant mean improved from 6.1 before the first procedure to 3.9 after the last treatment. Similarly, the mean IFT improved from 11.9 to 6.7.

The mean duration between injections in paper 2 was 5.3 ± 2.2 months (range, 7 weeks – 9 months).

VEGF and other inflammatory molecules as predictors of epistaxis severity in HHT

In paper 3, the inclusion period lasted from February 2012 to August 2013. A total of 109 blood samples from 75 patients (35 female [47%], mean age 56.8 years, ± 13.3) diagnosed with HHT were collected. Furthermore, samples were also obtained from healthy controls based on disease history (8 female [50%] mean age 47.7 years ± 14.5). The characteristics of the patients and controls are shown in Table 5.

Table 5: Descriptive characteristics of the included HHT patients and control group

	Patients n=75		Controls n=16
Gender	Females	35	8
	Males	40	8
Age	56.8 ±13.3		47.7 ±14.5
Gene mutation type	ACVRL1	41	N/A
	ENG	25	
	SMAD4	2	
	Non-ACVRL1, Non-ENG	4	
	Not tested	3	
Internal organ manifestations	None	40	N/A
	Pulmonary	25	
	Liver	4	
	CNS	4	
	GI tract	13	
	Multiple	8	

As the severity of HHT associated epistaxis fluctuates over time, it was desired to examine the correlation between the epistaxis severity and the levels of angiogenic factors, each time the patient visited the clinic during the observation period. This protocol also resulted in an increased total number of blood samples from the HHT patient group.

The investigated molecules were all related to angiogenesis or vascular inflammation.

Appendix 1 shows detailed description of the measured molecules.

HHT patient cohort vs control group

Of the measured mediators, PTX3, vWF and VCAM-1 showed significant difference between the HHT patients and controls. In the multivariable age adjusted analysis, only PTX3 was significantly higher in the patients compared to the controls (Table 6). Although VEGF and members of the TGF- β superfamily (TGF- β1 and Activin A) were higher in HTT patients than the controls, these differences did not reach statistical significance (Table 6).

Table 6: Comparison of the median levels of the angiogenic factors between patients group and control group

	Patients, n=75			Controls, n=16			P-Value	
	Median	25 Percentile	75 Percentile	Median	25 Percentile	75 Percentile	Mann-Whitney	Multivariate age adjusted
Ang2	0.77	0.57	0.95	0.81	0.74	1.09	0.088	0.142
Endostatin	122	107	142	126	114	133	0.862	0.463
OPG	3.72	3.02	4.49	3.45	2.81	3.90	0.225	0.780
VEGF	87	51	131	65	31	120	0.266	0.457
PTX3	1.42	1.07	2.13	0.82	0.54	1.03	**<0.001**	**<0.001**
SPARC	467	296	630	433	254	606	0.764	0.051
VCAM1	642	534	789	555	518	604	**0.024**	0.106
vWF	117	102	152	99	83	122	**0.028**	0.222
ActivinA	110	30	193	65	30	117	0.311	0.863
TGFβ-1	27	21	32	25	18	38	0.887	0.785
Tie2	53	46	63	51	48	57	0.853	0.556

SPARC= Secreted Protein Acidic and Rich in Cysteine; TGFβ1= Tumor Growth Factor β1; Tie2= Tyrosine kinase receptor 2; Ang2= Angiopoietin 2; OPG= Osteoprotegrin; VEGF= Vascular Endothelial Growth Factor; PTX3= Pentraxin 3; VCAM-1= vascular cell adhesion molecule -1; vWF= von Willebrand factor

Correlation of the grade of epistaxis and hemoglobin level with the angiogenic factors

Table 7 shows the correlation of parameters for epistaxis severity and Hgb level with the angiogenic factors.

As seen, PTX3 showed a significant correlation with all parameters of epistaxis severity, including IFT, ESS and Hgb, and had the strongest correlation coefficients with all these three parameters compared with the other mediators. SPARC, TGFβ-1 and VCAM-1 levels were significantly correlated with IFT and ESS scores. Ang-2 and Activin A were significantly correlated with ESS and Hgb levels, whereas VEGF showed a significant correlation only with IFT.

Table 7: Correlation of the IFT, ESS and Hgb with the levels of angiogenic factors

	IFT		ESS		Hemoglobin	
	Correlation coefficient	P value	Correlation coefficient	P value	Correlation coefficient	P value
SPARC	-0.30	0.005	-0.24	0.028	0.09	0.375
TGFβ-1	-0.23	0.033	-0.22	0.045	0.07	0.454
Tie2	0.03	0.773	-0.02	0.871	0.11	0.249
Ang2	0.18	0.101	0.36	0.001	-0.28	0.003
Endostatin	0.03	0.770	0.15	0.166	-0.24	0.012
OPG	0.04	0.716	0.19	0.087	-0.16	0.095
VEGF	-0.27	0.011	-0.20	0.068	-0.02	0.809
PTX3	**0.37**	**<0.001**	**0.42**	**0.001**	**-0.34**	**<0.001**
VCAM 1	0.32	0.003	0.25	0.024	-0.15	0.121
vWF	-0.14	0.219	-0.14	0.216	0.04	0.653
Activin A	0.19	0.084	0.27	0.016	-0.29	0.003

SPARC= Secreted Protein Acidic and Rich in Cysteine; TGFβ1= Tumor Growth Factor β1; Tie2= Tyrosine kinase receptor 2; Ang2= Angiopoietin 2; OPG= Osteoprotegrin; VEGF= Vascular Endothelial Growth Factor; PTX3= Pentraxin 3; VCAM-1= vascular cell adhesion molecule -1; vWF= von Willebrand factor

Correlation of internal organ involvement with the angiogenic factors

At OUH, screening for pulmonary AVMs is performed routinely for all the HHT patients after the age of 16 years. Screening for other internal organ involvement is done only in case of suspecting symptoms, in line with international accepted guidelines.[10] Therefore, the occurrence of internal organ involvement in the cohort is likely underestimated. Of the 75 patients with HHT, 40 patients did not have any identified internal organ involvement, 24 patients had pulmonary AVMs, 4 had CNS AVMs, 4 had liver involvement, 13 had gastrointestinal tract manifestations and 8 patients had two or more internal organ manifestations. However, no significant correlations were observed between the presence or the number of internal organs manifestations and the baseline level of any of the measured mediators.

6. METHODOLOGICAL CONSIDERATIONS

Paper 1

Paper 1 was based on a prospective, non-blinded, non-comparable study design. The cohort of patients treated with RISBI consisted of 33 patients. This has distinct limitations with respect to the validity and statistical power of the data. First, the placebo effect may contribute to some of the perceived improvement in many patients.

Second, "regression to the mean (RTM)" can arise in all studies without random allocation to a placebo and treatment group. This statistical phenomenon occurs when a natural variation in the repeated measured values look like a real change. Thus, an apparent regression that follows an intervention could be due to RTM rather than a real response to the intervention. The RTM can be reduced (but not eliminated) by taking multiple baseline measures from the same subjects, to help identify probable measurement errors.[86] The advantages to taking extra measurements are it gives better assessments of the mean, and the within-subject discrepancy. Barnet et al.[86] give an excellent overview of the RTM occurrence, and how to deal with it. In our study, we measured both ESS and IFT which reduces the RTM effect. Moreover, the long follow-up period with repeated epistaxis specific measurements after each treatment, makes the RTM effect less important.

Third, the cohort was not large enough to compute results with sufficient statistical power, increasing the chance of type II errors (false negative conclusions). This is a common problem in rare disease research, but can be solved by performing multi-center studies or longer inclusion periods to generate a larger study sample.

The 33 patients treated with RISBI had previously failed to show long-term benefit of other treatment options for epistaxis (including lasers, argon plasma or septodermoplasty). Consequently, the cohort was not representative for the "typical" HHT patient referred to our center, but rather consisted of "difficult to treat" patients. This selection bias should be considered when interpreting the results. It may partly explain the necessity for the frequent injections observed in the study population.

The effectiveness of RISBI was measured with the epistaxis specific scoring systems ESS and IFT, in addition to Hgb levels. The Hgb level alone may not be sufficient to monitor the epistaxis severity, as it can be affected by many other factors including GI hemorrhage and iron supplements.

There are some concerns regarding the design of ESS and IFT with respect to content-validity. The design of the IFT relied on the opinion of HHT experts[68], while the ESS was constructed on feedback from mostly Caucasian HHT patients in the United States.[69] In both instances, the data was collected by questionnaires, and no initial focus-interviews with patients were performed. Current standards in PROM development emphasize the gathering of qualitative data from patient interviews.[87-89] A multidisciplinary team (including clinicians, PROM development experts and representatives from the pharmaceutical industry) should process these results further. In addition, the ESS and IFT requires the patients to record events over the last 4 weeks. As recently pointed out by the group who published the ESS, this may lead to "recall bias", and give inaccurate data[90]. Thus, the current HHT epistaxis systems may be refined further. Hoag et al recently described the development of a new system, termed the epistaxis eDiary, which may give more accurate results than IFT and ESS for research purposes. The eDiary require the patients to give a daily classification of their epistaxis in order to omit the recall bias. However, the answers cannot be added to give a categorical value which somewhat limits its usefulness. Until the eDiary can be improved in this regard, we still prefer to measure the epistaxis severity with both IFT and ESS, in spite of the considerable overlap of both systems.

The study in paper 1 lacked a specific protocol to determine the intervals between injections. The subjective evaluation of the patients was used rather than specific ESS and IFT cut-off values, or Hgb levels. Consequently, some patients in the study may have incorrectly reported a worsening in epistaxis, hoping to receive the next treatment earlier. It can be argued that this contributed to the relatively short mean intervals between the injections. Given the decreased effectiveness of RISBI with subsequent injections in part of the cohort, together with the relatively serious complication affecting one patient in the study, it will be sensible to develop more qualitative and

specific criteria for additional injections other than subjective evaluations. Therefore, this was a limitation of the study.

An alternative to traditional ESS and IFT scoring is to examine the number and characteristics of intranasal telangiectasias after each treatment session by an endoscope. Although this approach may give an additional objective measure of the response to RISBI, it would be very time consuming and require the patient to travel to our hospital for clinical examination several times after each injection.

The patients who received RISBI in paper 1 were evaluated until after the third injection in 2015. Between 2015 and 2017 there was no ESS and IFT scoring, until the endpoint evaluation was performed in April 2017. Although Hgb was measured throughout the entire study period, the gap in epistaxis severity scores resulted in loss of potential valuable information concerning treatment effectiveness. The scoring gap arose from logistical issues with the study, as the senior author who collected the data at the time had other obligations. In retrospect, a research assistant or nurse could have helped to resolve this, but none was available at the time.

Paper 2

There are many instances where therapeutics can decrease mortality or serious morbidity. Nevertheless, in some cases patient symptoms, and the impact these symptoms have on patient functioning, are the most relevant clinical outcomes. Following the impact of epistaxis in HHT on patient QoL, PROMs have excellent value in evaluating the clinical benefit of novel therapeutics.

In this study, the 33 patients treated with RISBI were asked to complete and send in QoL questionnaires before, and 6-8 weeks after the last treatment during the observational period.

A potential source of bias in paper 2 was the possibility of respondents guessing the hypotheses of the study (i.e. RISBI is effective in treating HHT associated epistaxis), and faking the answers believing to receive prolonged treatment with RISBI. This possible effect could have been omitted or minimized if the questionnaires were anonymized.

Although the patients were asked to answer the questionnaires several times, the response rate (70%) was equal to our previous study were the same questionnaires were answered only once (cross-sectional survey). Thus, questionnaire fatigue was not evident in this study.

A strength of the study was the multiple measures used, including assessment of overall-, health-related- and disease specific QoL, in addition to psychological distress and epistaxis severity. The measures showed good convergent validity, increasing the value of the findings.

Paper 3

In this study, 10 molecules associated with angiogenesis and vascular inflammation was examined in the serum of HHT patients and healthy controls. The study has several limitations:

(a) The measurement of 10 different molecules increases the risk of random positive findings, even if there is none (i.e. wrongly rejecting the 0-hypohesis). Performing a multiple comparison test is one way to approach this. The Bonferoni correction is a multiple comparison test that adjusts p-values in proportion to the number of hypotheses tested. The Bonferroni is often criticized for being overly conservative, and it really increases the probability for type 2 (false negative) errors.[91] Despite this, when we perform the Bonferroni correction on our findings (making alpha 0.005, because 10 different levels of molecules are analyzed), PTX3 is still significantly correlated with epistaxis severity, measured by IFT and Hgb. Additionally; PTX3 levels are significantly raised compared to controls.

(b) Correlation data does not imply causality. The study was exploratory, and not designed to confirm a causal relationship between PTX3 and epistaxis severity. Moreover, there is no established theory to explain the observed increase in PTX3 in the HHT patients compared to controls, which leads to a speculative interpretation of the findings. The current paradigm in HHT pathogenesis mainly relies on the dysfunctional TGF-β /BMP9 pathway or SMAD4 dysfunction. It is therefore important to confirm the results in a larger sample, as with any novel finding.

(c) The investigated HHT patients were all scheduled for repetitive treatments to improve epistaxis. Blood samples were collected before any planned treatment was initiated. There is a theoretical possibility that previous performed treatments (like lasering of the nasal mucosa or RISBI) could have influenced the measured results. The probability for this is very low, because the blood samples were collected no sooner than 3 months after any previous treatment session.

(d) Plasma levels may not necessarily accurately reflect the levels at the site of the bleeding. Biopsies from the mucosa were not performed in the study, so the results could not be confirmed histologically.

(e) A reference value for PTX3 has not yet been established, and the levels vary based on demographics and measurement methods. Yamasaki et al. examined the normal range of plasma PTX3 in healthy Japanese subjects. In this study, normal values were ≤2 ng/ml.[92] A study from Denmark showed a median PTX3 plasma level of 0.0 ng/ml in 100 controls[93] (values <0.3 ng/ml were considered undetectable). The variations from these studies show the importance of including an age and gender matched control group from the same population, and using the same measurement methods in the control and study group.

7. ETHICAL CONSIDERATIONS

Paper 1 and 2

The decision to start RISBI was based on clinical assessment, in HHT patients who showed lack of benefit from other treatments, such as lasers, argon plasma diathermy and septodermoplasty. Thus, this was an observational rather than an interventional study. The patients were not subjected to any extra burdens from participating in the study. Hgb levels were collected as part of the routine clinical follow up. The exception was the QoL and HADS questionnaires used in paper 2. The regional research ethics committee (REK) approved this study. Oral and written informed consents were obtained from all patients in accordance with national regulations.

The clinical decision to initiate RISBI rested on evidence from the literature supporting the effectiveness and safety of intranasal bevacizumab injections. Previous research, including our own pilot study from 2012, showed promising results on improvement of epistaxis severity in many of the treated patients, and little or no side-effects (table 8, page 57 gives a comprehensive overview). Indeed, the only reported notable adverse effect was the onset of post-treatment septal perforation in one study. This only applied to some of the patients receiving concomitant laser and injections along the cartilaginous septum. When the authors changed the protocol, and avoided lasering and injecting the cartilaginous septum, no new septal perforations were observed.[94]

The studies described in paper 1 and 2 were limited by the absence of a control group. Although a randomized, placebo controlled trial might have produced higher quality evidence, it may not be ethically feasible to perform. First, in patients who receive placebo, an additional deterioration of epistaxis would be expected. The HHT patients eligible for RISBI at OUH usually have severe epistaxis, have failed other options and are considered a "difficult to treat group". Secondly, the goal of the study was to evaluate the impact of RISBI in a long-term manner. For these reasons, a long-term placebo-controlled evaluation in our cohort was considered unethical. The same principle can be seen in almost all of the other studies regarding intranasally injected

bevacizumab, where control groups are absent. The exception was the study by Riss et al. [95] who included a placebo control group. This design was possible due to the short-term follow up of only 3 months and relatively low mean ESS scores (4.0) of the patients at inclusion.

Paper 3

The blood samples collected from the patients in paper 3 were taken as part of the routine clinical follow up. All the patients were previously included in a national HHT research database, approved by the Norwegian regional ethical committee. In addition, they signed study-specific consent forms. The patients were not exposed to any additional risk or discomfort by participating in the study. The study was approved by the REK (REK south-east C number 1219).

8. DISCUSSION

The effect of RISBI on HHT associated epistaxis

Paper 1 and 2 examine the long-term effect of RISBI on HHT associated epistaxis. Compared to other relevant studies (Table 8), our studies included the highest number of patients and had the longest period of follow up. The evaluation of treatment effectiveness was based on multiple measurements, including ESS, IFT, Hgb levels and QoL questionnaires, which increased the validity of the findings.

As demonstrated in Table 8, a growing number of studies have sought to establish the optimal dose, administration method (spray, injection) and adverse effects of bevacizumab treatment in HHT. The first case report to describe an intransal injection of bevacizumab was published in 2009.[96] The patient had no epistaxis for about four months, before it gradually ensued. Interestingly, this first case had a treatment duration that approached our own mean duration of 5.3 months. It should be noted that this patient was injected with 100 mg bevacizumab, and the injection sites were non-specific. Then, a case series of 10 HHT patients treated with a single bevacizumab injection intranasally, in addition to KTP (potassium titanyl phosphate) laser was described.[97] These patients showed superior results when compared to another similar group treated by KTP laser alone. However, a later retrospective review by the same group showed that 8.6% of the 58 included patients sustained a septal perforation during the treatment period, most likely due to the combined lasering and injection of the cartilaginous septum.[94] In contrast, Rhorhmeier et al. described increased effectiveness of bevacizumab injected in very low doses combined with laser, versus laser alone, without observing any adverse effects. Karnezis et al examined 19 HHT patients who received one or more intranasal bevacizumab injections, without additional treatment.[98] The patients showed a significant improvement in the ESS, with a peak after 2 months. Then, Riss et al conducted the only placebo-controlled study with intransally injected bevacizumab.[95] A dose of 100 mg was applied, at areas with the most telangiectasias. The included patients had a variable degree of epistaxis at inclusion (the mean ESS was 4.0). A great variation in epistaxis severity pre- and post-treatment was noted, even in the placebo group. For this reason, the study ended

up underpowered. The results showed a trend towards improvement in ESS and VAS in the bevacizumab group (although not statistically significant), and the authors interpreted the results as supportive for using injected intranasal bevacizumab in treating HHT associated epistaxis.

Meanwhile, several studies showed promise in treating HHT associated epistaxis with topical bevacizumab (nasal spray).[99-102] However, these studies were limited by design features (retrospective, observational) and/or few numbers of patients. Unfortunately, two recent adequately powered, double blind, placebo-controlled studies showed that bevacizumab nasal spray was ineffective for treatment of HHT associated epistaxis.[103,104] Based on this, it is now hypothesized that bevacizumab fails to penetrate the mucosal membranes when administered as a topical agent, due to excessive nasal crusting in HHT patients and/or molecular properties of the medicine. Therefore, the research focus again shifted back to intranasally *injected* bevacizumab in treating HHT associated epistaxis. Dheyauldeen et al. developed a protocol for intranasal injection sites based on the vascular anatomy on the nose.[79] A significant improvement was shown in ESS and IFT scores. The patients were only followed up for 3 months. More patients and a longer observational period were warranted, leading to the study in Paper 1. It should be noted that the 8 patients described in the study by Dheyauldeen et al. also were included in this study.

Another recent study explored the effectiveness of sclerotherapy when injected together with bevacizumab intranasally, with promising results.[105] This study was retrospective, but it included relatively many patients and had a long follow up period.

Bevacizumab is relatively expensive. One vial with 4 ml of 25 mg/ml costs about 3600 NOK or 360 Euros. Despite the high cost, the benefit to the patient's quality of life, as well as the cost savings of fewer procedures, transfusions, and intravenous iron seems to justify the expense of treatment.[97]

Table 8: studies regarding intranasal bevacizumab

Author	Publication year	Study design	Number of patients	Age Mean(range)	Gender	Dose	Administration	Additional treatment	Observation time	Outcome measures
Simonds et al[97]	2009	Retrospective	10	58.3 y ± 8.2	3 F, 7 M		Injection	KTP laser	12 months	Epistaxis frequency & duration, blood transfusion and emergency room visit
Davidson et al[96]	2010	Case report	1	45 y	M	100 mg	Injection ± Spray	None	7 months	ESS
Chen et al[94]	2010	Retrospective	58	55 y (22–81)	26 F, 32 M	25-100 mg	Injection or Spray	± KTP laser	1.5 - 46 months	Safety (adverse effect).
Karnezis et al[102]	2011	Retrospective	32			25-100 mg	Injection or Spray	± KTP laser	10-12 months	ESS
Rohrmeier et al[106]	2011	Retrospective	11	65 y (46–77)	7 F, 4 M	0.3/ 0.6/ 3.75 mg	Injection	Nd:Yag laser		ESS + QoL + Hgb level
Brinkerhoff et al[99]	2012	Case report	1	55 y	F	100 mg	Spray	None	12 months	Hgb level + epistaxis frequency
Guldmann et al[100]	2012	Prospective	6	56.3 y	4 F, 2 M	50 mg	Spray	None	3 months	ESS
Karnezis et al[98]	2012	Prospective	19	60 y (40-80)	12 F, 7 M	100 mg	Injection ± Spray	None	10-12 months	ESS
Dheyauldeen et al[79]	2012	Prospective	8	56.5 y (35-69)	5 F, 3 M	100 mg	Injection	None	3 months	ESS + IFT + Hgb + QoL
Marglani et al[107]	2013	Case report	1	73 y	M	100 mg	Injection	Diode laser	10 months	Decrease in frequency of epistaxis and improvement in Hgb level and QoL
Alderman et al[101]	2013	Case report	3	(66-77)	1F+2 M	100 mg	Spray monthly	None	7 months	ESS + decrease bleeding duration

Dupuis-Girod et al[108]	2014	Double-blind, placebo-controlled Phase 1/3	40	-		12.5-100 mg	Spray	None	3 months	Safety (tolerance). Epistaxis frequency and duration + Need of transfusion + Hgb + Ferritin
Riss et al[95]	2015	Double-blind, placebo-controlled	15	-	6 F, 3 M	100 mg	Injection	None	3 months	ESS + VAS
Whitehead et al [103]	2016	Double-blind, placebo-controlled	106	52.8 y	43 F, 64 M	4 mg	Spray	None	6 months	Epistaxis frequency and duration + ESS + Hgb + ferritin + blood transfusion and emergency room visits
Dupuis-Girod et al[104]	2016	Double-blind, placebo-controlled Phase 2/3	80	60.5 y (SD10.7)	37 F, 43 M	25-75 mg	Spray	None	3 months	Epistaxis frequency and duration + Need of transfusion + Hgb + Ferritin
Steineger et al[109].	2018	Prospective	33	57.2 y (35-80)	17 F, 16 M	100-200 mg	Injection	None	2-66 months	ESS + IFT + Hgb + safety + QoL
Khoueir et al[105]	2019	Retrospective	31	60 y	16 F, 15 M	100 mg	Injection	Sclerotherapy	6 – 42 months	ESS + BSS + QoL

BSS= Bergler-Sadick score; ESS = epistaxis severity score; Hgb = hemoglobin; IFT = intensity, frequency and the need of blood transfusion score; KTP = potassium titanyl phosphate; Nd:Yag = neodymium yttrium aluminium garnet; QoL = quality of life; SD = standard deviation; VAS = Visual Analog Score

Resistance to intranasal bevacizumab

In our study, four of the patients who were treated with intranasal bevacizumab injection showed no improvement in epistaxis. This is in line with other studies regarding response to bevacizumab in HHT, where such resistance is reported in a minority of the treated patients.[79,95]

Further, eleven patients treated with RISBI developed gradually shorter lasting improvement, before the treatment eventually was discontinued. This decreased drug response is well-known in oncology, where monoclonal antibodies targeting VEGF have been used for many years in anti-neoplastic therapy.[110] In oncology, resistance to anti-VEGF therapy can be due to primary non-responsiveness, or linked to different escape mechanisms. Escape mechanisms can be upregulation of the existing VEGF pathway, recruitment of alternative growth factors or downstream changes in intracellular signal transduction.[111] Resistance to bevacizumab has also been described in patients treated with intravitreal injections to prevent development of macular degeneration.[112,113]

The bevacizumab resistance observed in our study may be due to changes in the physiology of the nasal mucosa, such as inflammatory changes. As shown in paper 3, raised levels of the vascular inflammation molecule PTX3 were present in the HHT cohort compared to healthy controls. The elevated PTX3 levels may reflect a local tissue inflammation in the endothelium of the nasal mucosa. This inflammatory mechanism might have a role in the development of resistance to bevacizumab. Performing histological examination of the nasal mucosa in the patients who develop resistance to RISBI could further explore this.

The majority of cases of decreased bevacizumab response occurred after multiple injections, suggesting that repeated exposure to the therapeutic agent is more likely to lead to an immune response against the treatment. It can be hypothesized that a formation of anti-bodies occurs after a repeated exposure to bevacizumab in these patients, as observed in other diseases. Forooghian et al. reported a trend towards increased levels of antibodies against bevacizumab in the serum of patients treated for macular degeneration[114]. These patients were treated with intravitreal injections, which

give much lower serological titers compared to intravenous administration. Consequently, measuring antibodies in serum of the HHT patients who develop resistance to RISBI could be considered.

RISBI dose

The dose of bevacizumab used in the study was 100 mg for the first eight procedures, and subsequently 200 mg for all the remaining treatments. The increase in dose was based on a written recommendation from a senior authority, rather than a response evaluation of the treatment. Interestingly, there was some evidence that the 100 mg dose of bevacizumab was more effective than 200 mg. This evidence was based on the degree of improvement in epistaxis severity scores and Hgb level after 100 mg injection compared to 200 mg. A possible explanation for this could be that a higher dose of bevacizumab leads to higher systemic level and is associated with more side effects. One of the known side effects of bevacizumab is epistaxis[115,116]. Therefore, 100 mg may be a more optimal dose for intranasal bevacizumab injection. Yet, the number of treatments performed with 100 mg in this study was eight, while the number of treatments performed with 200 mg was 202. This finding should therefore be carefully interpreted and further research to find the optimal dose is warranted. It is also of interest to study if a lower dose may prevent or delay the development of late resistance. Intranasal injections with lower than 100 mg doses have been used in other studies and evaluated to be less effective.[102]

Is the effect of RISBI local or systemic?

The procedure is carried out by injecting specific intranasal areas, which correspond to the entry points of the main arteries supplying the nasal cavity. This is based on our hypothesis that injecting these sites will improve the distribution of the active substance, leading to a reduced development of telangiectasias in the nasal mucosal membranes. Yet, this is just a hypothesis and not proven to be correct. In fact, the osteonecrosis observed in one patient who underwent RISBI suggests that the active substance is crossing into the systemic circulation. Part of the treatment response may therefore be due to a systemic effect. This assumption is supported by the case-series of Thompson and colleagues who described a significant improvement of epistaxis in six HHT patients treated with very low doses (0.125 mg/kg) of intravenous

bevacizumab.[117] These findings warrant further investigation, as very low doses of intravenous bevacizumab is associated with less morbidity and presumably the same low adverse effect profile as RISBI.

RISBI, HHT and osteonecrosis

To date, less than twenty cases of osteonecrosis associated with bevacizumab therapy is reported in the literature. Osteonecrosis occurred in cancer patients, when bevacizumab was administered intravenously in oncological doses (5 mg/kg) and with intervals of 2-3 weeks. The majority of cases occurred in the mandible, but recently osteonecrosis in the appendicular skeleton was also reported.[72-74]

The finding of osteonecrosis in the knees of an HHT patient during treatment with RISBI was therefore an unexpected adverse effect of RISBI. Since any known risk factors for osteonecrosis were absent (like corticosteroids, alcohol abuse and smoking), RISBI was suspected as the cause.

There is some evidence that HHT patients have an increased risk of bone infection[118] and thrombosis compared to the general population.[119,120] Thrombosis or ischemia (often resulting from direct cell injury) leading increased intraosseous pressure is a proposed pathogenic mechanism in avascular necrosis.[121] Based on this, it is possible that the osteonecrosis observed in our patient was due to a disturbance in the microcirculation related to HHT rather than RISBI. HHT patients in general may also have an increased susceptibility for developing bevacizumab-induced bone lesions due to the underlying disease process. Yet, it should be noted that many HHT patients have been treated with higher doses and with shorter intervals of bevacizumab *intravenously* without any reported incidences of osteonecrosis in the literature. Thus, further studies are warranted to uncover the incidence of osteonecrosis in HHT patients, both in general and during RISBI.

Pentraxin 3 as a potential biomarker for HHT associated epistaxis

Paper 3 examined several angiogenic and inflammatory molecules related to angiogenesis and vascular inflammation in HHT patients and healthy controls. PTX3, a mediator closely linked to vascular inflammation, was increased significantly

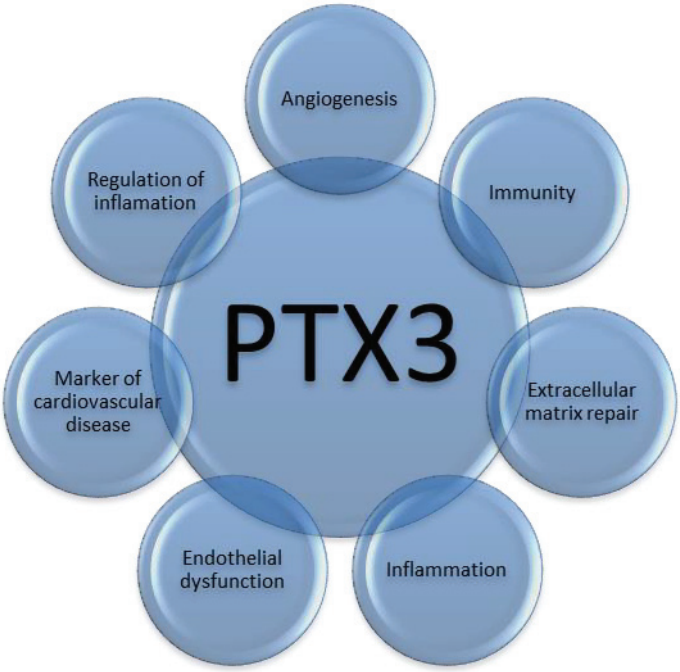
compared to the control group. Additionally, PTX3 was the only mediator examined which correlated significantly with all parameters of epistaxis severity (i.e. ESS, IFT and Hgb levels). This indicates PTX3 as a possible novel biomarker for the severity of epistaxis in HHT patients. In addition, the finding has the potential to unravel novel, underlying disease mechanisms in HHT.

Presently, there are no known specific biomarkers for HHT associated epistaxis. Due to the challenges in measuring epistaxis severity in HHT, biomarkers represent a potential valuable resource in clinical practice and research. PTX3 is already considered a useful biomarker in numerous other diseases.

High PTX3 levels correlate strongly with cardiovascular disease, heart failure and predict mortality after acute coronary syndrome.[122-124] Raised PTX3 also correlates with the severity of sepsis[93], preeclampsia[125] and systemic lupus erythematosus.[126] Available studies have failed to elucidate whether PTX3 actively promotes the inflammatory response or reflects a protective physiological response correlated with disease extent and severity.[127]

PTX3 is an inflammatory molecule and belongs to a family of molecules involved in innate immunity, termed the Pentraxin family. The Pentraxin family can be further divided into two groups: the short and long Pentraxins. C-reactive protein (CRP) is the best-known member in short Pentraxin group, whereas PTX3 is the prototype molecule from the long Pentraxin group.[128] CRP is produced in the liver and released into the bloodstream in response to systemic inflammation. PTX3 on the other hand, is produced locally at sites of inflammation by multiple different cells, including inflamed endothelial cells.[129] PTX3 is therefore more tissue specific than CRP, and may more directly mirror vascular inflammation (Figure 11).

Figure 11: PTX is involved in several processes



By interacting with several ligands, PTX3 can modulate angiogenesis, the complement system, inflammatory response, and vascular remodeling.[130]

Previous studies have demonstrated a close relationship between inflammation and angiogenesis. There is also rapidly accumulating evidence suggesting that inflammation and hemostasis are closely related processes. Each process amplifies and increases the other, creating the potential for a vicious cycle of thrombogenesis and inflammation.[131-133]

With this in mind, the increased levels of PTX3 in HHT patients with higher severity of epistaxis may reflect:

(a) Wound healing: The wound repair mechanism in the nasal mucosal membranes after bleeding from telangiectasias. The occurrence of vascular remodeling and tissue repair is related to increase in PTX3. PTX3 promotes wound healing and thrombogenesis by upregulating tissue factor expression in serum.[134,135] The high frequency of epistaxis observed in HHT patients may cause a state of repetitive coagulation and repair processes, which is accompanied by endothelial

inflammation. Consequently, locally produced inflammatory molecules such as PTX3 can become raised in the serum. Further, HHT is associated with increased risk of thromboembolism.[119,120] This may also partly be explained by the same hypothesis; that endothelial inflammation directly influences the pathogenesis in HHT. Further research is needed to examine this hypothesis.

(b) Coagulation activity: The repeated process of coagulation and clot formation in HHT patients with higher epistaxis severity scores may cause the raised PTX3 plasma levels. Activation of the coagulation cascade can promote inflammation. Inflammation, on the other hand, causes a variety of changes in the endothelium, leukocytes, and platelets, which stimulate the formation of a pro-coagulant, pro-thrombotic surface on the vessel wall. This can activate a vicious circle of inflammation and coagulation previously described.

(c) Dysregulated angiogenesis: Increase in PTX3 may mirror an upregulated disease process in the more serious affected patients, again predisposing to more severe epistaxis. The underlying mechanism is unknown, but may be related to the dysregulated angiogenesis in HHT. Fibroblast growth factors (FGFs) are multifunctional proteins involved in angiogenesis and wound healing, modulated by PTX3. An increase in FGF activity is accompanied by raised PTX3 levels, and evidence suggests that PTX3 diminishes the angiogenic response in vivo and in vitro.[136,137] FGF was not investigated in our present work, but should be considered for measurement in future studies.

Angiogenic factors and internal organ manifestations in HHT

We did not observe any correlations between the presence of internal organ manifestations and the levels of the measured mediators. However, the analysis was limited by the fact that the characteristics of each internal organ manifestations (e.g. size, multiplicity or flow) were unknown. It is possible that multiple AVMs in the lung with high flow may be correlated with inflammatory and angiogenic molecules, whereas small, clinically insignificant intrapulmonary manifestations are not.

9. CONCLUSIONS

The main conclusions from our work can be summarized as follows:

1. RISBI is an effective treatment for HHT-associated epistaxis, even in patients who are refractory to first-line treatment (like diode laser and argon plasma cautery).
2. A minority of HHT patients display primary treatment resistance, while others may develop late resistance to RISBI.
2. RISBI improves quality of life in patients treated for HHT related epistaxis.
3. RISBI is mostly a safe treatment option, but clinicians should be aware of the possible association of intranasal bevacizumab injections and osteonecrosis.
4. VEGF level is not good indicator for the severity of epistaxis in HHT.
5. PTX 3 can be considered as a potential biomarker for the severity of epistaxis in HHT.

Based on our conclusions the following recommendation in clinical practice can be made:

1. RISBI can be offered as a treatment option for HHT patients who are refractory to first line-treatment. Based on clinical experience and our current results, we recommend the following order of therapy methods: 1. Laser photocoagulation. 2. Argon plasma cautery. 3. Septodermoplasty. 4. RISBI. 5. Young's procedure (nasal closure). Table 9 shows the indication, advantages and disadvantages for each treatment method.

Table 9: The indications for treatment methods in HHT associated epistaxis

Therapy	Characteristics	Advantages	Disadvantages	Indication
Laser	-Yag, diode	-may be used together with endoscope -may reach posterior parts of nose -can be performed LA	-less effective against ongoing bleeding during procedure -less effective on grade 3 lesions	-first line treatment -telangiectasias in posterior nasal cavity
Argon plasma coagulation	-ionized gas	-effective against ongoing bleeding during procedure -more effective against grade 3 lesions	-more painful, GA or sedation usually required -limited range	-no effect of laser therapy -heavy bleeding during laser
Septodermoplasy	-replacement of nasal mucosa with skin transplant	-very long lasting effect (several years)	-GA required often extensive crusting and/or foul smell from the nose -requires daily cleansing of nose from patient for satisfactory result	-no effect of the above therapies -patient desires long-lasting treatment
RISBI	-repeated intranasal injections of bevacizumab	-effective in patients who are refractory to other therapy -can be performed in local anesthesia	-possible risk of systemic adverse effects -primary/late drug resistance -expensive	-no effect of the above therapies -patient declined septodermoplasty -long lasting GA contraindicated

Young's procedure	-surgical closure of the nose	-long lasting or permanent effect	-cause hyposmia -cause obligate breathing trough the mouth	-no effect of the above therapies

LA= Local anesthesia; GA= general anesthesia; RISBI = repeated intranasal submucosal injections

2. We recommend monitoring patients treated with RISBI for the possible development of drug resistance or the rare possibility of osteonecrosis. The patients are encouraged to report the occurrence of any persistent pain in the extremities or the jaw, and should be referred to imaging studies if a suspicion of osteonecrosis arises.

10. FUTURE STUDIES

This thesis examined the long-term effectiveness of RISBI, and plasma levels of angiogenic molecules in HHT patients. In the wake of our studies, several questions and ideas for future research projects arose. We still do not know why some patients develop primary or late resistance to RISBI. Measuring antibodies to bevacizumab pre- and post-treatment could be a possible next step to evaluate this phenomenon. Restarting RISBI in the patients who develop late resistance after a period of treatment discontinuation also merits consideration.

The possibility that RISBI can cause bone marrow edema or osteonecrosis should also be investigated further. As drug-induced osteonecrosis mainly manifests in the jaw, obtaining an Orthopantomogram (OPG) of all patients in the RISBI cohort, and then cross-checking against previous OPGs before initiation of treatment retrospectively is a possible study. This study could also reveal the potential increased risk of avascular necrosis in relation to thromboembolisms due to HHT itself.

There is sparse literature on the QoL in HHT patients. The studies to date mostly use SF-36 and other generic items (i.e. VAS scoring, HADS and so on) to measure disease specific QoL. These items may not be optimal for exploring the QoL in a rare disease, such as HHT. Indeed, they may underestimate the burden of the disease. Some aspects of the disease, such as internal organ manifestations, may potentially have a profound impact of the lives of HHT patients. Other parameters, such as time spent in sick leave due to severe epistaxis, are not considered with the present systems. Thus, the development of a novel validated, HHT disease specific QoL questionnaire is warranted. It is now recommended to obtain input from patients during focus-interviews as an initial step in the questionnaire development process.

For the first time, we observed a statistically significant elevation in PTX3 in the plasma of HHT patients compared to healthy controls. The finding was statistically significant even after adjusting for age in regression analysis. In addition, the severity of epistaxis in the HHT patients was correlated with the level of PTX3. Although these

findings were very intriguing, this was an exploratory pilot study that should be interpreted with caution and mainly serve as hypothesis-generating. The impact of PTX3 as a circulating protein marker on HHT disease management has yet to be determined.

Other molecules related to angiogenesis, such as FGF could also be measured. FGF is directly involved in angiogenesis, and modulated by PTX3, which may explain the raised levels of PTX3 observed in our study. A higher number of patients is needed to reinforce the possibility of using PTX3 as a marker for epistaxis severity in HHT. It is desired to repeat the sampling in a larger group of patients to perform a more extensive multivariate analysis. Based on our present results, effect sizes can be assumed and an adequately powered study could be designed. The data from our study were cross sectional. By performing repeated sampling we could examine if the measured mediators reflect previous epistaxis episodes, or predict the risk of future nosebleeds. It would also be of interest to investigate if the levels of inflammatory and angiogenic molecules correlate with other disease specific variables like internal organ manifestations and the occurrence of thromboembolic episodes. The internal organ manifestations should be further categorized to be of more value as a disease specific variable. For example, instead of only noting the presence of a lung AVM, we are also interested in the flow, any multiplicity and size.

APPENDIX 1

Table 10: Angiogenic and inflammatory molecules related to vascular inflammation measured in paper 3

Molecul4	Type	Function	Source
SPARC/osteonectin[138]	Matricellular	• Anti- matrix adhesion • Anti-angiogenic	Numerous cells
Vascular endothelial growth factor[139]	Growth factor	• Key pro-angiogenic	Platelets, macrophages, keratinocytes
Angiopoietin2/Tie 2 complex[140]	Growth factor	• Pro or anti-angiogenic	Endothelial cells
Endostatin[141]	Collagen derived protein	• Potent anti-angiogenic	Fragment located on collagen, enzyme generated
Transforming growth factor (TGF) β-1[142]	TGFβ superfamily, Growth factor	• Cell proliferation, differentiation and apoptosis • Homeostasis • Immunomodulation • Pro or anti-angiogenic	Lymphocytes, macrophages
Activin A[143]	TGFβ superfamily, Growth factor	• Pro or anti-angiogenic	Numerous cells
Pentraxin 3[128]	Long pentraxin group	• Vascular inflammation • Innate immunity • Acute-phase response	Endothelial cells, macrophages, smooth muscle cells, fibroblasts.

		• **Endothelial cell activation**	
Osteoprotegerin[144]	**Tumor necrosis receptor family**	• **Bone remodeling** • **Vascular inflammation** • **Atherogenesis**	**Osteoblasts, stromal cells, endothelial cells, VSMC**
Vascular cellular adhesion molecule-1[145]	**Transmembrane glycoprotein**	• **Vascular inflammation** • **Leukocyte adhesion and migration** • **Endothelial cell activation**	**Cytokine-activated endothelium**
von Willebrand factor[146]	**Blood glycoprotein**	• **Hemostasis** • **Platelet adhesion** • **Vascular inflammation** • **Endothelial cell activation**	**Endothelial cells, megakaryocytes**

VEGF:

Vascular Endothelial Growth factor is consistently up regulated in pro angiogenic states, and affects cell adhesion, endothelial permeability and cell migration. It acts as a critical modulator in pathological angiogenesis such as cancer, retinopathies and other diseases like HHT. It is previously described to be elevated in the serum of HHT patients[33,147], when compared to healthy controls. In spondylarthrosis, a chronic inflammatory joint disease where angiogenesis is involved, serum VEGF levels is

reported to be significantly higher in patients than healthy controls, and correlates with disease severity.[148,149]

Osteoprotegerin:

Osteoprotegerin, a member of the tumor necrosis factor receptor superfamily, is involved in bone remodeling, carcinogenesis, and atherogenesis as well as prothrombosis.[144,150] Additionally, correlation between elevated circulating Osteoprotegerin levels and severity of coronary artery disease, cerebrovascular disease, and peripheral vascular disease has been described in observational studies.

Angiopoietin (Ang2) and Tie2

Ang2 is part of the angiopoietin superfamily. The angiopoietins bind to the receptor tyrosine kinase Tie2 on endothelial cells where they act in accordance with VEGF to promote angiogenesis.[151] Ang2 is described to affect the endothelium directly, such as influencing permeability in peripheral tissues, depending on the context.[152] Thus, it is one of the key angiogenic regulators. The effect of Ang2 on angiogenesis is mediated by binding to endothelial-specific receptor tyrosine kinase 2 (TIE2).

TGFβ-1

TGFβ-1 belongs to a family of growth factors with a multitude of functions concerning cell proliferation, differentiation, and apoptosis. This cytokine plays an essential role in controlling homeostasis, growth and embryonic development. It is also implicated in chronic and acute disease, as well as wound healing.[153]

SPARC

SPARC is a multifunctional cytokine belonging to a group of matricellular proteins, involved in several biological processes, such as tissue repair, cell differentiation and matrix adhesion. This protein can promote anti-angiogenic action, and may also induce bone metastasis when expressed in highly aggressive tumors.

VCAM-1

Vascular cellular adhesion molecule -1 is a well-studied molecule, known for its ability to facilitate transmigration of leukocytes through the endothelial cell layer in immune responses.[154,155]

vWF

VWF mediates the adhesion of platelets to sites of vascular injury by binding to parts of exposed connective tissue and platelet membrane proteins.[156] VWF plays a crucial role in hemostasis as a carrier of coagulation factor VII, but is also involved in inflammatory pathways by facilitating leukocyte adhesion. Further, it is associated with angiodysplasia in patients with von Wilderbrand disease, and described to regulate angiogenesis.[157]

Activin-A

Activin-A is a cytokine belonging to the transforming growth factor TGF-β superfamily, involved in inflammation. It is associated with anti-angiogenic properties in vitro[158], but may in fact cause pro-angiogenesis in vivo through complex mechanisms.[159]

Pentraxin 3

The Pentraxin family of molecules is involved in innate immunity and the acute-phase response and can be divided into two groups: the short and long pentraxins. The short pentraxins consist of C-reactive protein (CRP) and serum amyloid P-component (SAP). PTX3 is the prototype molecule in the long pentraxin group.[128] The short pentraxins are produced in the liver and activated by interleukine-6. In contrast, PTX3 is produced locally at sites of inflammation by several different cells, including phagocytes, fibroblasts, smooth muscle cells and endothelial cells.[129] More recently, it has been demonstrated that high levels of PTX3 may induce dysfunction and alter the structure in the endothelial layer through a P-selectin/matrix metalloproteinase-1 pathway in vitro.[160] Likewise, PTX3 is associated with vascular remodeling and endothelial dysfunction in several diseases. Interestingly, high levels of PTX3 are reported to correlate strongly with risk factors for cardiovascular disease[122], may be

useful as a prognostic marker for patients with heart failure[123], and may predict short- and long-time mortality after acute coronary heart syndrome.[124] Increased levels of PTX3 are also observed in systemic lupus erythematosus[126], sepsis[93] and preeclampsia[125], and correlates with the severity of these diseases.

APPENDIX 2

HADS in English

The answer alternatives range from 0 (not present) to 3 (maximally present).

HADS anxiety

1. I feel tense and wound up.

3. I get sort of frightened as if something awful is about to happen.

5. Worrying thoughts go through my mind.

7. I can sit at ease and feel relaxed.

9. I get sort of frightened feeling like “butterflies in the stomach.

11. I feel restless as if I have to be on the move.

13. I get sudden feelings of panic

HADS Depression

2. I still enjoy the things I used to enjoy

4. I can laugh and see the funny side of things

6. I feel cheerful

8. I feel as if I have slowed down

10. I have lost interest in my appearance

12. I look forward with enjoy to things

14. I can enjoy a good book, or radio or TV program

HADS in Norwegian

Her kommer noen spørsmål om hvorledes du føler deg. For hvert spørsmål setter du kryss for ett av de fire svarene som best beskriver dine følelser den siste uka. Ikke tenk for lenge på svaret, de spontane svarene er best.

1.Jeg føler meg nervøs og urolig

- ☐ 3- Mesteparten av tiden
- ☐ 2- Mye av tiden
- ☐ 1- Fra tid til annen
- ☐ 0- Ikke i det hele tatt

2. Jeg gleder meg fortsatt over tingene slik jeg pleide før

- ☐ 0- Avgjort like mye
- ☐ 1- Ikke fullt så mye
- ☐ 2- Bare lite grann
- ☐ 3- Ikke i det hele tatt

3. Jeg har en urofølelse som om noe forferdelig vil skje

- ☐ 3- Ja, og noe svært ille
- ☐ 2- Ja, ikke så veldig ille
- ☐ 1- Litt, bekymrer meg lite
- ☐ 0- Ikke i det hele tatt

4. Jeg kan le og se det morsomme i situasjoner

- ☐ 0- Like mye nå som før
- ☐ 1- Ikke like mye nå som før
- ☐ 2- Avgjort ikke som før
- ☐ 3- Ikke i det hele tatt

5. Jeg har hodet fullt av bekymringer

- ☐ 3- Veldig ofte
- ☐ 2- Ganske ofte
- ☐ 1- Av og til
- ☐ 0- En gang i blant

6. Jeg er i godt humør

- ☐ 3- Aldri
- ☐ 2- Noen ganger
- ☐ 1- Ganske ofte
- ☐ 0- For det meste

7. Jeg kan sitte i fred og ro og kjenne meg avslappet

- ☐ 0- Ja, helt klart
- ☐ 1- Vanligvis
- ☐ 2- Ikke så ofte
- ☐ 3- Ikke i det hele tatt

8. Jeg føler meg som om alt går langsommere

- ☐ 3- Nesten hele tiden
- ☐ 2- Svært ofte
- ☐ 1- Fra tid til annen
- ☐ 0- Ikke i det hele tatt

T
T

T
T

9. Jeg føler meg urolig som om jeg har sommerfugler i magen

- ☐ 0- Ikke i det hele tatt
- ☐ 1- Fra tid til annen
- ☐ 2- Ganske ofte
- ☐ 3- Svært ofte

10. Jeg bryr meg ikke lenger om hvordan jeg ser ut

- ☐ 3- Ja, har sluttet å bry meg
- ☐ 2- Ikke som jeg burde
- ☐ 1- Kan hende ikke nok
- ☐ 0- Bryr meg som før

11. Jeg er rastløs som om jeg stadig må være aktiv

- ☐ 0- Ja, helt klart
- ☐ 1- Vanligvis
- ☐ 2- Ikke så ofte
- ☐ 3- Ikke i det hele tatt

12. Jeg ser med glede frem til hendelser og ting

- ☐ 0- Like mye som før
- ☐ 1- Heller mindre enn før
- ☐ 2- Avgjort mindre enn før
- ☐ 3- Nesten ikke i det hele tatt

13. Jeg kan plutselig få en følelse av panikk

- ☐ 3- Uten tvil svært ofte
- ☐ 2- Ganske ofte
- ☐ 1- Ikke så veldig ofte
- ☐ 0- Ikke i det hele tatt

14. Jeg kan glede meg over gode bøker, radio og TV

- ☐ 0- Ofte
- ☐ 1- Fra tid til annen
- ☐ 2- Ikke så ofte
- ☐ 3- Svært sjelden

APPENDIX 3

SF-36 Survey in English

INSTRUCTIONS: Please answer every question. Some questions may look like others, but each one is different. Please take the time to read and answer each question carefully by circling the number that best represents your response.

1. In general, would you say your health is?

Excellent (1)	Very Good (2)	Good (3)	Fair (4)	Poor (5)

2. <u>Compared to one year ago</u>, how would you rate your health in general <u>now</u>?

Much better now than one year ago (1)	Somewhat better now than one year ago (2)	About the same as one year ago (3)	Somewhat worse now than one year ago (4)	Much worse now than one year ago (5)

3. The following questions are about activities you might do during a typical day. Does <u>your health now limit you</u> in these activities? If so, how much: (circle one number on each line)

	Yes, Limited A Lot	Yes, Limited A Little	No, Not Limited At All
A. **Vigorous activities**, such as running, lifting heavy objects participating in strenuous sports	1	2	3
B. **Moderate activities**, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	1	2	3
C. Lifting or carrying groceries	1	2	3
D. Climbing **several** flights of stairs	1	2	3
E. Climbing **one** flight of stairs	1	2	3
F. Bending, kneeling, or stooping	1	2	3

G. Walking **more than a mile**	1	2	3
H. Walking **several hundred yards**	1	2	3
I. Walking **one hundred yards**	1	2	3
J. Bathing or dressing yourself	1	2	3

4. During the <u>past 4 weeks</u>, how much of the time have you had any of the following problems with your work or other regular daily activities <u>as a result of your physical health?</u> (Circle one number on each line)

	All the time	**Most** of the time	**Some** of the time	A **little** of the time	**None** of the time
A. Cut down on the **amount of time** you spend on work or other activities	1	2	3	4	5
B. **Accomplished less** than you would like	1	2	3	4	5
C. Were limited in the **kind** of work or other activities	1	2	3	4	5
D. Had **difficulty** performing the work or other activities (for example, it took extra effort)	1	2	3	4	5

5. During the <u>past 4 weeks</u>, how much of the time have you had any of the following problems with your work or other regular daily activities <u>as a result of any emotional problems</u> (such as feeling depressed or anxious)? (Circle one number on each line)

	All the time	**Most** of the time	**Some** of the time	**A little** of the time	**None** of the time
A. Cut down on the **amount of time** you spend on work or other activities	1	2	3	4	5
B. **Accomplished less** than you would like	1	2	3	4	5
C. Did work or activities **less carefully than usual**	1	2	3	4	5

6. During the past 4 weeks, to what extent has your physical health or emotional problems interfered with your social activities with family, friends, neighbours, or groups? (Circle one)

Not at all (1)	Slightly (2)	Moderately (3)	Quite a bit (4)	Extremely (5)

7. How much bodily pain have you had during the past 4 weeks? (Circle one)

None (1)	Very Mild (2)	Mild (3)	Moderate (4)	Severe (5)	Very Severe (6)

8. During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)? (Circle one)

Not at all (1)	Slightly (2)	Moderately (3)	Quite a bit (4)	Extremely (5)

9. These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time during the past 4 weeks… (Circle one number on each line)

	All the time	**Most** of the time	**Some** of the time	**A little** of the time	**None** of the time
A. did you feel full of life?	1	2	3	4	5
B. have you been very nervous?	1	2	3	4	5
C. have you felt so down in the dumps nothing could cheer you up?	1	2	3	4	5
D. have you felt calm and peaceful?	1	2	3	4	5
E. did you have a lot of energy?	1	2	3	4	5
F. have you felt downhearted and depressed?	1	2	3	4	5
G. did you feel worn out?	1	2	3	4	5
H. have you been happy?	1	2	3	4	5
I. did you feel tired?	1	2	3	4	5

10. During the <u>past 4 weeks</u>, how much of the time has your <u>physical health or emotional problems</u> interfered with your social activities (like visiting friends, relatives, etc.)?

All of the Time (1)	Most of the Time (2)	Some of the Time (3)	A Little of the Time (4)	None of the Time (5)

11. How TRUE or FALSE is each of the following statements for you? (Circle one number on each line)

	Definitely True	Mostly True	Don't Know	Mostly False	Definitely False
A. I seem to get sick a little easier than other people	1	2	3	4	5
B. I am as healthy as anybody I know	1	2	3	4	5
C. I expect my health to get worse	1	2	3	4	5
D. My health is excellent	1	2	3	4	5

SF 36 in Norwegian

Dette spørreskjemaet stiller spørsmål om hva du tenker om din egen helse, altså om hvor frisk du mener du er. Svaret ditt vil hjelpe oss å få vite hvordan du har det og klarer å være med på det du har lyst til.

Hvert spørsmål besvares ved at du setter et kryss i den boksen som passer best for deg. Hvis du synes det er vanskelig å svare, eller det egentlig ikke passer for deg, er det allikevel fint om du svarer så godt du kan. Ingen svar er rett eller galt, det som er viktig er hva som er riktig for deg.

1.Stort sett, vil du si at helsen din er

- ☐ Utmerket
- ☐ Meget god
- ☐ God
- ☐ Ganske god

2. Hvordan er helsen din nå sammenlignet med for ett år siden

- ☐ Mye bedre enn for ett år siden
- ☐ Litt bedre enn for ett år siden
- ☐ Omtrent som for ett år siden
- ☐ Litt dårligere enn for ett år siden

☐ Dårlig

☐ Mye dårligere enn for ett år siden

De neste spørsmålene handler om aktiviteter som du kanskje er med på i løpet av en vanlig dag. **Hindrer sykdommen deg** i å være med på disse aktivitetene **nå**?

3a. Hindrer sykdommen deg i å være med i anstrengende aktiviteter?

☐ Begrenser meg mye

☐ Begrenser meg litt

☐ Begrenser meg ikke i det hele tatt

3b. Hindrer sykdommen deg i være med i moderate aktiviteter?

☐ Begrenser meg mye

☐ Begrenser meg litt

☐ Begrenser meg ikke i det hele tatt

3c. Hindrer sykdommen deg i løfte eller bære handlekurv?

☐ Begrenser meg mye

☐ Begrenser meg litt

☐ Begrenser meg ikke i det hele tatt

3d. Hindrer sykdommen deg i å gå opp trapper flere etasjer?

☐ Begrenser meg mye

☐ Begrenser meg litt

☐ Begrenser meg ikke i det hele tatt

3e. Hindrer sykdommen deg i å gå trappen en etasje?

☐ Begrenser meg mye

☐ Begrenser meg litt

☐ Begrenser meg ikke i det hele tatt

3f. Hindrer sykdommen deg i å bøye deg eller sitte på huk?

☐ Begrenser meg mye

☐ Begrenser meg litt

☐ Begrenser meg ikke i det hele tat

3g. Hindrer sykdommen deg i å gå mer enn to kilometer?

- ☐ Begrenser meg mye
- ☐ Begrenser meg litt
- ☐ Begrenser meg ikke i det hele tatt

3h. Hindrer sykdommen deg i å gå noen hundre meter?

- ☐ Begrenser meg mye
- ☐ Begrenser meg litt
- ☐ Begrenser meg ikke i det hele tat

3i. Hindrer sykdommen deg i å gå hundre meter?

- ☐ Begrenser meg mye
- ☐ Begrenser meg litt
- ☐ Begrenser meg ikke i det hele tatt

3j. Hindrer sykdommen deg i å vaske deg eller kle på deg selv?

- ☐ Begrenser meg mye
- ☐ Begrenser meg litt
- ☐ Begrenser meg ikke i det hele tat

Her kommer noen spørsmål om det er ting du ikke har kunnet være med på **de siste 4 ukene på grunn av fysisk helse**.

4a. Har du redusert tiden du bruker på arbeidet vært med på færre ting ditt eller andre aktiviteter?

- ☐ Hele tiden
- ☐ Mesteparten av tiden
- ☐ Noe av tiden
- ☐ Litt av tiden
- ☐ Ikke i det hele tatt

4b. Har du utrettet mindre enn du ønsket?

- ☐ Hele tiden
- ☐ Mesteparten av tiden
- ☐ Noe av tiden
- ☐ Litt av tiden
- ☐ Ikke i det hele tatt

4c. Har du vært hindret i visse typer arbeid eller andre aktiviteter

- ☐ Hele tiden
- ☐ Mesteparten av tiden
- ☐ Noe av tiden
- ☐ Litt av tiden

4d. Har du hatt vanskeligheter med å utføre arbeidet ditt eller andre aktiviteter?

- ☐ Hele tiden
- ☐ Mesteparten av tiden
- ☐ Noe av tiden
- ☐ Litt av tiden

☐ Ikke i det hele tatt

☐ Ikke i det hele tatt

Har du, i løpet av **de siste 4 ukene** ikke har kunnet være med på **på grunn av følelsesmessige problemer**.

5a. Har du redusert tiden du bruker på arbeidet ditt eller andre aktiviteter?

☐ Hele tiden

☐ Mesteparten av tiden

☐ Noe av tiden

☐ Litt av tiden

☐ Ikke i det hele tatt

5b. Har du utrettet mindre enn du ønsker?

☐ Hele tiden

☐ Mesteparten av tiden

☐ Noe av tiden

☐ Litt av tiden

☐ Ikke i det hele tatt

5c. Har ikke utført arbeidet eller andre aktiviteter som vanlig like nøye som vanlig?

☐ Hele tiden

☐ Mesteparten av tiden

☐ Noe av tiden

☐ Litt av tiden

☐ Ikke i det hele tatt

I løpet av de **siste 4 ukene:**

6. I hvilken grad har dine fysiske eller følelsesmessige problemer hatt innvirkning på sosial omgang?

☐ Ikke i det hele tatt

☐ Litt

☐ En del

☐ Mye

☐ Svært mye

7. Hvor sterke kroppslige smerter har du hatt?

☐ Ingen

☐ Meget svake

☐ Svake

☐ Moderate

☐ Sterke

☐ Meget sterke

8. Hvor mye har smertene påvirket ditt vanlige arbeide hjemme og utenfor hjemmet de siste 4 uken?

- ☐ Ikke i det hele tatt
- ☐ Litt
- ☐ En del
- ☐ Mye
- ☐ Svært mye

Hvor ofte har du i løpet av **de siste 4 ukene**

9a. Følt deg full av tiltakslyst og hatt lyst til å ? være med på det meste?

- ☐ Hele tiden
- ☐ Nesten hele tiden
- ☐ Mye av tiden
- ☐ En del av tiden
- ☐ Litt av tiden
- ☐ Ikke i det hele tatt

9b. Følt deg veldig nervøs og engstelig?

- ☐ Hele tiden
- ☐ Nesten hele tiden
- ☐ Mye av tiden
- ☐ En del av tiden
- ☐ Litt av tiden
- ☐ Ikke i det hele tatt

9c. Vært så langt nede at ingenting kunne muntre deg opp?

- ☐ Hele tiden
- ☐ Nesten hele tiden
- ☐ Mye av tiden
- ☐ En del av tiden
- ☐ Litt av tiden
- ☐ Ikke i det hele tatt

9d. Følt deg rolig og harmonisk og glad?

- ☐ Hele tiden
- ☐ Nesten hele tiden
- ☐ Mye av tiden
- ☐ En del av tiden
- ☐ Litt av tiden
- ☐ Ikke i det hele tatt

9e. Hatt mye overskudd?

9f. Følt deg nedfor og trist?

☐ Hele tiden

☐ Nesten hele tiden

☐ Mye av tiden

☐ En del av tiden

☐ Litt av tiden

☐ Ikke i det hele tatt

☐ Hele tiden

☐ Nesten hele tiden

☐ Mye av tiden

☐ En del av tiden

☐ Litt av tiden

☐ Ikke i det hele tatt

Hvor ofte har du i løpet av **de siste 4 ukene**

9g. Følt deg sliten?

☐ Hele tiden

☐ Nesten hele tiden

☐ Mye av tiden

☐ En del av tiden

☐ Litt av tiden

☐ Ikke i det hele tatt

9h. Følt deg glad?

☐ Hele tiden

☐ Nesten hele tiden

☐ Mye av tiden

☐ En del av tiden

☐ Litt av tiden

☐ Ikke i det hele tatt

9i. Følt deg trett?

☐ Hele tiden

☐ Nesten hele tiden

☐ Mye av tiden

☐ En del av tiden

☐ Litt av tiden

☐ Ikke i det hele tatt

10. Hvor mye har din sosiale omgang blitt påvirket av fysisk helse eller følelsesmessige problemer de siste 4 ukene?

☐ Hele tiden

☐ Nesten hele tiden

☐ Mye av tiden

☐ Litt av tiden

☐ Ikke i det hele tatt

11a. Det virker som om jeg blir lettere syk enn andre

☐ Helt riktig

☐ Delvis riktig

☐ Vet ikke

☐ Delvis gal

☐ Helt gal

11b. Jeg er like frisk som de fleste jeg enn kjenner

☐ Helt riktig

☐ Delvis riktig

☐ Vet ikke

☐ Delvis gal

☐ Helt gal

11c. Jeg forventer at helsen min blir dårligere

☐ Helt riktig

☐ Delvis riktig

☐ Vet ikke

☐ Delvis gal

☐ Helt gal

11d. Helsen min er utmerket

☐ Helt riktig

☐ Delvis riktig

☐ Vet ikke

☐ Delvis gal

☐ Helt gal

APPENDIX 4

Epistaxis severity score (ESS)

Epistaxis Severity Score (ESS) for Hereditary Hemorrhagic Telangiectasia

Please answer each of the following questions as they pertain to your TYPICAL nosebleed symptoms DURING THE PAST 4 WEEKS. Please answer all questions.

1. How often did you TYPICALLY have nosebleeds during the past 4 weeks?

□ Less than monthly

□ One to three times per month

□ Once per week

□ Several per week

□ Once per day

□ Several per day

2. How long did each nosebleed TYPICALLY last for you during the past 4 weeks?

□ < 1 minute

□ 1-5 minutes

□ 6-15 minutes

□ 16-30 minutes

□ > 30 minutes

3. How would you describe your TYPICAL nosebleed intensity during the past 4 weeks?

□ Not Typically Gushing or Pouring

□ Typically Gushing or Pouring

4. Have you sought medical attention for your nosebleeds during the past 4 weeks?

□ No

□ Yes

5. Are you anemic (low blood counts) currently?

□ No

□ Yes

6. Have you received a red blood cell transfusion SPECIFICALLY for nosebleeds during the past 4 weeks?

□ No

□ Yes

ESS in Norwegian

Vennligst beskriv dine typiske symptomer ved neseblødning over de siste 4 uker.

1) Hvor ofte har du vanligvis neseblødning de siste 4 uker?

- Mindre enn én gang per måned
- Én gang per måned
- Én gang per uke
- Flere ganger per uke
- Én gang per dag
- Flere ganger per dag

2) Hvor lenge varer din **typiske** neseblødning de siste 4 uker?

- <1 minutt
- 1-5 minutter
- 6-15 minutter
- 16-30 minutter
- > 30 minutter
-

3) Hvordan vil du beskrive din **typiske** neseblødnings intensitet de siste 4 uker?

- Ikke vanligvis strømmende eller fossende
- Vanligvis strømmende eller fossende

4) Har du søkt lege for din neseblødning de siste 4 uker?

- Nei
- Ja

5) Har du mottatt blodoverføring spesielt for neseblødning de siste 4 uker?

- Nei
- Ja

6) Er du blodfattig (lav blodprosent) for tiden?

- Nei
- Ja
- Jeg vet ikke

APPENDIX 5

Epistaxis intensity, frequency and need or blood transfusion score (IFT)

Regarding nosebleeds:

1) During the past 4 weeks, how many times you got spot of blood from the nose or dripped a few drops of blood from nose?

□ None
□ 1-5 times
□ 6-10 times
□ 11 to 27 times
□ Daily or more

2) During the past 4 weeks, how many times you got blood soaked handkerchief?

□ None
□ 1-5 times
□ 6-10 times
□ 11 to 27 times
□ Daily or more

3) During the past 4 weeks, how many times you got blood soaked towel?

□ None
□ 1-5 times
□ 6-10 times
□ 11 to 27 times
□ Daily or more

4) During the past 4 weeks, how many times you got bleeding that fills so much as a bowl (1/2 liter) of blood?

□ None
□ 1-5 times
□ 6-10 times

□ 11 to 27 times

□ Daily or more

5) During the past 4 weeks, how many times did you get a blood transfusion?

□None

□ Once

□ Several times

Epistaxis intensity, frequency and need or blood transfusion score (IFT) in Norwegian

1) I løpet av de siste 4 uker, hvor mange ganger fikk du blodflekk fra nesen på lommetørkle eller dryppet noen dråper blod fra nesen?

☐ Ingen

☐ 1-5 ganger

☐ 6-10 ganger

☐ 11-27 ganger (nesten daglig)

☐ Daglig

2) I løpet siste 4 uker, hvor mange ganger fikk du blodgjennomtrukket lommetørkle?

☐ Ingen

☐ 1-5 ganger

☐ 6-10 ganger

☐ 11-27 ganger (nesten daglig)

☐ Daglig

3) I løpet siste 4 uker, hvor mange ganger fikk du blodgjennomtrukket håndkle?

☐ Ingen

☐ 1-5 ganger

☐ 6-10 ganger

☐ 11-27 ganger (nesten daglig)

☐ Daglig

4) I løpet siste 4 uker, hvor mange ganger fikk du blødning som fyller så mye som en bolle (1/2 liter) blod?
   - ☐ Ingen
   - ☐ 1-5 ganger
   - ☐ 6-10 ganger
   - ☐ 11-27 ganger (nesten daglig)
   - ☐ Daglig

5) I løpet av siste 4 uker, hvor mange ganger fikk du blodoverføring?
   - ☐ Ingen
   - ☐ Én gang
   - ☐ Flere ganger

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