Moving towards a personalized approach in the management of Vascular Malformations

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General introduction and outline of thesis

General introduction

Vascular malformations are complex congenital lesions of the vascular or lymphatic system. These congenital lesions consist of dilated and dysfunctional vessels that generally have a tortuous structure. Vascular malformations may appear as a mass or stain different in color and texture than normal skin and may be located anywhere in the body.

ISSVA classification

Historically, the management of peripheral vascular lesions was hampered by a bewildering nomenclature that was the result of unfamiliarity with the pathophysiology of vascular anomalies. For example, different definitions for the same type of lesions in different locations were used. In 1982, 'vascular anomalies' were first differentiated into vascular tumors and vascular malformations, based on cellular features of the vascular lesions.[1, 2]

Although vascular malformations and vascular tumors may both be congenital vascular lesions, they differ significantly from each other, mainly on the basis of endothelial cell function and the course of the disease.[2] Vascular malformations have normal endothelial cell turnover and progress during life without being able to regress spontaneously. On the contrary, vascular tumors are characterized by endothelial hyperplasia and the ability to regress spontaneously, for example infantile hemangiomas the most common form of benign vascular tumors. These discoveries formed the basis for the classification of vascular anomalies that we know nowadays.

In 1996, the International Society for the Study of Vascular Anomalies (ISSVA) further subdivided vascular tumors and vascular malformations in subgroups and provided the framework for great strides in research and treatment in the field (Table 1).[1, 3, 4]

Vascular *malformations* are divided into 'simple' and 'combined', based on the vessel types that are involved. Simple malformations consist of one affected vessel type (i.e., capillary, venous, lymphatic, and arteriovenous), and the combined vascular malformations are named based on the specific vessels involved.[1, 3, 4] Additionally, a distinction is made between low-flow vascular malformations and high-flow vascular malformations (those with an arterial component). Vascular *tumors* are divided into benign, locally aggressive, and malignant entities.[1, 3 4]

Table 1. Simplified ISSVA Classification of Vascular Anomalies ©2018 International Society for the Study of Vascular Anomalies. Available at "issva.org/classification", accessed on December 15th 2022.

Vascular anomalies				
Vascular tumors			Vascular malformations	
Benign	**Locally aggressive**	**Malignant**	**Simple**	**Combined**
Infantile hemangioma	Kaposiform hemangioendothelioma	Angiosarcoma	Capillary malformation (CM)	CVM, CLM
Congenital hemangioma	Retiform hemangioendothelioma	Epithelioid hemangioendothelioma	Lymphatic malformation (LM)	LVM, CLVM
Tufted hemangioma	Papillary intralymphatic angioendothelioma, Dabska tumor		Venous malformation (VM)	CAVM
Spindle-cell hemangioma	Composite hemangio-endothioma		Arteriovenous malformation (AVM)	CLAVM
Epithelioid hemangioma	Kaposi sarcoma		Arteriovenous fistula	
Pyogenic granuloma				

Etiology

Development of the vascular system

Vascular development consists of vasculogenesis and angiogenesis. The first step of the formation of the vascular system is vasculogenesis, taking place at the end of the second week of embryological development.[5]

Vasculogenesis is defined as vessel growth from embryonic cells, and the hemangioblasts (precursors derived from mesoderm) are the first cells that originate from embryonic cells. Hemangioblasts give rise to hemocytoblasts (blood cell precursors), and also give rise to angioblasts (endothelial precursors).[6] Subsequently, angioblasts fuse into "vascular islets" and from tube-like structures, inducing the formation of the primary capillary plexus. The primary capillary system extends and matures during angiogenesis to form the fully developed capillaries, veins, and arteries.[5, 7]

Angiogenesis occurs through various mechanisms: 1) small blood vessels can be formed by *sprouting* from preexisting vessels, 2) during *non-sprouting,* the preexisting vessels enlarge, fuse, or are split by transcapillary pillars, 3) the loss of endothelial cells and tubes by *pruning*, and 4) during *maturation,* recruitment of pericytes and smooth muscle cells take place.[7]

All these processes are controlled by interactions and ordered effects of various angiogenic and antiangiogenic factors. Angiogenic factors include VEGFs (vascular endothelial growth factors),

FGFs (fibroblast growth factors), PDGF-beta (platelet derived growth factor beta) and angiopoietins (ANGPT-1 and ANGPT-2).[7]

Due to the complexity of the various mechanisms and involvement of numerous factors, susceptibility to developmental defects exists.[8] These defects disturb normal blood vessel formation and may lead to the formation of vascular malformations.

The underlying genetics of vascular malformations

Two major signaling pathways are important regulators of cellular growth, proliferation, migration, and apoptosis, which are involved in endothelial cells (Figure 1).[9] The PI3K/AKT/mTOR is often called the anti-apoptosis pathway. PI3K is downstream of several tyrosine kinase receptors, such as TIE2 and VEGF-2. PI3K activates AKT and thereby mTOR, and regulates cell growth, apoptosis, proliferation, migration, and angiogenesis.

The other major signaling pathway is the RAS/MAPK/ERK pathway, generally called the proliferation pathway, it is involved in cell cycle regulation, proliferation, and migration. The pathway will activate when a growth factor binds to a receptor tyrosine kinase, which through several processes induces RAS with activation of RAF phosphorylation. Phosphorylated RAF activates MAPK, which in turn phosphorylates and activates ERK.

In recent years, it came to light that vascular malformations develop as a result of somatic or, more rarely, germline mutations in genes involved in the PI3K/AKT/mTOR or RAS/MAPK/ERK cell signaling pathways.[10, 11] Somatic mutations arise in the post-zygotic phase and are therefore not inherited as part of the germline DNA; the mutant cells act as progenitor cells and give rise to daughter mutant cells in specific tissues. Consequently, some cells contain the mutation, and other cells do not, hence, the tissue shows somatic mosaicism.[12] In contrary, a germline mutation is a mutation inherited from the spermatocyte or oocyte and will be present in all cells of the embryo and will be ultimately present in all tissues.[12]

Multiple genes are involved in the PI3K/AKT/mTOR and RAS/MAPK/ERK cell signaling pathways and are thought to contribute to distinct clinical manifestations of vascular malformations, although this has not been investigated on a large scale. Gaining insight into how the genotypes relates to the phenotypes of vascular malformations will lead to a better understanding of their pathophysiology.

The discovery of various mutated genes in vascular malformations uncovers that vascular malformations are even more heterogeneous than was known from clinical aspects alone. Therefore, the genotype should have a more prominent role in the classification and treatment of vascular malformations, resulting in a more personalized approach to their management.

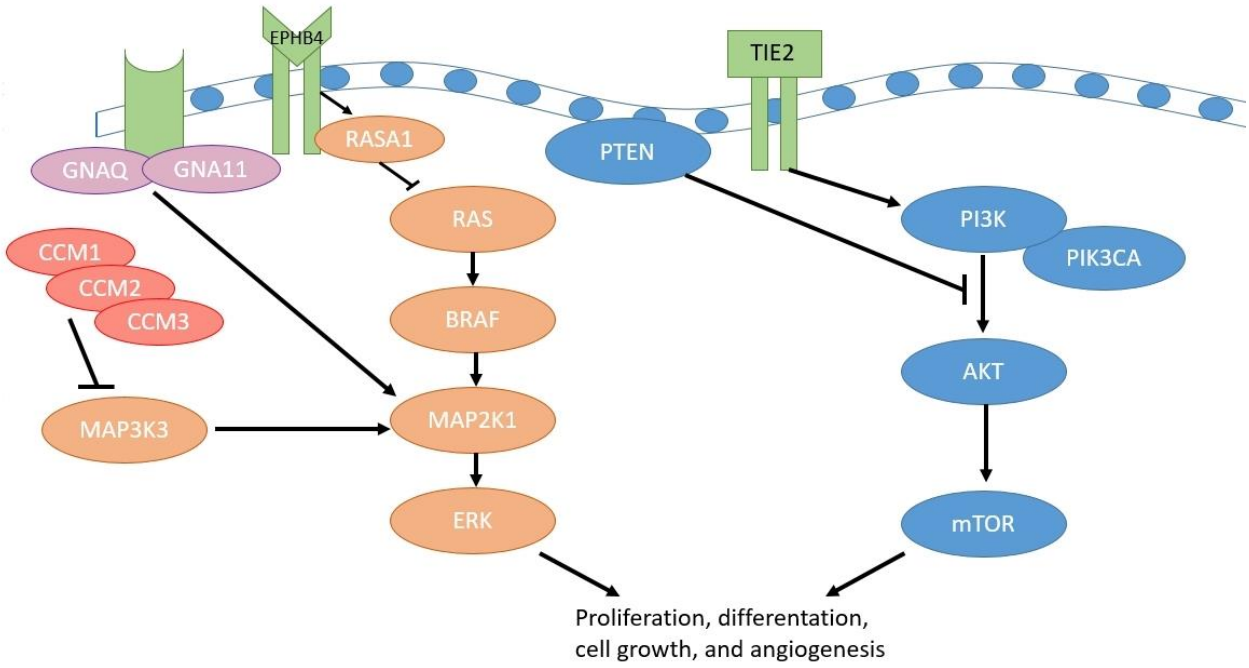

Figure 1 - Schematic diagram of the (simplified) RAS/MEK/ERK and PI3K/AKT/mTOR signalling pathways in endothelial cells, hyperactivated in peripheral vascular malformations.
Arrows indicate direct or indirect interactions and blunt lines indicate inhibition. The RAS/MAPK/ERK pathway is hyperactivated when mutations arise in GNAQ, GNA11, EPHB4, RASA1, BRAF, KRAS, HRAS, MAP2K1, MAP3K3, and KRIT1 (CCM1). The PI3K/AKT/mTOR pathway is hyperactivated when mutations arise in TIE2, PIK3CA, PTEN, AKT, and mTOR.

Clinical aspects of vascular malformations

Vascular malformations are congenital lesions that will grow simultaneously with the body during life. However, growth can increase because of trauma or hormonal influences such as puberty, pregnancy, and oral contraceptives.[13-15]

Vascular malformations may cause a wide variety of symptoms and may ultimately negatively affect patients' lives.[16, 17] Common symptoms caused by vascular malformations include pain, impaired physical functioning, a disfiguring appearance, thrombotic events, compression of functional structures such as the airway, nerves, or other organs, bleedings, fluid leakage, and compromised physical, mental, and social wellbeing.[16-20]

An important aspect in management of vascular malformations is that symptoms vary strongly in this patient population. Differences in lesions characteristics, such as the affected vessel types,

lesion localization, tissue types involved, and lesion size, are thought to contribute to the wide variety in symptoms.

For instance, venous malformations are known to cause thrombotic events due to venous stasis, while lymphatic malformations are known to cause fluid leakage.[21-24] Further, blood- and lymphatic vessels are present throughout (nearly) the whole body, and consequently, vascular malformations may be present in all anatomical locations and may affect various kinds of tissue. Reasonably, vascular malformations located in the head and neck area are more prone to cause disabilities concerning speaking and breathing, or facial distortion leading to appearance-related concerns, while vascular malformations located at the extremities are more prone to cause impaired physical functioning. At last, vascular malformations vary in size. The lesions may be small and confined to certain areas of the body, or on the contrary, vascular malformations may be diffuse and affect larger parts of the body. The common clinical aspects of the four most common types of vascular malformations are shown below. Figure 1 displays clinical pictures of the various types of vascular malformations.

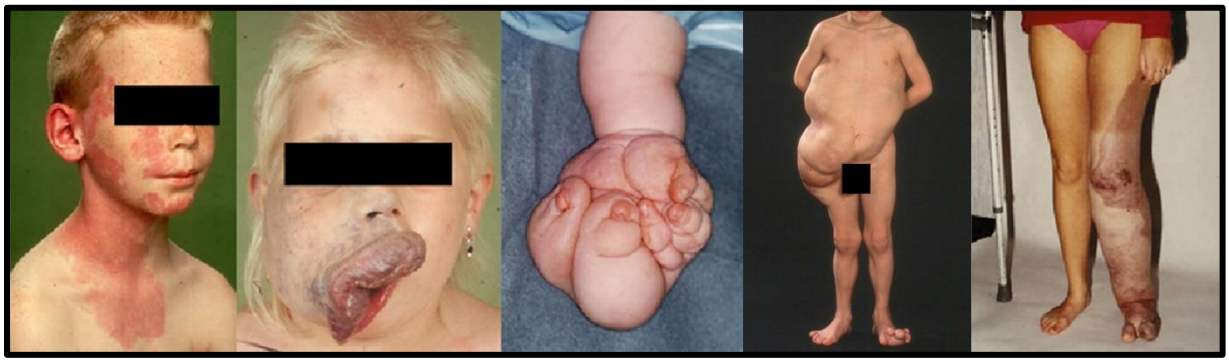

Figure 1 – clinical pictures of patients with various types of vascular malformations.
From left to right: capillary malformation, venous malformation, lymphatic malformation, and the latter two combined capillary-lymphatic-venous malformations with overgrowth of soft tissues.

Capillary malformations

Capillary malformations are generally visible as a flat pink, red, or purple stain during infancy, and may thicken during adult life as a result of progressive vascular ectasia.[25, 26] Additionally, with the increase of age, blebs and nodules may occur.[27] These lesions may appear anywhere in the body but are the most common in the head and neck region, possibly extending to the lips, gingiva, or oral mucosa.[28, 29] Capillary malformations may be accompanied by hypertrophy of soft tissue and are well known to cause disfigurement, asymmetry, and spontaneous bleeding.[30]

Capillary malformations affecting the skin in the distribution of the ophthalmic branch of the trigeminal nerve are associated with Sturge-Weber syndrome, which is further characterized

by venous-capillary abnormalities of the leptomeninges and occasionally ophthalmologic abnormalities, potentially leading to seizures and glaucoma.[31, 32]

Venous malformations

Venous malformations are present as a soft, compressible, non-pulsating mass that characteristically enlarges in a dependent position.[33] Superficial venous malformations located in subcutaneous tissue or the skin, generally have a bluish appearance. Inside the venous malformation, localized intravascular coagulopathy may develop due to venous stasis and the abnormal venous endothelium, which is associated with painful thrombotic episodes.[20, 34] The localized thrombi may bind to calcium deposits and form round, stone-like structures called phleboliths.[20]

Multiple, small (between 1-2 centimeters) venous malformations of the skin and within the gastrointestinal tract are associated with Blue Rubber Bleb Nevus syndrome.[35]

Lymphatic malformations

Lymphatic malformations are classified in three morphologic types of cystic lymphatic lesions: macrocystic, microcystic, and combined.[1] Macrocystic lymphatic malformations appear as large (≥2cm) compressible or non-compressible, smooth, and translucent masses under normal colored or bluish skin, they usually consist of various cysts that vary in size.[24, 36] On the contrary, microcystic lymphatic malformations are composed of multiple small vesicles with diffuse boundries.[37] Combined lesions contain a mixture of macro- and micro-cysts.

Lymphatic malformations can be present at any anatomical location, although, they are more commonly seen in lymphatic-rich areas, such as the head and neck, axilla, mediastinum, groin, and retroperitoneum.[24, 38] Intracystic hemorrhage or infection may rapidly enlarge the lymphatic malformation and lead to pain and compression of adjacent structures, possibly compromising breathing, swallowing or visual function in case the lesion is associated with the aerodigestive tract or eyes.[38, 39] Cutaneous involvement of the lymphatic malformation can be associated with spontaneous or trauma-triggered leakage of lymph fluid.[24]

Arteriovenous malformations

Arteriovenous malformations consist of a conglomerate of arteries with a direct connection to veins, bypassing the capillary network and nutritional exchange. Veins are incapable of maintaining high blood flow, and the draining veins undergo structural remodeling to expand themselves to the altered hemodynamics.[40]

Arteriovenous malformations can present as a pink to blue cutaneous stain or mass, feel warm, and may have a palpable thrill.[41] The reduced capillary oxygen delivery may lead to local ischemia, possibly resulting in pain and ulceration.[42] The high-flow vascular malformations can also cause uncontrollable bleeding and even cardiac high output failure in case of extensive

arteriovenous shunting. The lesions enlarge during life because of increased blood flow, causing collateralization, dilatation of vessels, and thickening of adjacent arteries and veins.[43] The Schobinger staging system classifies clinical severity and lesion progression (Table 2).[44]

Table 2. Schobinger staging system for arteriovenous malformations

Stage	Clinical findings
Stage I (Quiescence)	Cutaneous, blush, warmth
Stage II (Expansion)	Active growth, pulsations, bruit
Stage III (Destruction	Same as stage II but symptomatic (pain, bleeding, disfigurement)
Stage IV (Decompensation)	Same as stage II but with high-output cardiac failure

Tissue overgrowth

Vascular malformations may be accompanied by overgrowth of soft tissue or bone. The presence of tissue overgrowth next to vascular malformations historically merged into the diagnosis of a syndrome.

Parkes Weber syndrome is diagnosed based on the triad of capillary malformations, arteriovenous malformations or arteriovenous fistulas, and tissue overgrowth of a limb. In addition, venous, capillary, and lymphatic malformations may be a part of Klippel-Trenaunay syndrome, which is further characterized by localized bone or soft tissue hypertrophy of one or more limbs.[45]

The mutational discoveries have resulted in major advances in understanding the molecular etiology of vascular malformations and (associated) overgrowth. The triad in Parkes Weber syndrome is part of the wide clinical heterogeneity caused by RASA1 mutations.[46]

Furthermore, the somatic PIK3CA mutation is another gene mutation identified in vascular malformations, overgrowth disorders, as well as in syndromes consisting of both clinical features. The unraveling of the common pathophysiology of somatic PIK3CA mutations in phenotypically distinct disorders has led to a reappraisal of the historical clinical classification, and these overgrowth disorders are now grouped within the PIK3CA-Related Overgrowth Spectrum (PROS).[47] Various overgrowth phenotypes are included in PROS, ranging from localized tissue overgrowth of digits and limbs in macrodactyly to vascular malformations and more extensive overgrowth disorders such as Klippel-Trenaunay syndrome.[48-50]

Clinical heterogeneity

The heterogeneity among patients with vascular malformations with regard to the genotype and clinical manifestations is thought to be a critical factor in patients' perceived symptoms. However,

to date, it is unclear which clinical characteristics lead to which specific symptoms and how subsequently these symptoms relate to a decreased health-related quality of life. A better understanding of experienced symptoms in these patients may help to distinguish groups based on clinical severity. Furthermore, the clinical heterogeneity among patients with vascular malformations highlights the need for a more personalized approach in their management, where unsatisfactory treatment in clinically severely affected groups can be prevented, as well as excessive treatment in groups without symptoms.

Diagnosis

A correct diagnosis is essential when deciding about treatment methods and predicting the course of the disease. Clinical history, symptoms, and physical examination are commonly the basis on which the diagnosis is established. In atypical clinical cases, histopathology and radiological imaging can be necessary to determine the correct diagnosis.

Imaging

Magnetic resonance imaging (MRI) is ideal for the diagnosis and characterization of venous, lymphatic, and arteriovenous malformations because the great soft tissue resolution and anatomical detail, along with the absence of ionizing radiation.[51, 52] MRI findings aid therapeutic decisions and planning.

Histopathology

Vascular malformations have thin-walled vessels, with a large lumen lined and a thin layer of smooth muscle and pericytes.[53]

Molecular analysis

The recent discovery of somatic and germline mutations in endothelial cells of vascular malformations has brought out the importance of molecular analysis. Somatic mutations are restricted to a small fraction of cells within the vascular malformation tissue, and thus mostly not detected in surrounding healthy tissue, gingiva swab, or DNA isolated from peripheral blood.[54] Therefore, molecular analysis can solely be performed on vascular malformation tissue obtained during surgery or tissue biopsies.

Subsequently, these somatic mutations can be identified in DNA isolated from the tissue via Next Generation Sequencing (NGS) and Polymerase Chain Reaction (PCR).[55] However, only a small part of patients with vascular malformations are surgically treated and taking a tissue biopsy can be a troublesome process because of the high bleeding risk and unfeasibility in deep positioned vascular malformations. Consequently, a less invasive method for molecular analysis is required, which is also applicable for deep-seated vascular malformations.

Treatment

In general, treatment is deployed in patients with vascular malformations for symptom relief and improving physical function and health-related quality of life. In patients who experience minimal complaints and symptoms from the vascular malformation, a watchful waiting may suffice. In rare cases, life-threatening complications may occur, such as uncontrollable bleeding, airway compression, or high-output cardiac failure, and in these circumstances, a direct therapeutic response is needed. Common treatment methods used in patients with vascular malformations are outlined below.

Compression stockings can relief symptoms of vascular malformations of the extremities. The garment compresses the vascular malformation and thereby reduces blood stasis, which may result in lowered intravascular coagulation, reduction of pain and an improved appearance, diminished edema, and protection against minor trauma.[56]

Anticoagulants can hamper localized intravascular coagulopathy in venous malformations, and painful thrombotic episodes may be prevented.[35, 57]

Laser therapy is an effective treatment method for superficial vascular malformations, particularly capillary malformations. The Pulsed Dye Laser is the mainstay for laser therapy. The laser light is absorbed by hemoglobin, which is then converted to heat. The heat is transferred to the endothelia, where it destroys the vessel walls.[58] Adjacent structures are spared from the thermal destruction through a process called selective photothermolysis.[59]

The Pulsed Dye Laser only penetrates 1 mm in depth, and deeper and larger vessels are not reached. The long-pulsed Nd:YAG laser is able to penetrate deeper than the Pulsed Dye Laser and is therefore suitable for hypertrophic and treatment-resistant capillary malformations.[60]

Sclerotherapy is the first line of treatment for venous and lymphatic malformations. Sclerotherapy is performed through the administration of a sclerosing agent into the vascular malformation. Here, it disrupts the phospholipid bilayer of the endothelial cells, and subsequently, a cytotoxic effect is generated. Subendothelial collagen is exposed, which initiates activation of the coagulation cascade and results in thrombosis and fibrosis within the lumen and within the lesion as a whole.[61] However, results can be unpredictable and often multiple treatments are needed.

Embolization is a suitable treatment method for arteriovenous malformations since it stands on blocking the nidus, i.e., the abnormal vessels bridging the feeding artery to the draining veins. During embolization, an embolic agent is delivered through a catheter proximal to the arteriovenous malformation where it reduces arteriovenous shunting. The embolization can be

performed with both liquid agents (e.g., adhesive glue, Onyx) and solid agents (e.g., coils).[62] Additionally, embolization may lead to ischemia and scarring and may further reduce arteriovenous shunting, shrink the lesion, and improve symptoms.[41] Although, most arteriovenous malformations will ultimately re-expand after embolization.

Embolization is commonly combined with subsequent surgical resection because embolization will lead to a hardened block of the arteriovenous malformation that can be easier resected, and blood loss will be reduced during surgery.

Surgical resection can be effective, definitive, and safe for small vascular malformations where total resection can be achieved.[63] In contrast, subtotal resection may temporarily reduce symptoms but leaves vascular malformation tissue behind that has the potential to enlarge and cause recurrent problems.[64, 65]

However, total resection may be difficult to accomplish since lesions are difficult to distinguish from healthy tissue or the lesion is closely involved with functional structures. Additionally, a lot of soft tissue might need to be removed for total resection, which has the possibility to induce functional problems, and the resulting deformity can be worse than the initial appearance of the vascular malformation.[41]

The discovery of mutated genes in the endothelial cells of vascular malformations has led to the emergence of therapies targeting these molecular pathways. Most of the causative somatic mutations in vascular malformations are also noted in cancer, therefore, repurposing cancer targeted therapies based on molecular analysis provides a novel approach to the treatment of vascular malformations.[66]

The mTOR inhibitor sirolimus is most extensively studied in patients with vascular malformations, and there is compelling low-level evidence that it is effective in patients with venous and lymphatic malformations.[67, 68] Additionally, other targeted therapies, such as the PIK3CA-inhibitor alpelisib, the MEK-inhibitor trametinib, and the AKT-inhibitor miransertib, have been administered following a compassionate use protocol and are further being clinically investigated.[69-73]

Targeted therapies are based on the genetic profile of vascular malformations, therefore, molecular diagnostics must precede before initiating the targeted therapy. Although, molecular diagnostics of vascular malformations is currently a troublesome process, as it requires a lesion tissue biopsy or surgically resected tissue. With the increase in the use of targeted therapies, a less invasive method for molecular diagnostics is sought.

Targeted therapies have anti-proliferative and immunomodulatory effects and can be accompanied by severe adverse events. Consequently, targeted therapies are not eligible for every patient, and patients should be carefully selected before administering these therapies. Severely affected patients with intense problems and strongly decreased health-related quality of life

would be more eligible to be treated with targeted therapies. Currently, no guidelines exist to indicate severely affected patients, and patients that may benefit from treatment with targeted therapies are not easily identified.

Barriers in evaluating treatment effect

Patients with vascular malformations differ significantly with regard to lesion characteristics, such as lesion type, location, involved tissues, and size. Therefore, differences between patients in terms of experienced symptoms and reasons to seek treatment are expected.

Many treatment options are available for patients with vascular malformations. However, treatment effect is still regarded as unpredictable with great inter patient variability, and frequently accompanied by complications and the recurrence of symptoms.[26, 63, 74]

The high variability between patients regarding genotype, phenotype, symptoms, and quality of life does not allow for a uniform approach to treatment. However, an evidence-based personalized approach to treatment does not exist. Choosing the appropriate treatment method can be challenging because of the numerous therapeutic options and the variability in treatment outcome.

Ideally, individual lesion and patient characteristics should be used to form an evidence-based decision about which treatment option would lead to the desired outcome. Consequently, it can be more adequately investigated who will benefit from certain treatment methods, and ultimately treatment can be tailored more to the individual patient and their symptoms.

Since vascular malformations are not likely to resolve completely after treatment, and are therefore a lifelong burden for the patient, treatment generally aims to relieve symptoms and improve health-related quality of life. Consequently, the patient's view is crucial in treatment evaluation and should form the basis of assessing treatment effect. In current studies investigating treatment effect, most outcome measures focus on size reduction measured with MRI and other clinician-determined outcomes. Although, these outcomes, which are based on changes on imaging or clinician-determined, do not necessarily correlate with improvement in symptoms and quality of life from the patient's perspective.[75-77] Thus, to adequately evaluate treatment effect, the patient's perspective should not be omitted.

Besides the heterogeneity in clinical characteristics between patients, treatment guidelines are hampered by the various methods of how treatments are evaluated. The outcome measures evaluating treatment effects in patients with vascular malformations vary widely.[78] Consequently, the interpretation, comparison, and pooling of study results is problematic, and evidence based guidelines are challenging to develop.

The OVAMA-project

The Outcome measures for Vascular MAlformations (OVAMA) project was initiated in 2016 to establish uniformity in outcome reporting in the clinical research of vascular malformations. In a large e-Delphi study and subsequent consensus meetings with patients and experts worldwide, it was determined *what* outcomes should be measured when evaluating treatment effect in patients with vascular malformations.[19, 79] These outcome measures were combined in a Core Domain Set (CDS), and should be measured at minimum when evaluating treatment effect in patients with vascular malformations.[80] The CDS is displayed in Figure 2 and consists of patient-reported and clinician-reported outcome domains. In this thesis, we focus on the patient-reported outcome domains.

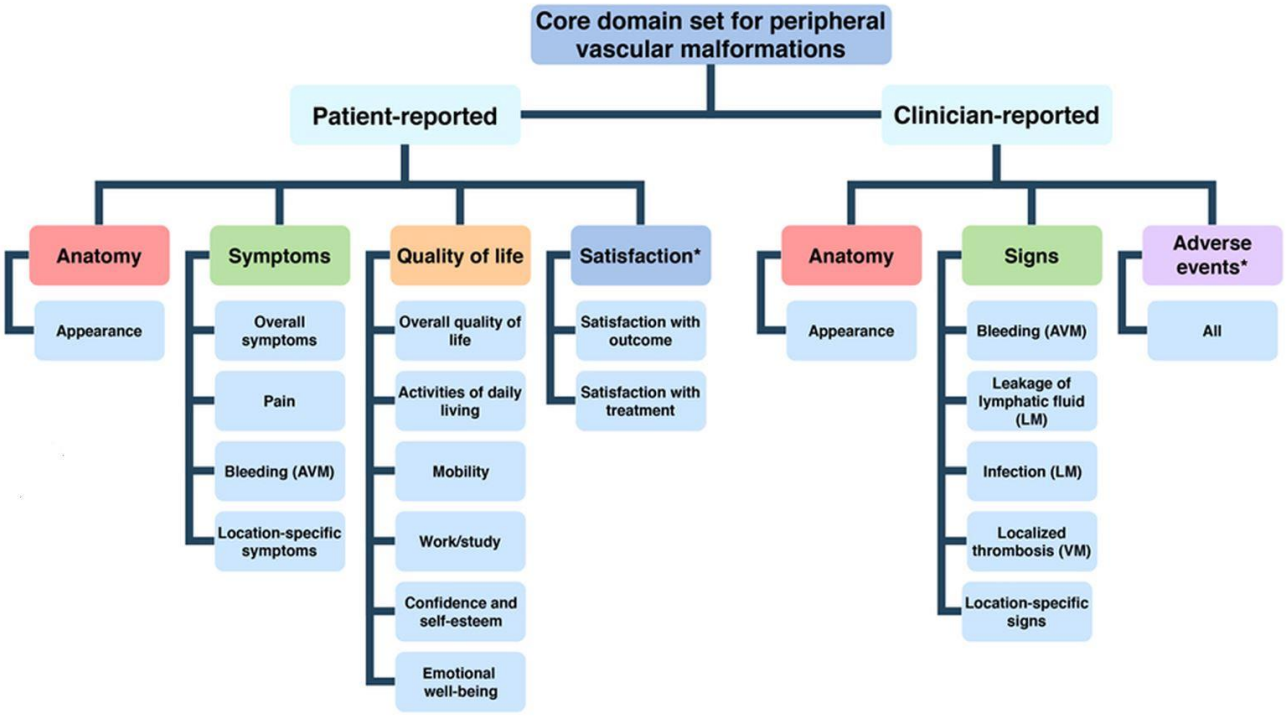

Figure 2 – Core domain set for peripheral vascular malformations.

The domains falling under the domain category 'Quality of life' are non-condition-specific domains. The domains falling under the domain-categories 'Anatomy', 'Symptoms', and 'Satisfaction' are condition-specific domains.

In the process to reach homogeneity in outcome measurement, the core outcome domains were now determined, and in the next phase, there was the need to establish *how* these core outcome domains should be measured. Measurement instruments should be selected or even developed to adequately measure the core outcome domains.

Patient-reported outcome measures

Patient-reported outcome domains are measured with patient-reported outcome measures (PROMs). PROMs are questionnaires designed to be filled in by the patient and seek to ascertain the patient's view of their symptoms, functional status, and quality of life.[81] Through the comparison of a patient's health at different times, the outcome of care received can be determined. Self-reported health has been demonstrated to be a powerful predictor of morbidity and mortality in various disorders.[82-85]

Non-condition-specific outcome domains

The concept quality of life is broad and encompasses functional status in physical, mental, and social domains, and general perceptions of well-being and life satisfaction.[86] The inclusion of quality of life measurement has allowed researchers to demonstrate the impact of the disease and treatment on patient's lives. Domains falling under quality of life are an example of non-condition-specific domains, as they apply to all disorders and all patients.

It is advised to measure non-condition-specific domains with generic measurement instruments.[87] With the use of generic measurement instruments, quality of life measurement can be compared across age groups, cultural, or regional groups, and may aid in the interpretation of results by allowing comparisons with the general population.[87]

In search of generic measurement instruments to measure quality of life in patients with vascular malformations, several PROMs were investigated. To adequately evaluate the effect of treatment on quality of life, PROMs need to be able to detect changes in quality of life before and after treatment, i.e., the PROMs should be responsive to changes. However, previous studies revealed that several PROMs ('Pediatric Quality of Life Inventory', 'Dermatology Life Quality Index', 'Medical Outcomes Study Short Form 36', and 'Skindex-29') were not responsive to changes in the quality of life of children and adults with peripheral vascular malformations.[88, 89] Hence, other generic measurement instruments to measure quality of life in patients with vascular malformations are required.

Condition-specific outcome domains

Condition-specific outcome domains refer to elements of health that are relevant to a particular patient group or condition, and are ought to be measured with condition-specific measurement instruments. Condition-specific measurement instruments are able to capture symptoms and health-status that are specific for a certain disorder and questions can be drafted to address the specific patient group, which are irrelevant for other conditions. For peripheral vascular malformations the condition-specific outcome domains included in the CDS are the domains falling under the domain categories 'anatomy', 'symptoms', and 'satisfaction' (see Figure 2). In a previous study the literature was systematically reviewed for outcome measures used in patients

with peripheral vascular malformations, however, validated PROMs to measure the condition-specific outcome domains were not identified.[78]

Measurement properties

Measurements are central to clinical practice and health research and form the basis of diagnosis, prognosis, and the evaluation of medical interventions. When using a measurement instrument, it is essential that the instrument is able to obtain accurate measurements and that the findings are truthful. Measurement properties represent the quality of the measurement instrument, and they indicate the accuracy of the measurements. Roughly, there are three main measurement properties.[90]

The first is *validity*, referring to the degree to which a measurement instrument measures the construct it intends to measure. For example, when measuring pain due to vascular malformations, pain due to headaches and other forms of pain non-related to vascular malformations should not be measured. The second measurement property is *reliability*, which refers to the consistency of a measurement instrument, i.e., if the measurements are consistent over time and consistent between researchers. The third is *responsiveness,* which refers to the ability of a measurement instrument to detect change over time, e.g., before and after treatment.[90-92]

In summary, vascular malformations portray a wide clinical spectrum with heterogeneity in vascular malformation type, anatomical location, tissue extension, and lesion size. Therefore, vascular malformations should be considered as various disease entities, and management should be adjusted to the individual patient. At the start of this thesis several major issues needed to be addressed to reach a more personalized approach to the management of vascular malformations.

Recent discoveries have pointed out that vascular malformations are caused by somatic and germline mutations in various genes regulating growth. It is suspected that the underlying mutated genes play an essential role in disease heterogeneity, although, this has not yet been investigated on a large scale.

Currently, it is unknown which treatment is most eligible for which patient, and the emergence of targeted therapies requires the identification of patients eligible for these therapies. In order to do so, molecular diagnostics should be easier to perform, more convenient and less invasive.

At last, adequate measurement instruments are not yet available to measure the non-condition-specific and condition-specific outcome domains established in the CDS for peripheral vascular malformations, hampering treatment evaluation from the patient's perspective. In this thesis, these knowledge gaps will be addressed, and the aims and outlines of this thesis are outlined below.

Aims and outline

The main aim of this thesis is to move towards a more personalized approach to the management of vascular malformations. The thesis is divided into five parts, covering distinct aspects of personalized medicine and laying the foundation to tailor treatment to the individual patient.

Part I

General introduction

Part II

Vascular malformations and overgrowth disorders: from genotype to phenotype.

Part III

Development and quality assessment of condition-specific patient-reported outcome measures in patients with peripheral vascular malformations.

Part IV

Defining disease severity in peripheral vascular malformations.

Part V

General discussion and future perspectives.

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Part II

Vascular malformations and overgrowth disorders: from genotype to phenotype.

Chapter 2

Genetic mutations and phenotype characteristics in peripheral vascular malformations: a systematic review

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Index of terms

AVM	Arteriovenous malformation
BRBN	Blue Rubber Bleb Naevus syndrome
CLAPO	Capillary malformation lower lip, lymphatic malformations, asymmetry and partial overgrowth syndrome
CLOVES	Congenital lipomatous overgrowth, vascular malformations, epidermal naevus, scoliosis syndrome
CM	Capillary malformation
ISSVA	International Society for the Study of Vascular Anomalies
KTS	Klippel-Trenaunay syndrome
LM	Lymphatic malformation
MCAP	Megalocephaly-capillary malformation
PROS	PIK3CA-related overgrowth spectrum
SWS	Sturge-Weber syndrome
VAF	Variant Allele Frequency
VeM	Venous Malformation
VM	Vascular malformation

List of genes

AKT	Somatic	Protein kinase B
BRAF	Somatic	B-raf proto-oncogene serine/threonine kinase
ENG	Somatic	Endoglin
ELMO2	Germline	Engulfment and cell motility 2
EPHB4	Germline	Ephrin type-B receptor 4
ERK		Extracellular signal-regulated kinase
CCM1	Germline	Cerebral cavernous malformation 1 (encodes KRIT1 protein)
GLMN	Germline	Glomulin
GNA11	Somatic	Guanine nucleotide-binding protein subunit alpha 11

GNAQ	Somatic	Guanine nucleotide-binding protein subunit alpha Q
HRAS	Somatic	Harvey RAS
KRAS	Somatic	Kirsten RAS
KRIT1	Germline	Krev interaction trapped protein 1 (encoded by the CCM1 gene)
MAP2K1	Somatic	Mitogen-activated protein kinase 1
MAP3K3	Somatic	Mitogen-activated protein kinase kinase kinase 3
mTOR	Somatic	Mammalian target of rapamycin
PI3K	Somatic	Phosphatidylinositol 3-kinase
PIK3CA	Somatic	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha
PTEN	Germline/somatic	Phosphatase and tensin homolog
RASA1	Germline	RAS protein activator 1
TIE2	Germline/somatic	Tyrosine Kinase With Ig And EGF Homology Domains-2

Abstract

Vascular malformations (VMs) are clinically diverse with regard to the vessel type, anatomical location, tissue involvement, and size. Consequently, symptoms and disease impact differ significantly. Diverse causative mutations in more and more genes are discovered and play a major role in the development of VMs. However, the relationship between the underlying causative mutations and the highly variable phenotype of VMs is not yet fully understood. In this systematic review, we aimed to provide an overview of known causative mutations in genes in VMs and discuss associations between the causative mutations and clinical phenotypes. PubMed and EMBASE libraries were systematically searched on November 9th, 2022 for randomized controlled trials and observational studies reporting causative mutations in at least five patients with peripheral venous, lymphatic, arteriovenous, and combined malformations. Study quality was assessed with the Newcastle-Ottawa Scale. Data was extracted on patient and VM characteristics, molecular sequencing method, and results of molecular analysis. In total, 5667 articles were found of which 69 studies were included, reporting molecular analysis in a total of 4261 patients and in 1686 (40%) patients with peripheral VMs a causative mutation was detected. In conclusion, this systematic review provides a comprehensive overview of causative germline and somatic mutations in various genes and associated phenotypes in peripheral VMs. With these findings, we attempt to better understand how the underlying causative mutations in various genes contribute to the highly variable clinical characteristics of VMs. Our study shows that some causative mutations lead to a uniform phenotype, while other causal variants lead to more varying phenotypes. By contrast, distinct causative mutations may lead to similar phenotypes and result in almost indistinguishable VMs. VMs are currently classified based on clinical and histopathology features, however, the findings of this systematic review suggest a larger role for genotype in current diagnostics and classification.

Introduction

Vascular malformations (VMs) are congenital anomalies of the vascular and lymphatic system histopathologically characterized by an increase in the number of vessels that can be tortuous, dilated, and dysfunctional. During early embryogenesis, primitive blood vessels are formed from mesoderm-precursor cells, known as vasculogenesis.[1] The primitive blood vessels expands and mature to generate functional vascular and lymphatic vessels, known as angiogenesis, which continues throughout life.[2] VMs arise during embryogenesis due to alterations in the vasculogenesis and angiogenesis cell signalling pathways. Post-zygotic somatic-mosaic mutations, and rarely, germline (hereditary) mutations in genes encoding proteins that are part of the RAS/MEK/ERK and/or PI3K/AKT/mTOR signalling pathways have been identified in endothelial cells of VMs. These genetic changes provoke altered endothelial cell proliferation, differentiation, and survival, resulting in VMs.[3]

VMs are classified by the International Society for the Study of Vascular Anomalies (ISSVA) and subdivided by the affected vessel type; venous (VeM), capillary (CM), arteriovenous (AVM), lymphatic (LM), or a combination thereof, leading to heterogeneity between patients.[4] Further, are vascular- and lymphatic vessels present throughout (nearly) the whole body, and VMs may, therefore, also be clinically heterogeneous because of their presence in various types of tissues and anatomical locations. Finally, lesions may be small and confined to a certain body area, or may affect large areas of the body and be accompanied by genetically-affected surrounding tissues, resulting in overgrowth.

Since the recent discovery of mutated genes in VMs, the perspective on how VMs are classified, diagnosed, and managed is shifting. VMs and associated syndromes are now more and more classified based on the genotype, e.g. the PIK3CA-Related Overgrowth Spectrum (PROS). Importantly, the revelation of genetic mutations in VMs also offers new opportunities for treatment. Several genes that are part of the RAS/MEK/ERK and/or PI3K/AKT/mTOR signalling pathways (associated with VMs) are also often implicated in cancers. Therefore, molecular inhibitors used in clinical oncology are being repurposed as targeted molecular therapies for VMs and molecular diagnostics is becoming inevitable.[5-7] It is suspected that the underlying mutated genes have a causative role in the highly variable phenotype among patients with VM. However, pooled evidence on how the mutated genes relate to the clinical phenotype is lacking. In search for associations between causative genetic mutations in VMs and the clinical features of peripheral VMs, we performed a systematic review of the literature.

Methods

We followed the checklist for reporting according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement 2020 for this systematic review.[8, 9] Our systematic review protocol was registered with PROSPERO on 11-12-2020 (registration number CRD42020219416).

Literature search and study selection

Literature was searched on November 9th, 2022, in PubMed (MEDLINE) and Embase (OVID). The search strategy was made with the help of a clinical librarian and is listed in Table 1. Inclusion- and exclusion criteria are shown in Table 2. Two researchers (M.S., E.T.) independently screened titles and abstracts, and further selected papers based on full-text.

Table 1. Search Strategy

Literature was initially searched on December 9th, 2020, in PubMed (MEDLINE) and Embase (OVID). However, the search in both databases was updated on November 9th, 2022, amending the protocol registered on PROSPERO. The search strategy was made with the help of a clinical librarian.

Database	Search Strategy
PubMed (Medline)	("Vascular Malformations"[Mesh:NoExp] OR "Arteriovenous Malformations"[Mesh:NoExp] OR vascular malformation*[tiab] OR venous malformation*[tiab] OR arteriovenous malformation [tiab] OR lymphatic malformation*[tiab] OR capillary malformation* [tiab] OR port wine stain* [tiab] OR vessel malformation*[tiab] OR congenital vessel malformation*[tiab] OR vascular anomal*[tiab] OR vascular system anomal*[tiab] OR cerebrovascular malformation*[tiab] OR blood vessel anomal*[tiab] OR disrupting vascular development*[tiab]) AND ("Mutation"[Mesh] OR "Genome"[Mesh] OR "genetics" [Subheading] OR mutation*[tiab] OR genom*[tiab] OR gene*[tiab]) NOT ("Letter"[Publication Type] OR "Editorial"[Publication Type] OR "Comment"[Publication Type] OR "Case Reports" [Publication Type] OR "Review" [Publication Type] OR letter[ti] OR editorial[ti] OR case report[ti]) NOT (("Animals"[Mesh] OR "Animal Experimentation"[Mesh] OR "Models, Animal"[Mesh] OR rat[tiab] OR rats[tiab] OR mice[tiab] OR mouse[tiab] OR dog[tiab] OR dogs[tiab] OR pig[tiab] OR pigs[tiab] OR cow[tiab] OR cows[tiab] OR monkey[tiab] OR monkeys[tiab] OR horse[tiab] OR horses[tiab]) NOT ("Humans"[Mesh] OR human*[tiab]))
Embase (OVID)	congenital blood vessel malformation/ or exp arteriovenous malformation/ OR (vascular malformation* or venous malformation* or cerebrovascular malformation* or arteriovenous malformation or lymphatic malformation* or capillary malformation* or port wine stain* or vessel malformation* or congenital vessel malformation* or vascular anomal* or vascular system anomal* or blood vessel anomal* or disrupting vascular development*).ti,ab,kw. AND

(mutation* or genom* or gene*).ti,ab,kw OR mutation/ or exp gene mutation/ or somatic mutation/ or exp gene/ or gene sequence/
NOT
letter/ or editorial/ or note/ or case report/ or "review"/ or conference paper/
or (letter or comment or editorial or case report).ti.
NOT
(exp animal/ or exp animal experiment/ or exp animal model/ or (rat or rats or mice or mouse or dog or dogs or pig or pigs or cow or cows or monkey or monkeys or goat or goats or horse or horses).ti,ab,kw.) not (human/ or human*.ti,ab,kw.)

Table 2. Inclusion and exclusion criteria

**Articles since 1994 were included, when the first genetic basis of familial forms of vascular anomalies were identified and the ISSVA classification system was generally accepted.*

Inclusion criteria	Exclusion criteria
Peripheral venous, lymphatic, arteriovenous, or combined malformations	Non-human subjects
Causative mutations (germline or somatic) in peripheral vascular malformations	Phenotypic characteristics are not described per person
Original studies (observational studies, cohort studies, case series, cross-sectional studies, case-control studies, randomized controlled trials)	Vascular malformations located in the central nervous system or purely in the visceral organs
Outcomes reported of at least 5 patients	Studies researching mutations in solely haemangiomas or other vascular tumours
Publication year ≥1994*	Studies researching mutations in solely capillary malformations.
	Non-Dutch or Non-English articles
	Short reports, letters, and conference abstracts

Data extraction

Data was extracted by one researcher (M.S.) and cross-checked by another researcher (E.T.). Data was extracted on study design, lesion characteristics, sequencing method, genetic test results, and associations between genotype and phenotype characteristics. Additionally, all patients were separately extracted from the included studies, lesion and patient characteristics and genetic test results were documented per individual patient and tabulated to synthesise data. Phenotypic variables comprised patient sex, VM type according to the ISSVA classification[4], lesion location, affected tissue types, lesion size, multiplicity of lesions, the diagnosis of an associated syndrome, overgrowth, and other documented characteristics. Lesion location was categorized as: head/neck, upper extremity, trunk, lower extremity, and multiple locations. If lesion size was reported in centimetres it will be categorized in 4 categories: very small (0-5cm), small (5-10 cm),

medium (10-30cm), and large (>30 cm), to allow data synthesis. Of all detected mutations the following data was extracted: germline/somatic mutation, affected gene, protein change, and variant allele frequency (VAF). Supplementary data of all articles were viewed, to avoid the missing of patient details.

The main outcome was genetic mutations causative of peripheral VMs and their associated phenotype characteristics in a descriptive manner.

Quality assessment

The quality assessment was independently performed by two researchers (M.S., E.T.) using the Newcastle-Ottawa Scale, adapted for cross-sectional studies.[10]

Results

The literature search yielded 5667 studies, of which 69 studies met the inclusion criteria (Figure 1). In supplementary table 1, details are shown on the study design, sequencing method, and documented phenotype characteristics of included studies. In the 69 observational studies, 4261 patients were included, of which 1686 (40%) patients with peripheral VMs had a causative mutation detected. All patients with peripheral VMs were a causative mutation was detected (n=1686) were included in the current study.

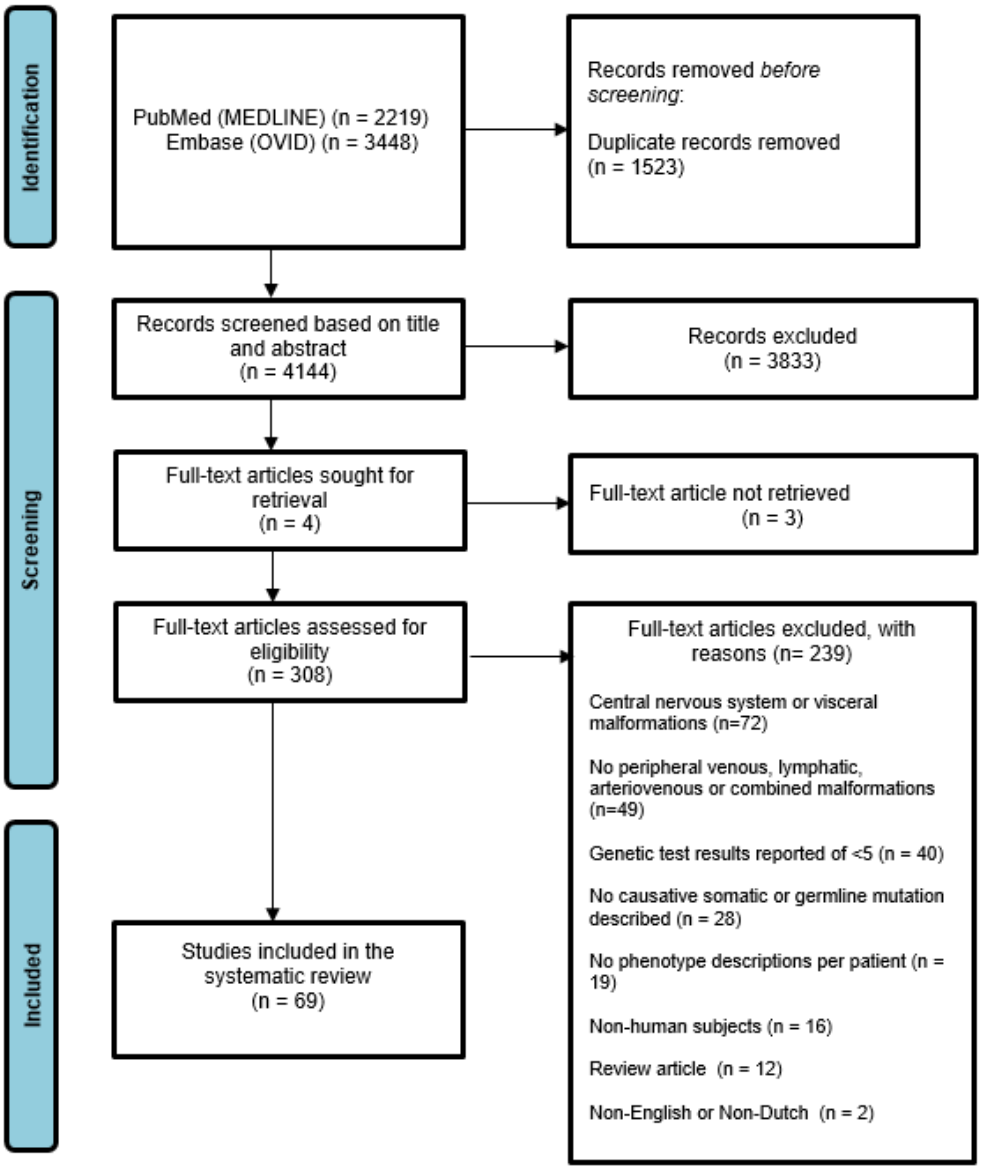

Figure 1 - Flow diagram of the literature search and study selection following the Preferred Reporting Items for Systematic Reviews (PRISMA).
The literature search yielded 5667 studies, of which 69 studies met the inclusion criteria.

Quality assessment

The study quality ranged from unsatisfactory to very good, however, most studies (82%) were rated satisfactory or good (Supplementary Table 2). Lower study quality was mainly caused by a selected or convenience sample (e.g. patients who already had a tissue sample available for molecular analysis or patients who underwent surgery where lesion tissue could be collected for molecular analysis), small sample size, and missing data on non-respondents.

Phenotypic and mutational details by gene.

An overview of the phenotype findings of the most frequently mutated genes is displayed in Table 3. A schematic diagram of the genes signalling pathways and associated general phenotypes is displayed in Figure 2. An overview of causative mutations in various genes and phenotype characteristics per study is listed in Supplementary Table 3. An overview of the distribution of causative genes per VM type is displayed in Figure 3.

Table 3. Frequently mutated genes with phenotype characteristics.

The table displays per gene a general vascular phenotype, associated non-vascular anomalies and frequencies of phenotype characteristics.

	TIE2	PIK3CA	PIK3R1	PTEN	EPHB4	RASA1	KRAS	MAP2K1	GNAQ	GNA11	KRIT1/CCM1	GLMN
Studies (n)	18	34	2	5	2	9	8	6	8	4	1	6
Patients (n)	293	537	14	39	105	268	40	30	26	14	33	203
VM phenotype	**Somatic TIE2**: solitary VeMs of various locations. **Germline TIE2**: multiple, deep blue, small (<5 cm), cutaneous or mucosal, deep or superficial, hereditary VeMs located at the head/neck or extremities.	**Somatic PIK3CA**: Heterogeneous phenotype with low-flow VMs, varying from a small solitary VM to large combined VMs with adjacent overgrowth and other non-vascular anomalies.	**Somatic PIK3R1:** low-flow VMs (CM, VeM, but mostly combined) with overgrowth. Phenotypes were described to be similar to PIK3CA phenotypes.	**Germline PTEN:** Mostly AVMs of the lower extremity, in one third intramuscular.	**Germline EPHB4**: Mainly isolated multifocal CMs and in a few cases an additional AVM. CMs were described pink/red, cutaneous, multifocal with geographic borders. Size varied from pinpoint to large lesions (15 cm).	**Germline RASA1**: Multifocal, round-to-oval, red/pink CMs of varying size, some with irregular borders and/or with a white/pale halo. Several CMs had an area of high flow (suggestive for an AVM) or a separate AVM was present.	**Somatic KRAS**: Phenotype with predominantly (extensive) AVMs. VMs are frequently located at the head/neck or lower extremity.	**Somatic MAP2K1**: Generally extra cranial AVMs located at the head/neck, with variable severity.	**Somatic GNAQ**: Mainly CM or combined malformations, frequently localized at the head/neck or in multiple locations.	**Somatic GNA11:** CMs or combined malformations with a capillary component.	**Germline KRIT1**: Alongside familial cerebral cavernous malformations a solitary, cutaneous CM, VeM, or combined malformation, mainly located at the extremities.	**Germline GLMN**: Glomuvenous malformations; hyperkeratotic, multifocal, bluish-purple, (sub)cutaneous hereditary lesions with a cobblestone-like appearance, mainly located at the extremities.
Associated non-vascular anomalies±		Extremities: overgrowth, macro-, poly-, and syndactyly, wide hand/feet, sandal gap, leg-length discrepancy, scoliosis Skin: epidermal naevus, abnormal pigmentation Internal: renal anomalies, ovarian cysts, gastro-intestinal bleeding, VTE Brain: hydrocephalus, macro-, and megalocephaly, developmental delay	Venous ectasias, red vascular stains, macrodactyly, sandal gap and lipoma.	Macrocephaly, intracranial developmental venous anomalies, penile freckling, developmental delays, overgrowth, gastrointestinal polyps, and thyroid involvement.	Bier spots (small, light macules) and telangiectasia's.	Varicose veins, tissue overgrowth, and telangiectasia.	Tissue overgrowth and varicose veins.		Tissue overgrowth. In Sturge Weber Syndrome: leptomeningeal angiomatosis, glaucoma, seizures, headache, and epistaxis.	Tissue overgrowth, subtle segmental hyper-pigmentation, and dermal melanocytosis.		
Mutation details												
Mutation type												
Somatic	204 (70%)	537 (100%)	14 (100%)	3 (8%)*	0 (0%)	2 (0.7%)* & **	40 (100%)	30 (100%)	26 (100%)	14 (100%)	0 (0%)	0 (0%)
Germline	89 (30%)	0 (0%)	0 (0%)	36 (93%)	105 (100%)	266 (99.3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	33 (100%)	192 (96%)
Germline/somatic	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	9 (4%)

Mutation position / protein change (frequencies)	L914F (n=119; 41%) R849W (n=71; 24%) Multiple(n=45;15%) Y897C (n=11; 4%) R915C (n=7; 2%)	H1047R (n=109; 22%), E545K (n=104; 21%), E542K (n=87; 18%), H1047L (n=20; 4%), C420R (n=22; 5%)	N564D (n=5; 36%) K567E (n=4; 29%) M582_D605del (n=3; 21%)	R130X (n=4; 11%), R233X (n=2; 5%). Other mutations are not repetitive	L12Wfs*10 (n=7, 7%) N745D (n=5; 5%) C268R (n=4; 4%) G516R (n=4; 4%) V469G (n=4; 4%)	R245fsX8 (n=11;4%) c.512delIT(n=11;4%) R679* (n=10; 4%) R711* (n=10; 4%)	G12D (n=14; 35%), G12V (n=8; 20%), Q61H (n=7; 18%), G12C (n=3; 8%)	K57N (n=19; 63%), Q56P (n=5; 17%), Q58_E62del (n=3; 10%)	R183Q (n=21; 81%) Other mutations are not repetitive	R183C(n=11;79%) Q209L (n=2; 14%) Q209H (n=1; 7%)	Missing data	157delAAGAA (n=88; 44%), 31delAA (n=12; 6%) 108C>A (n=10; 5%)
Phenotype characteristics												
Sex												
Reported in (n)	n=124	n=344	n=0	n=26	n=7	n=62	n=31	n=25	n=20	n=8	n=0	n=193
Male	43 (35%)	164 (48%)		14 (54%)	3 (43%)	31 (50%)	16 (52%)	15 (60%)	6 (30%)	6 (75%)		95 (49%)
Female	81 (65%)	180 (52%)		12(46%)	4 (57%)	31 (50%)	15 (48%)	10 (40%)	14 (70%)	2 (25%)		98 (51%)
Type Malformation												
Reported in (n)	n=293	n=523	n=14	n=37	n=105	n=266	n=40	n=30	n=26	n=14	n=33	n=198
Venous	253 (86%)	79 (15%)	1 (7%)	7 (19%)	0 (0%)	0 (0%)	5 (13%)	1 (3%)	3 (12%)	0 (0%)	6 (18%)	198 (100%)
Lymphatic	0 (0%)	163 (31%)	0 (0%)	2 (5%)	0 (0%)	0 (0%)	1 (3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Arteriovenous	9 (3%)	4 (1%)	0 (0%)	21 (57%)	0 (0%)	5 (2%)	22 (55%)	28 (93%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Capillary	3 (1%)	77 (15%)	2 (14%)	0 (0%)	84 (80%)	169 (64%)	4 (10%)	0 (0%)	17 (65%)	9 (65%)	12 (36%)	0 (0%)
Combined	14 (5%)	197 (38%)	11 (79%)	5 (14%)	21 (20%)	91 (34%)	8 (20%)	1 (3%)	6 (23%)	5 (35%)	15 (46%)	0 (0%)
Unclear	14 (5%)	3 (1%)	0 (0%)	2 (5%)	0 (0%)	1 (0.4%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Multiple lesions												
Reported in (n)	n=130	n=105	n=0	n=38	n=2	n=148	n=27	n=12	n=1	n=0	n=33	n=20
Yes	70 (54%)	32 (31%)		13 (34%)	2 (100%)	117 (79%)	4 (15%)	0 (0%)	0 (0%)		3 (9%)	11 (55%)
No	60 (46%)	73 (69%)		25 (66%)	0 (0%)	31 (21%)	23 (85%)	12 (100%)	1 (100%)		30 (91%)	9 (45%)
Localization												
Reported in (n)	n=191	n=320	n=1	n=38	n=18	n=73	n=38	n=29	n=20	n=8	n=32	n=1
Head and neck	48 (25%)	97 (30%)	0 (0%)	0 (0%)	9 (50%)	30 (41%)	13 (34%)	24 (83%)	6 (30%)	2 (25%)	3 (9%)	0 (0%)
Upper extremity	36 (19%)	14 (4%)	0 (0%)	4 (11%)	5 (28%)	12 (16%)	6 (16%)	0 (0%)	1 (5%)	1 (12.5%)	12 (38%)	1 (100%)
Trunk	15 (8%)	45 (14%)	0 (0%)	8 (21%)	0 (0%)	3 (4%)	3 (8%)	2 (7%)	2 (10%)	0 (0%)	0 (0%)	0 (0%)
Lower Extremity	43 (23%)	72 (23%)	1 (100%)	19 (50%)	4 (22%)	11 (15%)	14 (37%)	3 (10%)	5 (25%)	1 (12.5%)	17 (53%)	0 (0%)
Extremities, not specified	2 (1%)	37 (12%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Multiple locations	47 (25%)	55 (17%)	0 (0%)	7 (18%)	0 (0%)	15 (21%)	2 (5%)	0 (0%)	6 (30%)	4 (50%)	0 (0%)	0 (0%)
Tissue involvement												
Subcutaneous												
Reported in (n)	n=99	n=69	n=0	n=1	n=1	n=33	n=5	n=1	n=11	n=33	n=33	n=0
Yes	83 (84%)	59 (86%)		1 (100%)	1 (100%)	33 (100%)	5 (100%)	0 (0%)	11 (100%)	(100%)	33 (100%)	

No	16 (16%)	9 (13%)		0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)	
Intramuscular												
Reported in (n)	n=95	n=27	n=0	n=13	n=0	n=3	n=2	n=1	n=0	n=0	n=0	n=0
Yes	57 (60%)	8 (30%)		12 (92%)		0 (0%)	2 (100%)	1 (100%)				
No	38 (40%)	19 (70%)		1 (8%)		3 (100%)	0 (0%)	0 (0%)				
Intraosseous												
Reported in (n)	n=90	n=17	n=0	n=0	n=0	n=7	n=3	n=1	n=0	n=0	n=0	n=0
Yes	13 (14%)	35 (88%)				4 (57%)	3 (100%)	0 (0%)				
No	77 (86%)	5 (13%)				3 (43%)	0 (0%)	1 (100%)				
Overgrowth												
Reported in (n)	n=20	n=365	n=14	n=25	n=0	n=37	n=25	n=6	n=7	n=9	n=0	n=1
Yes	1 (5%)	236 (65%)	13 (93%)	13 (52%)		15 (41%)	10 (40%)	0 (0%)	5 (71%)	3 (33%)		0 (0%)
No	19 (95%)	129 (35%)	1 (7%)	12 (48%)		22 (59%)	15 (60%)	5 (100%)	2 (29%)	6 (67%)		1 (100%)
Syndrome												
Reported in (n)	n=107	n=419	n=13	n=16	n=7	n=105	n=32	n=5	n=19	n=7	n=0	n=1
Yes	26 (24%)	214 (51%)	0 (0%)	14 (87%)	7 (100%)	8 (8%)	3 (9%)	0 (0%)	5 (26%)	7 (100%)		0 (0%)
No	81 (76%)	205 (49%)	13 (100%)	2 (13%)	0 (0%)	97 (92%)	29 (91%)	5 (100%)	14 (74%)	0 (0%)		1 (100%)
Syndrome Type	BRBN (n=15), Bockenheimer disease (n=9)	CLOVES (n=72), KTS (n=76), CLAPO (n=7), Fibro adipose hyperplasia (n=3), MCAP (n=34), multiple lipomatosis (n=1)		Cowden (n=13), KTS (n=1)	Parkes Weber (n=7)	Parkes Weber (n=7), KTS (n=1)	KTS (n=2), Parkes Weber (n=1)		KTS (n=1) and Sturge Weber Syndrome (n=4)			

± Only the most important associated non-vascular anomalies are displayed, i.e., reported in >10% of cases per study.*Somatic mutation; the mutation was absent in blood/saliva.
** Somatic mutation; blood/saliva was not analysed for the mutation.

BRBN = Blue Rubber Bleb Naevus syndrome; CLAPO = Capillary malformation lower lip, Lymphatic malformations, Asymmetry and Partial Overgrowth syndrome; CLOVES = Congenital Lipomatous Overgrowth, Vascular Malformations, Epidermal Nevis, Scoliosis syndrome; KTS = Klippel-Trenaunay Syndrome; MCAP = Megalocephaly-Capillary malformation; VTE = Venous Thromboembolism.

The patient characteristic "lesion size" was not displayed in the table because of the high variability in outcome reporting, i.e. in descriptive names (e.g. small/localized/large/extensive/diffuse), millilitres, largest diameter in centimetres, and three-dimensional measurement in centimetre, and the synthesis of the data was infeasible.

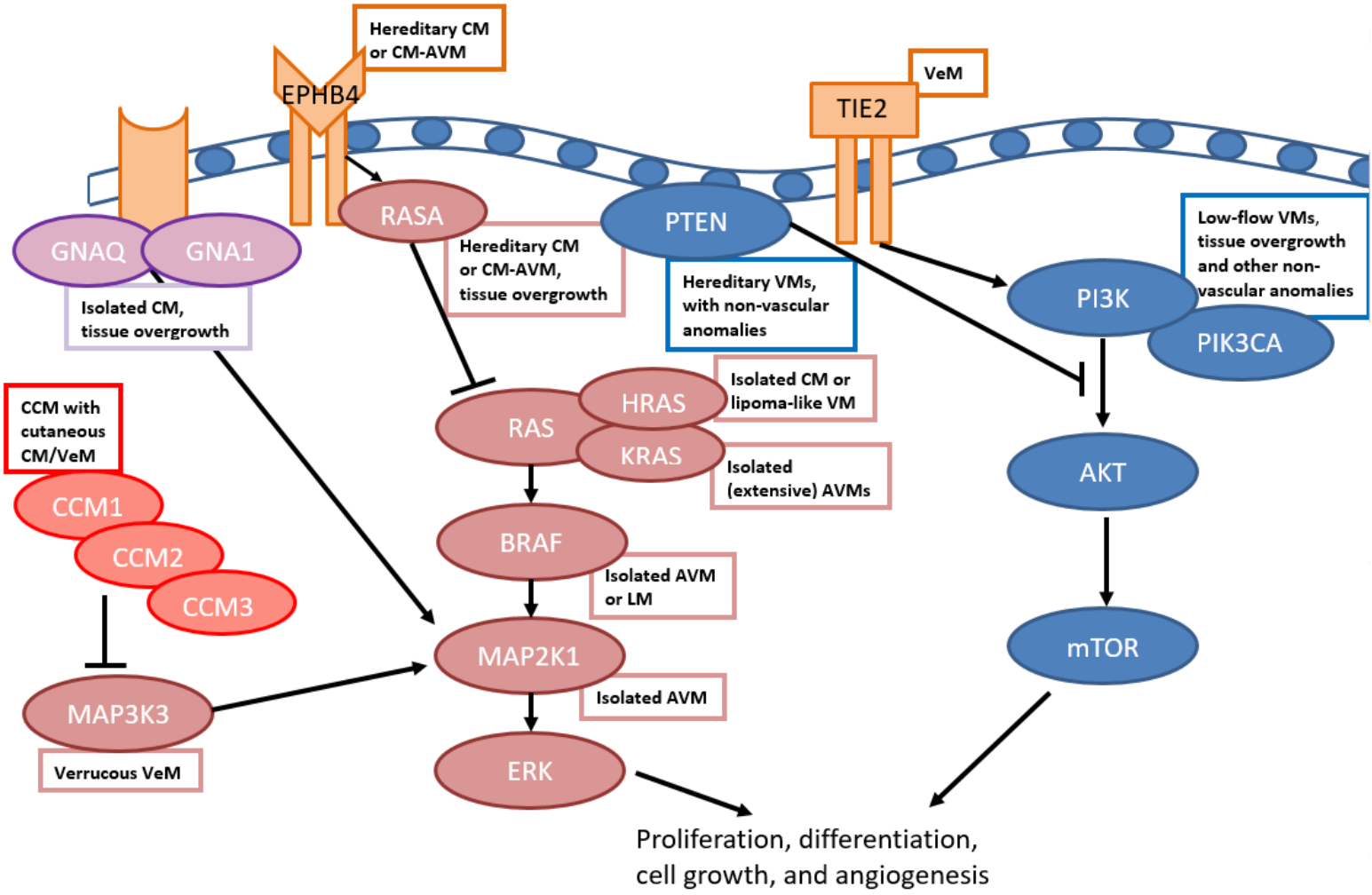

Figure 2 - Schematic diagram of the (simplified) RAS/MEK/ERK and PI3K/AKT/mTOR signalling pathways in endothelial cells, hyperactivated in peripheral vascular malformations, with the associated general phenotype per gene.

Arrows indicate direct or indirect interactions and blunt lines indicate inhibition. RAS/MAPK/ERK pathway hyperactivated when mutations arise in GNAQ, GNA11, EPHB4, RASA1, BRAF, KRAS, HRAS, MAP2K1, MAP3K3, and KRIT1 (CCM1). PI3K/AKT/mTOR pathway hyperactivated when mutations arise in TIE2, PIK3CA, PIK3R1, PTEN, AKT, and mTOR.

AVM= Arteriovenous malformation; CCM = Cerebral cavernous malformation; CM = Capillary malformation; LM = Lymphatic malformation; VeM = Venous malformation; VM = Vascular malformation

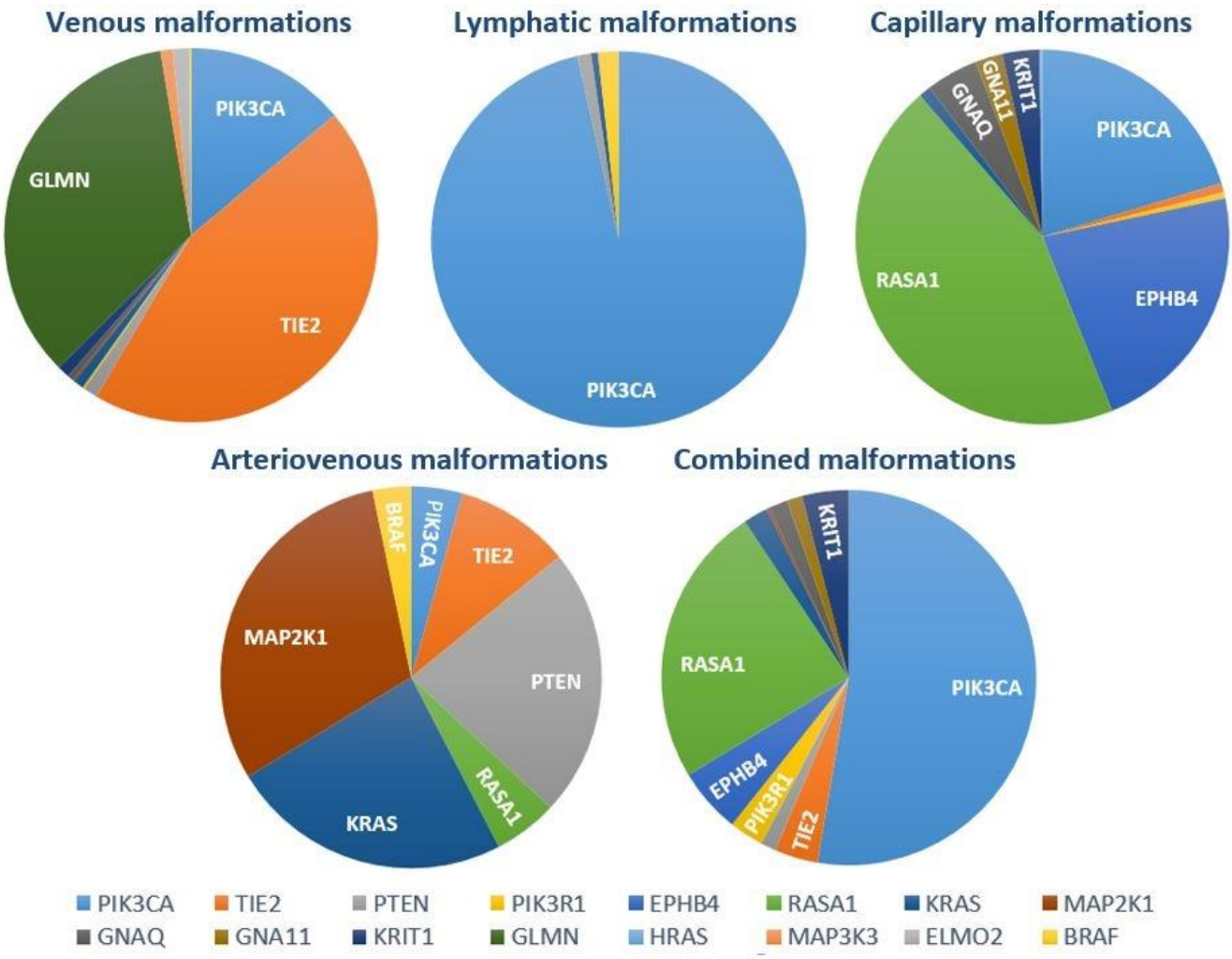

Figure 3 – Circle diagrams of the distribution of causative mutations per vascular malformation type. *The legend displays colours representing the causative mutations.*

TIE2

Eighteen studies reported 293 patients with somatic or germline TIE2 (activating) mutations.[11-29] The phenotype of germline TIE2 mutations was described as multiple, deep blue, small (<5 cm), cutaneous or mucosal, deep or superficial, hereditary VeMs located at the head/neck or extremities.[11-13] Several individuals gained new lesions during adulthood.[12] The phenotype of somatic TIE2 mutations was mainly reported as solitary VeMs of various locations.[14, 16, 17, 19, 21, 23-25] One study described VeMs mainly affecting the skin[16] and other studies found extensive lesions.[14, 21] Blue Rubber Bleb neavus was another phenotype associated with somatic TIE2 mutations, described as one large dominant VeM and >10 small (<2 cm) cutaneous hyperkeratotic (palmo-plantar) VeMs and the presence of gastrointestinal VeMs, mostly with intestinal bleeding

and chronic anemia.[20, 26] Bockenheimer disease with VeMs involving the length of an extremity (most frequently the upper extremity) affecting all tissue planes (subcutaneous tissue, muscle, bone) were also associated with somatic TIE2 mutations.[29]

PIK3CA

Thirty-four studies reported PIK3CA (activating) somatic mutations in 537 patients with peripheral VMs.[16, 18, 19, 21, 23, 25-28, 30-54] The phenotype of somatic PIK3CA mutations was generally described as heterogeneous, varying from a small solitary mainly low-flow malformation (venous, lymphatic, capillary, or a combination thereof) to large combined malformations with adjacent overgrowth and other non-vascular anomalies (listed in Table 3). In contrast, two studies described hypoplasia of subcutaneous tissue, muscles, or bones, either in combination with overgrowth.[27, 51] One study described a clinically distinct phenotype of generalized lymphatic anomaly (GLA) caused by somatic PIK3CA mutations: diffuse or multifocal LMs, mainly mixed micro/macrocystitic, with additional non-progressive lytic areas in the medullary cavity, and a high incidence of visceral and skin involvement.[43] One study found that hotspot-mutations on codons 542 and 545 were associated with more severe phenotypes[35], in other studies, such associations were not found.

PIK3R1

Two studies found (activating) somatic mutations in the PIK3R1 gene in 14 patients.[35, 55] The phenotype was described as low-flow VMs (mostly combined) with overgrowth and non-vascular anomalies (listed in Table 3). Phenotypes were described to be similar to PIK3CA phenotypes.

PTEN

Five studies found PTEN (inactivating) germline and somatic mutations in 39 patients with peripheral VMs.[22, 25, 49, 56, 57] The germline phenotype associated with multifocal intramuscular combinations of fast-flow channels (AVMs) with ectopic fat in one study.[56] Another study found arteriovenous, venous, lymphatic, and combined malformations that could be associated with non-vascular anomalies (listed in Table 3).[57] All PTEN phenotypes (e.g., Cowden syndrome) are included in the PTEN-hamartoma-tumour-syndrome.

EPHB4

Two studies reported germline EPHB4 (inactivating) mutations in 105 patients.[58, 59] The phenotype was described as mainly isolated multifocal CMs and in a few cases (18%) with an additional AVM, located in the head/neck region or extremities. CMs were pink/red, cutaneous, multifocal with geographic borders. Size varied from pinpoint to large lesions (15 cm). Several CMs had a surrounding white halo and a few CMs had a pale central zone.

RASA1

Nine studies reported RASA1 (inactivating) mainly germline mutations in 268 patients with VMs.[22, 26, 35, 59-64] The germline phenotype was described as multifocal, round-to-oval, red/pink CMs of varying size, some with irregular borders and/or with a white/pale halo that could be associated with non-vascular anomalies (listed in Table 3).[60-63] Several CMs had an area of high flow (suggestive for an AVM) or a separate AVM was present. One study found two other phenotypes of the CMs, described as (1) a single, moderately large, pink macular stain in the nucha or central forehead and (2) purple-red lesion of variable shape and size, often located on the face.[60]

KRAS

Eight studies reported somatic KRAS (activating) mutations in 40 patients.[23, 26, 35, 49, 64-67] Somatic KRAS mutations generally caused (extensive) AVMs, although all VM types were found. Another phenotype was described as relative large (5-10 cm) progressive vascular or lipoma-like malformations, occasionally with a high flow component.[66]

MAP2K1

MAP2K1 somatic (activating) mutations were described in six studies and found in 30 patients.[18, 35, 49, 64, 65, 68] The phenotype was generally described as solitary extracranial AVMs frequently located at the head and neck with variable severity[68], mostly with recurrent bleeding and progressive enlargement.[65]

GNAQ

Eight studies described somatic GNAQ (activating) mutations in 26 patients mostly causing CMs.[18, 22, 23, 25-27, 35, 39] One study described the phenotype as extensive CMs with overgrowth and three patients were diagnosed with Sturge-Weber Syndrome.[26]

GNA11

GNA11 (activating) somatic mutations were described in four studies and found in 14 patients with mostly CMs or combined malformations with a capillary component, in some patients cutaneous non-vascular anomalies were associated (listed in Table 3).[23, 26, 27, 35] In two studies the CMs were described as extensive and reticulated and were in combination with overgrowth.[26, 35]

KRIT/CCM1

One study investigated cutaneous VMs alongside familial cerebral cavernous malformations caused by germline (inactivating) mutations in the KRIT1/CCM1 gene.[69] In 33 patients (9%) with a germline CCM1 mutation, a cutaneous VM was present. The following phenotypes were reported: port-wine stains, punctate CMs (irregularly shaped red/brown lesions with telangiectatic/dotted edges), hyperkeratotic combined CVMs (plaque-like, irregularly shaped, and frequently solitary), or nodular VeMs.[69]

Glomulin (GLMN)

Six studies found GLMN (inactivating) mainly germline mutations in a total of 203 patients with glomuvenous malformations.[49, 70-74] The germline phenotype was described as hyperkeratotic, multifocal, bluish-purple, (sub)cutaneous hereditary lesions with a cobblestone-like appearance, mainly located at the extremities.[70-74] Less frequently, plaque-like glomuvenous malformations were seen, which were flat and purple, and darken over time.[74]

Other mutations

One study reported ELMO2 (inactivating) germline mutations in eight patients with intraosseous VeMs, localized in the skull and facial bones.[75]

One study reported MAP3K3 (activating) somatic mutations, harbouring the I441M protein change, in six patients with verrucous VeMs of the extremities.[76] The lesions were described as raised, reddish-purple, hyperkeratotic, and extending into the subcutis.[76]

HRAS (activating) somatic mutations were reported in one study and found in five patients causing solitary CMs or lipoma-like VMs of unclear classification, sometimes with high-flow, localized in the extremities (80%) and trunk (20%) often extending in subcutaneous (60%) and/or intramuscular (60%) tissue.[66] All lesions were relative large (5-10cm), progressive and caused pain.

Somatic (activating) mutations (protein change V600E) in the BRAF gene were found in three studies in 7 patients, causing AVMs (43%), LMs (43%), and VeMs (14%), frequently located at the head and neck (71%).[53, 64, 77] Other less frequent occurring mutations with phenotypes are listed in Supplementary Table 4.

Discussion

This systematic review provides a comprehensive overview of causative germline (hereditary) and somatic mutations and associated phenotypes in peripheral VMs. These insights are a valuable contribution to the rapidly evolving landscape of VMs caused by the elucidation of the underlying genetics. Our findings may aid the understanding of the highly varying clinical spectrum of VMs and how this derives from the underlying causal variants in genes. Furthermore, VMs are currently classified based on clinical and histopathologic features, however, the findings of this systematic review may support a more comprehensive classification also based on the genotype.

Many factors may influence the phenotype caused by somatic mutations.[35, 78] Firstly, somatic mutations that occur early during embryogenesis will generate many affected daughter cells and cell lines and may result in larger lesions affecting various tissues, a mutation later in embryogenesis will produce lower numbers of mutated cells and yield smaller lesions. Secondly, different mutations are able to activate genes to various rates.[78] In cancer, the same somatic mutations exists, and the mutation strength seems to derive from the location of the mutation (the protein change) and their consequent activation mechanism.[79] Thirdly, the cell type (e.g. capillary or lymphatic endothelial cell) that is affected by the mutation contributes to the variety in clinical features of VMs.

By contrast, distinct mutations may lead to similar phenotypes, e.g. somatic TIE2 and PIK3CA mutations may cause almost clinically indistinguishable VeMs, emphasizing that the PI3K/AKT/mTOR pathway is downstream of the tyrosine kinase receptor TEK (encoded by the TIE2 gene).[16] However, TIE2 mutations rarely lead to overgrowth or other abnormalities, which may be explained because the TIE2 receptor is almost exclusively expressed in endothelial cells.[80]

PIK3CA mutations were the most commonly found, causing a heterogeneous spectrum of phenotypes. The phenotype varied from a small isolated VM (generally low-flow) to syndromic large combined malformations with adjacent overgrowth and other abnormalities. These other abnormalities, frequently associated with syndromes, could be located anywhere in the body and included hand/feet abnormalities (e.g. syndactyly, polydactyly), skin abnormalities (e.g. epidermal naevus, hypopigmentation), renal abnormalities, scoliosis, and abnormalities associated with head/brain differences (e.g. gross motor delay, macrocephaly). PIK3CA encodes the 110-kD catalytic α subunit of PI3K (p110α) and PIK3CA mutations lead to a gain of function of PI3K, with consequent constitutional activation of AKT and thereby mTOR, involved in cellular proliferation, survival and growth, as well as in vascular development in the embryonic stage.[81] Somatic PIK3CA mutations may also be present in other isolated or syndromic overgrowth disorders that do not necessarily include VMs, such as macrodactyly or fibroadipose hyperplasia.[82, 83] However, all these PIK3CA-related phenotypes, including PIK3CA-originated VMs, are grouped within PROS.[31]

Several mutations led to a reasonably uniform phenotype; TIE2 mutations led predominantly to isolated VeMs, and MAP2K1 mutations resulted mainly in isolated AVMs. In the other few cases, these mutations led to distinct VMs, which can be considered unique and unusual. However, information was frequently lacking on how the diagnosis was established, e.g. based on clinical features, imaging, or histopathology, and accuracy of the diagnosis can be debated. A recent study showed that in more than half of the cases a discrepancy exists between clinical and histopathological diagnoses of soft tissue VMs, and emphasizes that a gold standard for diagnosis is lacking.[84]

Currently, VMs are classified based on their clinical and histopathologic characteristics.[4] However, the causative mutation of the VM reflects how the lesion was derived and is, therefore, a significant factor of VMs, which may enhance the current classification system.[3, 85] Although, it must not be forgotten that the same mutation may lead to various phenotypes and that, by contrast, different mutations may be responsible for similar phenotypes. One could assume that the genotype affects various aspects of VMs, such as symptoms, disease course, lesion progression, and treatment response. However, currently, there is no evidence available to support this hypothesis. Expanding the research and knowledge on genotype-phenotype correlations could hopefully fill this gap.

Yet, elucidating the genetic mechanisms has led to a better understanding of the pathophysiology of VMs and has resulted in novel treatment opportunities targeting molecular pathways. The mTOR inhibitor sirolimus is studied the most extensively in patients with VMs, and there is compelling low-level evidence that it is effective in VeMs and LMs.[5, 86] Furthermore, there is some evidence that other targeted therapies are effective, such as the PIK3CA-inhibitor alpelisib, the MEK-inhibitor trametinib, and the AKT-inhibitor miransertib, which are further investigated in clinical trials.[6, 87-89] Targeted therapies will play a more dominant role in VM management and may be used as a stand-alone treatment, but could also be used in combination with the 'classical' treatment modalities. Consequently, the genotype is becoming essential in the diagnosis and management of VMs nowadays.

The results of the current review show that VMs can differ significantly from each other and even more than could be known from the phenotype alone. The varying genetic bases of VMs emphasize the need for a multidisciplinary approach to their management, ensuring a comprehensive and efficient method for managing these complex and diverse lesions.[90, 91] However, our results and the emergence of targeted therapies argue for also including a clinical geneticist and oncologist in the multidisciplinary team, as they may understand the genetic bases of the disorder in-depth and may optimize the management of VMs.

It was notable that several included studies investigated the VM for only one (most common) gene, e.g. VeMs were analysed for TIE2 mutations. However, as VMs may be caused by various mutations, and the malformation type may not always be unequivocal, it is advised to analyse mutations using a gene panel in which multiple VM-associated genes, including the genes found in the current review. Although, frequently multiple tissue biopsies are needed to establish the

molecular diagnosis.[44] Whole exome sequencing can be initiated if a mutation is not detected using the gene panel. Given the contemporary diagnostic, clinical, and treatment implications of the genotype, it is advised to perform molecular analysis on a low-threshold basis.

Another significant and alarming finding is the proportion of missing phenotypic data. Peripheral VMs represent a broad clinical spectrum regarding vessel type, anatomical location, tissue extension, and size. Therefore, peripheral VMs should be considered as various disease entities, and in research detailed clinical information should be provided in order to show which patients are involved. Additionally, we found that some phenotypic characteristics were presented in various forms, e.g., the size of the VM was reported in descriptive names (e.g., small, localized, large, extensive, diffuse), millilitres, largest diameter in centimetres, and three-dimensional measurement in centimetres. The comparison of clinical data (e.g., lesion size) among different mutated genes could not be performed as was planned in the protocol due to the missing and heterogeneity in phenotype reporting. Variations in the outcome reporting of the genotype and especially the phenotype hamper the pooling of study results and the ability to investigate genotype-phenotype associations. Standardization in which phenotypic characteristics should be reported and how they should be reported could contribute to uniformity in the research of VMs. Subsequently, the comparison and aggregation of different studies can be more easily and correctly performed.

In this systematic review, the methodological quality of most included studies was satisfactory or good. Factors that decreased study quality were mainly small sample size, a selected or convenience sample (e.g., patients who underwent surgery where lesion tissue could be collected for molecular analysis), and missing data on non-respondents. These factors might have affected the results of the current study because patients with deep-positioned VMs where a tissue biopsy or surgery is not feasible were excluded, and these patients might have other causative mutations. Nevertheless, this review presents the best evidence currently available. In the current review, we did not include studies specifically investigating CMs. This decision was based on the extensiveness of the review and because the other VM types are more clinically overlapping as they are not confined to the skin. To complement the data presented in this review, we plan to systematically evaluate genetic mutations associated with CMs in the near future.

Conclusion

Peripheral VMs compromise a wide spectrum of clinical phenotypes. This systematic review provides a comprehensive overview of causative germline and somatic mutations and associated phenotypes in peripheral VMs. The results of the review reveal the varying genetic bases among VMs, championing a more prominent role for genetics in the diagnosis and classification of VMs and, consequently, rooting for the performance of molecular analysis on a low-threshold base. Additionally, molecular analysis has a therapeutic implication as targeted therapies are increasingly administered and have shown they could be patient-beneficial. These

insights emphasize the necessity of a multidisciplinary approach in which clinical geneticists and oncologists are included to improve the care of patients with VMs.

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Supplementary Materials

Supplementary table 1. Study characteristics

Characteristics per study in alphabetic order, including sequencing methods and documented phenotype characteristics.

Study	Study Year	Study type	Sample type	Control sample	Sequencing method	Sequencing platform (genes, exons etc.)	Patients included n	Patients with mutation n(%)	Mutation type (germline, somatic)	VAF (range)	VM types	Documented phenotype characteristics
Al-Olabi	2018	Observational, prospective	Lesion tissue	Blood	Targeted NGS	SureSeq solid tumor panel for high flow, custom overgrowth panel for low flow.	159	14 (9%)	Somatic	2-39%	All	Syndrome, localization, symptoms and overgrowth
Amyere	2013	Observational, prospective	Lesion tissue	Blood	PCR amplification, sequencing	19 exons and splice site of GLMN	26	26 (100%)	Germline and somatic	NA	VeM (Glomuvenous)	Sex, appearance, and tissue extension
Amyere	2017	Observational, prospective	Blood	None	Whole exome sequencing of some patients. Afterwards amplification and targeted deep sequencing of all patients.	All exons and exon-intron boundaries of RASA1 and EPHB4	414	103 (25%)	Germline	NA	CM-AVM	Syndrome, localization, and appearance
Blesinger	2018	Observational, prospective	Lesion tissue, isolating cell types	Healthy tissue	PCR amplification; direct sequencing	Coding exons 7,9 and 20 of PIK3CA	6	6 (100%)	Somatic	MD	LM	Sex
Bourgon	2022	Observational, retrospective	Lesion tissue	Healthy tissue	Targeted NGS	All coding sequences of PIK3CA	7	7 (100%)	Somatic	5-24%	LM, combined	Sex, localization, overgrowth, other abnormalities
Brahami	2013	Observational, prospective	Blood	None	PCR amplification; direct sequencing	All 23 coding exons of TIE2	10	0 (0%)	None detected	NA	VeM	Sex and localization
Brouillard	2002	Observational, prospective	Lesion tissue or blood	None	RT-PCR, 5'-Rapid amplification of cDNA	19 exons and splice site of GLMN	109	109 (100%)	Germline	NA	VeM (Glomuvenous)	Sex, appearance, and symptoms
Brouillard	2013	Observational, prospective	Blood	None	Allele-specific PCR for 3 most common mutations. Negative samples: high resolution melting analysis	19 exons and splice site of GLMN	207	69 (33%)	Germline	NA	VeM (glomuvenous, BRBN, sporadic)	Appearance
Brouillard	2005	Observational, prospective	Blood	None	SSCP, heteroduplex and size difference. Allele-specific PCR for 3 most common mutations.	GLMN	41	41 (100%)	Germline	NA	VeM (Glomuvenous)	Appearance, localization, tissue extension, and symptoms
Calvert	1999	Observational, prospective	Blood sample	None	PCR amplification; direct sequencing	TIE2 coding regions	36	16 (44%)	Germline	NA	VeM	Sex, and tissue extension
Castel	2016	Observational, prospective	Lesion tissue (archival samples)	Unmatched healthy samples	Targeted exome sequencing (MSK-IMPACT)	341 key cancer-associated genes	32	27 (84%)	Somatic	3.1-15.7%	VeM	Sex, syndrome, localization, and tissue extension
Castillo	2016	Observational, prospective	Lesion tissue	Blood	Targeted NGS sequencing of coding exons	23 TIE2 and 20 PIK3CA coding exons	13	9 (69%)	Somatic	4-13%	VeM	Sex, localization, and the absence overgrowth

Cetinkaya	2016	Observational, prospective	Skin biopsies and (peripheral) blood	None	Targeted massive parallel sequencing	Chromosome 20q13.12	8	8 (100%)	Germline	NA	VeM (intraosseous VM)	Sex, localization, tissue extension, and symptoms
Cottrell	2021	Observational, prospective	Skin biopsy affected tissue	None	Targeted NGS	Coding regions of cell-signaling and cancer-associated genes	108	17 (16%)	Somatic	1.1-39.8%	CM, LM, VeM, combined	Syndrome, overgrowth, skeletal abnormalities
Couto	2015	Observational, prospective	Lesion tissue (surgical resection)	Saliva	WES, with PCR amplification targeted sequencing.	Targeted sequencing MAP3K3 hotspots.	10	6 (60%)	Somatic	6-19%	Verrucous VeM	Sex, and Localization
Couto	2017	Observational, prospective	Lesion tissue and isolated EC from lesion tissue	Blood or saliva	WES and WGS, with ddPCR confirmation	Targeted sequencing MAP2K1 hotspots.	25	16 (64%)	Somatic	1-35%	AVM	Sex, Localization, symptoms, and AVM clinical stages
Delestre	2022	Observational, prospective	Lesion tissue or skin tissue	Unknown	Unknown	Unknown	6	6 (100%)	Somatic	1-10%	LM, combined	Sex, localization, size, symptoms.
De Wijn	2012	Observational, prospective	Peripheral Lymphocytes	None	PCR amplification and bidirectional sequencing.	All coding exons of RASA1	11	11 (100%)	Germline	NA	CM-AVM, LM	Sex, localization, size, overgrowth, and appearance
Diociaiuti	2022	Observational, retrospective	Lesion tissue biopsy	Blood	Targeted NGS	Genes included in their custom panel	43	43 (100%)	Somatic	1-46.5%	All	Sex, localization, tissue extension, overgrowth, brain and skeletal abnormalities.
Du	2020	Observational, retrospective	Lesion tissue	Blood	PCR amplification; direct sequencing	VM related gene panel (10 genes)	14	6 (43%)	Somatic	MD	VeM	Sex and localization
Eerola	2003	Observational, prospective	Blood	Healthy controls	PCR amplification, subsequent SSCP and heteroduplex analyses, and direct sequencing	25 exons and exon-intron boundaries of RASA1	107	45 (42%)	Germline	NA	CM-AVM, combined	Sex, syndrome, localization, tissue extension, overgrowth, and appearance.
Eijkelenboom	2019	Observational, prospective	Lesion tissue	None	Targeted NGS	smMIPs library to detect cancer associated genes (hotspots) and surrounding sequences.	299	108 (36%)	Somatic	4-13%	All	Sex, syndrome, localization, tissue extension, symptoms, and progression
El Sissy	2022	Observational, prospective	Surgically resected lesion tissue	Unaffected tissue or blood	Targeted BGS	Custom gene panel of 106 genes associated with hereditary vascular disorders and solid tumour-related.	23	18 (78%)	Somatic and germline	2-51%	AVM	Sex, location, extensiveness, Schöbinger, Yakes staging, and relapse.
Glaser	2018	Observational, prospective	Isolated EC from lymph fluid or lesion tissue	Blood or lesion non-endothelial cells	WES and verification with PCR amplification; direct sequencing	Targeted sequencing known mutation hotspots (exon 9, 20)	14	14 (100%)	Somatic	MD	LM, combined	Syndrome and localization
Goines	2018	Observational, prospective	EC (from solid tissue and blood samples during sclerotherapy)	Blood or saliva	qRT-PCR, NGS in undetected individuals	Exon 17 of TIE2, exons 7,9, and 20 of PIK3CA	9	9 (100%)	Somatic	47-59% (in isolated EC)	VeM	Sex, localization, and tissue extension
Gurunathan	2020	Observational, retrospective	No description	No description	No description	No description	22	22 (100%)	Germline	NA	All	Sex, localization, and overgrowth
Haefliger	2021	Observational, prospective	Blood	None	No description	RASA1 and EPHB4 genes	28	18 (64%)	Germline	NA	CM-AVM	Appearance, multiplicity of lesions

Hucthagowder	2017	Observational, retrospective	Lesion tissue, buccal swab and blood	None	NGS, following target hybrid capture	PIK3CA and related genes with known somatic involvement	12	8 (67%)	Somatic	7-47%	LM, CM, VeM	Sex, syndrome, and overgrowth
Keppler-Noreuil	2014	Observational, prospective	Lesion biopsy or lesion tissue (obtained during surgery)	Blood, saliva	PCR amplification; direct sequencing	PIK3CA hotspots	13	13 (100%)	Somatic	1-26%	LM, CM, VeM	Sex, syndrome, localization, tissue extension, size and overgrowth
Kurek	2012	Observational, prospective	Lesion tissue	Blood or saliva	Massive parallel sequencing with PCR amplification confirmation	exons of 77 genes involved in signaling for several growth factors	21	9 (43%)	Somatic	8-30%	All	Sex, overgrowth, syndrome, and associated abnormalities
Lalonde	2018	Observational, retrospective	Lesion tissue, buccal swab and blood	None	Amplification, targeted NGS	8 overgrowth-associated genes	48	26 (54%)	Somatic	2.2-51.3%	LM, CM, combined	Syndrome, overgrowth, macrodactyly, and other abnormalities
Le Cras	2020	Observational, prospective	Isolated EC from lesion tissue or lesion blood	Blood	PCR amplification; direct sequencing	PIK3CA exons 7,9 and 20	7	7 (100%)	Somatic	MD	Combined (CLVM)	Syndrome, localization, tissue extension, and overgrowth
Limaye	2009	Observational, prospective	Lesion tissue	Blood	PCR amplification; direct sequencing	Not clearly described.	57	28 (49%)	Somatic	4.5-48.3%	VeM	Syndrome, localization, tissue extension, and size
Limaye	2015	Observational, prospective	Lesion tissue (surgical resection)	Blood	Targeted NGS sequencing of coding exons	23 TIE2 and 20 PIK3CA coding exons	87	64 (74%)	Somatic	1-17.5%	VeM	Tissue extension
Luks	2015	Observational, prospective	Lesion tissue (surgical resection)	None	WES, with ddPCR confirmation	Targeted sequencing 5 PIK3CA hotspots.	71	65 (92%)	Somatic	0.8-25%	LM, combined	Sex, syndrome, localization, symptoms, and VTE
Martinez-Glaz	2022	Observational, retrospective	Lesion tissue	Blood	ddPCR	Hybridization-based custom gene panel of 45 vascular-related genes	20	13 (65%)	Somatic	1-15%	CM, VeM, combined	Sex, localization, undergrowth, other abnormalities.
Mattassi	2018	Observational, prospective	Blood sample (n=150), lesion tissue (n=17)	None	Targeted NGS	25 genes associated with VM	150	17 (11%)	Germline and somatic	MD	Not specified	Sex
Michel	2018	Observational, prospective	Urine	None	ddPCR	Targeted sequencing 5 PIK3CA hotspots.	41	6 (15%)	Somatic	0.25-7.69	Unclear	Syndrome
Mussa	2022	Observational, retrospective	Lesion tissue biopsy, blood or buccal swab	Blood or buccal swab	Targeted NGS	Custom panel including 21 genes	150	93 (62%)	Somatic	MD	Unclear	Syndrome, overgrowth
Nozawa	2022	Observational, prospective	Lesion tissue	None	Targeted NGS	Exonic regions of 29 genes associated with vascular anomalies or the PI3K signalling pathway	59	37 (63%)	Somatic	3.5-17.7%	LM, VeM, combined	Sex, localization, size, multiplicity of lesions, syndrome
O'Hagan	2006	Observational, prospective	MD	None	PCR with fluorescently labelled microsatellite marker and oligonucleotide primers.	19 exons and splice site of GLMN	19	19 (100%)	Germline	NA	VeM (Glomuvenous)	Sex, size, tissue extension, and symptoms
Osborn	2015	Observational, prospective	Isolated EC from lymph fluid or lesion tissue	Lesion non-endothelial cells	PCR amplification; direct sequencing	Targeted sequencing known mutation hotspots (exon 9, 20) PIK3CA	5	5 (100%)	Somatic	0.38-0.52%	LM, combined	Sex, syndrome, localization, and overgrowth
Palmieri (1)	2020	Observational, prospective	Liquid biopsy of Cell-free DNA	Lesion tissue	PCR amplification; direct sequencing	Oncomine Pan-Cancer Cell-Free Assay	7	7 (100%)	Somatic	0.18-1.47%	CM, LM, VeM	Sex, syndrome, localization, size, and overgrowth

Palmieri (2)	2020	Observational, prospective	Liquid biopsy of Cell-free DNA	None	NGS	Oncomine Pan-Cancer Cell-Free Assay	5	5 (100%)	Somatic	1.18-4.19%	AVM, combined	Sex, syndrome, localization, tissue extension, size, and overgrowth
Paolacci	2020	Observational, retrospective	Lesion tissue	Blood	NGS	Coding exons and flanking introns of 92 genes associated with VM	115	37 (33%)	Somatic	5-37%	All	Sex, syndrome, localization, tissue extension, symptoms, and overgrowth
Piacitelli	2018	Observational, retrospective	Lesion tissue	Blood	Targeted NGS	37 genes implicated in overgrowth and VM	11	3 (27%)	Somatic	1.9-21%	VeM, CM, LM	Sex, syndrome, localization, and overgrowth
Revencu	2014	Observational, prospective	Blood	None	PCR amplification, screening of amplicons and sequencing.	25 exons and exon-intron boundaries of RASA1	30	0 (0%)	None detected	NA	CM, LM, VeM, combined	Sex, syndrome, localization, and overgrowth
Revencu	2013	Observational, prospective and retrospective	Blood	None	PCR amplification, screening of amplicons and sequencing.	25 exons and exon-intron boundaries of RASA1	352	138 (39%)	Germline	NA	CM-AVM, CM	Syndrome, localization, appearance, and other clinical features.
Rodriguez-Laguna	2018	Observational, prospective	Lesion tissue	Blood, saliva	Next Generation Sequencing and confirmation with targeted ddPCR	3 gene panels	9	5 (56%)	Somatic	10-16%	CM, LM	Sex, syndrome, localization, size, and overgrowth
Rodriguez-Laguna	2019	Observational, retrospective	Lesion tissue, isolated EC (n=2)	Blood	Hybridization-based (HB) or Amplicon-based (AB) high-throughput sequencing	HB: 1370 genes associated with PI3K signaling. AB: PIK3CA and 19 related genes	9	5 (56%)	Somatic	3.5-33.7%	LM	Sex, syndrome, localization, tissue extension, and symptoms
Serio	2022	Observational, prospective	Lesion tissue or liquid biopsy of cell-free DNA	Blood	Targeted NGS	Oncomine-Pan-Cancer-Cell-Free Assay	53	37 (70%)	Somatic	0.1-49.9%	All	Sex, localization, multiplicity of lesions, syndrome, overgrowth
Shaheen	2022	Observational, prospective	Lesion tissue or lesion endothelial cells	None	Hybrid-capture targeted DNA sequencing	Exons of at least 324 cancer genes and select introns of 36 genes	26	21 (81%)	Somatic	1-38%	LM	Sex, tissue extension, size, and syndrome
Siegel	2017	Observational, prospective	Lesions tissue	None	Enrichment (hybrid capture); NGS	131 target loci in cancer associated genes	57	43 (75%(	Somatic	1-34.9%	All	Overgrowth, macrodactyly and severity.
Sirvente	2009	Observational, retrospective	Blood	None	Amplification, PCR-SSCP	12 exons and flanking intronic of CCM1 gene (KRIT1)	38	37 (97%)	Germline	NA	CM, VeM	Syndrome and localization
Soblet	2017	Observational, prospective	Lesion tissue	Blood	PCR amplification; direct sequencing	TIE2 coding regions	23	20 (87%)	Somatic	MD	VeM (BRBN)	Syndrome and localization
Sudduth	2021	Observational, prospective	Surgically resected lesion tissue	None	ddPCR	Variants in genes most frequently associated with sporadic VMs (TIE2, PIK3CA)	9	9 (100%)	Somatic	2-13.2%	VeM	Sex, localization, tissue extension, syndrome
Tan	2007	Observational, retrospective	MD	MD	MD	MD	13	13 (100%)	Germline	NA	AVM	Sex, Syndrome, Localization, Tissue Extension, and symptoms
Ten Broek	2019	Observational, prospective, retrospective	Lesion tissue	None	Targeted NGS	21 cancer associated genes (hotspots) and surrounding sequences.	319	132 (41%)	Somatic	2-32%	All	Sex, Syndrome, and Localization
Triana	2022	Observational, retrospective	Available affected tissue	None	Deep high-troughput sequencing	A hybridization-based custom panel of 83 vascular-related genes.	6	6 (100%)	Somatic	MD	VeM, CM, Combined	Sex, localization, syndrome, overgrowth, undergrowth

Ugwu	2021	Observational, prospective	Lesion tissue	Unaffected tissue	Paired WES; paired targeted sequencing	GJA4	5	3 (60%)	Somatic	MD	VeM	Sex, tissue extension, size
Vikkula	1996	Observational, prospective	Blood	None	PCR amplification, sequencing	Allele specific PCR for TIE2	47	47 (100%)	Germline	NA	VeM	Sex
Wang	2021	Observational, prospective	Lesion tissue	None	WES, digital PCR for verification of the mutation	IRS1, MTOR, TSC1, TSC2, PIK3CA, PIK3CD	6	2 (33%)	Somatic	MD	LM	Sex, localization, size
Wooderchak-Donahue	2018	Observational, retrospective	Blood	None	PCR amplification, direct (bi-directionally sequencing)	RASA1 coding regions and exon-intron boundaries. In several cases a 14-gene VM and 5-gene HHT panel was used.	281	60 (21%)	Germline	NA	CM-AVM	Sex, syndrome, localization, tissue extension, overgrowth, and other abnormalities
Wouters	2010	Observational, prospective	Blood	None	PCR amplification; direct sequencing	23 TIE2 exons	26	26 (100%)	Germline	N/A	VeM	Sex, localization, size, and other anomalies
Ye	2011	Observational, prospective	Lesion tissue	Blood or not-affected tissue	PCR amplification; direct sequencing (confirmation with allele-specific PCR)	Exon 17 of TIE2	106	35 (33%)	Somatic	MD	VeM, CM, AVM, combined	Localization, tissue extension, size, and severity
Yeung	2017	Observational, prospective	Lesion tissue	None	ddPCR	Targeted sequencing 5 PIK3CA hotspots.	5	5 (100%)	Somatic	4-31.6	CM, VeM, LM, and combined	Sex, syndrome, localization, size, symptoms, and overgrowth
Zenner	2019	Observational, prospective	Lesion tissue, lesion lymph fluid	Non-affected tissue	smMIP of the coding sequences in PIK3CA , afterwards ddPCR of 5 PIK3CA hotspot mutations in undetected individuals		81	64 (79%)	Somatic	0.1-13%	LM	Sex, syndrome, localization (including laterality), size, and de Serres staging
Zenner	2021	Observational, prospective	Lesion tissue	None	High-depth targeted sequencing	A 44 gene panel, called Vascular Anomaly sequencing	15	6 (40%)	Somatic	0.3-4.8%	LM	Sex, localization, multiplicity of lesions, de Serres staging
Zhou	2015	Observational, prospective	Lesion tissue	None	Real-time PCR amplification; direct sequencing	Exon 17 of TIE2	60	13 (22%)	Somatic	MD	VM (not specified)	Sex and localization

AVM = Arteriovenous Malformation; BRBN = Blue Rubber Bleb Naevus syndrome; CLVM = Capillary-Lymphatic-Venous Malformation; CM = Capillary Malformation; CVM = Capillary-Venous Malformation; EC = Endothelial cell; MD = Missing Data; NA = Not Applicable; NGS = Next Generation Sequencing; PCR = Polymerase Chain Reaction; VeM = Venous Malformation; VM = Vascular Malformation; WES = Whole Exome Sequencing

Supplementary table 2. Study quality assessment with the Newcastle-Ottawa Scale, adapted for cross-sectional studies.

Study	Newcastle-Ottawa Scale assessment
Castillo, 2016	Satisfactory
Glaser, 2018	Satisfactory
Osborn, 2015	Good
Couto, 2017	Good
Couto, 2015	Satisfactory
Le Cras, 2020	Satisfactory
Limaye, 2015	Satisfactory
Limaye, 2009	Very good
Luks, 2015	Good
Michel, 2018	Satisfactory
Soblet, 2013	Satisfactory
Zenner, 2019	Good
Zhou, 2015	Satisfactory
Yeung, 2017	Satisfactory
Ye, 2011	Good
Wouters, 2010	Satisfactory
Ten Broek, 2019	Very good
Tan, 2007	Satisfactory
Siegel, 2017	Good
Al-Olabi, 2018	Good
Blesinger, 2018	Good
Brahami, 2013	Unsatisfactory
Brahami, 2017	Satisfactory
Du, 2020	Satisfactory
Rodriguez-Laguna, 2018	Very good
Eijkelenboom	Good
Hucthagowder, 2017	Satisfactory
Keppler-Noreuil	Good
Lalonde, 2019	Good
Soblet, 2017	Good
Sirvente, 2009	Good
Guranathan,2020	Unsatisfactory
Palmieri, 2020	Satisfactory
Paolacci, 2020	Good
Piacitelli, 2018	Satisfactory
Rodriguez-Laguna, 2019	Satisfactory
Matassi, 2018	Satisfactory
Calvert, 1999	Unsatisfactory
Amyere, 2013	Unsatisfactory
Brouillard, 2002	Good
Brouillard, 2013	Satisfactory

Brouillard, 2005	Satisfactory
O'Hagan, 2006	Good
Castel, 2016	Satisfactory
Kurek, 2012	Satisfactory
Goines, 2018	Good
Cetinkaya, 2016	Satisfactory
Amyere, 2017	Good
De Wijn, 2012	Very good
Eerola, 2003	Good
Palmieri (AVM), 2020	Good
Revencu, 2014	Unsatisfactory
Revencu, 2013	Good
Wooderchak-Donahue, 2018	Good
Vikkula, 1996	Unsatisfactory
Bourgon, 2022	Good
Cottrell, 2021	Satisfactory
Diociaiuti, 2022	Good
El Sissy, 2022	Satisfactory
Haefliger, 2021	Unsatisfactory
Martinez-Glaz, 2022	Satisfactory
Massa, 2022	Satisfactory
Nozawa, 2022	Good
Serio, 2022	Satisfactory
Shaheen, 2022	Satisfactory
Sudduth, 2021	Satisfactory
Triana, 2022	Satisfactory
Ugwu, 2021	Satisfactory
Wang, 2021	Unsatisfactory
Zenner, 2021	Satisfactory
Delestre, 2021	Unsatisfactory

An amendment was made to the protocol registered on PROSPERO regarding the risk of bias and quality assessment tool. Ultimately, we have chosen to use the Newcastle-Ottawa Scale because of the comprehensiveness of the quality assessment tool, and it was more applicable to the studies included in this systematic review.

Supplementary table 3. Genetic mutations and phenotype characteristics per study

Gene	Study	Study Year	Mutations and phenotype characteristics
PIK3CA			
	Kurek	2012	**Somatic PIK3CA** mutations: CMs, LMs, VeMs, and combined malformations. In patients with Cloves syndrome other findings were: lipomatous overgrowth (100%), wide hands/feet (83%), sandal gap (67%), macrodactyly (83%), limb asymmetry (83%), scoliosis (67%), and renal abnormalities (33%)
	Keppler-Noreuil	2014	**Somatic PIK3CA** mutations: heterogeneous phenotype with LMs, CMs, VeMs, or combined malformations, all with fibroadipose overgrowth. Other findings were: kidney abnormalities (54%), skin abnormalities (54%), epidermal nevus (15%), polydactyly (15%), developmental or gross motor delay (15%), ovarian cysts (15%), scoliosis (15%), and other malformations (31%)
	Limaye	2015	**Somatic PIK3CA** mutations: univocal VeM without skin involvement.
	Luks	2015	No significant correlation between **somatic PIK3CA** mutations and disease phenotype was found. Findings associated with somatic PIK3CA mutations were: leg-length discrepancy (52%), macrodactyly (51%), syndactyly (14%), gastro-intestinal bleeding (21%), VTE (13%), portal vein thrombosis (6%), and Wilms tumor (1%).
	Osborn	2015	**Somatic PIK3CA** mutations: LMs, or combined LMs.
	Castel	2016	**Somatic PIK3CA** mutations: sporadic VeM.
	Castillo	2016	**Somatic PIK3CA** mutations: sporadic VeM without associated overgrowth.
	Hucthagowder	2017	**Somatic PIK3CA** mutations: heterogeneous phenotype with LMs, CMs, VeMs, or combined malformations, mainly with overgrowth. Other findings were: syndactyly (38%), developmental delay (25%), gross motor delays (13%), hydrocephalus (13%), and scoliosis (13%).
	Siegel	2017	**Somatic PIK3CA** mutations: mostly complex CM, VeM, LM or a combination thereof with non-proportional overgrowth. Hotspot PIK3CA mutations associated with more severe phenotypes. Other findings were: macrodactyly (56%), syndactyly (20%), sandal gap (16%), wide hand/feet (12%), scoliosis (4%), epidermal naevus (4%), macrocephaly (12%), megalocephaly (4%), hypotonia (4%), and hepatomegaly (4%)
	Yeung	2017	**Somatic PIK3CA** mutations: a heterogeneous phenotype with VeMs, CMs, LMs or combined VMs, mostly with adjoined overgrowth.
	Blesinger	2018	**Somatic PIK3CA** mutations: LMs, but no further phenotype descriptions.
	Glaser	2018	**Somatic PIK3CA** mutations: lymphatic or combined lymphatic malformations. Some with associated syndrome (PROS, Cloves, KTS).
	Goines	2018	**Somatic PIK3CA mutations:** lower extremity VeMs, mostly intramuscular.
	Lalonde	2018	**Somatic PIK3CA** mutations: LMs, CMs and combined malformations, frequently with overgrowth. Other findings were: other skin abnormalities (24%), brain abnormalities (22%), abnormal pigmentation (21%), macrodactyly (21%), megalocephaly (13%), and macrocephaly (9%).
	Michel	2018	**Somatic PIK3CA** mutations: venous malformations and Cloves syndrome.
	Piacitelli	2018	**Somatic PIK3CA** mutations: CMs or combined malformations, some with overgrowth.
	Rodriguez-Laguna	2018	**Somatic PIK3CA** mutations: CLAPO syndrome with CMs of the lower lip, LMs of the head and neck, and frequently CLVMs of the tongue. Other findings were asymmetry (as a consequence of the LM or asymmetric overgrowth).
	Rodriguez-Laguna	2019	**Somatic PIK3CA** mutations: diffuse or multifocal LMs (GLA), mainly mixed macro/microcystitic. Other findings were bone loss in the medullary cavity, chylous effusions, and visceral involvement.
	Ten Broek	2019	**Somatic PIK3CA** mutations: mainly low flow malformations (LM and VeM) with heterogeneous clinical aspects.
	Zenner	2019	**Somatic PIK3CA** mutations: isolated LMs. Higher genotype-adjusted VAFs* in LMs with more severe clinical characteristics including orofacial location or microcytic structure.
	Le Cras	2020	**Somatic PIK3CA** mutations: combined CLVM, mostly with overgrowth and a syndrome (Cloves, KTS)
	Palmieri (1)	2020	**Somatic PIK3CA** mutations: Extensive CVMs or CLVMs of one or both lower extremities with overgrowth (KTS). One patient with additional macrodactyly.

	Paolacci	2020	**Somatic PIK3CA** mutations: VeMs, LMs and combined malformations, some with overgrowth/adipose hypertrophy. Other findings include: neuromotor delay (5%), muscle retraction (5%), and dysmetria (5%)
	Delestre	2021	**Somatic PIK3CA** mutations: extensive LMs and combined malformations, causing severe symptoms: bleeding (83%), inflammatory flares (67%), fatigue (50%), venous thrombosis (50%), excessive swelling (50%), pain (33%), and pulmonary embolism (17%).
	Bourgon	2022	**Somatic PIK3CA** mutations**:** fetuses with extensive LMs, and combined malformations (CLM, CVLM). Other findings were: macrocephaly (43%), hemi megacephaly (43%), overgrowth (71%), polydactyly (14%), syndactyly (57%), macrodactyly (43%), wide hands (43%), sandal gap (43%).
	Diociaiuti	2022	**Somatic PIK3CA** mutations: Extensive (reticulate) CMs, LMs, and combined low-flow malformations with overgrowth, frequently in combination with other abnormalities: syndactyly (56%), sandal gap (40%), macrodactyly (24%), polydactyly (8%), scoliosis (12%), lipoma's (8%), epidermal naevus (4%), macrocephaly (24%), and psychomotor delay (24%). Patients have been diagnosed with MCAP (24%), CLOVES (12%), and KTS (8%).
	Martinez-Glaz	2022	**Somatic PIK3CA** mutations: VeMs or combined low-flow malformations of the lower leg, all with additionally bone and muscle undergrowth. Other clinical findings were: macrodactyly (13%), sandal gap (13%), lipoma's (13%), hypertrichosis (13%), and hyperpigmentation (13%).
	Mussa	2022	**Somatic PIK3CA** mutations: Isolated combined VMs (8%) or unspecified VMs part of a syndrome, which included MCAP (43%), KTS (38%), CLOVES (9%), and CLAPO (2%).
	Nozawa	2022	**Somatic PIK3CA** mutations: mostly solitary VeMs, LMs, or combined malformations, 30% was diagnosed with KTS. VMs were located at the legs (37%), head and neck (33%), trunk (22%), or upper extremity (11%). Most lesions were small and ≤5cm (53%) and fewer lesions were large and ≥10cm (42%).
	Serio	2022	**Somatic PIK3CA** mutations: solitary or multiple CMs, VeMs or combined malformations frequently located at the lower extremity, the majority of patients was diagnosed with KTS (80%).
	Shaheen	2022	**Somatic PIK3CA** mutations: localized (67%) or multifocal (33%) LMs, mainly extended to superficial soft tissues. Some patients were diagnosed with a syndrome (35%), which included CLOVES, KTS, and PTEN-like hamartoma.
	Triana	2022	**Somatic PIK3CA** mutations: VeM, CM, or combined malformations of the lower extremity with a combination of overgrowth and undergrowth in the same part of the body. Several patients were diagnosed with CLOVES (67%).
TIE2			
	Vikkula	1996	**Germline TIE2 mutations:** inherited VeMs.
	Calvert	1999	**Germline TIE2 mutations:** multiple deep blue superficial and deep VeMs affecting the skin and oral mucosa. Some individuals gained new lesions in adulthood.
	Limaye	2009	**Somatic TIE2** mutations: sporadic VeMs, mostly univocal and extensive.
	Wouters	2010	**Germline TIE2** mutations: hereditary cutaneousmuscosal VeMs, generally multiple, small (<5cm) lesions located in cervicofacial region (69%) and extremities (81%). Additionally, VeMs located in internal organs (15%) or brain (8%) can be seen. A VSD can be present (15%).
	Ye	2011	**Somatic TIE2** mutations: VeMs, AVMs, CMs and combined malformations. No associations were found between mutations and clinical characteristics.
	Limaye	2015	**Somatic TIE2** mutations: univocal VeMs often affecting the skin.
	Zhou	2015	**Somatic TIE2** mutations: spinal VMs and soft tissue VMs.
	Castillo	2016	**Somatic TIE2 mutations**: sporadic VeM without associated overgrowth.
	Soblet	2017	**Somatic TIE2** mutations: BRBN syndrome with gastrointestinal VeMs, one large dominant (sub) cutaneous VeM, and >10 small (<2 cm) cutaneous hyperkeratotic (palmo-plantar) VeMs, development of new lesions during life. Mostly with intestinal bleeding and chronic anemia. The other phenotype that was seen: multiple (10-20), sporadically occurring, (sub) cutaneous or intramuscular VeMs.
	Goines	2018	**Somatic TIE2** mutations: solitary VeMs, some extensive.
	Ten Broek	2019	**Somatic TIE2** mutations: non-circumscribed VeM, CM, LM or combined malformations without an arterial component.
	Du	2020	**Somatic TIE2** mutations: solitary VeMs
	Paolacci	2020	**Somatic TIE2** mutations: VeMs and CVMs, mostly solitary lesions.

	Sudduth	2021	**Somatic TIE2** mutations: Bockenheimer disease with VeMs involving most of the length of an extremity with all tissue planes affected (i.e., skin, subcutis, muscle, bone). Frequently the upper extremity is affected (78%).
	Diociaiuti	2022	**Somatic TIE2** mutations: BRBN syndrome with multiple cutaneous and subcutaneous bluish papules and nodules, which increased with age and also involved the palmoplantar surfaces, a congenital plaque-like larger lesion, and gastrointestinal venous malformations.
	Nozawa	2022	**Somatic TIE2** mutations: single (56%) or multiple (44%) VeMs located at head and neck (44%), legs (33%), or trunk (22%). Most lesions ranged from 3 to 5 cm in size (67%).
PTEN			
	Tan	2007	**Germline PTEN** mutations: multifocal intramuscular combinations of fast-flow channels (AVM) and ectopic fat. Other findings are macrocephaly (100%), penile freckling (100% of males), intracranial developmental venous anomalies (89%), thyroid involvement (31%), and gastrointestinal polyps (31%).
	Gurunathan	2020	**Germline PTEN** mutations: AVMs, VeMs, LMs, and combined malformations. Other findings were: overgrowth (48%), developmental delay (56%), macrocephaly (96%), penile freckling (86% of males), thyroid tumor, and gastrointestinal masses (20%).
PIK3R1			
	Cottrell	2021	**Somatic PIK3R1** mutations: low-flow VMs (CM, VeM, but mostly combined) with overgrowth. Other clinical features included: venous ectasias (92%), red vascular stains (83%), macrodactyly (31%), sandal gap (23%), lipoma (15%), syndactyly (8%), and developmental delay (8%). Phenotypes were similar to PIK3CA phenotypes.
MAP2K1			
	Couto	2017	**Somatic MAP2K1** mutations: Solitary, extracranial arteriovenous malformations with variable severity.
	Al-Olabi	2018	**Somatic MAP2K1** mutations: AVMs, mostly with recurrent bleeding and progressive enlargement.
	El Sissy	2022	**Somatic MAP2K1** mutations: limited AVMs located at the lips and face, mostly low-graded radiological Yakes classification (Type II). Relapse after surgery was rare (29%).
KRAS			
	Al-Olabi	2018	**Somatic KRAS** mutation: AVMs, CMs and VeMs.
	Eijkelenboom	2019	**Somatic inframe KRAS** mutations: relative large (5-10cm) progressive vascular or lipoma-like malformations, sometimes with high flow.
	Palmieri (1)	2020	**Somatic KRAS** mutations: extensive AVMs and CM-AVMs. Some with overgrowth (40%) and varicose veins (20%)
	Ten Broek	2019	**RAS mutations (KRAS, NRAS and RASA1)**: well-circumscribed CVMs and VeMs, noncircumscribed AVMs.
	Diociaiuti	2022	**Somatic KRAS** mutations: CM and CM-AVM with overgrowth of the lower limb, warmth to palpation. One patient with syndactyly. Other patient was diagnosed with PWS and had leg-length discrepancy.
	El Sissy	2022	**Somatic KRAS** mutations: extensive AVMs located at the face, frequently with high-graded radiological Yakes classification (Type IV). All AVMs relapsed after surgery.
	Serio	2022	**Somatic KRAS** mutations: AVMs frequently located at the lower extremity.
KRIT1 / CCM1			
	Sirvente	2009	**Germline KRIT1 (CCM1)** mutations: alongside cerebral cavernous malformations (CCM) several phenotypes of cutaneous VMs were seen: - Port wine stain (well demarcated red/purple CMs) - Punctate CMs (irregularly shaped red/brown CMs with telangiectatatic and dotted edges) - Hyperkeratotic cutaneous CVMs located on a limb, mostly solitary (plaque-like and irregularly shaped) - Nodular VeMs (solitary or multiple)
RASA1			
	Eerola	2003	**Germline RASA1** mutations: CM-AVMs. The CMs varied from a single, moderately large, pink macular stain in the nuchal or central forehead to a single, purple-red lesion of variable shape and size, often located on the face, to multiple small, round-to-oval, pink/red lesions, mainly on extremities. In some patients, AVMs and/or overgrowth were present.

	De Wijn	2012	**Germline RASA1** mutations: CM-AVMs. All patients had at least one CM of varying size, round-to-oval, red/pink lesions with at least one area of high flow (suggestive for AVM). Some CMs had a white halo. Other findings were: limb hypertrophy (18%), LM (9%), and varicose veins (36%)
	Revencu	2013	**Germline RASA1** mutations: CM-AVMs. Multifocal CMs, mainly located on the skin or some on mucosa (4%). The color varied from pale pink to red or brown, many surrounded by a pale halo. CMs were round, oval, or with irregular borders, and were either homogeneous or telangiectatic. 35% of patients had also an AVM/AVF, located on the extremities, intracranial, intraspinal and on the head/neck. Other findings were: PKWS (5%), renal abnormalities (1%), congenital heart defects (1%), and developmental delay (1%)
	Wooderchak-Donahue	2018	**Germline RASA1** mutations: mostly multifocal CMs and in nearly half of patients an AVM/AVF was present. Other findings were: telangiectasia (18%), overgrowth (15%), skin abnormalities (5%), macrocephaly (3%), epistaxis (3%), and congenital heart defect (2%)
	Haefliger	2021	**Germline RASA1** mutations: multiple high-flow cutaneous vascular stains (CM-AVM) that appear as geometric-shaped pink/red/brown macules with a pale ring, of which one predominant large vascular stain was present that was most commonly located at the limbs or face.
GLMN			
	Brouillard	2002	**Germline GLMN** mutations: glomuvenous malformations (cobblestone appearance, hard consistency, and painful on palpation). Not all individuals harboring a mutation are affected.
	Amyere	2013	**Germline GLMN** mutations: Glumuvenous malformations (hyperkeratotic bluish-purple cutaneous lesions, with often cobblestone-like appearance)
	Brouillard	2013	**Germline GLMN** mutations: glomuvenous malformations (pink to dark-blue, raised, nodular, multifocal, and hyperkeratotic). Also an atypical presentation was found of a plaque-like glomuvenous malformation (flat and purple, which darkens over time).
	Brouillard	2005	**Germline GLMN** mutations: glomuvenous malformations (nodular, multifocal, frequently hyperkeratotic with a cobblestone-like appearance. The color varies from pink to purplish dark blue. Lesions are mainly located on the extremities, involve skin and subcutis, and are often painful on palpation).
	O'Hagan	2006	**Germline GLMN** mutations: glomuvenous malformations (lesions varied from small solitary to more substantial, cosmetically noticeable lesions, all confined to the skin. Asymptomatic, except lesions in trauma-prone areas).
EPHB4			
	Amyere	2017	**Germline EPHB4** mutations: isolated multifocal CMs (63%) and CM-AVMs (37%). CMs were pink/red, cutaneous, and multifocal with geographic borders. Size varied from pinpoint to large lesions (15 cm). Some CMs (25%) had a surrounding white halo and some CMs had a pale central zone. Also bier spots (12%) and telangiectasia's (15%) were seen.
GNAQ			
	Siegel	2017	**Somatic GNAQ** mutations: CMs with mild overgrowth.
	Ten Broek	2019	**Somatic GNAQ** mutations: CVMs, VeMs, and combined malformation with an arterial component.
	Diociaiuti	2022	**Somatic GNAQ** mutations: CMs and extensive CMs with overgrowth. Three patients (38%) were diagnosed with SWS and brain MRI showed leptomeningeal angiomatosis, which resulted in glaucoma (100%), seizures (67%), headache (33%), and epistaxis (33%).
GNA11			
	Siegel	2017	**Somatic GNA11** mutations: diffuse, reticulated CM with mild overgrowth. Other findings: subtle segmental hyperpigmentation (33%) and dermal melanocytosis (17%).
	Ten Broek	2019	**Somatic GNA11** mutations: all were CVMs.
	Diociaiuti	2022	**Somatic GNA11** mutations: Extensive reticulate CMs with overgrowth and leg-length discrepancy.
ELMO2			
	Cetinkaya	2016	**Germline ELMO2** mutations: Intraosseous VeMs, mandibular and maxilla were affected in all individuals. Other findings were: gingival bleeding (100%), other recurrent bleeding (63%), and elevated Alkaline Phosphatase (50%).
MAP3K3			

	Couto	2015	**Somatic MAP3K3** mutations: verrucous VeMs (raised, reddish-purple, hyperkeratotic, and extending into the subcutis malformations. Mostly involving the extremities).
HRAS			
	Eijkelenboom	2019	**Somatic inframe HRAS** mutations: relative large (5-10cm) progressive vascular or lipoma-like malformations, sometimes with high flow.
BRAF			
	Al-Olabi	2018	**Somatic BRAF** mutations: AVM and VeM.
	El Sissy	2022	**Somatic BRAF** mutations: AVMs located at the face or lips.
	Zenner	2021	**Somatic BRAF** mutations: isolated LMs.
N/A			
	Brahami	2013	No mutations were detected.
	Revencu	2014	No mutations were detected.
	Mattassi	2018	No correlations between mutations and phenotype were identified.

AVF = Arteriovenous Fistula; AVM = Arteriovenous Malformation; BRBN = Blue Rubber Bleb Naevus syndrome; CLAPO = Capillary malformation lower lip, Lymphatic malformations, Asymmetry and Partial Overgrowth; CLOVES = Congenital Lipomatous Overgrowth, Vascular Malformations, Epidermal Nevis, Scoliosis syndrome; CLVM = Capillary-Lymphatic-Venous Malformation; CM = Capillary Malformation; CVM = Capillary-Venous Malformation; GLA = Generalized Lymphatic Anomalies; KTS = Klippel-Trenaunay Syndrome; MCAP = Megalocephaly Capillary Malformation Polymicrogyria syndrome; N/A = Not Applicable; PKWS = Parkes Weber Syndrome; PROS = PIK3CA-Related Overgrowth Spectrum; SWS = Sturge-Weber Syndrome; VeM = Venous Malformation; VM = Vascular Malformation; VTE = Venous Thromboembolism;

Supplementary table 4. Sporadically occurring mutations with phenotypes.

Gene	Protein change / mutation position	Mutation type	VAF	Phenotype	Study, year
AKT1	E17K	Somatic	8%	Male with VeM of the lower leg.	Ten Broek, 2019
AKT2	S31S	Somatic	51.3%	LM with overgrowth	Lalonde, 2019
AKT2	D46E	Somatic	44.5%	CM with overgrowth and Sturge Weber Syndrome was diagnosed.	Lalonde, 2019
AKT2	D46N	Somatic	50.5%	CM	Lalonde, 2019
AKT3	E17K	Somatic	13.9%	CM	Lalonde, 2019
AKT3	R247C	Somatic	MD	Male with intraosseous VeM of vertebra L5.	Castel, 2016
APC	Q1406*	Somatic	0.23%	Female with multiple VeMs located at the upper extremity.	Serio, 2022
ATM	G1818V	Somatic	MD	Female with intramuscular VeM of the thigh	Castel, 2016
BRAF, GNAQ	V600E, R183Q	Somatic	16.5%	CVM	Siegel, 2017
CCM2	MD	Germline	NA	Unclear type of VM located at the lower extremity in subcutaneous tissue. Diagnosed with FCCM.	Sirvente, 2009
CCM2	P55Rfs*9	Somatic	6%	Male with a single subcutaneous VeM of the right arm	Paolacci, 2020
CCM3	MD	Germline	NA	Unclear type of VM located at the lower extremity in subcutaneous tissue. Diagnosed with FCCM.	Sirvente, 2009
CCM3	MD	Germline	NA	Nodular VeM located at the upper extremity in subcutaneous tissue. Diagnosed with FCCM.	Sirvente, 2009
CCM3	MD	Germline	NA	Multiple, subcataneous VeM located over the whole body. Diagnosed with FCCM.	Sirvente, 2009
CHD11	V147D, V149D	Somatic	MD	Female with VeM at the mouth (lingual margin)	Du, 2020
CHD11	R226M	Somatic	MD	Female with VeM at the mouth (lingual ventrum)	Du, 2020
ENG	E468K	Somatic	MD	Female with VeM of the lower gum.	Du, 2020
FGFR2	S252L	Somatic	1.38%	Male with AVM located at the upper extremity and trunk.	Serio, 2022
FGFR3	F384L	Somatic	49.9%	Female with multiple VM of the lower extremity and overgrowth, diagnosed with KTS	Serio, 2022
FGFR3	F384L	Somatic	2.38%	Male with LVM of the head/neck.	Serio, 2022

FGFR3	CNV	Somatic	1.5%	Male with multiple VeM of the lower extremity.	Serio, 2022
FOXL2, BCOR	P257T, P326T	Somatic	MD	Male with a subcutaneous VeM of the finger.	Castel, 2016
GJA4	G41C	Somatic	MD	Male with small subcutaneous VeM (0.5 cm)	Ugwu, 2021
GJA4	G41C	Somatic	MD	Male with small subcutaneous VeM (1.4 cm)	Ugwu, 2021
GJA4	G41C	Somatic	MD	Male with small subcutaneous VeM (0.5 cm)	Ugwu, 2021
IDH1	R132C	Somatic	2%	Female with intramuscular CVM of the upper arm.	Ten Broek, 2019
IRS2	373_377del	Somatic	MD	Male with Intermuscular VeM of the thigh.	Castel, 2016
MAP2K1, KRAS	K57N, Q61H	Somatic	0.12%	Male with AVM of the trunk.	Serio, 2022
MAP3K1	H468Q	Somatic	MD	Female with intraosseous VeM of the skull.	Castel, 2016
MED12	Q2113_Q2114insQQHQ	Somatic	MD	Female with subcutaneous VeM of the finger.	Castel, 2016
MET	T1010I	Somatic	0.32%	Female with multiple LVMs of both hands, abdomen, and lower extremity.	Serio, 2022
MET, FGFR3	D1028N; F364L	Somatic	0.59%	Paravertebral, chest, and forearm complex CLVM, infiltrating muscles.	Serio, 2022
MET, FGFR3	T1010I; F384L	Somatic	0.97%	Upper right limb and chest LVM with overgrowth.	Serio, 2022
MET, FGFR3	T1010I; F384L	Somatic	1.34%	Left upper limb and hemithorax VeM.	Serio, 2022
mTOR	F1888L	Somatic	4.2%	LVM, accompanied by macrodactyly and sandal gap	Siegel, 2017
NRAS	Q61R	Somatic	14%	Male with CM of the thorax	Ten Broek, 2019
NRAS	Q61R	Somatic	21.7%	CM	Siegel, 2017
NRAS	Q61R	Somatic	0.1%	Male with multiple VeMs of the trunk.	Serio, 2022
NRAS	Q61R	Somatic	6%	Male with multifocal visceral LM.	Shaheen, 2022
PIK3CA, CDKN1C	Y897H, R918L, I143V, K181M	Somatic	45.8%	CM	Lalonde, 2019
PIK3CA, GNAQ	H1047R, Q209H	Somatic	26%	Male with CVM of the skull.	Ten Broek, 2019
PIK3CA, GNAQ	Q542K, Q209H	Somatic	15%	Male with CVM	Ten Broek, 2019
PIK3CA, GNAQ	E453K, Q209H	Somatic	34.9%	CLVM with additionally overgrowth, syndactyly, and macrodactyly.	Siegel, 2017
PIK3CA, GNA11	H1047R, Q209H	Somatic	17%	Male with the CVM located at the trunk (periumbilical).	Ten Broek, 2019

PIK3CA, GNA11	E545K, Q209H	Somatic	7%	Male with CVM of the nose.	Ten Broek, 2019
PIK3CA, MDC1	Y897N, R918C, Q2819R	Somatic	7.74%	Male with intramuscular VeM of the thigh.	Castel, 2016
PIK3CA, TIE2	Q546K, R915C	Somatic	MD	Female with an extensive subcutaneous VeM of the thigh, calf, and ankle.	Goines, 2018
PIK3CA, TIE2	E545K, L914F	Somatic	4%	Male with VeM of the upper extremity, 5cm in size.	Nozawa, 2022
PIK3CD	L666P	Somatic	MD	Male with macrocystic LM of the head/neck, 3.7x6.5x6.3 cm in size.	Wang, 2021
PTEN, RASA1	L757*, PTEN: c.1026+1G>A	Germline	NA	Combined LM-AVM of the lower extremity. Diagnosed with Parkes Weber.	Wooderchak-Donahue, 2018
TGFBR2, PHOX2B	S527I, 247_252del	Somatic	MD	Female with subcutaneous and intramuscular VeM of the elbow.	Castel, 2016
TIE2, MDC1	L914F, 207_214del	Somatic	4%	Female with subcutaneous VeM of the face.	Castel, 2016
TIE2, MED12	L914F, 441_442del	Somatic	8.17%	Female with intramuscular VeM Para spinal.	Castel, 2016
TIE2, MLL2	Y897H, R918L, I143V, K181M	Somatic	8.93%	Female with intramuscular VeM of the calf.	Castel, 2016
TIE2, NF1	L914F, C324S	Somatic	7.37%	Female with subcutaneous VeM of the face.	Castel, 2016
TIE2, PIK3CA, AKT2	E542K, E56G, L712P	Somatic	15.65%	Female with intramuscular VeM of the buttock.	Castel, 2016
TIE2, TERT	E542K, C1599G	Somatic	14.11%	Female with intramuscular VeM of the thigh.	Castel, 2016
TP53	P191fs	Somatic	0.1%	Male with intramuscular VeM of the lower extremity and overgrowth.	Serio, 2022

AVM = Arteriovenous malformation; CM = Capillary Malformation, CLVM = combined Capillary Lymphatic Venous malformations; CVM = combined Capillary Venous malformation; FCCM = Familial Cerebral Cavernous Malformation; LM = Lymphatic malformation; LVM = combined Lymphatic Venous malformation; MD = Missing Data; NA = Not Applicable; VeM = Venous malformation.

Chapter 3

Cell-free DNA obtained during sclerotherapy as a novel method for molecular analysis of venous and lymphatic malformations.

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Submitted

Abstract

Objective

Vascular malformations (VMs) are usually caused by post-zygotic somatic mutations in genes regulating tissue growth. The mutational discoveries in VMs have provoked a transition in the diagnosis, classification, and management of VMs. Therefore, molecular analysis of VMs is becoming inevitable, which generally requires interventional-obtained lesion tissue. We hypothesized that cell-free DNA (cfDNA) obtained from the VM during sclerotherapy could be a minimally-invasive alternative for specimen collection for molecular analysis.

Methods

In a prospective case series, blood and lymph fluid were collected locally from venous, lymphatic, and combined malformations during sclerotherapy. cfDNA was isolated from the collected samples and analyzed for VM-associated genes with Next-Generation Sequencing. If a mutation could not be detected in lymphatic malformations, the cfDNA was analyzed using a cfDNA assay with molecular barcodes, allowing for a lower detection limit of 0.10%.

Results

Somatic mutations were detected in cfDNA of patients with venous (5/14) and lymphatic malformations (5/8). However, in three patients with lymphatic malformations, the somatic mutation could only be detected using the cfDNA assay. We did not detect any somatic mutations in two patients with combined malformations.

Conclusions

Somatic mutations were detected in the cfDNA obtained during sclerotherapy of venous and lymphatic malformations and thus cfDNA is an excellent alternative for tissue biopsies. Particularly for deep-positioned VM or other unenforceable tissue biopsies, cfDNA provides a solution. The development of a cfDNA-assay for all VM-associated genes may further enhance molecular analysis. The findings in this study are a valuable contribution to a field in which genetics is becoming increasingly important, and where molecular diagnostics are becoming inevitable.

Introduction

Vascular malformations (VMs) are congenital anomalies of the vascular system, resulting in dilated and dysfunctional vessels, which can be of arteriovenous, venous, lymphatic, capillary, or mixed origin.[1] VMs are associated with a broad spectrum of problems such as pain, extensive bleeding, impairment of physical function, and thrombotic complications.[2-4] Furthermore, their disfiguring appearance may cause psychosocial problems, and VMs lead to an overall decreased quality of life.[1, 5-7]

VMs are caused by post-zygotic somatic mosaic, and rarely germline, mutations in the vasculogenesis and angiogenesis cell signaling pathway. Mutations associated with VMs often occur in genes encoding proteins that are part of the PIK3CA-mTOR and RAS-MAPK pathways.[8] Approximately twenty genes are known to be involved in these pathways and are responsible for distinct clinical manifestations.[9]

The discovery of the same mutated genes in VMs and other overgrowth disorders and syndromes has led to a revaluation of the current classification. PIK3CA-mutated VMs, macrodactyly, and Klippel-Trenaunay syndrome are now all classified within the PIK3CA-Related Overgrowth Spectrum (PROS), and equivalent entities are increasingly classified based on the genotype.[10-12]

Current treatment methods for VMs include sclerotherapy, surgery, embolization, and laser therapy. Despite the variety of available treatment options, they are mostly invasive and only provide a partial and temporary effect. Therefore, multiple targeted therapies are being repurposed for the treatment of VMs, which are investigated in clinical trials. Currently, an mTOR inhibitor (sirolimus) is widely used off-label for lymphatic and venous malformations, and recently a PI3K inhibitor (alpelisib) was proven effective in a phase II study, following a compassionate use protocol.[13, 14]

Ultimately, targeted therapies will play a more dominant role in VM management and may be used to prevent lesion progression, decrease lesion size before surgical intervention, as well as reduce the risk of recurrence following “classical” interventions. However, the molecular diagnosis should be established before initiating targeted therapies. All advances in the field of genetics of VMs have resulted in molecular diagnostics being increasingly performed.

Somatic mosaic mutations in VMs are only present in the affected tissue, and they are generally not detectable in DNA isolated from regular blood cells or the surrounding normal tissue (e.g., skin tissue overlying the lesion).[15] Consequently, it is difficult to detect the mosaic mutation, and multiple tissue biopsies are often needed to establish a molecular diagnosis.[15] Furthermore, a tissue biopsy of a VM may lead to cosmetically undesirable scarring and may be challenging because of a high risk of bleeding, and it is known to be a troublesome process in

children. Occasionally, a tissue biopsy cannot be obtained at all because the VM is located too deep in the body or is only possible in combination with surgical procedures.

In oncologic management, liquid biopsies using cell-free DNA (cfDNA) from plasma is rapidly emerging as a minimally invasive alternative approach to standard tumor biopsies. CfDNA is released into the bloodstream through apoptosis, necrosis, autophagy and, active secretion.[16] CfDNA can be used for genetic analysis of VMs because the somatic mutation is present in the endothelial cells of VMs, which have intimate contact with blood and lymph fluid. However, the molecules of cfDNA are rapidly cleared from the circulation, with a half-life of an hour or less.[17] Consequently, the concentration of the VM cfDNA is very low in plasma from a regular venipuncture. Therefore, the cfDNA should be collected locally from the VM.

Sclerotherapy provides a convenient way to collect plasma or lymph fluid from the VM. Before injecting the sclerosing agent, blood or lymph fluid is aspirated from the VM to confirm the correct location of the needle and to prevent dilution of the sclerosing agent. Consequently, the aspirated blood or lymph fluid can be used to obtain cfDNA for molecular analysis of VMs. Moreover, sclerotherapy provides a unique opportunity to reach deep-positioned venous and lymphatic malformations, which are difficult to access with regular tissue biopsies. The minimally invasive liquid biopsy using cfDNA can be a tremendous improvement for the currently troublesome process to collect material for molecular analysis. In this study, we aim to investigate if cfDNA obtained during sclerotherapy is an appropriate method to perform molecular analysis of VMs.

Methods

This prospective case series was performed in the Amsterdam University Medical Centers, a tertiary vascular anomaly expertise center in the Netherlands. The study adhered to the declaration of Helsinki, and written informed consent was obtained from each participant or parent of the participant before data and sample collection. The Medical Ethics Committee reviewed the study protocol and exempted the study from full ethical review since disposed blood and lymph fluid samples were collected, and patients were not subjected to interventions or rules of conduct.

Participants and sample collection

Patients with venous, lymphatic, or combined venous-lymphatic malformations who underwent sclerotherapy between June 2021 and September 2022 were contacted to participate in the study. In all patients, molecular diagnostics had not yet been performed. During sclerotherapy treatment, patient inclusion took place. Guided by ultrasound, blood and lymph fluid was aspirated from the VM before the sclerosing agent was injected, in order to prevent dilution of the sclerosing agent and to confirm the correct position of the needle. Instead of disposing the blood or lymph fluid, the sample was collected for molecular diagnostics. A minimum of 3 mL blood or lymph fluid was needed to perform molecular diagnostics and the samples were collected in Cell-Free DNA BCT® CE collection tubes, "streck tubes" (Streck Corperate, Omahe, NE). Patient data was collected on sex, age, VM type, lesion localization, and lesion size (measured on Magnetic Resonance Imaging). The primary outcome was the presence of a somatic mutation detected in the cfDNA.

Sample processing

Whole blood and lymph fluid in Streck tubes were centrifuged at 1711 x g for 10 minutes at room temperature. The upper layer (plasma and lymph fluid supernatant) was removed to a new tube and centrifuged for 16000 x g for 10 minutes. Subsequently, cfDNA was extracted from plasma and lymph fluid supernatant using the QIAamp Circulating Nucleic Acid Kit (Qiagen, Venlo, Netherlands).

cfDNA Sequencing

The cfDNA was analyzed with Next-Generation Sequencing (NGS) using a custom targeted NGS amplicon panel, which included hotspots of the following VM-associated genes: AKT1, AKT2, AKT3, BRAF, FGFR2, FGFR3, GNA4, GNA11, GNAQ, GNAS, HRAS, IDH1, IDH2, KRAS, KRT1, MAP2K1, MTOR, NRAS, PIK3CA, PIK3R2, PTEN, RASA1, and TIE2. cfDNA libraries were prepared using the Ion AmpliSeq Library Kit 2.0 according to the manufacturer's instructions. Libraries were quantified using the Qubit 3.0 Fluorometer. DNA libraries were sequenced on a Ion 540 chip in the Ion GeneStudio S5 System (ThermoFisher). The target sequencing depth was minimally 1,500X per amplicon. Sequences were analyzed using SeqNext software v4.1.2 (JSI Medical Systems GmbH, Ettenheim, Germany). Of all detected mutations the Variant Alle Frequency (VAF) was noted, which is a surrogate measure of the proportion of DNA molecules

in the sample carrying the mutation. If a mutation could not be detected in lymphatic malformations, the cfDNA of lymph fluid was analyzed using the Oncomine™ Lung cfDNA Assay with molecular barcodes, which includes the PIK3CA gene. The cfDNA Assay has a lower detection limit of 0.1%.

Results

In total, 28 patients were contacted to participate in the study. All patients were willing to participate in the study; however, in four patients collection of blood or lymph fluid collection failed. Of 24 patients, blood and lymph fluid were collected from the VM during sclerotherapy before injecting the sclerosing agent (Table I). Fourteen patients with venous malformations were included, of which five patients (36%) had a somatic mutation detected in the cfDNA. In two patients, a TIE2 mutation was detected, and in three patients a PIK3CA mutation. Eight patients with lymphatic malformations were included, and in five (63%) of them, a somatic PIK3CA mutation was detected in the cfDNA. In three patients with lymphatic malformations, the somatic PIK3CA mutation could only be detected using the cfDNA assay, which has a lower detection limit. Two patients with a combined lymphatic-venous malformation were included, however, in both patients, a somatic mutation could not be detected. In a total of ten patients (42%), a somatic mutation was detected in the cfDNA. The cfDNA VAF ranged from 0.07% to 8.0% (mean 2.48%; SD 2.4).

Comparison with tissue biopsy

Patient 1 was simultaneously surgically treated for the venous malformation located in her labia, therefore, surgically resected lesion tissue was also taken for molecular analysis. In both the lesion tissue and the cfDNA, the pathogenic variant TIE2 p.L914F was found. However, differences in VAF were noted; the VAF of the micro-dissected lesion tissue was 16%, while the cfDNA contained a VAF of 3.66%. Patient 24 had a large macrocystic lymphatic malformation located intra-abdominal, from diaphragm to the top of the bladder, extending to the mesentery. In the cfDNA the somatic PIK3CA mutation p.H1047R was found (VAF 1.50%). He was also diagnosed with inflammatory bowel disease (ulcerative colitis), unresponsive to therapy. However, his bowel symptoms improved since the start of sirolimus treatment, and also micro-dissected duodenum vessel tissue was sequenced; revealing the same somatic PIK3CA mutation p.H1047R (VAF 0.41%). In a peripheral blood sample the somatic PIK3CA mutation could not be detected.

Table I. Somatic mutations in cfDNA from vascular malformations obtained during sclerotherapy.

** = Somatic mutation could only be detected using the cfDNA assay, which has a lower detection limit.*

*** = Somatic mutation could not be detected, even with the cfDNA assay.*

ND = Not detected; VAF = Variant Allele Frequency; VM = Vascular malformation

Patient ID	Sex	Age	VM type	VM location	Tissue extension	VM size (cm)	Blood / lymph fluid drawn (ml)	Details blood / lymph fluid collection	Mutation	VAF
1	V	22	Venous	Whole left leg, from the left labia majora to the foot	Subcutaneous, intramuscular, intraosseous	100 x 20	9	Blood collection from a large convolute located lateral of the upper leg.	TIE2 p.L914F (c.2740C>T)	3.66%
								Lesion tissue: Surgically resected lesion tissue of the left labia majora.	TIE2 p.L914F (c.2740C>T)	16%
2	V	14	Venous	Right lower leg	Subcutaneous, intramuscular	31 x 7.5 x 5	8	Blood collected from proximal region, large draining veins to central venous system were present.	PIK3CA p.E545K (c.1633G>A)	0.95%
3	V	22	Venous	Cheek	Subcutaneous	4 x 3 x 3.5	4	Blood collected from large venous cysts, minimal central venous outflow.	ND	ND
4	V	3	Lymphatic (macrocystic)	Right side neck	Subcutaneous	5.2 x 3.9 x 3	15	Lymph fluid aspirated from a large cyst, in total 25 ml lymph fluid was collected.	PIK3CA* p.E545K (c.1633G>A)	0.57%
5	V	3	Lymphatic	Left Forearm	Subcutaneous	2 x 3 x 2	2	Lymphatic malformation contained large blood clot, complicating lymph fluid collection.	ND	ND
6	M	16	Venous	Left knee	Subcutaneous, intramuscular	3 x 4.1 x 2.6	4	Minimal central venous outflow.	PIK3CA p.E545K (c.1633G>A)	4.85%
7	V	13	Combined	Right upper and lower leg	Subcutaneous, intramuscular	16 x 5 x 8	10	Blood was collected from a large cyst located at the lower leg.	ND	ND
8	M	22	Venous	Right forearm	Intramuscular	16 x 2.9 x 3	10	Venous malformation consisted of large cysts that were not connected to each other.	PIK3CA p.E545K (c.1633G>A)	0.87%
9	V	30	Lymphatic (mixed)	Abdominal wall	Subcutaneous	12.5 x 12 x 4.9	10	Lymphatic malformation consisted of multiple cysts that were connected to each other.	PIK3CA p.E542K (c.1642G>A)	8%
10	V	22	Venous	Left upper arm, triceps muscle	Intramuscular	7.5 x 2.1 x 1	10	Minimal central venous outflow.	ND	ND

11	V	2w	Lymphatic (mixed)	Right side neck	Subcutaneous	5.9 x 5.5 x 3.2	15	Lymph fluid aspirated from 2 large lymphatic cysts located submandibular.	PIK3CA* p.H1047R (c.3140A>G)	0.07%
12	V	34	Venous	Right shoulder	Subcutaneous, intramuscular	2.2 x 2.1 x 3.7	7	Minimal central venous outflow.	ND	ND
13	V	21	Lymphatic (macrocystic)	Left chest, flank and axillary	Subcutaneous	20 x 7.5 x 5.2	15	Large macrocystic cavities and in total 200 mL lymph fluid could be drained out of the lesion.	ND**	ND
14	M	24	Venous	Left lower leg, calf	Subcutaneous, intramuscular	4.4 x 1.1 x 1.2	9	High central venous in- and outflow.	ND	ND
15	V	33	Venous	Right hand	Subcutaneous, intramuscular, intraosseous,	5.3 x 4 x 1.1	10	High central venous outflow.	ND	ND
16	M	36	Venous	Right knee	Subcutaneous, intramuscular	8.1 x 2.6 x 2.6	11	High central venous outflow.	ND	ND
17	V	18	Venous	Left upper leg	Subcutaneous	3.1 x 6.3 x 6.7	10	Venous stasis within the VM and minimal central venous outflow.	ND	ND
18	V	61	Venous	Right cheek	Subcutaneous	7 x 3.3 x 3.5	4	A lot of septum's were dividing the VM in small cavities and there was minimal central venous outflow of the VM.	TIE2 p.R918S (c.2752C>A)	3.6%
19	V	43	Venous	Right side chin, submandibular	Subcutaneous	2.1 x 1.5	5	Difficult blood collection because the malformation was small	ND	ND
20	M	26	Lymphatic (macrocystic)	Right side chin, submandibular	Subcutaneous, intramuscular	8.2 x 4.3 x 4.4	15	The malformation consisted of one prominent macrocyst, in total 60 ml lymph fluid was collected	ND**	ND
21	V	24	Venous	Left knee and upper leg	Subcutaneous, intramuscular	16 x 3.9 x 7.2	12	Easy blood collection, high ventral venous outflow.	ND	ND
22	M	23	Venous	Left knee and upper leg, vastus intermedius and medialis muscles	Intramuscular	8.4 x 4.6 x 4.6	7	Difficult blood collection because the malformation consisted of multiple small cavities that were connected to each other.	ND	ND
23	M	47	Lymphatic (macrocystic)	Intra-abdominal, in retroperitoneal space	Intra-abdominal extending to the mesentery	20 x 17 x 15	15	Large macrocystic cavities with connection to each other, in total 1 liter lymph fluid was collected	PIK3CA* p.E545K (c.1633G>A)	0.77%
24	M	2	Lymphatic (macrocystic)	Intra-abdominal, from diaphragm to the top of the bladder	Intra-abdominal extending to the mesentery	18 x 18.6 x 11	15	Large macrocystic cavities with connection to each other, in total 1.5 liters lymph fluid was collected	PIK3CA p.H1047R (c.314A>G)	1.5%
								Lesion tissue: tissue biopsy of duodenum.	PIK3CA p.H1047R (c.314A>G)	0.41%
								Peripheral blood sample.	ND	ND

Unfeasibility to collect a sample

Patient 25 had a large subcutaneous lymphatic malformation located at the left flank, consisting of numerous microcysts in an area of 10x15 cm. The microcysts were, however, so small that collection of any lymphatic fluid could not be performed.
Patient 26 had an intramuscular venous malformation located at the right cheek in the masseter muscle. The malformation had a small size of 1.6x0.8x1.0 cm, and it was, therefore, impossible to collect blood from the malformation.
Patient 27 had a venous malformation located at the right knee extending subcutaneous and intramuscular in the vastus medialis muscle with a size of 2.8x3.7x4.7 cm. He was previously successfully treated with sclerotherapy in 2016. During the current sclerotherapy treatment, on ultrasound a lot of acoustic shadowing was seen within the VM, due to calcifications and scar tissue as a result of the previous sclerotherapy. Therefore, blood collection could not be performed.
Patient 28 had a subcutaneous lymphatic malformation of the right cheek (4.0x3.5x2.0 cm) and around the ortbita (2.0x1.0x1.5 cm), in connection with each other. When he lied sedated in the intervention room, the superficial and easily accessible cavities of the malformation drained to deeper positioned unreachable cavities, and it was impossible to collect any lymph fluid.

Discussion

In this study, we demonstrate that cfDNA obtained during sclerotherapy is a convenient and suitable technique to detect somatic mutations in venous and lymphatic malformations, in which patients are not bothered or harmed by specimen collection for molecular analysis. A total of 24 patients were included, and we were able to find a somatic mutation in 42%. Hence, cfDNA obtained out of the VM is a minimally invasive and adequate alternative to perform molecular analysis of VMs in contrast to tissue biopsies, which are associated with a high bleeding risk, may lead to cosmetically undesirable scarring, or cannot be obtained at all if the VM is located too deep in the body or are only possible in combination with surgical procedures.

CfDNA is released into the bloodstream through a variety of natural processes including necrosis, apoptosis, autophagy, and active secretion.[16] In addition, the cfDNA in the bloodstream is derived partly from vascular endothelial cells.[18] Previous research has revealed that somatic mutations are primarily present in the endothelial cells of VMs and that this is the origin of VM development.[19-22] Intimate contact between the endothelial cells and blood or lymph fluid may have led the ability to detect somatic mutations in cfDNA collected locally from the VM.

However, in some patients we were not able to detect any mutation, which might have different explanations. First, the affected gene may not be present in the used gene panel, or the VAF was below the detection limit of the assay. Secondly, several venous malformations had high central venous outflow, and cfDNA containing the somatic mutation was possibly quickly washed out, which made detection of the mutation more complicated. Thirdly, a few patients had large macrocystic lymphatic malformations where up to 1.5 liters of lymph fluid could be collected. It might be that due to the largeness of the cysts there was minimal contact between the endothelial cells and the lymph fluid, and consequently, the proportion of cfDNA containing the somatic mutation was low. Therefore, it is advised to collect the lymph fluid from multiple and preferable also smaller lymphatic cysts.

On the contrary, the detection of somatic mutations in lesion tissue and tissue biopsies is also known to be difficult because of the mosaic nature of mutations. Therefore, multiple tissue biopsies are generally needed to establish the molecular diagnosis.[15] In a large study including 319 patients with peripheral VMs, somatic mutations were detected in lesion tissue of 41%, corresponding to the detection rate of 42% of cfDNA in the current study.[23]

In a few patients, we were not able to collect any blood or lymph fluid due to the small size of the lesion or lymphatic cysts, scar tissue within the VM, or because lymph fluid drained to deep unreachable cysts. In advance, it is difficult to predict whether blood or lymph fluid collection will be successful. However, patients do not experience any inconvenience from the blood or lymph fluid collection during sclerotherapy, therefore, the clinician should always make an attempt to collect cfDNA for molecular analysis.

In previous studies, cfDNA for molecular analysis of vascular malformations has been investigated in a small number of patients. In a study among seven patients with Klippel-Trenaunay syndrome, a somatic PIK3CA mutation could be found in all patients (100%) with low VAFs (range 0.18%-1.23%) compared to VAFs of tissue biopsies (range 5.1%-22.7%).[24] Another study from the same research group found somatic KRAS mutations in five out five patients (100%) with arteriovenous malformations (VAF range 0.19%-4.19%).[25] Both studies support cfDNA collection from the efferent vein of the VM rather than peripheral blood collection because of higher mutational load, i.e., higher VAFs.[24, 25] In a study among four patients with combined lymphovenous malformations all patients (100%) carried a somatic MET mutation with low VAFs (range 0.09%-0.95%).[26]

Zenner et al., included 18 patients with arteriovenous, venous, and lymphatic malformations and detected causative somatic mutations in ten patients (55%).[27] In all seven patients with lymphatic malformations somatic PIK3CA mutations were detected in the locally collected cfDNA, and surprisingly, five cfDNA samples had a VAF equal to or greater than their corresponding tissue-detected VAF.[27] In contrast, venous and arteriovenous malformations in the same study with somatic TIE2 and MAP2K1 mutations respectively, contained lower VAFs in cfDNA (range 0.40-2.1%) than in tissue.[27]

In the current study, we also found slightly low VAFs, i.e., proportion of DNA molecules in the sample carrying the mutation, and five out of ten VAFs were <1%. Consequently, the low VAF in cfDNA may hamper the detection of somatic mutations in VMs. Due to low VAFs in the current study, in three out of five lymphatic malformations, somatic PIK3CA mutations could only be identified using the cfDNA assay, which has a lower detection limit of 0.10%.

Somatic PIK3CA mutations are also associated with oncologic disorders, resulting in the development of cfDNA assays for tumor-derived PIKC3A mutations. Pathogenic variants in the TIE2 gene are primarily present in VMs, and a cfDNA assay for the TIE2 gene is currently not available. The development of a cfDNA assay for the TIE2 and other VM-associated genes would allow for a lower detection limit of somatic mutations in cfDNA, and thereby somatic mutations in cfDNA of VMs could be more readily found.

In order to determine the genotype of VMs, a tissue biopsy or surgically resected tissue is regularly needed to perform molecular analysis, resulting in genotyping of only superficial VMs or lesions demanding surgery, ultimately leading to selection bias of genotype analysis. CfDNA obtained during sclerotherapy provides a beneficial and minimal-invasive approach for the molecular analysis of VMs. Patients receiving sclerotherapy are generally not surgically treated, and therefore lesion tissue is not routinely available, hence, cfDNA offers a solution for molecular analysis. Furthermore, a tissue biopsy of deep-positioned VMs, intramuscular lesions, or lesions in the proximity of nerves or other vital structures can be problematic or not feasible. For these patients, cfDNA collected from the VM provides a breakthrough for molecular analysis. Next to the collection of cfDNA during sclerotherapy, cfDNA may be obtained from superficial VMs during outpatient visits or guided by ultrasound for deeper VMs.

Several limitations to the current study should be considered when interpreting the results. First, molecular analysis of cfDNA was not compared with molecular analysis of lesion tissue in all patients, which could have provided further insight into the ability to detect mutations in cfDNA. However, in the current study, children and patients with facial, deep, and intramuscular VMs were included, in whom a tissue biopsy is undesirable or not feasible. In addition, a tissue biopsy in combination with sclerotherapy is troublesome since the sclerosing agent may leak out of the lesion. Another limitation was the small sample size of the study. Although this is always a challenge in rare diseases, and despite the small sample size, we were able to perform a proof of principle.

The discovery of mutated genes causative of VMs has triggered changes in research, and in recent years the molecular discoveries have been translated into clinical practice. Subsequently, a transition in the diagnosis, classification, and management of VMs has eventuated. Molecular analysis can now be used to identify lesions with an unclear diagnosis and VMs are increasingly classified based on the genotype, e.g., PROS.[10] The revelation of mutated genes in VMs has also led to the repurposing of targeted therapies based on molecular profile, which are now being used off-label for VMs and are further investigated in clinical trials.[13, 14, 28, 29] Consequently, molecular analysis of VMs is becoming increasingly important and will be more routinely performed.

This study demonstrated that cfDNA from VMs can be easily collected, and it is a minimally-invasive technique to perform molecular analysis, preparing for the wide use of targeted therapies in VMs. The convenience of cfDNA will allow that molecular analysis can effortlessly be performed in children and other patients with VMs undergoing sclerotherapy. Therefore, a routine collection of cfDNA samples for molecular analysis during sclerotherapy would be advised whenever genetic analysis is desired. In order to keep up with the evolving landscape of genetics and targeted therapies, future studies need to optimize molecular analysis of cfDNA and ideally develop a cfDNA assay for all VM-associated genes.

In this study, somatic mutations were detected in cfDNA obtained during sclerotherapy of venous and lymphatic malformations. The novel technique represents an opportunity to perform molecular analysis conveniently and minimally invasive. The findings of this study are a valuable contribution to a field in which genetics is becoming increasingly important in the diagnosis, classification, and management of VMs.

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Chapter 4

Characterization of patient-derived GNAQ mutated endothelial cells from capillary malformations

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ABSTRACT

Capillary malformations (port-wine stains) are congenital skin lesions that are characterized by dilated capillaries and post-capillary venules. Capillary malformations are caused by altered functioning of the vascular endothelium. Somatic genetic mutations have predominantly been identified in the endothelial cells of capillary malformations, providing an opportunity for the development of targeted therapies. However, there is currently limited in-depth mechanistic insight in the pathophysiology and a lack of pre-clinical research approaches.

In a mono-centre exploratory study of 17 adult patients with capillary malformations, we found somatic mutations in the *GNAQ* [p.R183Q, p.R183G or p.Q209R] or *GNA11* [p.R183C] genes. We applied an endothelial-selective cell isolation protocol to culture primary endothelial cells from skin biopsies from these patients. We demonstrate that patient-derived cells can be expanded in culture, while maintaining endothelial specificity as demonstrated by Vascular Endothelial (VE)-cadherin immunostainings. In addition, we find that the angiogenic capacity of the endothelial cells from a patient with a *GNAQ* [p.R183G] mutation is increased compared to control endothelial cells expanded from normal skin.

These proof-of-principle results reveal that primary cells isolated from capillary malformations may represent a highly valuable research model to investigate the role of endothelial somatic mutations in the aetiology of capillary malformations.

INTRODUCTION

Capillary malformations (CMs), also known as port-wine stains, are congenital vascular lesions affecting the skin and sometimes the underlying tissues. Their typical appearance as red or purple skin stains are caused by hyperdilated capillaries and post-capillary venules (Mulliken et al., 2013; Schneider et al., 1988). Sporadically, spontaneous bleeding may occur, and patients may develop nodules or (bone/soft tissue) overgrowth, which could lead to tissue asymmetry and dysmorphosis (Enjolras and Mulliken, 1993; Geronemus and Ashinoff, 1991; van Drooge et al., 2012). Furthermore, CMs can instigate significant psychological burden resulting in a decreased health-related quality of life, particularly when lesions are located visibly in the face (Lanigan and Cotterill, 1989; Masnari et al., 2012). CMs may be accompanied by glaucoma and epilepsy, or by soft tissue and/or bone hypertrophy and venous malformations in genetic disorders such as Sturge-Weber and Klippel-Trenaunay syndrome (Lee et al., 2005; Thomas-Sohl et al., 2004). Currently, no cure has been found yet for CMs, and complete vascular normalization is seldom achieved. The current gold standard for treatment is laser therapy, yet despite technological advancements treatment outcomes in terms of lesional lightening are still not optimal, and lesions recur frequently (Huikeshoven et al., 2007; van Raath et al., 2019).

There is currently a lack of understanding of the molecular and cellular mechanisms that drive CM pathogenesis, although somatic and germline mutations are often found in the endothelial cells of CMs (Nguyen et al., 2019). Specifically, in patients with CMs, recurrent somatic pathogenic variants have been detected in the *Guanine nucleotide-binding protein G(q) subunit alpha (GNAQ)* gene, the *Guanine nucleotide-binding protein subunit alpha-11 (GNA11)* gene and the *phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA)* gene, and germline mutations in the *RAS p21 protein activator 1 (RASA1)* gene (Couto et al., 2017; Couto et al., 2016; Eerola et al., 2003; Revencu et al., 2008; Shirley et al., 2013; Siegel et al., 2018; Vahidnezhad et al., 2016). These genes encode for proteins central to molecular signaling pathways that, when activated, drive cellular growth, proliferation and survival. The mutations in these genes may lead to modified endothelial cell proliferation, differentiation, survival and inflammatory status (Huang et al., 2022; Nguyen et al., 2019; Shirley et al., 2013). Confirmatory, at a cellular level, CMs are characterized by hyperactive and proliferative endothelial cells, enlarged vessel lumens and disorganized perivascular cells (Couto et al., 2016; Le Cras et al., 2020; Nguyen et al., 2019). However, it is still unclear if and how the genetic mutations lead to vascular lesions, and whether targeting the pathways downstream of these genetic variants may cure CMs. With this exploratory prospective study, we aimed to address the functional aspects of primary endothelial cells with genetic mutations, isolated from CM skin lesion of patients.

RESULTS

Patient characteristics

Based on the appointment lists of the outpatient clinic at the department of Dermatology (Amsterdam UMC, the Netherlands), a total of 59 potentially eligible CM patients were identified. By screening the electronic patient files, 24 patients (41%) were considered not eligible based on the predefined exclusion criteria (Table 1). The remaining 35 patients (59%) were subsequently contacted to participate. Of these patients, eventually 17 patients (49%) agreed to participate in this study and skin biopsies were taken accordingly.

Table 1. Patient eligibility criteria.

Inclusion criteria	*Exclusion criteria*
Adult patients (>17 years) with a CM of any sex	Patients with a CM younger than 17 years
CMs of all anatomical locations, except facial CMs not extending in the hairline	Facial CMs not extending into the hairline
CMs as part of the Sturge Weber syndrome	Mix of vascular malformation
	Known coagulation disorders leading to prolonged bleeding
	Anticoagulant use (excluding NSAIDs)
	Cognitively impaired patients

CM = capillary malformation

Table 2 lists all patient characteristics. Most patients were female (77%, *n*=13), the mean age was 35 years (SD +/- 17) and Fitzpatrick type 2 was the most frequent skin type (77%, *n*=13). The majority of the patients had a CM in the head and neck region (77%, *n*=13). Local hypertrophy, including blebs, was present in 8 patients (47%), and soft tissue overgrowth was present in 9 patients (53%). Telangiectasia was found in only 3 patients (18%). 13 patients had another laser therapy session planned after the biopsies were taken; mean time to follow-up was 16 weeks (SD +/- 4).

Table 2. Somatic mutations and phenotype characteristics.

The table displays an overview of the somatic mutations and associated phenotypic characteristics per patient in this study. No clear phenotypic differences (regarding CM anatomic location, size, tissue overgrowth, nodules/blebs, and telangiectasia) were found between GNAQ and GNA11 mutations.

Patient number	Gene	Mutation	VAF	Sex, age (in years)	Fitzpatrick Skin type	Color	Anatomic location	Size (cm)	Tissue overgrowth	Nodules and blebs	Telangi-ectasia	Biopsy site	Time to follow-up (weeks)
1	GNAQ	Exon 4 c.548G>A p.R183Q	8%	Female, 18	Type 2	Red to purple	The left chest	8x5.5	No	No	Yes	Border left and right mammae	15
2	GNAQ	Exon 4 c.548G>A p.R183Q	11%	Female, 25	Type 2	Light red	Right side of face (cheek, lips, neck, ear)	20x15	Lower lip	No	No	Hairline behind right ear	N/A**
3	GNA11	Exon 4 c.547C>T p.R183C	2.05%	Female, 43	Type 2	Light red to red	Left side of face (skull, orbita, cheek, and lip)	10x6	Upper lip	No	No	Hairline left	N/A**
4	GNAQ	Exon 5 c.626A>G p.Q209R	2.48%	Female, 21	Type 2	Dark red to purple	Chest: left breast towards left flank	20x15	No	No	No	Left flank close to IMF	18
5	GNAQ	Exon 5 c.626A>G p.Q209R	11%	Male, 40	Type 2	Dark red	Left side of head: beyond hairline towards ear and neck	6.1x3.6	No	Yes	Yes	In hairline	21
6	GNAQ	Exon 4 c.547C>G p.R183G	3.68%	Female, 54	Type 3	Red	Left side of face, above upper lip, cheek, temporal region, ear and neck	7x15	No	Yes	Yes	Behind left ear	20
7	GNAQ	Exon 4 c.548G>A p.R183Q	9%	Male, 30	Type 3	Dark red to purple	Face and neck: lower lip, chin, both cheeks and neck continuing to chest	20x30	Chin and lower lip	Yes	No	Right side chest	18

8	GNAQ	Exon 4 c.548G>A p.R183Q	7.2%	Female, 19	Type 1	Red and purple	Head and neck: right face continuing in hairline, ear and neck.	15x10	Soft tissue right side face zygoma / infra orbital, possibly also bony overgrowth	Yes	No	Behind right ear	12
9	GNAQ	Exon 4 c.548G>A p.R183Q	12%	Female, 76	Type 2	Red	Head and neck: left front face continuing into hairline left side	10x20	Upper lip and eyebrow left	Yes	No	Frontotemporal left in hairline Healthy tissue: left upper leg	14
10	GNAQ	Exon 4 c.548G>A p.R183Q	2.63%	Female, 26	Type 2	Light red to red	Neck: right anterior of carotis artery	8x3	No	No	No	Neck, cranial site of the CM	11
11	Not found	N/A	N/A	Female, 24	Type 4	Red	Head and neck: right front face, upper eye lid, temporal, check and neck.	Unreported	Upper and lower lip and supra orbital right	Yes	No	Behind left ear	11
12	Not found	N/A	N/A	Female, 66	Type 2	Red	Head and neck: right side face, nose, lips and chin continuing in hairline right	30	Soft tissue overgrowth of upper lip, nose, cheek right	No	No	Preauricular right in hairline Healthy tissue: right upper arm	N/A**
13	GNA11	Exon 4 c.547C>T p.R183C	12%	Female, 28	Type 2	Light red	Head and neck, upper and lower extremity left and trunk: complete face, neck, chest left side and left arm and leg	150x30	No	No	No	Left upper arm	9
14	Not found	N/A	N/A	Male, 38	Type 2	Light red to red	Head and neck: right side front face until midline, both cheeks,	15x20	No	No	No	Behind right ear	11

							temporal region, skull and neck both sides						
15	Not found	N/A	N/A	Female, 37	Type 2	Dark red to purple	Trunk, upper and lower extremity: right arm, half side back right, buttocks both sides, both legs back side	140x50	No	Yes	No	Right lower leg laterally	23
16	GNAQ	Exon 4 c.548G>A p.R183Q	11%	Male, 36	Type 2	Dark red to purple	Head, neck, trunk: multiple CMs face right into hairline, pre auricular, cheek, jaw line and neck. Small CM shoulder/back	25x10	Soft tissue overgrowth of face	Yes	No	Right shoulder/back	N/A**
17	GNAQ	Exon 4 c.548G>A p.R183Q	15%	Female, 23	Type 2	Light red	Upper extremity and trunk: right side chest to infra mammary fold, continuing to back midway. Complete right arm affected.	80x80	Soft tissue overgrowth of arm and hand right	No	No	Right upper arm	20

**Wound healing of the skin biopsies sites was assessed at follow-up at the next laser treatment.*

***In four patients time to follow-up could not be determined, as these patients completed laser therapy and thus no following laser treatment were scheduled.*

IMF = inframammary fold, N/A = not applicable, VAF = Variant Allele Frequency

Genetic and histological analysis

By sequencing of the CM biopsies, we detected somatic mutations in 13 patients (76%): the *GNAQ* gene was mutated in 11 patients (85%) and the *GNA11* gene was mutated in 2 patients (15%). The detected allele frequency of the point mutations ranged between 2% to 15% within the lesions, with a mean of 8.2%. The most predominant somatic mutation was the *GNAQ* [c.548G>A; p.R183Q] (*n*=8), confirming previous findings (Shirley et al., 2013). Moreover, we identified the *GNAQ* [c.626A>G; p.Q209R] mutations in two patients, one *GNAQ* [c.547C>G; p.R183G] mutation, and two patients with a *GNA11* [c.547C>T; p.R183C] mutation. In 4 patients no pathogenic mutation was found. In this study, we did not observe a clear genotype-phenotype correlation between CM characteristics and somatic mutations. Histological analysis of the CM coupes showed enlarged vessel lumens in the skin specimens (Figure 1A). In addition, we observed vascular leakage in a patient with lesions containing a *GNAQ* [c.626A>G; p.Q209R] mutation (biopsy 5).

Isolation and expansion of patient-derived primary endothelial cells.

Recently, a novel endothelial cell isolation protocol was successfully developed for low-flow vascular malformations (Kobialka et al., 2022). To assess the possibility to obtain primary cells from CM patient lesions, we aimed to isolate and expand cells from the 4 mm biopsies of the patient cohort. Fresh surgical biopsies of CMs were tissue digested and endothelial cells were purified from the homogenate through using anti-CD31 conjugated magnetic beads as described before for *PIK3CA*-related vascular malformations (Kobialka et al., 2022). The remaining cell fractions were taken into culture to expand patient-derived primary skin fibroblasts. Initially the CD31-enriched cell fraction formed small colonies that in the course of 2 to 4 weeks grew into cobblestone-shaped monolayers, a typical characteristic of cultured endothelial cells (Figure 1B). Of note, during tissue culture expansion, 5 patient-derived endothelial lines were lost due to cell senescence and/or the overgrowth of remaining fibroblasts. Attempts to eradicate fibroblast contaminations from the endothelial cultures through a second CD31-magnetic bead selection step did not fully remove fibroblasts. In addition, some endothelial cultures expanded inefficiently (for instance biopsy 9 GNAQ p.R183Q cells). The size and shape of the proliferating endothelial cells did not differ between CM and healthy tissues, indicating that overall endothelial cell morphology is maintained in CMs. To further investigate the characteristics of the patient-derived cells we performed immunofluorescence stainings for the endothelial-specific marker Vascular Endothelial (VE)-cadherin, the F-actin cytoskeleton (phalloidin) and the nucleus (DAPI) in the expanding cultures (Figure 2). These immunostainings confirmed that bona fide endothelial monolayers were expanded from patient biopsies harboring a GNAQ p.R183Q (biopsy 2 and 8) mutation and GNAQ p.R183G (biopsy 6), as well as from the 2 healthy control biopsies.

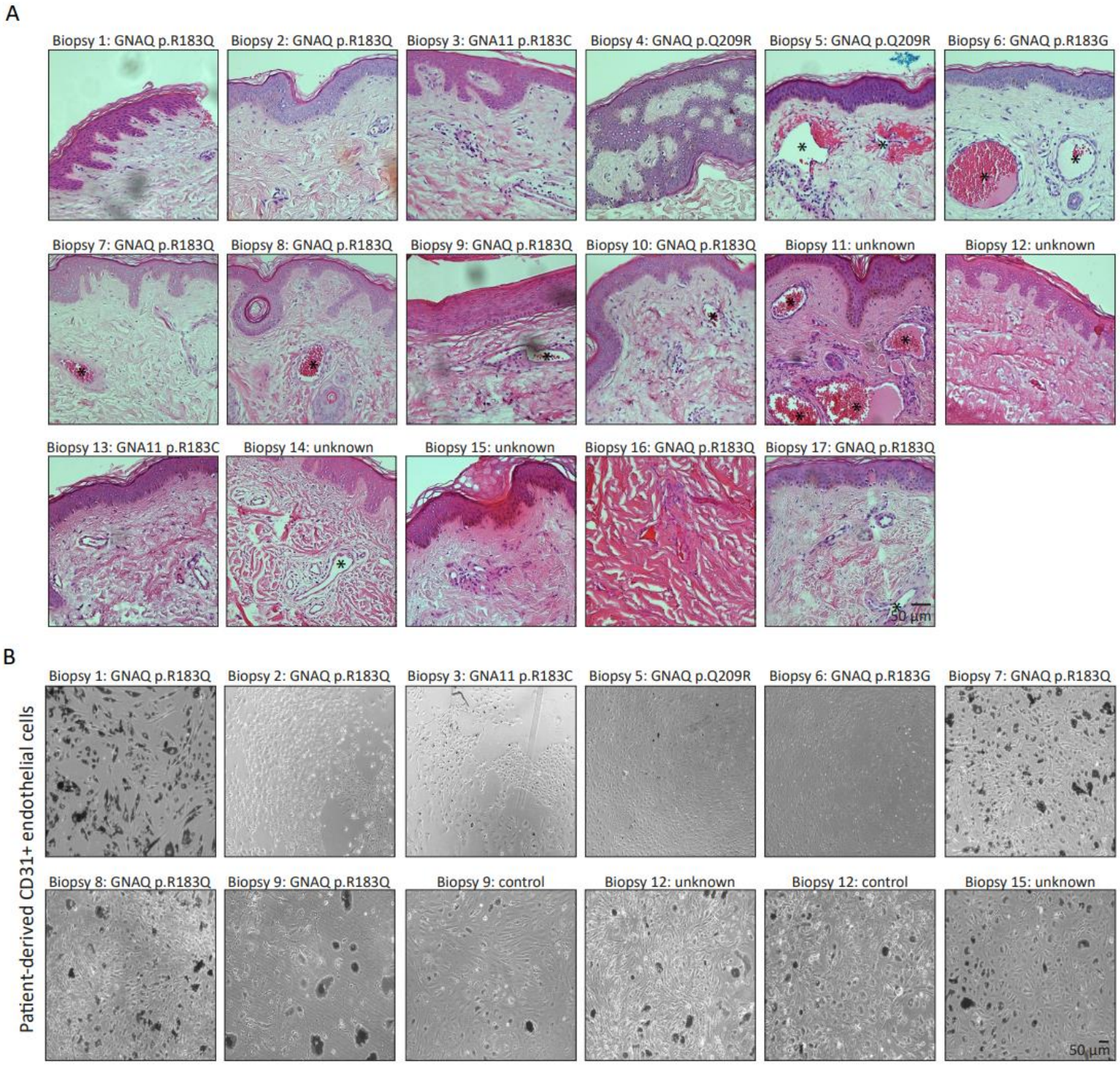

Figure 1. Characterization of skin capillary malformations and patient-derived endothelial cells. **(a)** Bright-field images of H&E stained tissue section from patient skin. Clear dilated capillaries are visible in biopsy samples 5, 6, 7, 8, 9, 10, 11, 14 and 17 (indicated by *). In addition, there was vascular leakage from the CMs in a GNAQ [c.626A>G; p.Q209R]-positive lesion (biopsy 5). Scale bar 50 µm. **(b)** Phase-contrast images of patient-derived primary endothelial cell cultures, which were selected by anti-CD31 coated magnetic beads. Black aggregates are remaining magnetic beads that were used to enrich the endothelial cells from patient tissue. Scale bar 50 µm.

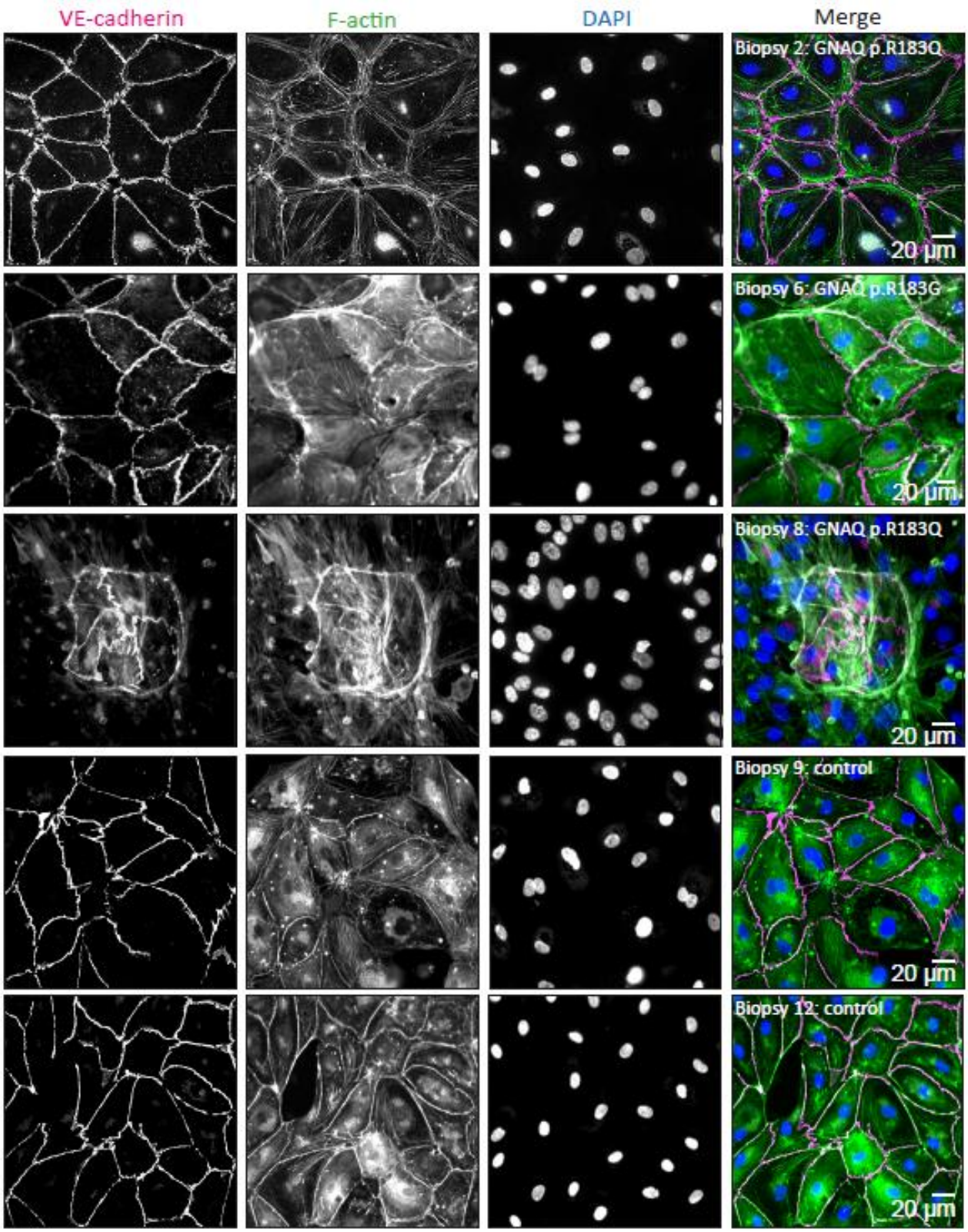

Figure 2. CD31-selected patient-derived cells are endothelial cells. Representative widefield immunofluorescence images taken from culture expanded CD31-selected patient-derived primary endothelial cell cultures stained for the endothelial marker VE-cadherin (magenta), F-actin cytoskeleton (phalloidin, green) and nucleus (DAPI, blue). Scale bar 20 µm.

Angiogenic sprouting capacity of patient-derived GNAQ [p.R183G] endothelial cells

The formation of vascular malformations depends on sprouting angiogenesis (Kobialka et al., 2022). Thus far, it has not been possible to assess the function of CM-derived endothelial cells. To functionally test the angiogenic capacity of CM-derived endothelial cells we first sequenced the DNA of the culture expanded endothelial cells from biopsy 6, which confirmed the *GNAQ* [c.547C>G; p.R183G] mutation. The allelic frequency of the mutation increased to 20% (from 3.7% in the original CM tissue). The increased VAF might indicate that the somatic mutation increased endothelial proliferation rate compared to the normal endothelial cells in a mosaic culture or that the mutation is enriched in the endothelial cell isolated fraction. To investigate the angiogenic potential of the CM-derived GNAQ p.R183G endothelial cells, we performed angiogenic growth factor-induced sprouting assays based on endothelial multicellular spheroids in 3-dimensional collagen matrix. The experiments showed that there was no difference in the number of induced sprouts in the CM-derived GNAQ p.R183G endothelial cells (mean number of sprouts per spheroid 13.1; SD ± 5.7) compared to control (mean number of sprouts per spheroid 12.9; SD ± 4.6) (Figure 3a, b). The length of forming sprouts was significantly higher in CM-derived GNAQ p.R183G endothelial cells (mean sprout length 60.3 µm; SD ± 32.0) and healthy skin-derived endothelial cells from biopsy 12 (mean sprout length 49.3 µm; SD ± 24.8) (Figure 3a, b). Taken together, these data indicate that the GNAQ p.R183G mutation in capillary endothelial cells potentially increases their angiogenic sprouting capacity, although a larger sample size is needed to be able to draw definitive conclusions. Due to the limited growth of the patient-derived endothelial cultures, follow-up experiments to assess endothelial barrier function, scratch wound migration and biochemical signals in lysates could not be determined.

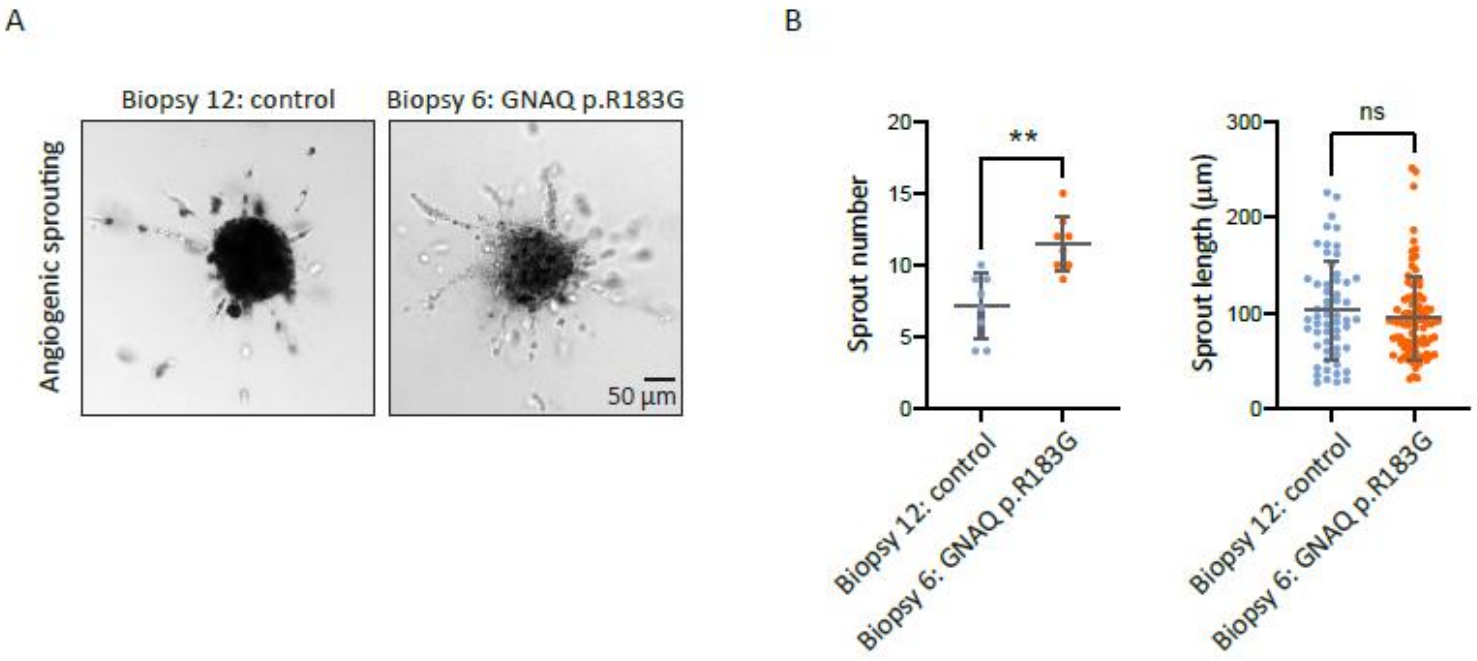

Figure 3. Capillary malformation-derived GNAQ p.R183G endothelial cells display increased angiogenic sprouting. (a) Representative phase-contrast images of sprouting spheroids 24 hours after VEGF stimulation of patient-derived endothelial cells. The sprouting capacity of control endothelial cells were compared with capillary malformation-derived GNAQ p.R183G endothelial cells. **(b)** Graphs show the mean ±SD sprout length and number of sprouts. Data represents n=13 spheroids from control endothelial cells (from one biological replicate) and n=35 spheroids from GNAQ p.R183G endothelial cells (from two biological replicates; indicated by distinct colors of the data points in the graph) were analyzed. **** P<0.0001, n.s. non-significant.

DISCUSSION

In this study, we showed that primary cells isolated from cutaneous CMs may represent a highly valuable research model to investigate the importance of endothelial somatic mutations in the etiology of CMs. As a proof-of-principle small size study, our results suggest that CM-derived GNAQ p.R183G endothelial cells could have changed angiogenic sprouting capacity, however, more evidence is needed to assess whether that explains the increased number of dilated capillaries in CMs. It is expected that future larger studies will define the relevance of specific mutations in the *GNAQ* and *GNA11* genes for the development of cutaneous CMs and related clinical features.

Expansion of patient-derived primary cells.

This study indicates that it is possible to isolate and expand primary endothelial cells from CM lesions. The experimental approach enables the identification of key perturbed endothelial functions in CMs, and whether such dysfunctions associate with specific genetic predispositions and/or clinical features. The patient-derived cells are useful as a disease model in which the somatic mutations, molecular signaling pathways and cellular functions from CMs are recapitulated. We expect that the use of patient-derived primary cells, once expanded sufficiently, will spur the development of compound or therapeutic screens that are aimed at restoring endothelial function in CMs. Furthermore, identifying the effector pathways of the mutated genes that underlie CM formation will allow for the potential development of targeted therapies. Our results also indicate that the efficiency of expansion of endothelial cells from CMs is very low, perhaps less efficient than endothelial cells derived from PIK3CA-related vascular malformations (Kobialka et al., 2022), which is coherent with the notion that those lesions are highly proliferative and more associated with tissue overgrowth (Angulo-Urarte and Graupera, 2022). In this study, only the GNAQ p.R183G mutated endothelial cells expanded sufficiently to be able to perform angiogenic sprouting assays. We posit that the success in expanding the endothelial cells cannot be attributed to specific CM characteristics exhibited by the respective patient. This conclusion is drawn from the observation that the clinical characteristics of the CMs (i.e. located in head and neck region and featuring local blebs) in the other patients were found to be similar and shared commonalities. In addition, the biopsy was taken from the skin behind the ear, which was also a frequent tissue biopsy site in the other patients. Whether the GNAQ p.R183Q, GNAQ p.Q209R or GNA11 p.R183C mutations have a potential negative impact on endothelial cultures is currently unclear. Expression of the closely related hyperactive GNAQ p.Q209L mutation in endothelial cells has been shown to induce vascular malformations in mice (Sasaki et al., 2022; Schrenk et al., 2023), providing proof-of-principle that mutations in GNAQ are disease drivers. Of note, skin biopsies may also be used to expand patient-derived fibroblasts, which is much more efficient in terms of collecting large numbers of cells. These fibroblasts, provided they carry the somatic mutation, can serve as a viable source for generating induced pluripotent stem (iPS) cells. Endothelial cells, pericytes, and smooth muscle cells can be differentiated from these iPS cells (Orlova et al., 2014; Vila Cuenca et al., 2021), to generate genetically identical CM vascular cell types.

Genetic mutations

Somatic and germline mutations in genes regulating cell growth are known to cause CMs. However, in four patients we were not able to detect any genetic mutation. This might have several reasons: (1) the allele frequency is below the sequencing detection limit, (2) the mutation is in a gene that was not included in the applied gene panel, or (3) the mutation is present in another part of the gene. In this study we most frequently found somatic pathogenic mutations in the *GNAQ* gene (85%), which is in line with previous findings (Couto et al., 2016; Shirley et al., 2013). *GNA11* was another mutated gene found in some of our included patients. Both genes code for G proteins (i.e. the G protein q polypeptide [Gαq] and the G protein α11 [Gα11]), which are part of heterotrimeric G protein complexes that mediate signaling through G-protein-coupled receptors (GPCR). The somatic mutations induce expression of hyperactive Gαq and Gα11 protein variants (Shirley et al., 2013).

Currently, the presence of clinical distinctions between patients harboring GNAQ and GNA11 mutations remains uncertain; however, it is notable that GNAQ mutations have been closely correlated with facial capillary malformations (CMs), while GNA11 mutations have been associated with extremity CMs and tissue overgrowth (Couto et al., 2017; Couto et al., 2016; Lian et al., 2014; Shirley et al., 2013). In addition, a recent study among 32 patients with CMs found that port-wine stains, ipsilateral segmental overgrowth, varicose veins and macrocephaly were associated with *GNAQ* mutations, whereas cutis marmorata, nevus anemicus, and ipsilateral hypotrophy were associated with *GNA11* mutations (Jordan et al., 2020). Due to the low number of patients with *GNA11* mutations (n=2) in the current study, we were not able to establish phenotypic differences between *GNAQ* and *GNA11* mutations. Somatic mutations in *PIK3CA* are widely present in low-flow malformations and overgrowth disorders, and also have been recently identified in patients with diffuse multifocal CMs and overgrowth (Goss et al., 2020). Lastly, CMs have been associated with germline *RASA1* and *EPHB4* mutations, resulting in a phenotype with multiple, round-to-oval, pink, hereditary CMs, some with a pale halo (Amyere et al., 2017; Revencu et al., 2013). Patients included in the current study did not portray these phenotypic descriptions and no *RASA1* or *EPHB4* mutations were identified in this patient cohort. Future larger-scale studies are needed to investigate how mutations in the *GNAQ* and *GNA11* genes contribute to the development of CMs and associated clinical features in patients, and explore possible genotype-phenotype correlations.

Patient study limitations

Several limitations to the current study need to be mentioned. First, the number of samples was small due to the exploratory character of this case series, which prevented comparative statistical analysis, and thus no correlations could be made between patient phenotype and genotype. The initial sample size was decided arbitrarily, based on the limited availability of funding for the genetic analysis and cellular experiments. Owing to the COVID-19 pandemic and rarity of the disease the inclusion rate was slow, after which it was decided to stop inclusion

at *n*=17 participants. Second, selection bias in this study cannot be fully ruled out since only patients were included who opted for laser therapy for their CM. Therefore, more severely affected patients with larger and thicker CMs or in highly visible areas, such as the head and neck region, could be overrepresented. Third, to be able to definitively conclude whether a somatic mutation leads to a different cellular phenotype, paired control cells should be compared to mutated endothelial cells from the same patient. Therefore, developing approaches that may separate mutated from normal endothelial cells from the biopsies would be preferred. Finally, we were only able to assess angiogenic sprouting capacities in endothelial cells from one patient in two biological replicates, as the cells of the majority of the patients did not continue to proliferate in culture. An innovative experimental protocol was applied for the derivation of endothelial cells for which no practical guideline is yet available. To improve the expansion of patient-derived endothelial cells, culture protocol optimizations may be required. The addition of more growth factors, in the current study we increased FCS to 12% in the culture medium, already improved the expansion of the primary cultures. Alternatively, CD31 selected cells from biopsies may be directly assessed for their sprouting capacity in the angiogenesis assay, provided that the biopsies contain a sufficient number of endothelial cells to generate spheroids. Future studies may focus on identifying and defining essential growth factors, which improve the isolation and expansion protocol. This study therefore serves as a first practical guide for other researchers aiming to derive endothelial cells from skin biopsies in CM patients and as a stepping stone for future studies.

In conclusion, our study showed that primary cells isolated from cutaneous CMs are able to expand while maintaining endothelial cell specificity, resulting in a valued new research model to assess the endothelial cell function of CMs. Using this model, we found that the angiogenic capacity of endothelial cells carrying a somatic GNAQ [R183G] mutation was enhanced compared to endothelial cells from normal skin tissue. Increased angiogenic activity may contribute to CM formation and/or progression. Future research efforts should focus on the use of patient-derived primary cells in the search for therapeutic treatments that restore endothelial function in CMs.

MATERIALS AND METHODS

Study design

The prospective case series was performed at the department of Dermatology of a tertiary vascular anomalies center at Amsterdam University Medical Centers (Amsterdam UMC) in Amsterdam, the Netherlands. The STROBE (Strengthening the Reporting of Observational Studies of Epidemiology) checklist for cross-sectional studies was followed (Cuschieri, 2019). The study adhered to the Declaration of Helsinki, and written informed consent was obtained from all patients. The study was approved by the Medical Ethics Committee from the Amsterdam UMC (Case number NL75128.018.20), and the study was registered at the National Trial Register in the Netherlands on February 23th 2021 (trial ID NL9295).

Participants

Study participants encompassed adult patients (>17 years of age) with a CM receiving laser therapy at the department of Dermatology at the Amsterdam UMC location AMC. CMs of all anatomical locations were eligible for inclusion; in patients with a facial CM the lesion had to extend into the hairline, so that the tissue biopsy could be taken from a non-visible site. Patients with a mix of vascular malformations were excluded from this study. Table 1 summarizes all patient eligibility criteria. For this explorative study, the aim was to include 20 patients in total, which was based on estimated patient flow and budgetary constraints.

Study outcomes

Intended study outcomes included the assessment of the histology, biochemical activity profile in endothelial lysates, endothelial barrier function, and angiogenic sprouting and scratch wound healing capacities of endothelial cells from CMs. Furthermore, we assessed the presence of somatic mutations in CMs, and explored its correlations with CM phenotypic characteristics.

Data and sample collection

Patients with a CM visiting the outpatient clinic for laser therapy between April 2021 and July 2022 were screened for eligibility. Before the laser sessions, patient and lesion characteristics (i.e. gender, age, Fitzpatrick skin type, previous therapies, lesion size, color, location, presence of soft tissue/bone overgrowth and nodules/blebs, telangiectasia) were collected. Of each patient, two skin tissue biopsies were taken from a not recently lasered area of the CM. Of these tissue biopsies, one biopsy of 3 mm in diameter was taken for histological and molecular analysis and preserved in saline until further processing. The second tissue biopsy of 4 mm was taken and preserved in EGM2 complete medium in an incubator at 37°C prior to endothelial cell isolation. Also, a control biopsy of normal skin (4 mm) was taken from two CM patients to enable comparison of cells from CM and healthy tissue. Next, laser sessions commenced, and the treatment course occurred as planned. Patients were followed up during their regular appointments for their CM laser treatment (approximately 3-4 months after the last treatment).

Next-generation sequencing (NGS) for genetic analysis

The 3 mm tissue biopsies were processed for histology, embedded in paraffin, and cut into coupes. Subsequently, DNA was isolated from the coupes using 400 µg proteinase K. The extracted DNA was analyzed with NGS for common mutations in vascular malformation-associated genes, including the *GNAQ*, *GNA11*, *PIK3CA*, and *RASA1* genes. These mutations are known from previously published studies on patients with CMs or vascular tumors (Cai et al., 2019; Couto et al., 2016; Fjær et al., 2021; Jansen et al., 2021; Shirley et al., 2013). Supplementary file 1 shows a complete overview of the applied gene panel (Supplementary file 1).

Histological analysis

CM tissue sections from the 3 mm biopsies were Hematoxylin and Eosin (H&E) stained. The histological images were assessed by an experienced pathologist and compared with histological images of healthy skin.

Endothelial cell isolation and culture

Endothelial cells were isolated from the 4 mm tissue biopsies, based on a previously developed protocol for low-flow vascular malformations (Kobialka et al., 2022). Cells were immediately isolated from the biopsies when obtained (of note, biopsy 15 was stored overnight in Endothelial cell Growth Medium 2 (EGM2) in an incubator at 37°C prior to processing). Biopsies were homogenized using scalpels and incubated with dispase II (0.050mg/ml; #04942078001 Roche Diagnostics GmbH) and collagenase A (10 mg/ml; #10103578001, Roche Diagnostics GmbH) in Hank's Balanced Salt Solution (no magnesium, no phenol red; #14175053 Gibco) supplemented with penicillin/streptomycin for 1 hour at 37°C. During the incubation the sample was vortexed every 10 minutes. Next, the tissue homogenate was mixed by pipetting using a P1000 pipette until aggregates were disintegrated. The sample was filtered over a 40 µm cell strainer and washed with Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal calf serum to deactivate the digestive enzymes. The cells were centrifuged at 1200 rpm for 5 minutes (RT), resuspended in 5 ml PBS + 0.5% BSA, centrifuged at 1200 rpm for 5 minutes, resuspended in 1 ml PBS + 0.5% BSA and transferred to a 1.5 ml tube, centrifuged at 1200 rpm for 5 minutes and resuspended in 100 µl PBS + 0.5% BSA. For each biopsy. 6.4 x 10^6 (16 µl) magnetic dynabeads (Pan mouse IgG, #11041, Invitrogen) were washed 5X in 1 ml PBS + 0.5% BSA and coupled to 2.5 µl anti-CD31 antibody (monoclonal mouse anti-human CD31 clone JC70A, #M0823, Agilent Dako) in 16 µl PBS + 0.5% BSA in low binding Eppendorf tubes for 1 hour at room temperature, protected from light while mixing the beads every 5 – 10 minutes by gently tapping the bottom of the tube. Beads were resuspended in 100 µl PBS + 0.5% BSA. Next, the bead suspension was added to the isolated cells and incubated for 1 hour, mixed head over head, at room temperature, protected from light. Following the incubation, the bead-cell suspension was resuspended in EGM2 and placed on a magnet. The supernatant (CD31-) fraction was taken in culture to obtain skin fibroblasts and the pellet (CD31+) fraction was

resuspended in EGM2 and cultured in 0.5% gelatin-coated 12-wells plates. Endothelial cells and fibroblasts were cultured in EGM2 supplemented with growth medium 2 supplement pack (PromoCell) and fetal calf serum (FCS). Initially, 2% FCS was used, which resulted in slow growth of the primary endothelial cells. As a result, the FCS concentration was adjusted to 12% FCS to improve growth of endothelial cells.

Immunofluorescence stainings

For immunofluorescence stainings, cells were cultured on 5 µg/ml fibronectin-coated coverslips. Cells were fixed by 10-minute incubation with 4% paraformaldehyde in PBS^{++} (PBS with 1 mM $CaCl_2$ and 0.5 mM $MgCl_2$). Fixed cells were permeabilized for 5 minutes with 0.5% Triton X100 in PBS and blocked for 30 minutes in 2% bovine serum albumin (BSA) in PBS. Antibody (AlexaFluor-647 conjugated anti-human VE-cadherin, clone 55-7H1, #561567, BD Biosciences diluted 1:100) and markers (AlexaFluor 568-Phalloidin diluted 1:1000, #A12380 and DAPI #1306 diluted 1:1000 were from Invitrogen) were diluted in PBS + 0.5% BSA and incubated for 45 minutes. Stained cells were washed three times with PBS + 0.5% BSA and coverslips were mounted in Mowiol4-88/DABCO solution (Sigma).

Microscopy

H&E slides were imaged using a Leica DM6 upright microscope with 20x objective. Cell cultures and sprouting assays were imaged with an EVOS M7000 imaging system using 4X and 10x objectives. Immunofluorescently stained samples were imaged using an inverted NIKON Eclipse TI equipped with a 20x objective, a lumencor SOLA SEII light source, standard DAPI, mCherry and Cy5 filter cubes and an Andor Zyla 4.2 plus sCMOS camera. Images are enhanced for display using ImageJ.

Sprouting assay

For the VEGF-induced sprouting angiogenesis assay, cells were resuspended in EGM-2 medium with 0,1% methylcellulose (4.000 cP, Sigma). Spheroids were formed by seeding 750 cells per 100 µl methylcellulose medium in a 96 U-bottom-wells plate and incubation for 24 hours at 37°C and 5% CO_2. Subsequently, spheroids were collected and resuspended in 1.7 mg/ml collagen type I rat tail mixture (#50201, ibidi) and plated in a glass bottom 96 wells plate as described previously (Korff and Augustin, 1999; van der Stoel et al., 2020). After stimulation with 50 ng/ml VEGF to induce sprouting, spheroids were incubated for 24 hours at 37°C and 5% CO_2. Sprout length was assessed using the ImageJ plugin NeuronJ (Meijering et al., 2004) and sprout number was counted manually.

Statistical analysis

Categorical data are expressed as numbers (n) and percentages (%), continuous variables are presented as means with standard deviations. All dot graphs represent the mean ± SD. Data was checked for normal distribution by visually inspecting histograms and qq plots. Data was

analyzed using Prism GraphPad V9 and statistically analyzed by an unpaired parametric Student's T-test.

Data availability statement

Datasets from this study are available upon request to SH.

Competing interests

The authors declare no competing interests.

Author contributions

G.B.L., M.S., C.v.d.H., A.W. and S.H. conceived and designed the study. G.B.L. and M.S. took the biopsies from patients and performed clinical assessments. G.B.L., M.S., S.E.R.H., C.v.N. and S.H. analyzed sequencing and histological data. A.d.H. and V.J. isolated cells from biopsies and performed the cell-based in vitro experiments and analyzed the data. M.G. contributed to the development of the methodology for isolation of patient-derived cells. C.v.d.H., A.W. and S.H. supervised the research. G.B.L., M.S., and S.H. wrote the manuscript. All authors reviewed and edited the manuscript.

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Supplementary Materials

Supplementary file 1. Gene panel applied for Next-Generation Sequencing

AKT1
AKT2
AKT3
BRAF
FGFR2
FGFR3
GNA11
GNA14
GNAQ
GNAS
HRAS
IDH1
IDH2
KRAS
KRT1
MAP2K1
MTOR
NRAS
PIK3CA
PIK3R2
PTEN
RASA1
TEK

Chapter 5

The long-term progression of macrodactyly.

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Abstract

Background: Macrodactyly is a rare congenital disorder of overgrowth affecting the digits of the upper or lower extremity. Mostly, patients are surgically treated during childhood to reduce the digit or to stop growth. There are no standardized guidelines for treatment and follow-up of macrodactyly. Consequently, follow-up may not be regularly scheduled into adulthood.

Methods: A retrospective, descriptive analysis of patients with long-term progression of macrodactyly who presented at our tertiary referral hospital between July 2018 and March 2020 was performed. All patients from our local macrodactyly database were screened for progression of macrodactyly since adulthood; this resulted in four patients. The aim of these case series is to highlight the clinical features and disease course at long-term follow-up.

Results: All patients were surgically treated during childhood and showed progression of tissue overgrowth during adult life. All patients developed severe secondary degenerative bone changes in macrodactyly affected digits, such as ankyloses of joints, new bone formation, and bony spurs. Subsequently, tissue overgrowth and degenerative bone changes led to functional problems.

Conclusion: Patients with macrodactyly may experience growth during adult life, which may progress to deforming changes. Consequently, patients should be informed about the possible growth and the progressive growth should be monitored.

Keywords: Macrodactyly; macrodystrophia lipomatosa; overgrowth; PIK3CA

Introduction

Macrodactyly is a rare congenital disorder of overgrowth affecting the digits of the upper or lower extremity.[1] Digital enlargement may involve all types of mesenchymal tissue, which involve muscle, bone, and predominantly fibro-adipose tissue. Not only fingers and toes, also adjacent parts of the hand or foot may be affected. Macrodactyly can be classified by the rate of growth, either static, growing proportionally with the hand or foot, or progressive, growing faster than the rest of the limb. Thus, macrodactyly encompasses a wide range of clinical phenotypes, with the growth rate, location, and extent of overgrowth varying greatly between patients.

Patients may encounter functional problems and difficulty in walking due to enlargement of the foot in length and width.[2] Additionally, complaints of cosmetic disfigurement are often experienced.[3] At a later stage, secondary functional problems may develop such as secondary osteoarthritis and compression of neurovascular structures.[4]

Recently, somatic gain-of-function mutations in the PIK3CA gene were detected in the affected tissue in patients with macrodactyly.[5-8] Mutations in the PIK3CA gene were also found in several overgrowth disorders, which are now grouped as PIK3CA-Related Overgrowth Spectrum (PROS) disorders.[9,10]

Current medical management of macrodactyly is mainly ablative and not based on molecular targets. Treatment consists of debulking a part of the enlarged digit, epiphysiodesis to stop longitudinal skeletal growth, and amputation when the enlarged parts are no longer functional.[11] However, due to the rarity of the disease and the highly variable manifestations of macrodactyly, there are no standardized guidelines for treatment and follow-up of macrodactyly.[11] As often a stable situation is achieved during childhood, follow-up may not be regularly scheduled into adulthood. However, to date, little is known about the long-term disease course of macrodactyly and the possible progression of overgrowth. The aim of this study is therefore to highlight the clinical features and disease course of macrodactyly at long-term follow-up.

Methods

Patients with long-term progression of macrodactyly who presented at our tertiary referral hospital between July 2018 and March 2020 were included. All patients from our local macrodactyly database were screened for progression of macrodactyly since adulthood; this resulted in four patients. Data was extracted from the electronic patient files on: age, gender, anatomical location of macrodactyly, presence of symptoms, comorbidities, previous treatments, imaging results, histopathology results, and genetic test results. Our institutional review board approved a waiver of consent for this study and written informed consent was obtained from all patients. The guidelines of the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) Statement were followed.[12]

Results

All patients are listed in Table 1.

Table 1. Patient characteristics

Case (gender, age)	Affected body part	First surgery	Age at first surgery	Additional surgeries	Age at additional surgeries	Reason for consultation	Imaging	Following treatment	Genetic analysis
Case 1 (male,59)	1st, 2nd, 3rd toe of the left foot	Amputation 1st, 2nd toe through MTP-joint	1	Soft tissue debulking	3, 4, 12, 17	Plantar swelling and functional problems	Bony spurs MT II towards plantar side and synostosis with MT I. Ankylosis Lisfranc and partial ankylosis Chopart.	Amputation through the Chopart joint (planned)	Not yet
Case 2 (female, 33)	1st, 2nd toe of the left foot	Amputation 2nd toe through MTP-joint	7	None	-	Progressive growth of the 1st toe.	1st toe showed bone overgrowth and bone deformation of the phalanxes and around MTP-joint. Ankylosis IP-joint.	Removal bone exostosis and soft tissue debulking (planned)	Not yet
Case 3 (female, 44)	2nd and 3rd toe of the left foot	Amputation 3rd toe through MTP-joint	4	None	44	Progressive growth of the 2nd toe and pain forefoot	Bone deformation MT and proximal phalanx of 2nd toe. Ankylosis MTP-joint of the 2nd and 3rd toe.	Amputation 2nd toe and removal bone deformation	PIK3CA mutation
Case 4 (female, 47)	Thumb and index finger of the right hand	Soft tissue debulking of the thumb	18	Shortening proximal phalanx, arthrodesis IP and MCP-joint CTR and correction osteotomy thumb	38 38	Progressive swelling and worsening of function of the thumb and index finger	Juxta-articular new bone formation CMC, MCP and PIP-joint of the thumb and index finger, and around scaphoid, trapezium and trapezoid.	Splint	No

D = digit, MTP = Metatarsalphalangeal, MT = Metatarsal, IP = Interphalangeal, CMC = Carpometacarpal, MCP = Metacarpophalangeal, PIP = Proximal Interphalangeal.

Case 1

A 59-year old male with macrodactyly of the first, second and third toe of the left foot presented at our hospital. At the age of 1, the first and second toe were amputated through the metatarsophalangeal (MTP) joint. At the age of 3, 4, 12 and, 17 years he underwent additional soft tissue debulking surgery. These procedures took place at another hospital, thus comprehensive information is lacking.

At the age of 54, he noted severe enlargement of the left forefoot. He experienced a total loss of sensory function of the forefoot and therefore did not feel any pain. He encountered functional problems and was unable to walk far distances. Additionally, he suffered from recurrent erysipelas of the foot, once complicated with osteomyelitis. On clinical examination, a deformed left forefoot was seen with a massive swelling at the plantar side and substantial hyperkeratosis. Also, he developed psoriatic lesions on the medial and lateral side of the foot (Figure 1).

Conventional radiograph and Computed Tomography (CT) scan of the left foot showed substantial hypertrophy of fat tissue, muscles and bone tissue in comparison with the right foot. In particular, metatarsal II showed bone overgrowth with bony spurs towards the plantar side and synostosis with metatarsal I. Ankyloses of the Lisfranc joint was visible between the medial, intermediate and lateral cuneiform bones and metatarsals I,II and III. Additionally, ankyloses of the Chopart joint was visible, with partial fixation between the talus and navicular bone and partial fixation between the calcaneus and cuboid bone (Figure 2&3).

Because of the substantial growth of his left foot and mobility disabilities, he needs additional surgical treatment. Currently, a Chopart amputation is planned, because of the best functional advantages.

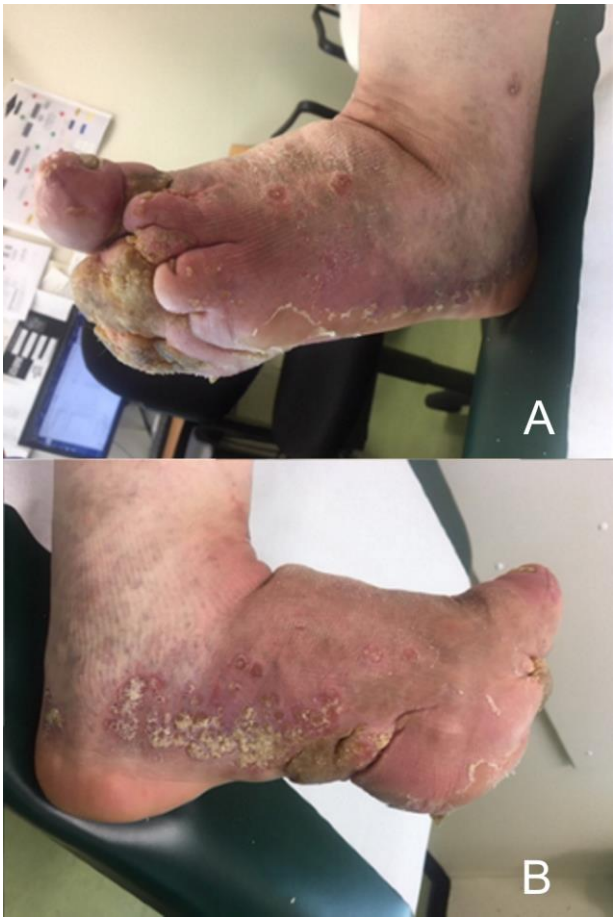

Figure 1 – Lateral (A) and medial (B) view of the left foot with macrodactyly of case 1. Note the massive plantar swelling, hyperkeratosis and psoriatic lesions.

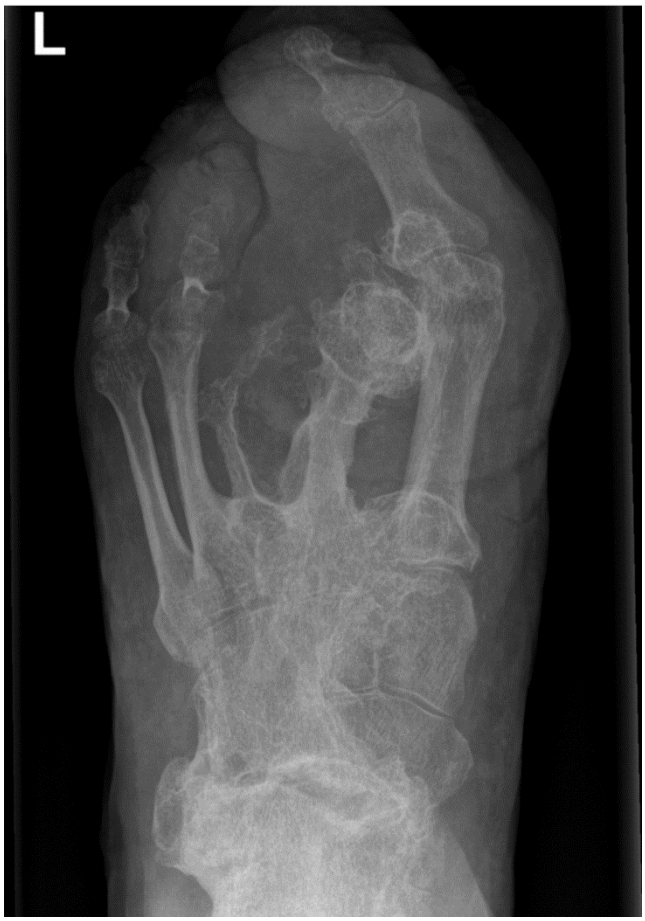

Figure 2 – Conventional A-P radiograph of the left foot of case 1 showing a synostosis between metatarsal I and II.

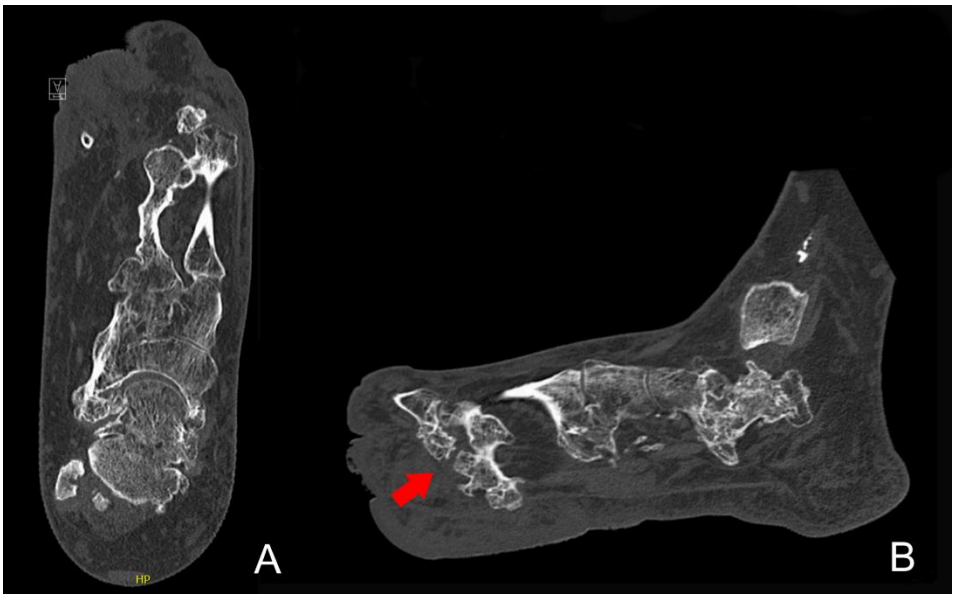

Figure 3 – CT-scan of the left foot of case 1. 3A: Transversal view showing a synostosis between metatarsal I and II. 3B: Sagittal view with a visible bone spur from metatarsal II towards the plantar side (arrow).

Case 2

A 33-year old female with macrodactyly of the first and second digit of the left foot. At the age of 7, the second toe was amputated through the MTP joint and the soft tissue of the first toe was debulked.

Since age 30, the first digit grew progressively. She felt slight discomfort lateral of the second digit without experiencing any real pain. On clinical examination, the first digit was enlarged with a substantial swelling of the plantar side (Figure 4). The MTP joint and the interphalangeal (IP) joint were unable to move. Imaging showed an enlarged and deformed first digit with ankyloses of the IP joint and striking soft tissue overgrowth. Further, a severe bone deformation was visible, especially of the phalanxes and around the MTP joint (Figure 5).

Because of the rapid growth of the first digit and the additional functional problems, she will undergo another surgery.

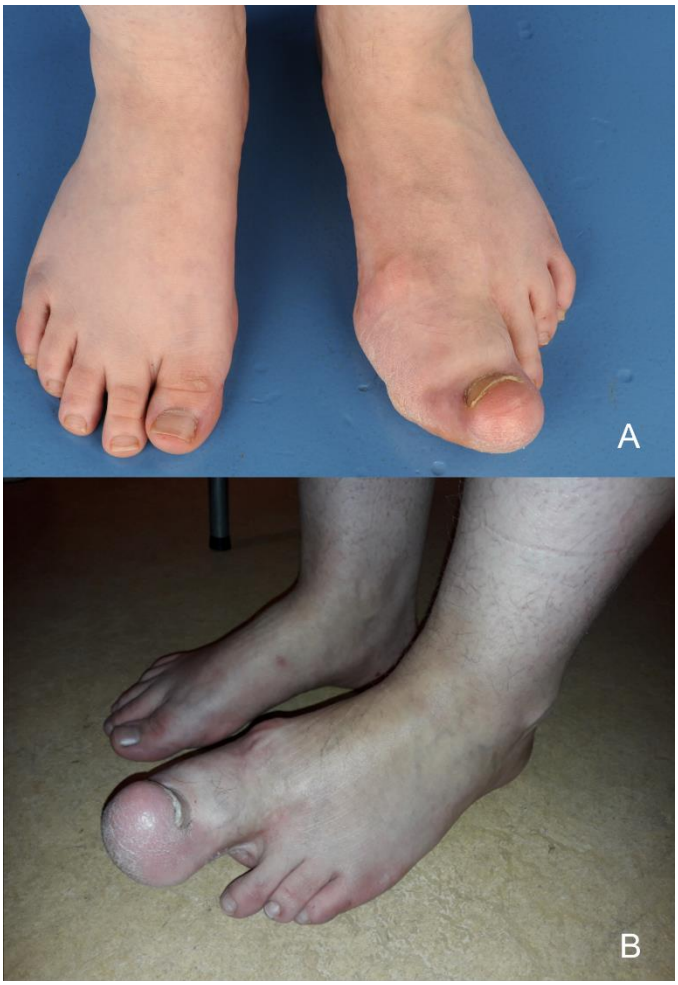

Figure 4 – Macrodactyly of the first digit of the left foot of case 2. Progression of overgrowth between age 26 years (4A) and age 31 years (4B).

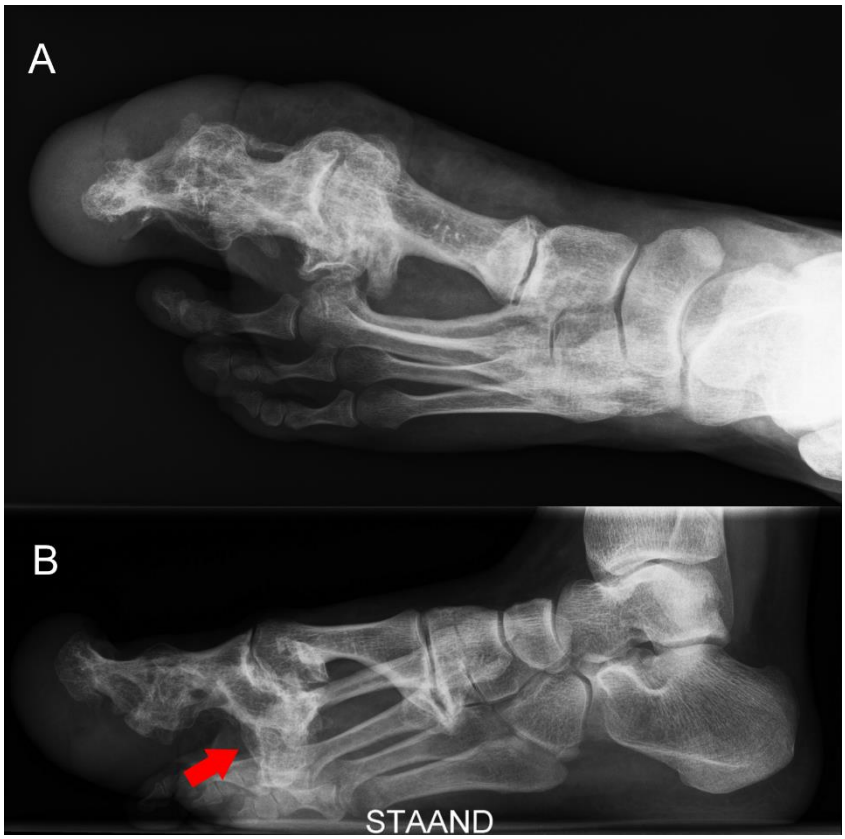

Figure 5 – Conventional radiographs of the left foot of case 2. A: A-P radiograph showing significant bone overgrowth and deformation of the phalanxes, and around the MTP-joint of the first toe. B: lateral radiograph in standing positions, notice the substantial bony formation on the plantar side of the foot (arrow) and thereby tilt of the foot.

Case 3

A 44-year old female with macrodactyly of the second and third toe of the left foot and concomitant syndactyly between these toes. Previously, at the age of 4 years, the third toe was amputated through the MTP joint and simultaneously the syndactyly was resolved.

Several years before she visited our clinic she experienced increasing pain in her forefoot and was unable to wear her orthopedic shoes because of fast growth of the foot. The pain occurred dorsal and lateral of the second toe and radiated to the fourth and fifth toe. On clinical examination, her second toe was enlarged with a plantar swelling (Figure 6) and her left foot was three cm wider in comparison with her right foot. Her gait was impaired by the enlargement of her second toe, which was not able to touch the ground. Conventional radiographs of the left foot showed severe osteoarthritis and ankyloses of the MTP joint of the second and third toe. Additionally, exostosis of the metatarsal II towards the third toe was visible (Figure 7).

Considering the progressive growth of the second toe and accompanying complaints, she underwent further surgery of her left foot. The second toe was amputated distally from the MTP joint and osteoarthritic deformed bone was removed. The second toe was sent in for genetic analysis, which showed a somatic mosaic mutation in PIK3CA. The pathogenic variant c.3140A>T p.(His1047Leu) was detected with a variant allele frequency (VAF) of 25%. The surgical intervention led to pain reduction and increased functioning of her left foot.

Case 4

A 47-year old

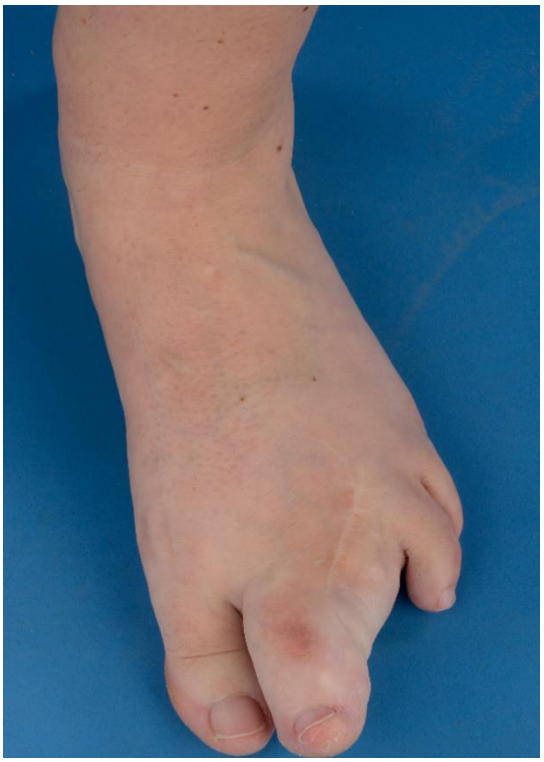

Figure 6 – Left foot of case 3 with macrodactyly of the second toe.

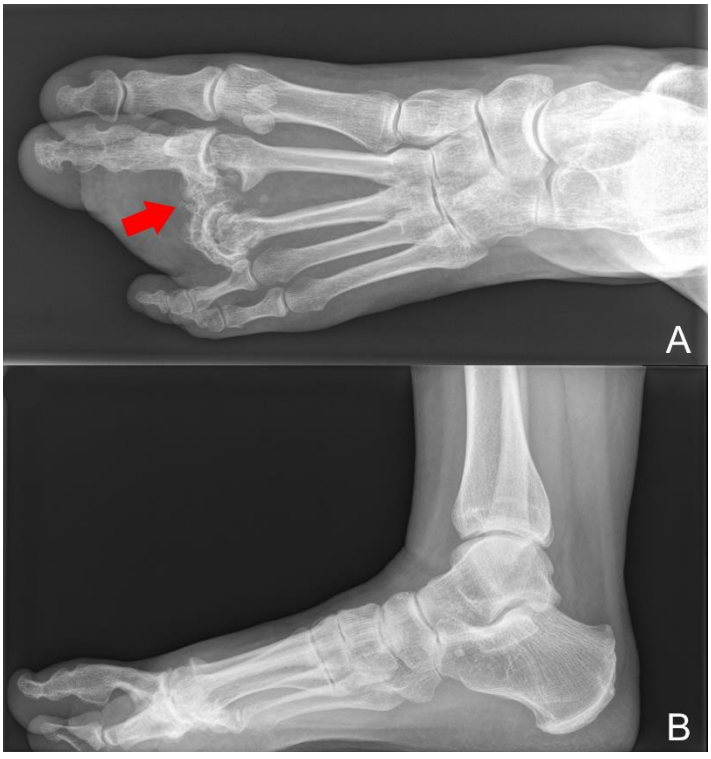

Figure 7 – Conventional radiographs of the left foot of case 3. 7A: A-P radiograph showing bone exostosis and osteoarthritic deformed bones of the second and third toe (arrow). 7B: lateral radiograph showing elevation of the second toe because of the plantar swelling.

female, known with macrodactyly of the thumb and index finger of the right hand. At the age of 18, the first surgery took place with soft tissue debulking of the thumb. However, post-operatively she experienced more functional problems. At the age of 38, she underwent further surgery of the thumb including soft tissue debulking, shortening of the proximal phalanx, and arthrodesis of the IP joint and metacarpophalangeal (MCP) joint. In the same year, a carpal tunnel release and a correction osteotomy of the thumb was performed, which led to an improved position of the IP joint arthrodesis. Nevertheless, she noticed an increase in swelling and tingling of the thumb and index finger.

At age 47, she presented at our hospital because of the progression of swelling and worsened functioning of the thumb and index finger. Since a few months, her thumb, index finger and wrist were painful. On examination, a swelling was seen of the volar, radial side of the hand. Solid nodules were present at the MCP joint and proximal interphalangeal joint (PIP) of the index finger. Her wrist showed a position of 20 degrees ulnar deviation, and flexion and extension were limited.

Conventional radiographs of her right hand showed complete consolidation of the IP and MCP joint arthrodesis of the thumb. Remarkable juxta-articular new bone formation was present in the thumb and index finger of the carpometacarpal (CMC) joint, MCP joint, and PIP joint (index finger). Also, new bone formation was visible of the scaphoid, trapezium, and trapezoid (Figure 8).

Given her functional problems, she was referred to a rehabilitation specialist. Subsequently, a splint was made for her right hand to improve her abilities. Additionally, she was referred to a clinical geneticist. However, she was lost in follow-up and genetic analysis of the affected tissue did not take place.

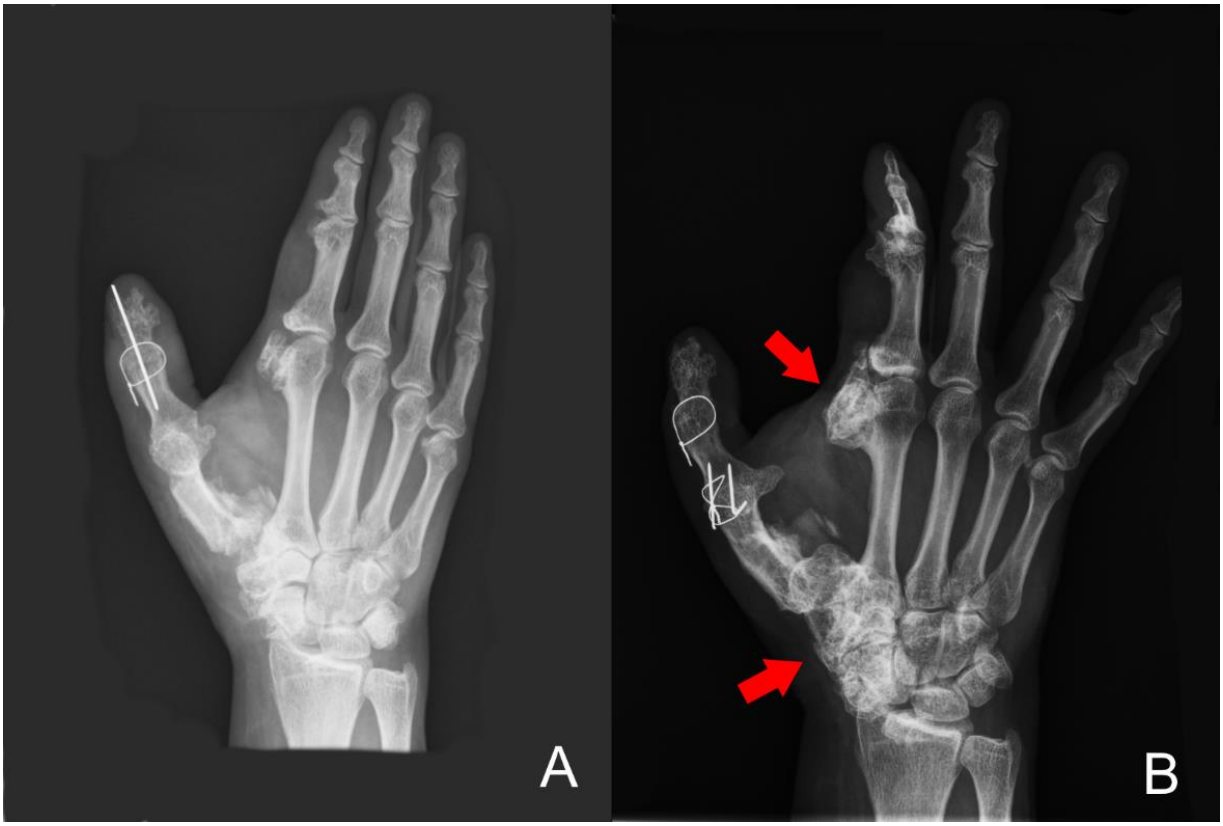

Figure 8 – Conventional radiograph of the right hand of case 4, showing progression of juxta-articular new bone formation between age 38 (8A) and age 47 (8B). In particular, the thumb and index finger (upper arrow) and the scaphoid, trapezium, and trapezoid (bottom arrow) show severe progression.

Discussion

Four macrodactyly cases with severe long-term progression of overgrowth were described. All patients experienced rapid growth of the affected digit long after treatment. The results of this study show that tissue overgrowth can progress excessively during adult life. The long-term follow-up of these cases of macrodactyly provides useful insights into the condition.

A remarkable finding was the degenerative and deforming bone changes. The phalanges and metacarpals in our patients were expanded and deformed at their distal ends. This may be explained by the periosteum being studded with nodules consisting of chondroblasts and osteoblasts, which are more numerous towards the end of the phalanges and account for the distal osseous enlargement.[13] Furthermore, our cases demonstrated new bone formation and the development of bony spurs. A possible explanation for these bony changes could be a misalignment of articular surfaces, which results in severe secondary degenerative joint changes with new bone formation.[14]

In particular, patient 1 developed exceptional bony changes, however, other factors may have contributed to the deformation of the foot. Chronic osteomyelitis may lead to reactive new bone formation, bone deformation, and ankylosis.[15] Other longstanding cases, described in literature, showed comparable severe bone changes and newly formed bone synostosis between multiple digits.[16,17] It is unclear if surgical treatment during childhood also contributes to the expanded growth and degenerative bone changes. All of our cases were surgically treated during childhood and showed severe overgrowth during adult life. However, patient 4 showed also osteoarthritic changes and juxta-articular new bone formation of unoperated areas, such as the index finger and carpal bones.

Not all patients with macrodactyly develop secondary degenerative joint changes. Ishida et al. investigated long-term term results of surgical treatment for macrodactyly of the hand.[18] They reported that 2 out of 23 patients developed early degenerative changes in the affected joints, after a mean follow-up of 23 years.

Unilateral involvement in macrodactyly is the most common, which was also noted in our patients. Hands and feet are affected with almost equal frequency. In both hands and feet, third digit involvement is the most prevalent, followed by second digit enlargement.[8] In macrodactyly patients, syndactyly, polydactyly, and clinodactyly may simultaneously be present,[8] as was also seen in patient 3 with syndactyly.

For a long time, the etiopathogenesis of macrodactyly was poorly understood. However, now it is clear that postzygotic somatic mutations in the PIK3CA/AKT/mTOR pathway may be a cause of macrodactyly.[5] This pathway is involved in cell signaling, cell growth, differentiation, and proliferation. PIK3CA encodes the p110α catalytic subunit of phosphoinositide 3-kinase (PI3K), which activates AKT and mTOR signaling to promote tissue growth.[19]

However, between patients, growth trajectories vary greatly for unknown reasons. While some patients exhibit excess growth limited to childhood, others have progressive tissue growth during adult life. As the somatic mutation in PIK3CA remains present in the affected tissue, this may promote tissue growth continuously. Eventually, resulting in advanced stages

of overgrowth. These cases are an example of continuous growth during adult life and illustrate the deforming changes the overgrowth can entail.

After the discovery of somatic mutations in isolated macrodactyly[6], PIK3CA mutations were observed in multiple patients with macrodactyly. Wu et al., identified a PIK3CA mutation in nine of twelve patients, indicating that a high proportion of isolated macrodactyly patients carry a pathogenic PIK3CA mutation.[7] However, a negative result of PIK3CA mutations does not necessarily exclude the presence of a PIK3CA mutation. The patient may have another mutation beyond the targeted genomic regions or the mutation may be below the detection rate. In macrodactyly the highest mutation detection rate is found in adipose tissue, followed by nerve and skin tissue.[7]

PIK3CA somatic mutations have been found in various overgrowth disorders, such as megalencephaly-capillary malformation syndrome, muscular hemihypertrophy, Klippel-Trenaunay syndrome and CLOVES syndrome.[20-22] The presenting phenotype of the PIK3CA-Related Overgrowth Spectrum disorders seems to depend on the timing of the somatic mutation, the tissue localization of the mutations, and the location of the mutation in the embryo. Mutations that occur early during embryogenesis will generate many affected daughter cells, potentially of distinct differentiation routes (stroma, fat, smooth muscle, endothelium, etc.), which may result in larger and multiple body segments that are affected, such as in CLOVES syndrome.[10] A mutation later in embryogenesis will produce lower numbers of mutated cells and yield smaller lesions, such as in macrodactyly.

There exist several limitations in these case reports. These cases are not representative of all longstanding macrodactyly cases. However, this is inherent to the study design, since primarily patients who experience complaints or growth of macrodactyly would revisit the outpatient clinic. Secondly, the natural course of the disease could not be assessed, as the patients visited the outpatient clinic a couple of years after they experienced growth. Yet, this is unavoidable since patients are not monitored regularly during adult life. Unfortunately, we did not have genetic information on all patients. Two patients will undergo surgery soon, where the tissue will be taken for genetic analysis.

In conclusion, our study shows that tissue overgrowth can continue and progress excessively in patients with macrodactyly without monitoring during adult life. Somatic mutations in PIK3CA were identified in patients with macrodactyly and may be responsible for this continuous growth. Clinicians should be aware of the deforming changes the progressive overgrowth can entail. Monitoring during adulthood may lead to earlier intervention, which may prevent excessive overgrowth and may preserve function. Therefore, clinicians should inform patients that growth may occur in a later stadium, and patients should receive instructions to revisit the outpatient clinic if worsening of function or growth occurs. Currently, no follow-up guidelines exist. Monitoring patients with macrodactyly regularly every three years is recommended.

Follow-up should consist of an evaluation of the function, size and degenerative changes of the affected digits by physical examination and conventional radiography.

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Part III

Development and quality assessment of condition-specific patient-reported outcome measures in patients with peripheral vascular malformations.

Chapter 6

Development of a condition-specific patient-reported outcome measure for measuring symptoms and appearance in vascular malformations: the OVAMA questionnaire

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Summary

Background

The symptoms and appearance of vascular malformations can severely harm a patient's quality of life. The aim of treatment of vascular malformations generally is to improve condition-specific symptoms and/or appearance. Therefore, it is highly important to start testing treatment effects in clinical studies from the patient's perspective.

Objectives

The objective of this study was to develop a patient-reported outcome measure (PROM) for measuring symptoms and appearance in patients with vascular malformations.

Methods

A first draft of the PROM was based on the previously internationally developed core outcome set. The qualitative part of this study involved interviews with 14 patients, which led to a second draft. The second draft was field-tested cross-sectionally, after which groups of items were evaluated for adequate internal consistency (Cronbach's alpha >0.7) to form composite scores. Construct validity was evaluated by testing 13 predefined hypotheses on known-group differences.

Results

The patient interviews ensured adequate content validity and resulted in a general symptom scale with 6 items, head/neck symptom scale with 8 items and an appearance scale with 9 items. Cronbach's alpha was adequate for two composite scores: a general symptom score (0.88) and an appearance score (0.85). Ten out of 13 hypotheses on known-group differences were confirmed, confirming adequate construct validity.

Conclusions

With the development of the OVAMA questionnaire, outcomes of patients with vascular malformations can now be evaluated from the patients' perspective. This may help improve the development of evidence-based treatments and the overall care for patients with vascular malformations.

What's already known about this topic?

- Vascular malformation symptoms and appearance may severely impact the patient's physical, mental and social functioning
- Condition-specific symptoms and appearance are the main drivers for treatment of vascular malformations
- Symptoms and appearance are determined to be core outcome domains and should be measured in all clinical research on vascular malformations
- No instrument exists for measuring patient-reported symptoms and appearance problems in vascular malformations
- Vascular malformation research is hampered by heterogeneity in outcome measures

What does this study add?

- With this study, a condition-specific patient-reported outcome measure was developed for measuring symptoms and appearance in patients with vascular malformations: the OVAMA questionnaire
- This study confirms adequate content and construct validity

What are the clinical implications of this work?

- Problems that matter most to patients with vascular malformations can now be evaluated from the patients' perspective
- Treatments can be evaluated and compared for effects on these core outcome domains
- This study is a big step in tackling current heterogeneity in outcome measures
- Clinically distinct groups can be determined based on disease severity
- The many applications of the OVAMA questionnaire may significantly improve research, and ultimately, the care for patients with vascular malformations

Introduction

Vascular malformations are congenital deformities, characterized by dilated and tortuous vessels. These benign tangles can occur anywhere in the soft tissues, grow proportionally with the body, and are often visible as a mass differing in colour and texture compared to normal skin. Subtypes are distinguished by the kind of vessel involved: capillary (CM), venous (VM), lymphatic (LM), arteriovenous (AVM) and combined malformations.[1,2]

Clinical presentation varies widely depending on type, localization, extensiveness and involved tissues. Apart from a distorted appearance, patients frequently experience pain, swelling, bleeding, fluid leakage, physical impairment and functional problems.[2-4] These symptoms can severely harm the patient's quality of life, impacting physical, mental and psychosocial well-being.[5] The aim of treatment is generally to improve condition-specific symptoms and quality of life. Treatment can additionally be imperative to preserve or recover vital functions. However, despite the abundance of treatment options, treatment remains challenging as it rarely leads to a complete cure. Many vascular malformations can therefore be seen as a chronic condition, with patients experiencing lifelong symptoms and appearance issues.

A strong contributing factor to current treatment difficulties is the lack of knowledge on the treatments' effect from the patient's perspective.[4] Additionally, contemporary evaluation of treatment is impeded by heterogeneous outcome measures.[4,6,7] This hampers the development of evidence-based treatments and treatment guidelines, which are urgently needed to improve outcomes for patients with vascular malformations.

The mission of the Outcome measures for VAscular MAlformations (OVAMA) project is to establish homogeneity in outcome use and reporting. This collaboration includes clinical experts and patient/parent contributors from all over the world. The first step was deciding what to measure. In previous studies, the OVAMA collaborative developed a core domain set (CDS) for evaluating treatment in vascular malformations (Figure 1).[8,9] A CDS is a set of outcome domains that should be measured at the minimum when evaluating treatment effect in a certain health condition.[10]

The next step towards homogeneity in outcome use and reporting was determining how to measure these core domains. Non-condition-specific domains are advised to be measured by noncondition-specific outcome measurement instruments.[11] However, broadly used instruments such as the Short Form-36 and Skindex-29 seem to fall short for detecting changes in outcome over time in this specific patient population.[12] Newer instruments such as the PROMIS (Patient-Reported Outcomes Measurement Information System13) item banks may be used in this patient population as they are more likely to adequately capture small differences in the domains falling under quality of life.[14,15] To fully capture those domains, the following PROMIS scales were identified: 'pain interference', 'physical functioning', 'anxiety', 'depression' and 'social participation'.

However, no patient-reported outcome measures (PROMs) were available for the patient-reported domain categories 'symptoms', 'anatomy' (including appearance) and 'satisfaction'.[16] We therefore developed a condition-specific PROM to measure vascular

malformation symptoms and appearance, called the OVAMA questionnaire. Satisfaction with treatment and outcome is only relevant at follow-up and thus follows a different development process on which we will report in a separate publication ('OVAMA follow-up questionnaire'). It is highly important to start testing treatment effect in clinical studies from the patient's perspective since the aim of treatment of vascular malformations is to improve the patient's symptoms or appearance-related issues. Here we report on the development and field-test of the OVAMA questionnaire, measuring symptoms and appearance in vascular malformations.

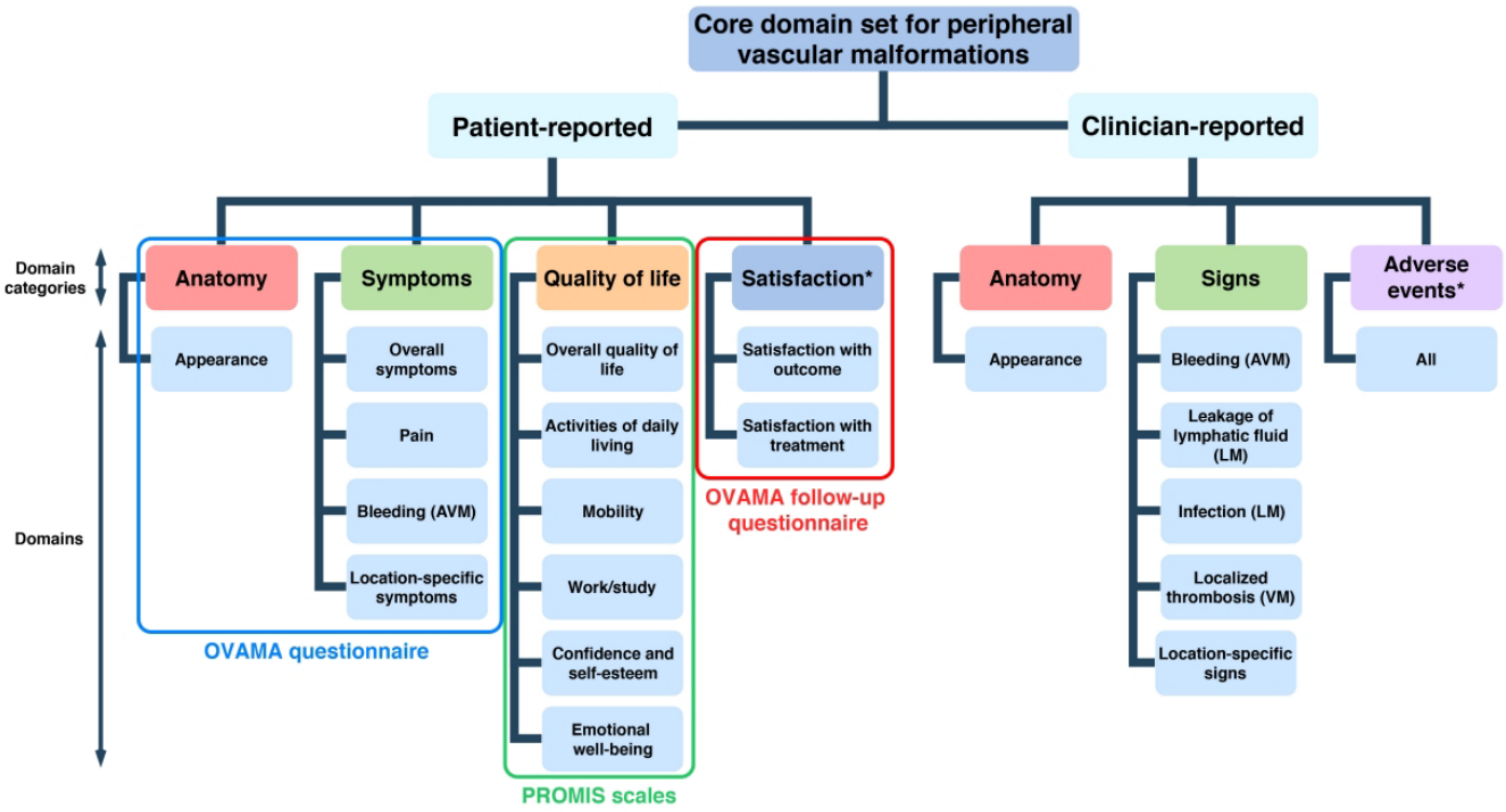

Figure 1 - Core domain set for vascular malformations. AVM = arteriovenous malformation, LM = lymphatic malformation, VM = venous malformation.

Methods

The COSMIN (COnsensus-based Standards for the selection of health Measurement Instruments) 'study design for PROMs' checklist was followed for this study.[17] This study adhered to the Declaration of Helsinki, and was exempted from full ethical review by the Medical Ethics Committee of the Amsterdam UMC, since patients were not subjected to interventions or rules of conduct. Informed consent was obtained from all participants. A flowchart of the methods is presented in Supplement 5.

First draft development

Concepts of interest were identified in previous studies.8,9,16 At first, the literature was searched extensively to determine all outcome domains measured in research on peripheral vascular malformations.[16] Based on these outcome domains, via an international e-Delphi study and two consensus meetings, a CDS was developed wherein outcome domains were defined (Figure 1).[8,9] In total, 167 physicians and 134 patients/parents of younger patients participated to ensure inclusion of the patient's perspective.

No instruments were available for the condition-specific domains falling under 'anatomy', (including 'appearance') and 'symptoms' (including 'pain', 'location-specific symptoms', and 'type-specific symptoms'). Hence, based on these core domains, a first Dutch draft of the OVAMA questionnaire was made with the vascular anomaly expert group of the Amsterdam UMC. It followed the definitions of the domains as determined in the first consensus study and consisted of 5 items on vascular malformation symptoms, 9 items on head/neck symptoms and 7 items on appearance. Symptom items were structured in a way that a patient first answers if they experienced the symptom in the past 4 weeks. If yes, two additional items were presented on frequency and severity, as was determined in the first international consensus study.[8]

Second draft development: concept elicitation and cognitive interviews

Hybrid concept elicitation with cognitive interviews were conducted in patients with vascular malformations. This allowed for immediate matching of emerging concepts of interest to the concepts already included in the first draft.[11] Participants were recruited at the outpatient clinic and from the vascular malformation database of the Amsterdam UMC. Demographic data were collected on age, gender, ethnicity, level of education, type of vascular malformation, lesion localization, lesion size, tissue involvement of lesion, previous treatments. Regarding sample size for the interviews, 5-10 participants were considered sufficient according to a rare disease PROM workgroup and ≥7 according to the COSMIN guidelines.[11,17] At first, 11 patients ≥18 years with a diagnosed peripheral vascular malformation were interviewed. Secondly, 3 adolescents (age 14-17) were additionally interviewed to evaluate if the concepts of interest were the same and/or if the items were also comprehensible for this age group. We aimed for a heterogeneous group by including at least one of the following subtypes: venous, arteriovenous, lymphatic, capillary, combined; one of the maximal diameter categories: <5, 5-15, 15-30, >30 cm; one of the localization categories: head/neck, upper extremity, trunk, lower extremity; and one of the

tissue involvement categories: skin/subcutaneous, muscle, bone. All interviews were conducted by two Medical Doctors, both conducting a PhD on outcome measures in vascular malformations; M.M. Lokhorst (male) and M.L.E. Stor (female). Both were trained in conducting qualitative interviews. A semi-structured interview guide was followed with a standard set of questions.

The first part of the one-on-one, in-person or telephone-based, interviews involved concept elicitation. The interviewer asked open-ended questions to identify the most important problems each patient experiences from their vascular malformation. Further open-ended questions were directed at what patients considered the most important aspects of the spontaneously raised concepts of interest and of the previously established concepts of vascular malformation symptoms (including pain, bleeding, fluid leakage and location-specific symptoms) and appearance.

The second part involved cognitive interviews during which the patients extensively reviewed the draft. Patients evaluated the appropriateness of concepts of interest, domains, items, response options, recall period, and ability to understand the instructions, items and response options. Only the patients in which the head/neck area was affected reviewed the head/neck symptoms scale.

The interviews were then coded by two independent researchers (M.L. and M.S.). All concepts were coded and it was scored if a concept was mentioned by the patient spontaneously, after probing or when reading the questionnaire.

After each interview, recall periods, wording of the items and response options were changed according to relevant patient feedback. All interviews were audio-recorded and transcribed. The version after the last interview was translated to English (using two forward and two backwards translations) and evaluated by the international OVAMA Steering Group, after which the second draft was finished in both Dutch and English.

Field-testing the second draft

The Dutch second draft was distributed among patients who were identified through the vascular malformation database of the Amsterdam UMC. Adult patients and parents of children with a vascular malformation received an invitation by email to complete the questionnaire on the KLIK PROM portal. This is an online secure platform for patients to fill in PROMs and to receive feedback of their scores using a personal account.[18] Parents of children 14-17 years old were instructed to let the child complete the questionnaire themselves. Parents of children 0-13 years old were instructed to help their child (where needed). The version for children (0-17) only differed from the adult version in form of address (informal and formal). Patients completed the vascular malformation symptom scale, appearance scale and if the head/neck region was affected, also the head/neck symptom scale. If the patients created an account on KLIK but did not fill in the questionnaire, they received a reminder after seven days. Descriptive statistics were analysed for each item individually. All items were scored ordinally. Most items refer to a separate outcome domain and should therefore be evaluated individually. However, we additionally evaluated if groups of items had adequate internal consistency (Cronbach's

alpha >0.7) to also form a composite score. Such composite scores may function as a quick indication for disease severity. The following groups of items were analysed: 1. all items from the general symptoms scale, 2. severity and frequency items for every single symptom individually, 3. all items from the head/neck symptoms scale, 4. all items from the appearance scale. If internal consistency was adequate to form a composite score, the scores were converted to a 0-100 scale for easy interpretation (in which higher scores mean more symptom severity).

Construct validity (known-groups validity)

Beforehand, hypotheses on differences in outcome between known-groups were defined (Table 1). Definition of known-groups was based on clinical characteristics (such as lesion localization or maximal diameter as measured with MRI) and clinician-reported outcomes (such as clinician-reported presence of pain in the medical file) from our vascular malformation database. Hypotheses on clinical characteristics were formulated based on common knowledge and patterns we encountered in our database.[1,2,19]

All data were analysed with IBM SPSS (version 26).

Results

Concept elicitation and cognitive interview results

Fourteen patients were interviewed, of whom the baseline characteristics are shown in Table S1 (see Supporting Information). An overview of the interview results is shown in Table S2 (see Supporting Information). The interviews showed that pain and appearance were the most relevant concepts according to patients. Bleeding, fluid leakage and several head and neck symptoms were also mentioned spontaneously by patients in the concept elicitation phase of the interview. It became apparent that temporary enlargement of the vascular malformation, which was not yet included, was a major problem for patients. Regarding appearance, most patients thought of the swelling or mass of the lesion as the major aspect of appearance, followed by colour and texture. Additionally, being stared at by other people appeared to be a major problem related to appearance. Patients mentioned that several of these issues were generally not discussed by physicians during regular follow-up, although they are important to their daily functioning.

After probing or during the revision of the questionnaire, all items were noted to be relevant by patients except for problems with the sense of smell. This item was therefore removed. No further concepts of interest were identified.

The 4-week recall period was deemed the most appropriate by patients, since several symptoms were experienced only once a month, but were considered relevant nonetheless. One patient preferred a recall-period of 6 months, however, this was not considered to be appropriate for measuring and evaluating treatment effect. No patient wished for a shorter recall-period, since several symptoms that were considered relevant for measuring treatment effects occur sporadically or with longer symptom-free periods.

The second draft consisted of a general symptom scale with 6 items, head/neck symptom scale with 8 items and an appearance scale with 9 items (Supplement 3). All responses are scored in ordinal fashion to allow for statistical analysis. For example, items with two options as 1-2, or items with five options as 1-2-3-4-5.

Field-test

A total of 475 patients were invited by email to complete the final concept version. One-hundred-thirty-four patients (28%) completed the questionnaire including 98 adults and 36 children. Baseline characteristics of participants in the field-test are shown in Supplement 3. An overview of the results of the field-test is presented in Supplement 4.

Scoring

Cronbach's alpha was adequate for two composite scores: using the severity and frequency of general problems of vascular malformation items (0.88) and the nine-item appearance scale (0.85). Cronbach's alpha was inadequate for a composite score for the items on pain frequency and severity (0.54), and a composite score for the items on temporary enlargement frequency and severity (0.45). There were too few cases to calculate Cronbach's alpha for a composite

score of all items on symptoms, a composite score of frequency and severity of bleeding and fluid leakage or a composite score of all items on head/neck symptoms.

Construct validity (known-groups validity)

An overview of the results of the hypotheses is shown in Table 1. Ten out of 13 hypotheses were confirmed.

Table 1. Hypotheses on known-group differences.

	Hypothesis	Group size	Result	Confirmation
1	Higher presence of pain in patients with *clinician*-reported (medical history of) pain	80 vs 54	73% vs 20% (p<0.000)	Confirmed
2	Higher presence of pain in patients with intramuscular lesions	61 vs 73	69% vs 37% (p<0.000)	Confirmed
3	Higher presence of pain in patients with lower extremity lesions	48 vs 86	67% vs 43% (p=0.009)	Confirmed
4	Higher presence of bleeding in patients with *clinician*-reported (medical history of) bleeding	22 vs 111	23% vs 6% (p=0.013)	Confirmed
5	Higher presence of fluid leakage in patients with lymphatic component	19 vs 115	16% vs 3% (p=0.025)	Confirmed
6	Higher presence of temporary lesion enlargement in patients with venous or lymphatic component	96 vs 38	68% vs 38% (p=0.001)	Confirmed
7	High correlation (>0.5 Spearman's rho) between clinician-reported lesion size and patient-reported lesion size	134	Spearman's rho: 0.558	Confirmed
8	Less swelling/mass in patients with *pure* capillary malformations	13 vs 121	2.08 vs 2.69 (p=0.079)	Rejected
9	Less color difference with skin in patients with *pure* lymphatic malformations	13 vs 121	2.00 vs 2.88 (p=0.053)	Rejected
10	More color difference with skin in patients with skin/subcutaneous tissue involvement	109 vs 25	3.09 vs 1.48 (p<0.000)	Confirmed
11	More texture difference with skin in patients with skin/subcutaneous tissue involvement	109 vs 25	2.54 vs 2.08 (p=0.14)	Rejected
12	More facial distortion in patients with head and neck lesions	55 vs 79	2.58 vs 1.15 (p<0.000)	Confirmed
13	More bodily distortion in patients with arm, trunk and leg lesions	86 vs 48	2.65 vs 1.38 (p<0.000)	Confirmed

Discussion

With this extensive international project, including comprehensive input from patients and leading clinical experts worldwide, a condition-specific PROM for patients with vascular malformations was developed. The OVAMA questionnaire enables measurement of symptoms and appearance in cross- sectional and prospective research. With the addition of the OVAMA follow-up questionnaire (measuring satisfaction) and the PROMIS scales, this will cover all patient-reported core outcome domains as previously determined by the international vascular malformation community.

International consensus with patients and experts had previously been reached on core outcome domains for measuring treatment effect in vascular malformations. The same domains emerged in our cognitive patient interviews.[8,9] We believe that the participation of patients throughout several steps in the process was essential, and has led to excellent content validity of the PROM according to the COSMIN checklist. By including a clinically representative and heterogeneous group, we incorporated the most common problems for all types of patients with vascular malformations.

The field-test showed that the symptoms of pain and temporary lesion enlargement are common, while bleeding, fluid leakage and head/neck symptoms are rare but relevant nonetheless. As for appearance, the problems seem fairly normally distributed. Since bleeding, fluid leakage and the head/neck symptoms were included in the CDS, and also emerged in the interviews, we decided to keep them in the final instrument. In a later stadium, we may be able to tailor the questionnaire more to the specific characteristics of the patient, so that only questions specifically relevant to that 'type' of patient and lesion are presented to patients. In the current situation, patients who do not experience a certain symptom can skip the frequency and severity items for that symptom.

Construct validity was considered to be good since most known-group hypotheses were confirmed. Results of the three rejected hypotheses were in the expected direction, however, not statistically significant. Furthermore, this concerned small subgroups, thus the hypotheses may potentially be confirmed with an increased sample size in future studies. Formulation of hypotheses was limited since there is a paucity of knowledge on what clinical characteristics determine disease and symptom severity, and appearance problems. One of the goals of the OVAMA questionnaire is to investigate such clinical patterns and thereby define clinically distinct groups, which will also be evaluated in future studies.

The e-Delphi study and consensus meetings involved both adult patients and parents of children with vascular malformations. Thus, the core domains pertain for both groups, making the questionnaire suitable for both adults and children, and allowing for comparison between groups.

Below the age of 8, it is generally advised to let parents fill in questionnaires.[20] Since one of the main goals of the OVAMA project is to increase comparability, we chose to let parents fill in the PROM for patients up to 14 years, instead of developing a separate PROM for children between the ages of 8 to 14. This this would have resulted in two different PROMs and comparison would then be impossible.

Scoring

The ordinal rating of the response options allows for statistical analysis. Since most items refer to a separate outcome domain, the individual item outcome is relevant. All items should be analysed and reported separately. Additionally, two composite scores can be calculated reliably: general problems and appearance. These scores will quickly give the clinician or researcher an idea of disease severity. Subsequent evaluation of the individual items will then reveal specifically what causes the severity. However, for evaluating treatment effect, we urge to only evaluate changes in the individual items, since the composite scores are still rough, and clinically important changes can occur in separate symptoms or aspects of appearance. In the future, after refining the scoring model based on more data, it could potentially become possible to form additional composite scores for other symptoms.

We chose to develop a questionnaire for all patients with vascular malformations for several reasons. Currently, there is little evidence of what problems are subtype-specific. With this questionnaire, we can compare the presence and severity of symptom and appearance problems between the different subtypes, which will provide evidence on what problems are more relevant for the specific subtypes. Also, the lesions are often of combined origin, clinical diagnoses show discrepancy with histopathological diagnoses, and future classification is likely to change based on genetic mutations.[21]

In this study, we interviewed 14 patients and reached saturation, so we consider this sample to be adequate for draft development. In contrast, the response rate of the field-test was low in certain subgroups but adequate for the overall group. A large group of eligible patients were treated years ago. Such patients may not have felt prompted to participate. However, we believe that by avoiding a selection of certain patients of our database, we were able to investigate a relatively large representative sample size which reflects the whole group in the best possible way. The OVAMA questionnaire will be freely available online (www.ovama.org) to stimulate wide use.

The final version is available in Dutch and English, after it was translated into English following the COSMIN linguistic validation standards.[17] A protocol for translation to other languages is being developed, enabling easy and correct translation by local groups independently.

The OVAMA questionnaire will allow us to tackle the current heterogeneity in outcome measures within the field of vascular malformations and thereby allow for comparison of treatments. This PROM allows us to identify which treatment options affect which specific symptom or appearance problem. Treatments can then be tailored more to the individual patient, since the clinician has more scientific evidence at hand on how treatments affect certain subgroups or specific symptoms differently. This is especially important in this heterogeneous patient group. Additionally, the OVAMA questionnaire enables definition of clinically distinct groups, which allows for classification on disease severity based on the severity of symptoms and appearance problems. This is even more pressing with the emerging

gene-targeted therapies, which will predominantly play a role in more severe cases, for which a proper definition is currently lacking.

To conclude, with the development of the OVAMA questionnaire, problems that matter most to patients with vascular malformations can be studied scientifically. The many applications of the OVAMA questionnaire may significantly improve research, and ultimately, the care for patients with vascular malformations.

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Supplementary Material

Supplement 1. Baseline characteristics of the interview participants.

Total n=14		
	Median (range)	**Median, IQR (25^{th}-75^{th} percentile)**
Age at baseline	33.3 (14-58)	32.0 (17.8-49.5)
	Frequency	**Percentage**
Gender		
Female	7	50.0
Ethnicity		
Dutch	10	71.4
Dutch/Indonesian	1	7.1
Aruban	1	7.1
Syrian	1	7.1
Chinese	1	7.1
Education		
High school	4	28.6
MBO	6	42.9
HBO	2	14.3
Bachelor's University	1	7.1
Master's University	1	7.1
Type		
Venous	3	21.4
Arteriovenous	3	21.4
Venous, capillary	2	14.3
Lymphatic	2	14.3
Venous, lymphatic	2	14.3
Capillary, venous, lymphatic	1	7.1
Capillary	1	7.1
Overgrowth		
Yes	2	14.3
Localization		
Head/neck	6	42.9
Lower extremity	3	21.4
Upper extremity	2	14.3
Trunk	1	7.1
Upper extremity, trunk	1	7.1
Trunk, upper extremity, lower extremity	1	7.1
Size (largest diameter)		
<5 cm	3	21.4
5-10 cm	5	35.7
10-20 cm	1	7.1
20-30 cm	1	7.1
≥30 cm	3	21.4
Tissues involved		
Skin/subcutaneous tissue	5	35.7
Skin/subcutaneous tissue, muscle	3	21.4
Muscle	2	14.3

Skin/subcutaneous tissue, muscle, bone	1	7.1
Muscle, intra-articular	1	7.1
Skin/subcutaneous tissue, muscle, airway involvement	1	7.1
Skin/subcutaneous tissue, muscle, intra-abdominal	1	7.1
Treatment history included		
No prior treatment	2	14.3
Surgery	6	42.9
Elastic stockings	3	21.4
Embolization	2	14.3
Laser therapy	2	14.3
Sclerotherapy	5	35.7
Rapamycin	1	7.1
Anticoagulants	1	7.1
Tracheostomy	1	7.1

Supplement 2. Coding results of the interviews. S = mentioned spontaneously, P = mentioned after probing, Q = mentioned during questionnaire review. *Added later after spontaneous mention in first interviews.

Patient	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total
Type	VM	CM	LVM	VM	CVM	AVM	VM	AVM	LM	LM	CLVM	AVM	CVM	LVM	
Size	5-15 cm	5-15 cm	>30 cm	5-15 cm	15-30 cm	5-15 cm	<5 cm	5-15 cm	15-30 cm	5-15 cm	>30 cm	5-15 cm	>30 cm	<5 cm	
Localization	Abdomen	Head/neck	Head/neck	Knee	Head/neck	Head/neck	Wrist	Head/neck	Arm/trunk	Leg	Arm/trunk/abdomen/leg	Arm	Leg	Head/neck	
Symptoms															
Pain	S	-	-	S	-	-	S	S	P	S	S	S	S	S	71%
Bleeding	P	-	P	-	Q	-	-	S	-	-	P	-	P	-	43%
Fluid leakage	-	-	S	-	Q	-	-	-	P	-	S	Q	-	S	43%
Temporary enlargement*	P	-	S	S	-	-	S	S	-	P	S	S	S	S	71%
Breathing problems	-	-	S	-	-	-	-	-	-	-	-	-	-	-	7%
Vision problems	-	-	Q	-	-	S	-	Q	-	-	-	-	-	-	21%
Hearing problems	-	-	-	-	-	-	-	Q	-	-	-	-	-	-	7%
Smelling problems	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0%
Swallowing problems	-	-	-	-	Q	-	-	-	-	-	-	-	-	Q	14%
Speech problems	-	-	-	-	Q	-	-	-	-	-	-	-	-	-	7%
Chewing problems	-	-	-	-	Q	-	-	Q	-	-	-	-	-	Q	21%
Tasting problems	-	-	-	-	Q	-	-	-	-	-	-	-	-	-	7%
Drooling	-	-	-	-	Q	-	-	-	-	-	-	-	-	Q	14%
Appearance															
Appearance in general	-	S	S	S	S	S	P	S	S	-	S	S	S	S	86%
Swelling/mass	-	-	S	S	S	S	S	S	P	-	S	S	S	Q	79%
Colour	-	S	P	S	S	-	P	S	S	-	-	S	Q	Q	71%
Texture	-	S	Q	S	P	-	-	-	S	-	Q	Q	-	Q	64%
Distortion of face	-	Q	Q	-	S	S	-	S	-	-	-	-	-	Q	43%
Distortion of body	-	-	-	Q	-	-	-	-	S	-	S	S	-	-	29%
Staring*	-	S	S	-	-	S	-	Q	S	-	-	Q	Q	Q	57%
Reduced self-confidence*	-	S	S	-	-	S	-	S	S	-	P	Q	-	-	50%

Supplement 3. Baseline characteristics of the field-test participants.

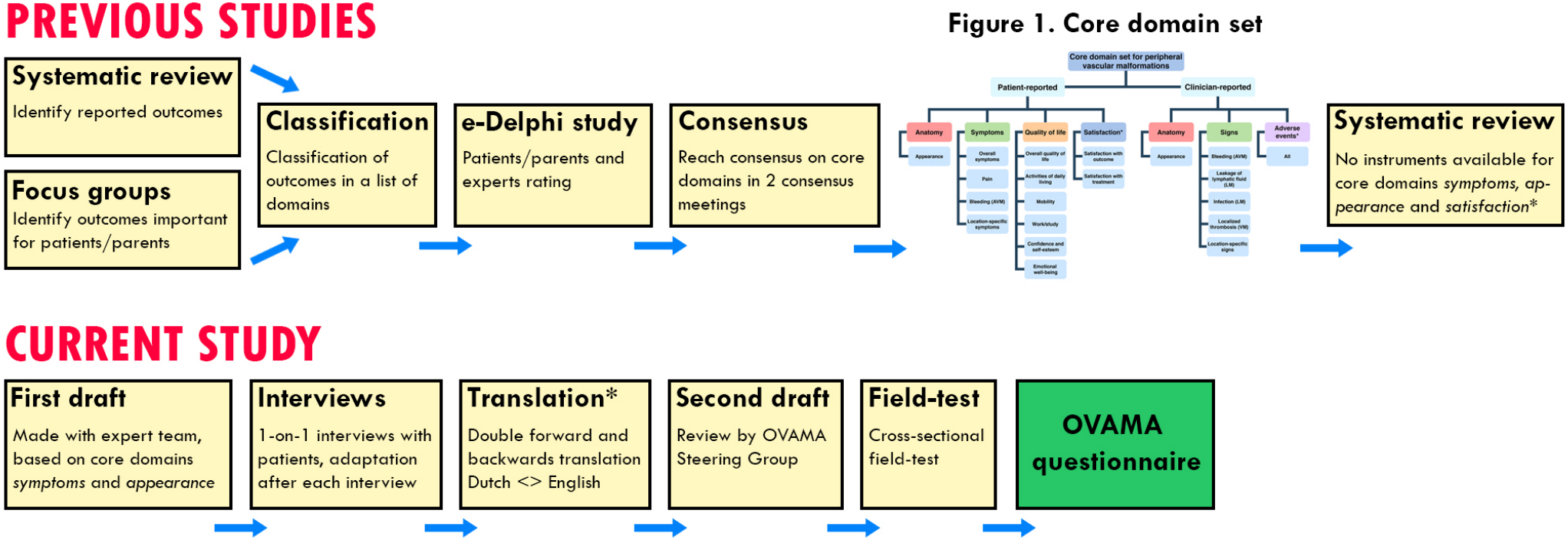
Total n=134		
	Mean (range)	Median (IQR (25^{th}-75^{th} percentile))
Age at baseline	32.6 (1-78)	28 (17.0-51.0)
	Frequency	Percentage
Gender		
Female	87	64.9
Type		
Venous	61	45.5
Arteriovenous	19	14.2
Lymphatic	13	9.7
Capillary	13	9.7
Combined	26	19.4
Overgrowth		
Yes	18	13.4
Localization involved		
Head/neck	55	41
Lower extremity	48	35.8
Upper extremity	27	20.1
Trunk	37	27.6
Size (largest diameter)		
<5 cm	41	30.6
5-10 cm	27	20.1
10-30 cm	38	28.6
≥30 cm	24	18.0
Tissues involved		
Skin/subcutaneous tissue	62	46.3
Skin/subcutaneous tissue, muscle	23	17.2
Skin/subcutaneous tissue, muscle, bone	17	12.7
Muscle	16	11.9
Skin/subcutaneous tissue, bone	5	3.7
Unclear	4	3.0
Muscle, bone	3	2.2
Bone	2	1.5
Skin/subcutaneous tissue, muscle, airway involvement	2	1.5
Treatment history included		
No prior treatment	19	14.2
Surgery	57	42.5
Elastic stockings	36	26.9
Embolization	23	17.2
Laser therapy	22	16.4
Sclerotherapy	55	41.0
Rapamycin	1	0.7
Anticoagulants	6	4.5

Supplement 4. Results and descriptive statistics of the field-test in which a total of 134 patients participated. All questions on symptoms referred to the past 4 weeks.

General symptoms n=134 (100%)												**Mean (SD)**	**Median**
Frequency	*1. Never*		*2. <1 a week*		*3. +-1 a week*		*4. Several times*		*5. Every day*			2.91 (1.51)	3
n (%)	39 (29%)		15 (11%)		25 (19%)		29 (22%)		26 (19%)				
Bother	*1. Not at all*		*2. A little bit*		*3. Moderately*		*4. A lot*		*5. Extremely*			2.37 (1.12)	2
n (%)	37 (28%)		36 (27%)		40 (30%)		16 (12%)		5 (4%)				
Pain n=69 (51%)													
Frequency	*1. <1 a week*			*2. +-1 a week*			*3. Several times a week*			*4. Every day*		2.55 (0.99)	3
n (%)	13 (19%)			17 (25%)			27 (39%)			12 (17%)			
Severity	*0*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	4.62 (2.12)	5
n (%)	0 (0%)	5 (7%)	8 (12%)	11 (16%)	8 (12%)	10 (14%)	14 (20%)	7 (10%)	4 (6%)	2 (3%)	0 (0%)		
Bleeding n=12 (9%)													
Frequency	*1. <1 a week*			*2. +-1 a week*			*3. Several times a week*			*4. Every day*		1.42 (1.00)	1
n (%)	10 (83%)			0 (0%)			1 (8%)			1 (8%)			
Duration	*1. <1 minute*			*2. 1-5 minutes*			*3. >5 minutes*			*4. Medical assistance needed to stop*		1.67 (0.78)	1.5
n (%)	6 (50%)			4 (33%)			2 (17%)			0 (0%)			
Fluid leakage n=7 (5%)													
Frequency	*1. <1 a week*			*2. +-1 a week*			*3. Several times a week*			*4. Every day*		2.43 (0.98)	3
n (%)	2 (29%)			0 (0%)			5 (71%)			0 (0%)			
Bother	*1. Not at all*		*2. A little bit*		*3. Moderately*		*4. A lot*		*5. Extremely*			2.29 (0.49)	2
n (%)	0 (0%)		0 (0%)		5 (71%)		2 (29%)		0 (0%)				
Temporary enlargement n=80 (60%)													
Frequency	*1. <1 a week*			*2. +-1 a week*			*3. Several times a week*			*4. Every day*		2.50 (1.04)	3
n (%)	19 (24%)			16 (20%)			31 (39%)			14 (18%)			
Bother	*1. Not at all*		*2.A little bit*		*3. Moderately*		*4. A lot*		*5. Extremely*			2.99 (1.10)	3
n (%)	8 (10%)		19 (24%)		24 (30%)		24 (30%)		5 (6%)				
Appearance n=134 (100%)													
Size	*1. Very small*		*2. Small*		*3. Medium-sized*		*4. Large*		*5. Very large*			3.25 (1.06)	3
n (%)	8 (6%)		22 (16%)		49 (37%)		39 (29%)		16 (12%)				
Swelling/mass	*1. Not visible*		*2. Small*		*3. Medium-sized*		*4. Large*		*5. Very large*			2.63 (1.21)	3
n (%)	29 (22%)		35 (26%)		34 (25%)		28 (21%)		8 (6%)				
Color difference with normal skin	*1. Not at all*		*2. Slightly*		*3. Moderately*		*4. Very*		*5. Extremely*			2.79 (1.55)	3
n (%)	43 (32%)		19 (14%)		24 (18%)		19 (14%)		29 (22%)				

Texture difference with normal skin	*1. Not at all*	*2. Slightly*	*3. Moderately*	*4. Very*	*5. Extremely*	2.46 (1.40)	2
n (%)	50 (37%)	25 (19%)	20 (15%)	26 (19%)	13 (10%)		
Facial distortion	*1. Not at all*	*2. Slightly*	*3. Moderately*	*4. Very*	*5. Extremely*	1.74 (1.27)	1
n (%)	91 (68%)	16 (12%)	7 (5%)	11 (8%)	9 (7%)		
Bodily distortion	*1. Not at all*	*2. Slightly*	*3. Moderately*	*4. Very*	*5. Extremely*	2.19 (1.39)	2
n (%)	64 (48%)	22 (16%)	18 (13%)	18 (13%)	12 (9%)		
Staring	*1. Not at all*	*2. Rarely*	*3. Sometimes*	*4. Frequently*	*5. All the time*	2.44 (1.25)	2
n (%)	43 (32%)	26 (19%)	36 (27%)	21 (16%)	8 (6%)		
Self-esteem reduction	*1. Not at all*	*2. A little bit*	*3. Moderately*	*4. A lot*	*5. Extremely*	2.04 (1.14)	2
n (%)	56 (42%)	39 (29%)	23 (17%)	10 (7%)	6 (4%)		
Satisfaction with appearance	*1. Very satisfied*	*2. Satisfied*	*3. Not satisfied or dissatisfied*	*4. Dissatisfied*	*5. Very dissatisfied*	2.87 (1.18)	3
n (%)	20 (15%)	29 (22%)	46 (34%)	26 (19%)	13 (10%)		
Head and neck symptoms n=51 (38%)							
	1. Not at all	*2. A little bit*	*3. Moderately*	*4. A lot*	*5. Extremely*		
Breathing problems n=6 (12%)						2.67 (0.82)	2.5
Bother n (%)	0 (0%)	3 (50%)	2 (33%)	1 (17%)	0 (0%)		
Eyesight problems n=7 (14%)						2.71 (0.49)	3
Bother n (%)	0 (0%)	2 (29%)	5 (71%)	0 (0%)	0 (0%)		
Hearing problems n=4 (8%)						3.75 (1.50)	4
Bother n (%)	0 (0%)	1 (25%)	1 (25%)	0 (0%)	2 (50%)		
Swallowing problems n=4 (8%)						3.00 (1.41)	2.5
Bother n (%)	0 (0%)	2 (50%)	1 (25%)	0 (0%)	1 (25%)		
Speech problems n=5 (10%)						2.40 (0.55)	2
Bother n (%)	0 (0%)	3 (60%)	2 (40%)	0 (0%)	0 (0%)		
Chewing problems n=5 (10%)						3.20 (1.30)	3
Bother n (%)	0 (0%)	2 (40%)	1 (20%)	1 (20%)	1 (20%)		
Taste problems n=2 (4%)						4.50 (0.71)	4.5
Bother n (%)	0 (0%)	0 (0%)	0 (0%)	1 (50%)	1 (50%)		
Saliva leakage n=5 (10%)						2.00 (0.00)	2
Bother n (%)	0 (0%)	5 (100%)	0 (0%)	0 (0%)	0 (0%)		
Composite scores n=134 (100%)							
General problems (0-100)						52.84 (25.09)	60.00
Appearance (0-100)						49.80 (17.14)	48.89

Supplement 5. Flowchart of methods. *Will be reported in a separate study.



Supplement 6. The OVAMA questionnaire.

The OVAMA questionnaire

This questionnaire is intended for patients with a vascular malformation and assesses the symptoms they may experience. 'Vascular malformation' is the medical term for a group of vascular anomalies that one can be born with. If you are 14 years or older, you can fill in the questionnaire by yourself. For children of the age 0-13 years, parents can complete the questionnaire together with the child as much as possible.

General symptoms scale

1. General problems

How often were you bothered by the vascular malformation in the past 4 weeks?

□ Never
□ Less than once a week
□ On average once a week
□ Several times a week
□ Every day

2. General problems

How much were you bothered by the vascular malformation in the past 4 weeks?

□ Not at all
□ A little bit
□ Moderately
□ A lot
□ Extremely

3. Pain because of the vascular malformation

In the past 4 weeks …

□ I did not have this problem (go to question 4)

□ I had this problem
↓
If you had this problem:

A. How often did you have pain in the past 4 weeks?

□ Less than once a week
□ About once a week
□ Several times a week
□ Every day

B. If you were in pain, how severe was this on average in the past 4 weeks?

0 – 1 – 2 – 3 – 4 – 5 – 6 – 7 – 8 – 9 – 10

No pain — Most severe pain imaginable

4. Bleeding because of the vascular malformation

In the past 4 weeks …

□ I did not have this problem (go to question 5)

□ I had this problem
↓
If you had this problem:

A. How often did you have bleeding episodes in the past 4 weeks?

□ Less than once a week

□ On average once a week
□ Several times a week
□ Every day

B. How long did these bleeding episodes last?

□ The bleeding stopped within a minute
□ The bleeding stopped within 5 minutes
□ The bleeding stopped but lasted longer than 5 minutes
□ The bleeding could not be stopped without medical assistance

5. Leakage of fluid (other than blood) from the vascular malformation

In the past 4 weeks …

□ I did not have this problem (go to question 6)

□ I had this problem
↓

If you had this problem:

A. How often did the vascular malformation leak fluid in the past 4 weeks?

□ Less than once a week
□ On average once a week
□ Several times a week
□ Every day

B. How much did the leakage of fluid bother you?

□ Not at all
□ A little bit
□ Moderately
□ A lot
□ Extremely

6. Temporary enlargement of the vascular malformation

For example: after exercise, in warm weather or in a certain pose.

In the past 4 weeks …

□ I did not have this problem (go to question 7)

□ I had this problem
↓

If you had this problem:

A. How often did you experience enlargement of the vascular malformation in the past 4 weeks?

□ Less than once a week
□ About once a week
□ Several times a week
□ Every day

B. If the vascular malformation enlarged, how much did this bother you?

□ Not at all
□ A little bit
□ Moderately
□ A lot
□ Extremely

Head and neck symptoms scale

The following questions are about possible head and neck symptoms of the vascular malformation. You only have to fill in the following questions if the vascular malformation affects the head and neck region. If you experience a problem as a result of *treatment* of the vascular malformation, you can also fill this in.

1. Breathing problems because of the vascular malformation

In the past 4 weeks …

□ I did not have this problem
(go to question 2)

□ I had this problem
↓
If you had this problem:

A. How much did this problem bother you?

□ Not at all
□ A little bit
□ Moderately
□ A lot
□ Extremely

2. Eyesight problems because of the vascular malformation

In the past 4 weeks …

□ I did not have this problem
(go to question 3)

□ I had this problem
↓
If you had this problem:

A. How much did this problem bother you?

□ Not at all
□ A little bit
□ Moderately
□ A lot
□ Extremely

3. Hearing problems because of the vascular malformation

In the past 4 weeks …

□ I did not have this problem
(go to question 4)

□ I had this problem
↓
If you had this problem:

A. How much did this problem bother you?

□ Not at all
□ A little bit
□ Moderately
□ A lot
□ Extremely

4. Problems with swallowing because of the vascular malformation

In the past 4 weeks …

□ I did not have this problem
(go to question 5)

□ I had this problem
↓
If you had this problem:

A. How much did this problem bother you?

□ Not at all
□ A little bit
□ Moderately

□ A lot
□ Extremely

5. Speech problems because of the vascular malformation

In the past 4 weeks …

□ I did not have this problem (go to question 6)

□ I had this problem ↓

If you had this problem:

A. How much did this problem bother you?

□ Not at all
□ A little bit
□ Moderately
□ A lot
□ Extremely

6. Problems with chewing because of the vascular malformation

In the past 4 weeks …

□ I did not have this problem (go to question 7)

□ I had this problem ↓

If you had this problem:

A. How much did this problem bother you?

□ Not at all
□ A little bit
□ Moderately
□ A lot
□ Extremely

7. Problems with taste because of the vascular malformation

In the past 4 weeks …

□ I did not have this problem (go to question 8)

□ I had this problem ↓

If you had this problem:

A. How much did this problem bother you?

□ Not at all
□ A little bit
□ Moderately
□ A lot
□ Extremely

8. Saliva leakage from the mouth because of the vascular malformation

In the past 4 weeks …

□ I did not have this problem

□ I had this problem ↓

If you had this problem:

A. How much did this problem bother you?

□ Not at all
□ A little bit
□ Moderately
□ A lot
□ Extremely

Appearance scale

The following questions are about the appearance of the vascular malformation. If the vascular malformation has completely disappeared (for example after surgical removal), please assess the spot where the vascular malformation used to be.

1. Size of the vascular malformation

How large is your vascular malformation?

□ Very small
□ Small
□ Medium-sized
□ Large
□ Very large

2. Visible swelling/mass

Do you have a visible swelling or mass of the vascular malformation?

□ No visible swelling
□ Small swelling
□ Medium swelling
□ Large swelling
□ Very large swelling

3. Color of the vascular malformation

Is the color of the vascular malformation different from that of your normal skin?

□ Not at all
□ Slightly different
□ Moderately different
□ Very different
□ Extremely different

4. Surface/texture of the vascular malformation

Is the surface/texture of the vascular malformation different from your normal skin? For example irregular, rough, or bumpy.

□ Not at all
□ Slightly different
□ Moderately different
□ Very different
□ Extremely different

5. Alteration of facial features

Do you find that your face looks different or distorted because of the vascular malformation?

□ Not at all
□ Slightly distorted
□ Moderately distorted
□ Very distorted

□ Extremely distorted

6. Alteration of bodily features

Do you find that your body (except your face) looks different or distorted because of the vascular malformation?

□ Not at all
□ Slightly distorted
□ Moderately distorted
□ Very distorted
□ Extremely distorted

7. Staring

Do other people stare at you because of your vascular malformation?

□ Not at all
□ Rarely
□ Sometimes
□ Frequently
□ All the time

8. Self-confidence

Is your self-confidence reduced because of the vascular malformation?

□ Not at all
□ A little bit
□ Moderately
□ A lot
□ Extremely

9. Satisfaction with the appearance of the vascular malformation

How satisfied are you with the appearance of the vascular malformation?

□ Very satisfied
□ Satisfied
□ Not satisfied or dissatisfied
□ Dissatisfied
□ Very dissatisfied

Calculation of the composite scores:

General problems: ((item 1 of general symptoms scale + item 2 of general symptoms scale) / 2) * 20

Appearance: ((sum of all 9 items of appearance scale) / 9) * 20

Chapter 7

Responsiveness of the condition-specific OVAMA questionnaire to measure symptoms and appearance in patients with vascular malformations.

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Under revision, British Journal of Dermatology

Abstract

Background

Evidence-based guidelines for the treatment of vascular malformations are not readily available, possibly due to the diversity in methods used to evaluate treatment efficacy in clinical research, complicating the aggregation and comparison of study results. The OVAMA (Outcome Measures for VAscular Malformations) questionnaire was developed to uniformly measure symptoms and appearance, i.e., condition-specific core outcome domains, in patients with vascular malformations. However, the OVAMA questionnaire needs to be responsive to changes in these constructs in order to assess whether the disease status has altered since treatment.

Objectives

To assess the responsiveness of the OVAMA questionnaire in patients with vascular malformations.

Methods

In a prospective longitudinal study, patients completed the OVAMA questionnaire at baseline and eight weeks follow-up since treatment or watchful waiting policy. Additionally, patients completed the Global rating of change (GRC) scales at follow-up. Responsiveness was evaluated following the criterion approach of testing predefined hypotheses about expected relationships between the OVAMA questionnaire and GRC scales, measuring the same constructs. The OVAMA questionnaire was considered responsive if ≥ 75% of the hypotheses were confirmed.

Results

Between July 2020 and September 2022, 89 patients were recruited in a vascular anomaly centre in the Netherlands, of which 63 patients completed the questionnaires at baseline and follow-up. In total, fifteen constructs of the OVAMA questionnaire were assessed for five hypotheses. Of these 75 hypotheses, 63 (84%) hypotheses were confirmed and thereby providing evidence that the OVAMA questionnaire is responsive to change.

Conclusion

Our study found convincing evidence that the OVAMA questionnaire is responsive to changes in symptoms and appearance in patients with vascular malformations. In addition to determining a baseline of symptoms and appearance, the OVAMA questionnaire can now be used to evaluate the effect of treatment from the patient's perspective. The responsive OVAMA questionnaire allows for uniform evaluation and comparison of the effects of treatment on the condition-specific core outcome domains, tackling heterogeneity in outcome measurement and improving the clinical research of vascular malformations.

What is already known about this topic?

- There exists considerable heterogeneity in outcomes used in clinical research on vascular malformations.
- Symptoms and appearance are determined to be core outcome domains and should be measured in all clinical research on vascular malformations.
- The OVAMA questionnaire is a condition-specific patient-reported outcome measure developed for measuring symptoms and appearance in patients with vascular malformations.
- To use the OVAMA questionnaire to evaluate treatment effect, it needs to be responsive to change.

What does this study add?

- This study showed that the OVAMA questionnaire is responsive to changes in symptoms and appearance in patients with vascular malformations.
- In addition to determining a baseline of symptoms and appearance, the OVAMA questionnaire can now be used to evaluate the effect of treatment from the patient's perspective.

What are the clinical implications of this work?

- The condition-specific core outcomes domains for vascular malformations can now be measured with the OVAMA questionnaire to evaluate the effect of treatment.
- Treatments are generally initiated to relieve symptoms and improve appearance. Now, the effect of treatment on these outcomes can be measured from the patient's perspective.
- Treatments can be uniformly evaluated and compared for effects on the core outcome domains, tackling heterogeneity in outcome measurement and improving clinical research.

Introduction

Peripheral vascular malformations, congenital anomalies of the vascular and lymphatic system, can negatively impact a patient's health-related quality of life (HRQOL).[1, 2] Since vascular malformations are clinically heterogeneous with regard to involved vessel type, anatomical location, tissue extension, and size, the tortuous vessels are known to cause a wide spectrum of symptoms.[3-5] The clinical diversity and varying symptoms among patients have led to the development of diverging treatment methods.

However, evidence-based guidelines for the treatment of vascular malformations are not readily available. A reason for the absence of treatment guidelines could be the diversity in methods used to evaluate treatment efficacy in clinical research, complicating the aggregation and comparison of study results.[6-8] To reach uniformity in outcome reporting, the OVAMA (Outcomes measure for VAscular MAlformations) project was initiated.[9, 10] With (clinical) experts and patients worldwide, the Core Domain Set (CDS) for peripheral vascular malformations was determined,[9, 10] i.e., a set of core outcome domains that should be measured at a minimum when evaluating treatment effect in a certain condition.[11] Since vascular malformations have a lifelong disease course and complete remission is unlikely, treatments are usually deployed to reduce symptoms and improve HRQOL. However, in current treatment evaluation the patient's perspective of treatment efficacy is often omitted. Therefore, the OVAMA project also emphasizes on patient involvement.

The patient-reported outcome domains included in the CDS consist of condition-specific and generic outcome domains, e.g., domains falling under HRQOL, which apply to diverse health conditions and are advised to be measured with generic outcome measurement instruments.[12] For the measurement of condition-specific outcome domains, the patient-reported outcome measure (PROM) OVAMA questionnaire was developed to assess symptoms and appearance in patients with vascular malformations, which showed good construct and content validity and reliability.[13]

The assessment of whether the disease status of patients has changed after treatment is generally the most crucial measurement in treatment evaluation, therefore, PROMs need to be responsive to change. The identification of an instrument that is able to adequately measure these changes involves assessment of the measurement property *'responsiveness',* defined as "the ability of an instrument to detect change over time in the construct to be measured".[14] In this study we aim to assess the responsiveness of the OVAMA questionnaire in patients with peripheral vascular malformations and evaluate if it is an adequate PROM to assess treatment effect in this patient population.

Methods

The prospective longitudinal study was conducted at the Amsterdam University Medical Centres, a tertiary vascular anomaly expertise centre. Written informed consent was obtained from all patients, and full ethical review by the Medical Ethics Committee was exempted because patients were not subjected to interventions or rules of conduct. We followed the COSMIN (COnsensus-based Standards for the selection of health Measurement INstruments) checklist for PROM measurement instruments.[15]

Patient and data collection

All patients with peripheral vascular malformations who were treated in our centre between July 2020 and September 2022 were contacted to participate, including patients with an explicit watchful waiting policy. Patient details were extracted from electronic patient files and included age at start of treatment, sex, VM type according the International Society for the Study of Vascular Anomalies classification[16], lesion maximal diameter (radiologically determined), lesion localization, tissue extension, overgrowth, diagnosis of an associated syndrome, treatment method, and treatment date.

Adult patients and parents of patients were asked to complete the OVAMA questionnaire before visiting the outpatient clinic to establish a baseline of symptoms and appearance. Two months post-treatment or two months after visiting the outpatient clinic in case of a watchful waiting policy, patients were asked once more to complete the OVAMA questionnaire and Global Rating of Change (GRC) scales. A follow-up period of two months was chosen since most treatments are accompanied by a recovery period, and sclerotherapy induces an inflammatory phase of approximately six weeks before the vessels regress.[17] Questionnaires were proxy-reported for children ≤13 years and self-reported above the age of 13 years, as determined in the OVAMA-project.[13]

Measurement instruments

OVAMA questionnaire

The OVAMA questionnaire is a condition-specific PROM for measuring symptoms and appearance in patients with peripheral vascular malformations (supplement 1). The first two questions refer to the frequency and intensity of general problems, both asked on a five-point textual interval scale. The next four questions address other symptoms, i.e., 'pain', 'bleeding', 'leakage of fluid', and 'temporary enlargement'. If patients indicate that they experience a specific symptom, additional questions are administrated regarding the frequency and intensity of that symptom on a four or five-point textual interval scale, or eleven-point numeric rating scale in case of pain intensity.

Furthermore, the questionnaire includes nine questions referring to the appearance of the vascular malformation and includes: 'size', 'visible swelling', 'colour', 'surface/texture', 'facial distortion', 'bodily distortion', 'staring', 'self-confidence', and 'satisfaction with the appearance of the vascular malformation'. All appearance questions are posted on a five-point

textual interval scale. A comprehensive appearance score was generated by ((the sum of all 9 appearance outcomes) / 9) ∗ 20).

Finally, the questionnaire includes questions concerning head and neck symptoms, which are only addressed to patients who indicated head or neck involvement of the vascular malformation. The eight head and neck questions refer to problems with 'breathing', 'eyesight', 'hearing', 'swallowing', 'speech', 'chewing', 'taste', and 'saliva leakage'. Patients indicating a specific symptom will receive another question concerning the intensity of that problem on a five-point textual interval scale. All questions of the OVAMA questionnaire refer to issues that occurred within the last four weeks.

Global Rating of Change scales

Patients completed simultaneously the GRC scales at follow-up, questioning the change in symptoms and appearance since treatment or watchful waiting policy (supplement 2). GRC scales are designed to quantify a patient's improvement or deterioration over time, generally, to determine the effect of treatment.[18, 19] GRC scales have high face validity and may therefore be considered a gold standard to measure change if the GRC scales assess the same constructs as the measurement instrument under study.[20]

Therefore, we formulated fifteen GRC scales corresponding with the constructs measured with the OVAMA questionnaire, which consisted of items that asked about changes in 'general problems', 'pain', 'bleeding', 'leakage of fluid', 'temporary enlargement', 'appearance', 'visible swelling', 'colour', 'surface/texture', 'facial distortion', 'bodily distortion', 'depression symptoms', 'anxiety symptoms', 'physical functioning', and 'social activities'. Additionally, eight GRC scales were formulated to assess changes in head and neck symptoms, which corresponded with the head and neck constructs measured with the OVAMA questionnaire. All GRC scales captured change on a seven-point Likert scale, based on previously reported GRC scales.[19, 21-23] The GRC scales included seven response options ranging from 'much worse' to 'much better', or patients had the option to state that they never had this problem. In that case, the question was considered not applicable to the patient and was made a missing value.

Evaluating responsiveness

Responsiveness was evaluated following the criterion approach of testing predefined hypotheses about expected relationships between the OVAMA questionnaire and GRC scales, measuring the same constructs.[20, 24] Two researchers (MS and ML), both experienced with PROMs and measurement properties, formulated five hypotheses beforehand (Table 1). The hypotheses were based on previous studies evaluating responsiveness and the COSMIN guidelines.[15, 20, 22, 23, 25-27] The first two hypotheses were based on correlation strength between the changed scores of the OVAMA questionnaire and change measured with GRC scales of similar constructs or dissimilar constructs (to assess the specificity of the OVAMA constructs and preclude random correlations). The next three hypotheses refer to the mean OVAMA change score of a construct and improved, unchanged, and worsened patients according to the

associated GRC scale. If ≥75% of the hypotheses were confirmed, according to the methodological COSMIN guidelines, this provides convincing evidence that the OVAMA questionnaire is responsive to change.[15, 24]

Table 1. Predefined hypotheses for assessing responsiveness of the OVAMA questionnaire.
If ≥75% of the hypotheses were confirmed, according to the methodological COSMIN guidelines, this provides convincing evidence that the OVAMA questionnaire is responsive to change.

	Hypotheses
1.	High correlation (≥0.5) between the OVAMA questionnaire change scores and GRC scale measuring a similar construct.
2.	Low correlation (<0.3) between the OVAMA questionnaire and GRC scale measuring a dissimilar construct.
3.	Patients indicating improvement on the associated GRC scale should have a positive mean score of the OVAMA questionnaire measuring the same construct.
4.	Patients indicating worsening on the associated GRC scale should have a negative mean score of the OVAMA questionnaire measuring the same construct.
5.	The mean change score (of a construct of the OVAMA questionnaire) of patients indicating improvement (on associated GRC scale) should be higher than the mean change score of unchanged patients, which in turn should be higher than the mean change score of worsened patients

Minimal important change.

The minimal important change (MIC) was defined as a threshold for a minimal within-person change above which patients perceive themselves as importantly changed. The MIC of a sample can be approached as the mean of these individual thresholds.[28] COSMIN advises estimating the MIC using anchor-based methods[29], where scores are compared to an external variable in order to 'anchor' it with results that are clinically relevant. We used the GRC scales as patient-reported anchors, distinguishing an improved, unchanged, and worsened status of the patient. We focused on determining the MIC for improvement since that is the goal of treatment. The MIC is defined as the mean change of a construct score of patients rating themselves as improved minus the mean change of patients rating their status as unchanged in the associated GRC scale. A condition for determining the MIC is that there must be a correlation coefficient of at least 0.3 between the outcome and the associated anchor.[30]

Data analyses

Statistical differences in baseline characteristics between patients who participated in the study and patients who only completed the OVAMA questionnaire at baseline were explored; chi-square was used for categorical variables and Mann-Whitney U test for nonparametric continuous variables. Correlation strength between the OVAMA change scores and GRC scales were measured using Spearman's rank correlation. Subsequently, disattenuated correlations were calculated based on Cornbach's Alpha to rectify measurement errors. Correlation was interpreted as high (≥0.5), moderate (0.3-0.5) or low (<0.3), based on previous studies and

guidelines for assessing responsiveness.[15, 20, 22, 31, 32] In order to investigate if the GRC scales can correctly assess change and are able to discriminate between patients who experience minimal change and patience who experience significant change, differences between the conservative group (watchful waiting, compression stockings, and anticoagulants) and invasive treatment group (sclerotherapy, surgery, and embolization) were assessed using the Mann–Whitney U-test.

Results

In total, 208 patients were invited to participate in the study. Of these patients, 98 (47%) completed the OVAMA questionnaire at baseline, and 63 (64% of baseline responders) completed the questionnaires at two months follow-up. Baseline characteristics of the included patients are presented in Table 2. Comparing patients who participated in the study and who did not participate, i.e., by completing only questionnaires at baseline, patients who participated were statistically significant older, more frequently diagnosed with an associated syndrome, more frequently had truncal lesions, and more frequently had intraosseous lesions (Table S1).

Responsiveness

An overview of the disattenuated correlations between the OVAMA change scores and the GRC scales is presented in Table 3. A total of fifteen constructs of the OVAMA questionnaire were assessed for all five hypotheses. Of these 75 hypotheses, 63 (84%) hypotheses were confirmed and thereby providing evidence that the OVAMA questionnaire is responsive to change according to the COSMIN guidelines.[15, 24] The constructs 'bleeding', 'leakage of fluids', and all head and neck symptoms were evaluated by less than 30 patients, and therefore the responsiveness of these constructs could not be assessed according to the COSMIN guidelines.[15]

High correlations were expected between the OVAMA questionnaire and GRC scales measuring similar constructs, and these were found for the constructs 'frequency of general problems', 'temporary enlargement', 'texture', 'facial distortion' and 'bodily distortion', the other constructs showed moderate or even low correlations. Furthermore, the construct 'pain intensity' (measured on a an eleven-point numeric rating scale) was lowly correlated with pain on the GRC scale, therefore 'pain intensity' was converted into a six-point numeric rating scale, resulting in moderate correlations with pain. All OVAMA constructs had low correlations with the GRC scales for which a low correlation was expected (GRC measuring an unrelated construct). Patients who indicated improvement or worsening on the GRC scale had corresponding changes measured with the OVAMA questionnaire for almost all constructs.

Minimal important change

The MIC-values of all OVAMA constructs are displayed in Table 4. However, the construct 'pain intensity' had a correlation <0.30 with the associated GRC scale and therefore did not meet the criteria for estimating the MIC.

Global Rating of Change

An overview of the differences in change measured with the GRC scales between the conservative and invasive treatment groups is presented in Table S2. Patients who received an invasive treatment indicated more improvement than the conservative group in all GRC scale domains, of which the following were statistically significant: general problems (p=0.006),

temporary enlargement (p=0.008), appearance (p<0.001), visible swelling (p=0.002), and colour (p=0.006).

Table 2. Baseline characteristics of the included patients (n=63)

Patient, lesion and treatment characteristics of all included patients.
IQR = Interquartile range.

Patient Characteristics	*Case number (%)*
Male	29 (46%)
Age in years (median, IQR)	32 (22-46)
Syndrome (%)	7 (11%)
Overgrowth	7 (11%)
Lesion characteristics	
Vascular malformation type	
Venous malformation	32 (51%)
Lymphatic malformation	9 (14%)
Capillary malformation	3 (5%)
Arteriovenous malformation	4 (6%)
Combined malformation	14 (22%)
Unclear	1 (2%)
Localization	
Head and neck	19 (30%)
Upper extremity	21 (33%)
Lower extremity	63 (34%)
Trunk	17 (27%)
Tissue extension	
(sub)cutaneous	46 (73%)
Intramuscular	28 (44%)
Intraosseous	16 (25%)
Maximal diameter in cm (median, IQR)	7.2 (4-19)
Size groups	
<5 cm	22 (35%)
5-10 cm	15 (24%)
10-30 cm	14 (22%)
>30 cm	10 (16%)
Unclear	1 (2%)
Treatment	
Watchful waiting	9 (14%)
Compression stockings	5 (8%)
Sclerotherapy	35 (56%)
Surgery	9 (14%)
Embolization	3 (5%)
Anticoagulants	2 (3%)
Time to follow-up in months (median, IQR)	2 (2-4)

Table 3. Predefined expected relations between the change score of the OVAMA questionnaire and Global Rating of Change scales (GRC)

Confirmed hypotheses are displayed in bold. In total, 63 (84%) of the 75 hypotheses were confirmed.

The constructs 'bleeding' and 'leakage of fluids' were evaluated by <30 patients, therefore the responsiveness of these constructs could not be assessed according to the COSMIN guidelines. Due to high random error of pain intensity measured on an eleven-point numeric rating scale, pain intensity was converted into a six-point numeric rating scale. Correlations between pain intensity measured on a six-point numeric rating scale and GRC scales are displayed at the bottom in grey.

NRS = numeric rating scale.

OVAMA construct	GRC measuring similar construct. Expected high (≥0.5) correlations		GRC measuring dissimilar construct. Expected low (≤0.3) correlations		Improvement GRC scale similar construct should have positive mean score	Worsening GRC scale similar construct should have negative mean score	GRC mean change score improvement > same > worsening
General problems (frequency)	General problems	**High**	Texture	**Low**	**Positive**	**Negative**	**Yes**
General problems (intensity)	General problems	Moderate	Colour	**Low**	**Positive**	**Negative**	**Yes**
Pain (presence)	Pain	Moderate	Bleeding	**Low**	**Positive**	**Negative**	**Yes**
Pain (frequency)	Pain	Moderate	Appearance	**Low**	**Positive**	**Negative**	**Yes**
Pain (intensity)	Pain	Low	Appearance	**Low**	**Positive**	Positive	**Yes**
Temporary enlargement (presence)	Temporary enlargement	**High**	Pain	**Low**	**Positive**	**Negative**	**Yes**
Temporary enlargement (frequency)	Temporary enlargement	**High**	Pain	**Low**	**Positive**	**Negative**	**Yes**
Temporary enlargement (intensity)	Temporary enlargement	**High**	Pain	**Low**	**Positive**	**Negative**	**Yes**
Appearance (satisfaction)	Appearance	Moderate	Pain	**Low**	**Positive**	**Negative**	No
Visible swelling	Visible swelling	Moderate	Colour	**Low**	**Positive**	Positive	**Yes**
Colour	Colour	Moderate	Temporary enlargement	**Low**	**Positive**	**Negative**	**Yes**
Texture	Texture	**High**	Physical functioning	**Low**	**Positive**	**Negative**	**Yes**
Facial distortion	Facial distortion	**High**	Leakage of fluids	**Low**	**Positive**	**Negative**	**Yes**

Bodily distortion	Bodily distortion	High	Pain	Low	Positive	Negative	Yes
Appearance composite score	Appearance	Moderate	Physical functioning	Low	Positive	Positive	Yes
Pain (intensity) six-point NRS scale	Pain	Moderate	Appearance	Low	Positive	Positive	Yes

Table 4. Minimal Important Changes (MIC) of the OVAMA questionnaire

The minimal important change (MIC) is defined as the mean change of a construct score of patients rating themselves as improved minus the mean change of patients rating themselves as unchanged in the associated Global Rating of Change (GRC) scale.

The MIC for the presence of pain and the presence of temporary enlargement could not be calculated since these are dichotomous variables. A condition for determining the MIC is that there must be a correlation coefficient of at least 0.3 between the outcome and the associated anchor (the GRC scale measuring a similar construct), therefore the MIC of pain intensity could not be calculated.

SD = Standard deviation

OVAMA construct	Mean change unchanged (SD)	Mean change improved (SD)	MIC
General problems (frequency)	0.09 (0.79)	1.13 (1.46)	1.04
General problems (intensity)	0.13 (0.62)	0.87 (1.17)	0.74
Pain (frequency)	0.26 (1.39)	1.16 (1.72)	0.90
Temporary enlargement (frequency)	0.44 (1.45)	1.30 (1.75)	0.86
Temporary enlargement (intensity)	0.70 (1.27)	1.90 (2.24)	1.20
Appearance (satisfaction)	0.45 (0.88)	0.92 (1.11)	0.47
Visible swelling	0.28 (0.79)	0.83 (0.96)	0.55
Colour	0.12 (0.88)	1.0 (1.12)	0.88
Texture	0.11 (1.15)	1.28 (1.13)	1.17
Facial distortion	0.19 (0.68)	1.0 (0.71)	0.81
Bodily distortion	0.05 (0.79)	1.08 (1.56)	1.03
Appearance composite score	5.65 (10.65)	8.0 (10.14)	2.35

Discussion

In this study, we evaluated the responsiveness of the OVAMA questionnaire, a condition-specific PROM to measure symptoms and appearance in patients with vascular malformations, and found convincing evidence that the OVAMA questionnaire is responsive to change. In addition to determining a baseline of symptoms and appearance, the OVAMA questionnaire can now be used to evaluate the effect of treatment from the patient's perspective.

Eighty-four percent of predefined hypotheses about expected relations with the GRC scales, measuring change after treatment, were confirmed. Approximately half of the expected high correlations between changes in OVAMA scores and GRC scales measuring similar constructs were found, which is a satisfactory result since lower correlations can be expected in responsiveness studies because a change score consists of two measurements, both accompanied by a certain degree of measurement error, as stated by the COSMIN guidelines.[20] Additionally, the GRC scales are also subjected to measurement error and recall bias since patients might have difficulties recollecting symptoms and appearance before treatment.[33] Almost all hypotheses were confirmed regarding the mean change scores of the OVAMA constructs and associated worsening/improvement on GRC scales, indicating that all questions of the OVAMA questionnaire are capable of discriminating between patients who unchanged, worsened, or improved on that construct.

A low correlation was found between the change in 'pain intensity' measured with the OVAMA questionnaire and the GRC scale measuring pain, while a high correlation was expected. The low correlation might have occurred because 'pain intensity' was measured on an eleven-point numeric rating scale, and a 1-point change might be attributable to random error rather than a real change.[34] Additionally, previous studies have pointed out that a 2-point change is considered clinically meaningful.[34, 35] Pain intensity was converted into a six-point numeric rating scale, leading to a moderate correlation. These findings are supportive of adjusting the OVAMA questionnaire measuring 'pain intensity' into a six-point verbal rating scale ranging from no pain to extreme pain.

The constructs bleeding, leakage of fluids, and all head and neck symptoms could not be assessed for responsiveness since the majority of patients indicated that they never experienced these symptoms and skipped these questions. The rarity of these symptoms was also noted during the development of the OVAMA questionnaire[13], and the presence of these questions should be carefully reconsidered, although these outcome domains emerged from the international vascular malformation community.[10] Conceivably, these questions are only relevant for a subgroup of patients, however; to date, it is not clear which subgroups of patients experience which symptoms. According to the COSMIN group, responsiveness is a continuous process of accumulating evidence[20] and the responsiveness of the OVAMA questionnaire ought to be repeatedly studied, preferably in larger and international cohorts allowing for the evaluation of responsiveness of these rare symptoms. Nonetheless, with our findings, we are

now able to use the OVAMA questionnaire to evaluate treatment effects in patients with vascular malformations.

The MIC refers to the smallest change in score that patients consider clinically important, and in this study, we found fairly low MIC-values (generally around 1.0) of the OVAMA constructs. Although, this is not surprising since most questions are asked on a five-point verbal rating scale, and a one-point change (e.g., from 'a lot bothered' to 'extremely bothered') is considered clinically important by patients, and thus the five-point seems accurate. It is necessary to have a proportion of unchanged patients to estimate MIC-values, and therefore our study population was very appropriate since it also included patients with a watchful waiting policy or other conservative treatments.

The COSMIN guidelines consider the GRC scales to be a gold standard to assess change[20], however, it is difficult to establish evidence that this is the correct assessment of change. In the current study, patients treated invasively indicated more improvement in all GRC scales than patients treated conservatively, which supports the accuracy of the GRC scales. Nonetheless, measurement with the GRC scales are retrospective and for treatment evaluation prospective measurement remains superior, which is now achievable with the responsive OVAMA questionnaire.

Several limitations of this study should be considered when interpreting the results. Approximately one-third of patients did not complete the questionnaires at follow-up, which may indicate that some patients did not feel the importance of completing the questionnaires or found it too much of an effort. Nevertheless, treatment evaluation from the patient's perspective was considered essential by the international vascular malformation community.[9, 10] Second, multiple differences were found between participants and non-participants, however, as the study aim was to assess responsiveness rather than to measure treatment effect, selective loss to follow-up is not likely to cause bias. Thirdly, due to the COVID pandemic a lot of treatments were postponed, hampering patient inclusion and resulting in a moderate sample size (n=63). However, a sample size >50 is considered adequate for studies on responsiveness[24], especially in light of the rarity of the disorder.

The clinical heterogeneity among patients with vascular malformations resulted in a variety of treatment methods to manage this diverse patient group, and the revelation of mutated genes causative of vascular malformations has precipitated other novel treatment techniques through targeted therapies.[36-38] Particularly, when various treatments are used, uniformity of outcome measurement is needed so that treatment outcomes can be adequately compared. The first steps to uniformity in outcome measurement were established with the CDS, and the subsequent development and responsiveness evaluation of the OVAMA questionnaire provides a measurement instrument to assess the condition-specific outcome domains determined in the CDS.[9, 10, 13]

Additionally, the OVAMA questionnaire enables treatment evaluation from the patient's perspective, which was considered crucial by the vascular malformation community.[10] Treatments are generally initiated to relieve symptoms and improve appearance, which can now be measured with the OVAMA questionnaire.

In conclusion, the constructs of the OVAMA questionnaire correlated well with the GRC scales, and 84% of predefined hypotheses were confirmed, indicating sufficient responsiveness of the OVAMA questionnaire. Our study supports the use of the OVAMA questionnaire to measure treatment effect on symptoms and appearance in patients with vascular malformations.

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Supplementary Materials

Supplement 1. Global Rating of Change scales

The following questions are on *changes* in problems caused by the vascular malformation.

How did the following problems change since the start of the treatment? If you did not receive any treatment (wait-and-see policy), you can assess the change since it was decided that you would not receive a treatment.

	Much worse	Moderately worse	A little worse	No change	A little better	Moderately better	Much better	Never had this problem
General problems of the vascular malformation	-3	-2	-1	0	+1	+2	+3	
Pain because of the vascular malformation	-3	-2	-1	0	+1	+2	+3	X
Bleeding because of the vascular malformation	-3	-2	-1	0	+1	+2	+3	X
Leakage of fluid (other than blood) from the vascular malformation	-3	-2	-1	0	+1	+2	+3	X
Temporary enlargement of the vascular malformation	-3	-2	-1	0	+1	+2	+3	X
Appearance of the vascular malformation	-3	-2	-1	0	+1	+2	+3	X
Visible swelling/mass of the vascular malformation	-3	-2	-1	0	+1	+2	+3	X
Color of the vascular malformation (difference with normal skin)	-3	-2	-1	0	+1	+2	+3	X
Surface/texture of the vascular malformation, for example: irregular, rough or bumpy (difference with normal skin)	-3	-2	-1	0	+1	+2	+3	X
Alteration of facial features because of the vascular malformation	-3	-2	-1	0	+1	+2	+3	X
Alteration of bodily features (excluding face) because of the vascular malformation	-3	-2	-1	0	+1	+2	+3	X
Depression, helplessness, unhappiness	-3	-2	-1	0	+1	+2	+3	
Anxiety, worries, nervousness	-3	-2	-1	0	+1	+2	+3	
Physical functioning, for example: chores around the house, walking, climbing stairs, carrying groceries.	-3	-2	-1	0	+1	+2	+3	
Ability to participate in social roles and activities, for example: regular leisure activities, work, activities with friends or family.	-3	-2	-1	0	+1	+2	+3	

Supplementary Table 1. Baseline characteristics of participants versus non-participants.

Participants completed the OVAMA questionnaire at baseline and at follow-up the GRC scales and OVAMA questionnaire. Non-participants only completed the OVAMA questionnaire at baseline.

IQR = Interquartile range.

	Participants		
	Case number or Median (IQR)		
	No	Yes	p-value
Male			
No	22	34	0.70
Yes	16	29	
Age	24 (17-36)	32 (22-46)	**0.03**
Syndrome			
No	38	56	**0.03**
Yes	0	7	
Overgrowth			
No	36	56	0.32
Yes	2	7	
Vascular malformation type			
Venous	24	32	
Lymphatic	5	9	
Capillary	4	4	0.57
Arteriovenous	1	3	
Combined	4	14	
Lesion localization			
Head and neck			
No	26	44	0.88
Yes	12	19	
Upper extremity			
No	29	47	0.85
Yes	9	16	
Trunk			
No	35	46	**0.02**
Yes	3	17	
Lower extremity			
No	21	42	0.25
Yes	17	21	
Tissue extension			
(sub)cutaneous			
No	7	17	0.38
Yes	31	46	
Intramuscular			
No	20	35	0.78
Yes	18	28	
Intraosseous			
No	35	47	**0.03**
Yes	3	16	
Size			
<5 cm	17	22	
5-10 cm	11	15	
10-30 cm	5	14	0.27
>30 cm	2	10	
Unclear	2	1	
Maximal diameter (cm)	6.9 (3.5-9)	7.2 (4-19)	0.21

Treatment			
Watchful waiting	9	9	
Sclerotherapy	23	35	
Surgery	2	9	0.48
Compression stockings	3	5	
Embolization	1	3	
Anticoagulants	0	2	

Supplementary Table 2. Differences in change measured with the Global Rating of Change scales between the conservative and invasive treatments groups.

The global rating of changes scales captured change on a seven-point textual interval scale, ranging from 'much worse' to 'much better'. A score of 0 indicated 'no change' and a score of 1 indicated 'a little bit better'. GRC = Global Rating of Change scale; IQR = Interquartile range

GRC constructs	Conservative treatment group *Median (IQR)*	Invasive treatment group *Median (IQR)*	p-value
General problems	0 (0-0)	1 (0-2)	**0.006**
Pain	0 (0-0)	1 (0-2)	0.30
Bleeding	0 (0-0)	0 (0-2)	0.38
Leakage of fluids	0 (0-0)	0 (0-1.25)	0.51
Temporary enlargement	0 (0-0)	0 (0-2)	**0.008**
Appearance	0 (0-0)	1 (0-2)	**<0.001**
Visible swelling	0 (-0.75-0)	1 (0-2.75)	**0.002**
Colour	0 (0-0)	0 (0-1)	**0.033**
Texture	0 (-0.75-5.25)	0 (-0.75-2)	0.89
Facial distortion	0 (0-0)	0 (0-2)	0.17
Bodily distortion	0 (-0.75-0)	0 (0-2)	0.06
Depression symptoms	0 (0-0)	0 (0-1.5)	0.15
Anxiety symptoms	0 (0-0)	0 (-1 - 2)	0.30
Physical functioning	0 (0-0)	0 (0-2.25)	0.092
Social functioning	0 (0-0)	0 (0-1.5)	0.11

Chapter 8

Development of a condition-specific patient-reported outcome measure for measuring treatment outcome in peripheral vascular malformations: the OVAMA Treatment Outcome questionnaire.

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Submitted

Abstract

Background

Patients with vascular malformations (VMs) may experience various symptoms and a diminished quality of life. Many treatment options are available, although it is difficult to compare them due to the lack of applicable validated outcome measures. Additionally, the patient's perspective is often omitted in treatment assessment. Therefore, it is crucial to evaluate the effect of treatment from the patient's perspective.

Objectives

To develop a patient-reported outcome measure (PROM) to measure satisfaction with the treatment outcome in patients with VMs, and to investigate relevant measurement properties of the PROM, and to present preliminary results of satisfaction with treatment outcomes.

Methods

Based on an internationally developed core domain set, a first draft of the PROM was formulated, called the OVAMA-Treatment Outcome questionnaire (OVAMA-TO). In interviews with 14 patients, content validity was assessed, which led to a second draft. In a cross-sectional study, construct validity of the OVAMA-TO questionnaire was investigated by testing nine predefined hypotheses about expected relationships with the Global Rating of Changes (GRC) scales, measuring similar constructs. In univariate analysis using Kruskal-Wallis test, satisfaction with treatment outcome was compared between patients who received different treatments.

Results

Adequate content validity was found in the patient interviews, and resulted in five items referring to satisfaction with the treatment outcome and change in various symptoms, and two items referring to tolerability of treatment. 104 patients completed the OVAMA-TO questionnaire and the GRC scales, and all nine hypotheses on expected relationships with the GRC scales were confirmed, hence, construct validity was considered good. Patients treated with surgery were the most satisfied overall with the treatment outcomes.

Conclusions

Satisfaction with the treatment outcome can now be adequately measured from the patient's perspective, and the OVAMA-TO questionnaire can be used in clinical research to achieve consistency in outcome reporting, allowing for adequate comparison of treatments. These are crucial steps for evidence-based guidelines for patients with VMs.

What is already known about this topic?

- Symptoms and appearance of vascular malformations may negatively impact the patient's quality of life.
- Treatments are deployed to improve these subjective outcomes, therefore, treatment evaluation from the patient's perspective is crucial.
- Satisfaction with treatment and outcome are core domains and should be measured in all clinical research on vascular malformations.
- No instrument exists for measuring satisfaction with treatment and outcome in vascular malformations, and heterogeneity is present in outcome measurement.

What does this study add?

- In this study, a condition-specific patient-reported outcome measure was developed to measure satisfaction with treatment and outcome in patients with vascular malformations called the OVAMA Treatment Outcome questionnaire (OVAMA-TO)
- The content and construct validity of the OVAMA-TO were considered adequate in this study.
- This study showed that patients treated with surgery were the most satisfied overall with the treatment outcomes.

What are the clinical implications of this work?

- The core domains satisfaction with the treatment and outcome in vascular malformations can now be adequately measured from the patient's perspective.
- The OVAMA-TO questionnaire can be used in clinical research to achieve consistency in outcome reporting, allowing for adequate comparison of treatments.
- Consistency in outcome reporting with the use of the OVAMA-TO may significantly improve research and ultimately lead to evidence-based guidelines for patients with vascular malformations.

Introduction

Vascular malformations (VMs) arise due to defects during embryologic development and result in defects of the vascular and/or lymphatic system. These anomalies consist of tangled, dilated, and dysfunctional vessels that grow proportionally with the patient. VMs are classified according the types of vessels involved: capillary, venous, lymphatic, arteriovenous, and combined malformations.[1, 2] Since blood and lymphatic vessels are present throughout the whole body, VMs may occur anywhere and involve different tissues.

VMs often present as a mass that alters the skin texture and/or colour and may cause deformity and affect appearance in this patient population.[3] Additionally, patients may experience a wide range of symptoms, including pain, swelling, bleeding, fluid leakage, thrombosis and functional impairment.[4-7] Consequently, patients with VMs often experience impaired health-related quality of life (HRQOL), affecting both physical and mental well-being.[8, 9]

Various treatment options can be applied to improve the symptoms. The traditional treatment modalities include surgery, sclerotherapy, embolization, and laser therapy.[10-13] However, the discovery of gene mutations involving in the proliferation pathways of endothelial cells in VMs has led to the use of inhibitors targeting these signalling pathways.[14, 15] Since complete eradication of VMs is generally not feasible, therapies are mostly deployed to manage symptoms and improve appearance and HRQOL. These are subjective outcomes and therefore evaluation from the patient's perspective is crucial.

Irrespective of the diversity of treatment options, evidence-based guidelines are lacking. An important reason for this lack is the heterogeneity in outcome reporting in clinical research, complicating the aggregation of study results.[10, 11, 16] This highlights the need for standardized outcome measures to assess treatment outcomes and to properly compare all these diverse treatment methods.[17]

The OVAMA (Outcome measures for VAscular MAlformations) project attempts to establish homogeneity in outcome reporting. With patients and experts worldwide it was determined in a Core Domain Set (CDS) which outcomes should be measured when evaluating treatment outcome (Figure 1).[6, 18] The CDS consists partially of non-condition-specific domains, such as the domains falling under HRQOL, which are measured with generic outcome measurement instruments.[19] To measure the condition-specific domains 'symptoms', 'appearance', and 'satisfaction' adequate measurement instruments were sought, although, these were not available.[20] Therefore, the patient-reported outcome measure (PROM) the OVAMA-questionnaire was developed to measure the outcome domains 'symptoms' and 'appearance'.[21]

The domain category 'satisfaction' referring to satisfaction with outcome and treatment was not included in the OVAMA questionnaire since it is only relevant at follow-up. It is crucial that patients are able to evaluate the effect of treatment from their perspective and express their satisfaction with treatment outcomes in clinical studies since the ultimate goal of treatment is to improve subjective outcomes. Here we describe the development of the

Treatment Outcome questionnaire (OVAMA-TO) to measure satisfaction with outcome and treatment in patients with VMs and report on the assessment of the measurement properties content and construct validity. Finally, we provide preliminary results of treatment outcomes measured with the OVAMA-TO questionnaire.

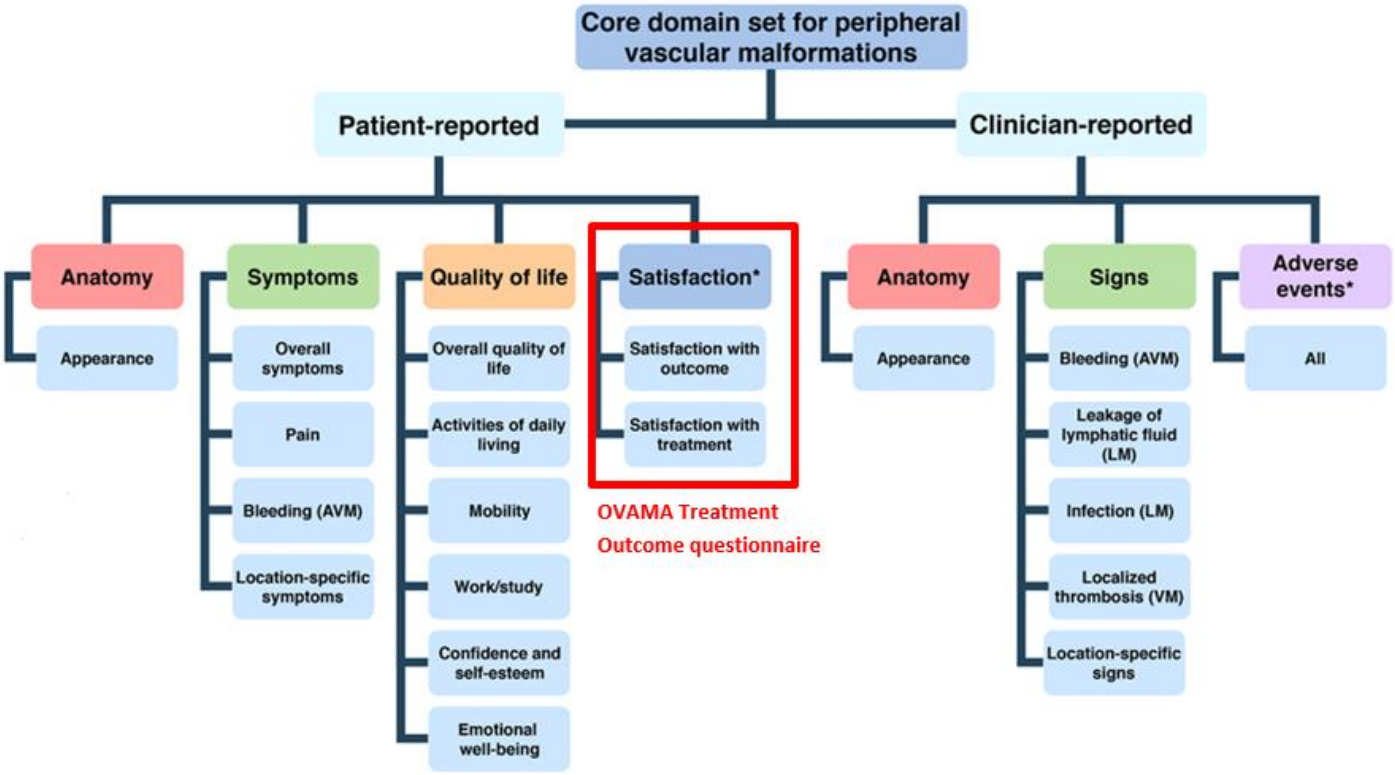

Figure 1 – Core domain set for peripheral vascular malformations.

The domain category 'satisfaction' consist of the outcome domains 'satisfaction with outcome' and 'satisfaction with treatment'. The OVAMA Treatment Outcome questionnaire was developed to measure the domain category 'Satisfaction'.

AVM = arteriovenous malformations; LM = lymphatic malformations; VM = venous malformation.

Methods

Study design

The COSMIN (COnsensus-based Standards for the selection of health Measurement INstruments) 'study design for PROMs' checklist was followed for this study.[22] The study adhered to the Declaration of Helsinki, and informed consent was obtained from all patients.

Development

In an international e-Delphi study, including 167 physicians and 134 patients/parents of patients, and two consensus meetings a CDS was developed wherein outcome domains were defined (Figure 1).[6, 18] The condition-specific domain category 'satisfaction' and subjected outcome domains 'satisfaction with outcome' and 'satisfaction with treatment' could not be measured with existing measurement instruments since these were not available.[20] The outcome domain 'satisfaction with outcome' was defined as satisfaction with cosmetic outcome, functional outcome, and symptom relief. The outcome domain 'satisfaction with treatment' was defined as tolerability of treatment and willingness to undergo the same treatment again.[6] Fully based on these outcome domains, the first draft of the OVAMA-TO questionnaire consisted of items referring to the satisfaction with outcome on 'general problems', 'appearance', and 'physical functioning', and items referring to 'change in the size of the VM', 'satisfaction with the chosen treatment', 'tolerability of treatment', and 'willingness to undergo treatment again', the latter three belonging to the satisfaction with treatment.

Content validity

In patient interviews, concept elicitation and extensive review of the first draft were conducted. According to COSMIN guidelines, a sample size of ≥7 patient interviews was considered sufficient.[22] A heterogeneous group of 14 patients with VMs were approached and included, as described in a previous study.[21] Three adolescents (aged 14-17 years) were interviewed to assess if the questions were comprehensible for this age group and if the concepts of interest were the same. Two Medical Doctors (MS and ML), both experienced with outcome measures in VMs, conducted the interviews.

The first part of one-on-one interviews consisted of concept elicitation, and formulation of open-ended questions to identify what the patients considered the most relevant aspects to measure after treatment. In the second part, patients extensively reviewed the draft questionnaire in the interviews, to assess the comprehensibility, relevance, and completeness of the concepts of interest and response options.

All interviews were audio-recorded and transcribed. The interviews were coded by the two researchers independently, and scored to evaluate how the patient responded to the concept (spontaneously, after probing, or after reading the questionnaire). After each interview, the items and the response options were revised according to relevant patient feedback.

We evaluated if the items referring to satisfaction with treatment and outcome in the OVAMA-TO questionnaire had adequate internal consistency (Cronbach's alpha >0.7) to form a composite score that represented the overall satisfaction with treatment outcome.

Construct validity

Patients from the Amsterdam University Medical Centres VM database were invited to participate in the cross-sectional study assessing construct validity. Patient data was extracted from electronic patient files including age, sex, VM type according the International Society for the Study of Vascular Anomalies classification[2], lesion localization, types of tissues involved, lesion size (maximal diameter), the presence of overgrowth and/or diagnosis of a syndrome, treatment(s) received, and date of most recent treatment.

Between September 2020 and April 2022, adult patients and parents of children identified from our local VM database who were managed for their VM (including explicit watchful waiting policy) in the past five years were called and asked to participate in the study. Patients were sent an invitation by email if they could not be reached by telephone (n=14). Patients were asked to complete the OVAMA-TO questionnaire to evaluate the outcome of their most recent treatment. Parents of children 0-13 years old were instructed to guide their child (where needed) in completing the questionnaire.

The commonly way to investigate construct validity of PROMs is to test predefined hypotheses about expected relationships with other outcomes measures of good quality, measuring the same constructs.[22] In this case, the Global Rating of Change (GRC) scales, since it can be considered the gold standard for measuring change since treatment.[23, 24] Simultaneous with the final version of the OVAMA-TO questionnaire, patients filled in the GRC scales, assessing change in symptoms since treatment or watchful waiting policy (supplement 1). GRC scales are designed to quantify a patient's improvement or deterioration of their symptoms over time, usually to determine the effect of treatment.[24, 25] However, the scales were adapted to capture the relevant and desired constructs.[24] We formulated nine GRC scales corresponding with the constructs measured with the OVAMA-TO, that captured change in these constructs on a seven-point Likert scale. Additionally, patients had the option to state if they had never had this problem. In that case, the question was considered not applicable to the patient and was made a missing value.

Nine hypotheses were formulated beforehand by the two independent researchers (Table 1), based on the methodology guidelines of COSMIN and previous studies assessing construct validity.[22, 23, 26-29] Correlation strength was calculated for the items of the OVAMA-TO questionnaire with the GRC scale measuring change in a similar or a related but dissimilar construct, referred to in hypotheses 1 to 6. Correlation was interpreted as high (≥ 0.5), moderate (0.3 – 0.5) or low (≤ 0.3), based on previous studies and guidelines.[23, 26, 28] Correlation strength between similar constructs were expected to be high (hypotheses 2, 3, 4 and 6).

Correlation strength between related but dissimilar constructs were expected to be moderate (hypotheses 1 and 5). Hypotheses 7, 8, and 9 refer to the overall treatment satisfaction and how it relates to the GRC scale 'general problems'. The OVAMA-TO questionnaire construct validity was considered good in relation to the GRC scales if ≥75% of the hypotheses were confirmed.[22, 26]

Treatment Outcome

Satisfaction with treatment outcome was explored with the OVAMA-TO questionnaire between groups receiving different treatments (e.g., watchful waiting, sclerotherapy, surgery). Among different treatment groups, percentages of patients who were satisfied with the effect of treatment on various symptoms measured with the OVAMA-TO questionnaire were displayed in a histogram.

Data analyses

Statistical differences in all baseline characteristics between responders and non-responders were explored; Chi-square test was used for categorical variables and Mann-Whitney U test for nonparametric continuous variables. Correlation strength between the OVAMA-TO questionnaire and GRC scales were measured using Spearman's rank correlation. Statistical differences in satisfaction with treatment outcome among different treatment groups were measured with the Kruskal-Wallis test for numerical outcome data, for numerical differences between subgroups a post hoc Bonferroni correction was applied. All statistical analyses were performed with SPSS, 26.0 (IBM, Armonk, NY, U.S.A.).

Table 1. Hypotheses for measuring construct validity.

Hypotheses about expected relationships between the OVAMA-TO questionnaire and GRC scales, measuring corresponding constructs.
Correlation strength between related but dissimilar constructs were expected to be moderate (hypotheses 1 and 5). The size of the vascular malformation is highly similar to the visible swelling or mass of the vascular malformation. However, the surface of flat capillary malformations or the volume of interior vascular malformations that are not visible are also attributable to size. Therefore, only a moderate correlation was expected between the size and visible swelling. The appearance-related aspects visible swelling, colour, and surface/texture are only a subject of appearance, while overall appearance is more extensive. Therefore, only moderate correlations were expected for this hypotheses.

	Hypotheses
1.	Moderate to high correlation (≥ 0.3) between "size of the vascular malformation" and the GRC scale "visible swelling/mass".
2.	High correlation (≥ 0.5) between "satisfaction with effect on general problems" and the GRC scale "general problems".
3.	High correlation (≥ 0.5) between "satisfaction with effect on pain" and the GRC scale "pain".
4.	High correlation (≥ 0.5) between "satisfaction with effect on appearance" and the GRC scale "appearance".
5.	Moderate to high correlation (≥ 0.3) between "satisfaction with effect on appearance" and at least one of the GRC scales "visible swelling", "color", and "surface/texture".
6.	High correlation (≥ -0.5) between "satisfaction with effect on physical functioning" and the GRC scale "physical functioning".
7.	Patients indicating improvement on the GRC scale "general problems" should have a mean score >3 of the OVAMA-TO questionnaire (corresponding with being (very) satisfied with overall treatment effect).
8.	Patients indicating worsening on the GRC scale "general problems" should have a mean score <3 of the OVAMA-TO questionnaire (corresponding with being (very) dissatisfied with overall treatment effect).
9.	Patients indicating improvement on the GRC scale "general problems" should have a higher mean OVAMA-TO score than unchanged patients on the GRC scale "general problems", which in turn should be higher than worsened patients on the GRC scale "general problems".

Results

Content validity

Fourteen patients were interviewed (supplement 2), and an overview of the interview results are shown in supplement 3. The effect of treatment on appearance, the size of the lesion and pain were considered the most relevant for the patients, therefore, an item referring to the effect of treatment on pain was added to the OVAMA-TO questionnaire. Patients found the items 'satisfaction with the chosen treatment' and 'satisfaction with the effect on general problems' too much overlap, therefore, these two items were merged into one. Some patients deemed the item 'tolerability of the treatment' vague or interpreted it as the side-effects of treatment, and others missed an item referring to the side-effects, therefore, 'tolerability of treatment' was changed accordingly.

Patients considered the quantifying response to treatment on a five-point scale (ranging from very dissatisfied to very satisfied) appropriate. However, the patients also stated a response option should be added in which patients had the ability to indicate that they had never experienced that particular symptom. Additionally, some patients mentioned that they were unable to assess the size of the VM because it was located internally. Finally, patients reported that they would like to explain in more detail if they were unwilling to undergo the same treatment again. These changes to the response options were implemented. The interviewed patients deemed the questionnaire comprehensible for children older than 14 years, and advised the guidance of parents in completing the questionnaire for children 13 years and younger (proxy-reporting).

The final draft of the OVAMA-TO questionnaire consisted of seven items (supplement 4), with four items referring to the satisfaction with treatment outcome on 'general problems' 'appearance', 'physical functioning', and 'pain', and items referring to 'change in the size of the VM', 'side-effects', and 'willingness to undergo treatment again'. All responses were scored in ordinal fashion to allow for statistical analysis. Cronbach's Alpha (0.86) was adequate for a cumulative score to measure the overall satisfaction with the treatment outcome.

Construct validity

In total, 143 patients including 118 self-reported and 25 proxy-reported, were invited to complete the questionnaires. Of the invited participants, 73% (n=104) completed the OVAMA-TO questionnaire and GRC scales (86% (n=89) self-reported and 14% (n=15) proxy-reported). The baseline characteristics of all included patients are listed in Table 2. A statistically significant difference was found in the age between non-responders and responders, with the responders being older (p = .004) (supplement 5).

Table 3 shows the results of hypotheses testing the expected relationships between the OVAMA-TO and the GRC scales, measuring related constructs. All hypotheses were confirmed and good construct validity of the OVAMA-TO questionnaire was proven.

Treatment outcome

Patients being satisfied or very satisfied with treatment outcome among different treatment groups are shown in Figure 2. Statistical analysis of treatment outcomes between different treatment groups is shown in Table 4. A statistically significant difference was found between the different treatment options regarding satisfaction with treatment outcome on the size of the VM (p = .001), general problems (p=0.04), and physical functioning (p=0.013). All other treatment outcomes were not statistically different between the various treatment options.

Bonferroni adjusted post hoc analyses showed statistically significant more satisfaction with the effect on the size of the lesion in patients receiving surgery compared to patients who received sclerotherapy (p = .023), compression stockings (p = .001), or watchful waiting policy (p = .001).

Post hoc analyses showed more satisfaction with the effect on general problems in patients receiving surgery compared to patients who received sclerotherapy (p = .015) or watchful waiting policy (p = .009).

Finally, Bonferroni adjusted post hoc analyses showed statistically significantly more satisfaction with the effect of treatment on physical functioning in patients receiving surgery compared to patients who received sclerotherapy (p= .043).

Table 2. Baseline characteristics

**Time to follow-up refers to time in months between the last treatment and completing the questionnaires. IQR = Inter Quartile Range.*

Patient characteristics	Case number (%)
Females	63 (60.6%)
Age in years (median, IQR)	31 (19-49)
Children (0-17 years)	23 (22.1%)
Vascular malformation type	
Venous	48 (46.2%)
Lymphatic	8 (7.7%)
Arteriovenous	14 (13.5%)
Capillary	9 (8.7%)
Combined	25 (24.0%)
Localization	
Head and neck	40 (38.5%)
Upper extremity	25 (24.0%)
Lower extremity	33 (31.7%)
Trunk	23 (22.1%)
Tissue extension	
(Sub)cutaneous	87 (83.7%)
Intramuscular	44 (42.3%)
Intraosseous	19 (18.3%)
Unclear	7 (6.7%)
Maximal diameter in cm	
<5 cm	37 (35.6%)
5-10 cm	24 (23.1%)
10-30 cm	28 (26.9%)
> 30 cm	13 (12.5%)
Unclear	2 (1.9%)
Previous therapy	
Watchful waiting	11 (10.6%)
Sclerotherapy	50 (48.1%)
Surgery	23 (22.1%)
Laser therapy	8 (7.7%)
Embolization	5 (4.8%)
Compression stockings	6 (5.8%)
Radiofrequency ablation	1 (1.0%)
Time in months to follow-up* (median, IQR)	17 (2-38.5)

Table 3. Hypotheses results for the evaluation of construct validity of the OVAMA-TO questionnaire.

**Spearman's rank correlation coefficients between OVAMA follow-up questionnaire and GRC scales. SD = Standard deviation.*

	Hypothesis	n	Correlation Coefficient*	Confirmation
1	Moderate to high correlation (≥0.3) between "size of the vascular malformation" and the GRC scale "visible swelling/mass".	86	0.765	Confirmed
2	High correlation (≥0.5) between "satisfaction with effect on general problems" and the GRC scale "general problems"	101	0.748	Confirmed
3	High correlation (≥ 0.5) between "satisfaction with effect on pain" and the GRC scale "pain".	86	0.663	Confirmed
4	High correlation (≥ 0.5) between "satisfaction with effect on appearance" and the GRC scale "appearance".	88	0.607	Confirmed
5	Moderate to high correlation (≥ 0.3) between "satisfaction with effect on appearance" and at least one of the GRC scales "visible swelling", "colour", and "surface/texture".			Confirmed
	"Visible swelling"	84	0.544	
	"Colour"	74	0.311	
	"Surface/texture"	71	0.438	
6	High correlation (≥0.5) between "satisfaction with effect on physical functioning" and the GRC scale "physical functioning".	62	0.718	
			Mean (SD)	
7	Patients indicating improvement on the GRC scale "general problems" should have a mean score >3 of the OVAMA-TO questionnaire (corresponding with being (very) satisfied with overall treatment effect).	55	3.9 (±0.76)	Confirmed
8	Patients indicating worsening on the GRC scale "general problems" should have a mean score <3 of the OVAMA-TO questionnaire (corresponding with being (very) dissatisfied with overall treatment effect).	12	2.36 (±0.90)	Confirmed
9	Patients indicating improvement on the GRC scale "general problems" should have a higher mean OVAMA-TO score than unchanged patients on the GRC scale "general problems", which in turn should be higher than worsened patients on the GRC scale "general problems".	Improved Unchanged Worsened	3.9 (±0.76) 3.34 (±0.83) 2.36 (±0.90)	Confirmed

Table 4. Univariate analysis of treatment satisfaction among different treatments.

*Statistically significant p-values are displayed in bold. IQR = Inter Quartile Range. *Same treatment refers to willingness to receive the same treatment again.*

Change in the size of the vascular malformation was questioned on an eight-point verbal rating scale, ranging from 1 'much larger' to 8 'completely disappeared'. Satisfaction with the effect on general problems, pain, appearance, and physical functioning were questioned on a five-point verbal rating scale, ranging from 1 'very dissatisfied' to 5 'very satisfied'. Bothering by side-effects was questioned on a five-point verbal rating scale, ranging from 1 'extremely' to 5 'not at all'.

		Size		General problems		Pain		Appearance		Physical functioning		Side effects		Same treatment*		
		Median		*Median*		*Median*		*Median*		*Median*		*Median*		*Case number*		
Treatment	*n*	*(IQR)*	*P*	*(IQR)*	*P*	*(IQR)*	*P*	*(IQR)*	*P*	*(IQR)*	*P*	*(IQR)*	*P*	*Yes*	*No*	*P*
Watchful waiting	11	4 (3.5-4.5)		3 (3-4)		3 (3-4)		3.5 (3-4)		3 (3-4)		5 (4-5)		8	3	
Sclerotherapy	50	5 (4-6)		4 (3-4)		4 (3-4)		3 (2-4)		4 (3-4)		4 (3-5)		37	13	
Surgery	23	7.5 (5-8)		4 (4-5)		4 (3-5)		4 (4-4.75)		4 (4-5)		4 (3-5)	.	20	3	
Laser therapy	8	5 (4-6)	**<0.001**	3 (3-4)	**.004**	4 (2.5-4)	0.29	3 (2-4)	.069	4 (4-4.75)	**.013**	5 (3.25-5)	.18	6	2	0.62
Embolization	5	6 (4-6)		4 (3-4)		4 (3-4.5)		3 (2-3)		4 (2.5-3.5)		3 (2-5)		5	0	
Compression stockings	6	3.5 (2-4)		3.5 (2.75-4)		3 (2.5-3.5)		3 (3-3)		3.5 (2.75-4)		5 (4-5)		4	2	

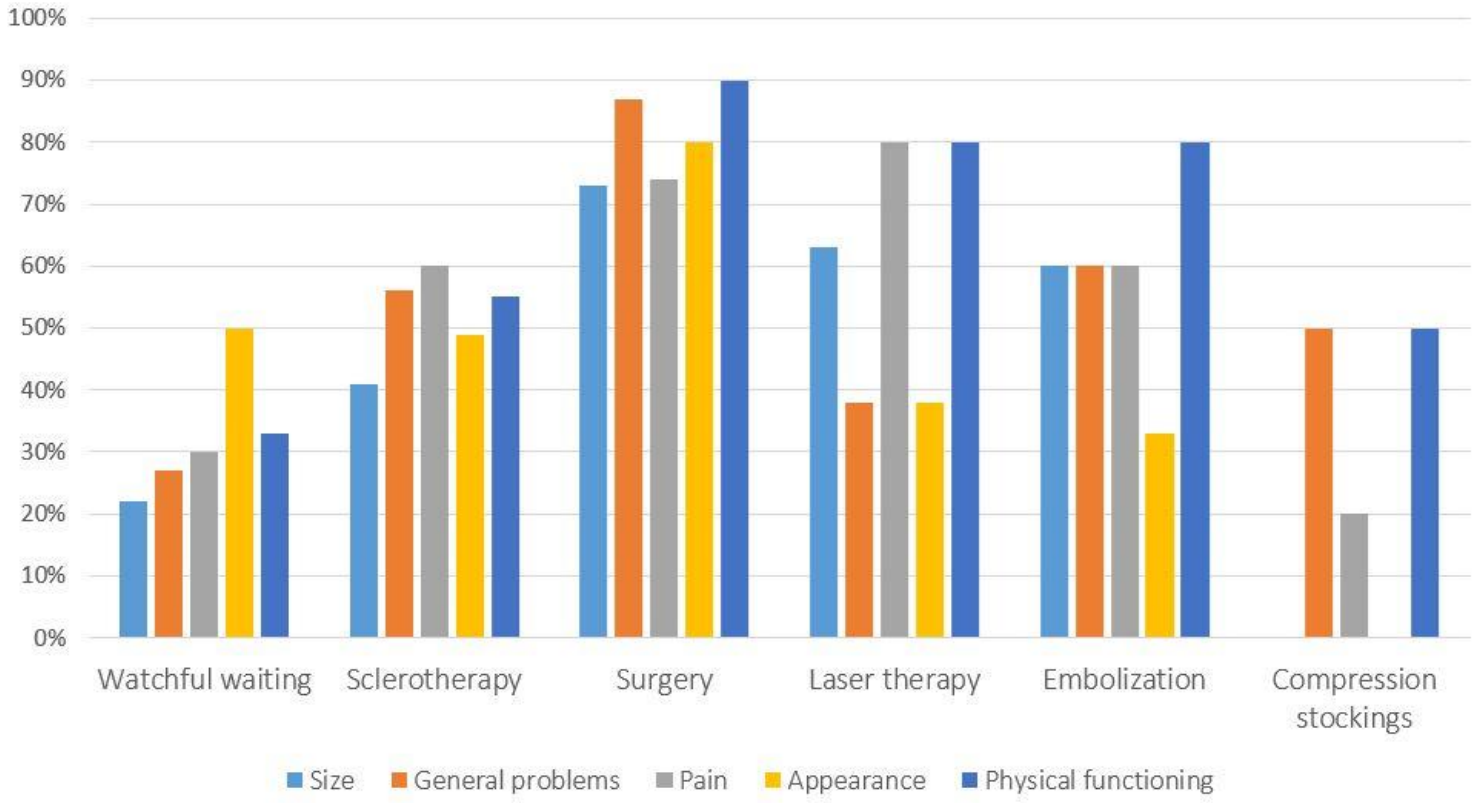

Figure 2 – Percentages of patients being satisfied or very satisfied with treatment outcome

The figure displays the percentages of patients who were satisfied or very satisfied with the effect of treatment on each symptom (e.g., size and pain) among the different treatment groups. Showing that patients who received surgery were most frequently satisfied with the effect of treatment on size, general problems, appearance, and physical functioning. On the contrary, patients who followed a watchful waiting policy or were treated with compression stockings were overall the least frequently satisfied with treatment outcomes.

Discussion

With extensive input from patients and clinical experts in multiple international studies, a condition-specific PROM was developed to evaluate treatment outcome in patients with VMs: the OVAMA-TO questionaire.[6, 18, 21] Additionally, this study demonstrated that the OVAMA-TO questionnaire provides good content and construct validity in patients with peripheral VMs. Therefore, the OVAMA-TO questionnaire is suitable for evaluation of treatment outcome in patients with VMs.

All hypotheses were confirmed and construct validity was considered good. A moderate to high correlation between the constructs 'size of the VM' of the OVAMA-TO questionnaire and 'visible swelling/mass' of the GRC scale was expected since these were related but dissimilar constructs. However, we found that these constructs were very highly correlated, implying that patients relate the visible swelling to the size of the VM. Although, only a few patients with capillary malformations were included, in whom the VM is usually flat without visible swelling and therefore these patients probably had a different interpretation of the size of the lesion.

The GRC scale 'colour' was moderately correlated with satisfaction with the effect of treatment on 'appearance', while 'visible swelling' was highly correlated with 'appearance', suggesting that changes in visible swelling contribute more to satisfaction with the effect of treatment on appearance than changes in colour. This might be because colour changes could be more difficult to determine for patients than changes in visible swelling, or because colour changes might be easier to conceal with make-up.

Construct validity was confirmed by testing predefined hypotheses on relations between satisfaction with treatment outcome (OVAMA-TO) and change after treatment (GRC scales), because these are separate but highly correlated constructs.[30-34] Furthermore, studies among patients with VMs showed that satisfaction with the treatment outcome seems to be strongly dependent on the subjective change in symptoms, and other factors, such as sex, the type, location and extent of the VM, and the number of treatments, showed not to be contributable to the satisfaction with treatment.[35-38]

Treatment outcome

Patients who underwent surgery were overall the most satisfied with treatment outcome and were significantly more satisfied with the effect on 'general problems', the 'size' of the lesion, and 'physical functioning'. They were also most frequently satisfied with the effect of treatment on the 'appearance' and second most satisfied with 'pain'. These findings show that surgery is a greatly appreciated treatment from the patients' perspective. However, these results should be interpreted with caution, because surgery is only performed in selected patients, excluding patients with large, intramuscular/intraosseous, or inoperable VMs. A recent systematic review revealed similar findings that surgery can be effective and safe, but that it is mostly performed in smaller lesions, and that large or deeply extending lesions are associated with subtotal resections and recurrences.[10] However, our results clearly show that for cases when surgery is feasible, it should be considered because of the high satisfaction with the treatment outcome.

For each item addressed in OVAMA-TO questionnaire, consistently half of the patients who received sclerotherapy indicated that they were satisfied with the treatment outcome. This is consistent with previous research showing sclerotherapy results in improvements in health and HRQOL in about half of treated patients[39], which would consequently improve satisfaction with outcomes.

Merely 20% of the patients treated with compression stockings reported being satisfied with the effect on pain, while they were generally prescribed to relieve pain and to prevent painful thrombotic events. A previous study also showed that compression stockings fail to eliminate pain and currently there is no high-quality evidence available to justify its use.[4, 16]

Laser therapy is generally used to improve the colour and nodules/blebs of capillary malformations, and only 38% of this patient cohort was satisfied with its effect on appearance. This might be explained by unrealistic expectations of laser therapy, as its efficacy is limited and has not improved in the past decades.[13]

Patients who followed a watchful waiting policy were generally the least satisfied with all treatment outcomes. Although, this possibly depicts a biased result since patients not undergoing treatment probably did not have severe symptoms. Therefore, in observational studies where interventions are compared with a watchful waiting policy, the study results should be interpreted with care.

There exist several limitations in our study. The inclusion of all types of malformations resulted in a low number of patients with rare VM types, and these patients might be underrepresented. However, we have intentionally chosen to develop and validate the OVAMA-TO questionnaire for all VM types to enable adequate comparison of outcomes among all subgroups. Additionally, subgroups are currently primarily based on the VM type, although, the emergence of the genetic bases of VMs might shift the view on the subgroup classifications. In the assessment of treatment outcome, the choice of treatment was discussed with the patients, although patients were mostly eligible for a limited treatment options or advised to follow a watchful-waiting policy in patients experiencing minimal symptoms, and this might have influenced the patient's satisfaction with treatment outcome. Nevertheless, this is inevitable since some treatments are not feasible for certain types of VMs, e.g., laser therapy is only suitable for superficial VMs. We included various lesion characteristics per treatment, resulting in heterogeneous treatment groups for the evaluation of treatment outcome. However, as the different treatment groups were already small, further subgroup analyses were not desirable from a statistical point of view.

In clinical setting, satisfaction with treatment outcome, a core outcome domain that emerged from the international VM community[6, 18], can now be evaluated from the patient's perspective using the OVAMA-TO questionnaire. The OVAMA-TO is complementary to the OVAMA questionnaire, which was developed to measure changes in symptoms and appearance before and after treatment.[21] Additionally, the OVAMA-TO questionnaire may be used separately in retrospective studies to describe outcomes in a standardized manner. The GRC scales may be

used alongside the OVAMA-TO questionnaire to capture the change in symptoms more comprehensively.

In research, both the OVAMA and OVAMA-TO questionnaires may provide more consistent outcome reporting, allowing for adequate comparison of treatments, which are crucial steps to evidence-based guidelines. International collaboration in the evaluation of therapeutic strategies, including less frequently applied treatments such as targeted therapies, using the OVAMA and OVAMA-TO questionnaires, could lead to a more enhanced and patient-based assessment. To encourage widespread use, the OVAMA and OVAMA-TO questionnaires are available in various languages at www.OVAMA.org.

Conclusion

To evaluate satisfaction with treatment outcome the OVAMA-TO questionnaire was developed, and good construct validity was proven. The OVAMA-TO questionnaire can now be utilized in clinical research to measure treatment outcome from the patient's perspective and possibly to reach consistent reporting of treatment outcome, paving the way for evidence-based guidelines.

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Supplementary Materials

Supplement 1 - Global rating of change scales

The following questions are on *changes* in problems caused by the vascular malformation.

How did the following problems change since the start of the treatment? If you did not receive any treatment (wait-and-see policy), you can assess the change since it was decided that you would not receive a treatment.

	Much worse	Moderately worse	A little worse	No change	A little better	Moderately better	Much better	Never had this problem
General problems of the vascular malformation	-3	-2	-1	0	+1	+2	+3	X
Pain because of the vascular malformation	-3	-2	-1	0	+1	+2	+3	X
Bleeding because of the vascular malformation	-3	-2	-1	0	+1	+2	+3	X
Leakage of fluid (other than blood) from the vascular malformation	-3	-2	-1	0	+1	+2	+3	X
Temporary enlargement of the vascular malformation	-3	-2	-1	0	+1	+2	+3	X
Appearance of the vascular malformation	-3	-2	-1	0	+1	+2	+3	X
Visible swelling/mass of the vascular malformation	-3	-2	-1	0	+1	+2	+3	X
Color of the vascular malformation (difference with normal skin)	-3	-2	-1	0	+1	+2	+3	X
Surface/texture of the vascular malformation, for example: irregular, rough or bumpy (difference with normal skin)	-3	-2	-1	0	+1	+2	+3	X

Supplement 2: Baseline characteristics of the interview participants.

Total n=14		
	Median (range)	**Median, IQR (25th-75th percentile)**
Age at baseline	33.3 (14-58)	32.0 (17.8-49.5)
	Frequency	**Percentage**
Gender		
Female	7	50.0
Ethnicity		
Dutch	10	71.4
Dutch/Indonesian	1	7.1
Aruban	1	7.1
Syrian	1	7.1
Chinese	1	7.1
Education		
High school	4	28.6
Post-secondary vocational education	6	42.9
Higher vocational education	2	14.3
Bachelor's University	1	7.1
Master's University	1	7.1
Type		
Venous	3	21.4
Arteriovenous	3	21.4
Venous, capillary	2	14.3
Lymphatic	2	14.3
Venous, lymphatic	2	14.3
Capillary, venous, lymphatic	1	7.1
Capillary	1	7.1
Overgrowth		
Yes	2	14.3
Localization		
Head/neck	6	42.9
Lower extremity	3	21.4
Upper extremity	2	14.3
Trunk	1	7.1
Upper extremity, trunk	1	7.1
Trunk, upper extremity, lower extremity	1	7.1
Size (largest diameter)		
<5 cm	3	21.4
5-10 cm	5	35.7
10-20 cm	1	7.1
20-30 cm	1	7.1
≥30 cm	3	21.4
Tissues involved		
Skin/subcutaneous tissue	5	35.7
Skin/subcutaneous tissue, muscle	3	21.4
Muscle	2	14.3
Skin/subcutaneous tissue, muscle, bone	1	7.1
Muscle, intra-articular	1	7.1
Skin/subcutaneous tissue, muscle, airway involvement	1	7.1
Skin/subcutaneous tissue, muscle, intra-abdominal	1	7.1
Treatment history included		
No prior treatment	2	14.3
Surgery	6	42.9
Compression stockings	3	21.4
Embolization	2	14.3
Laser therapy	2	14.3
Sclerotherapy	5	35.7
Sirolimus	1	7.1
Anticoagulants	1	7.1
Tracheostomy	1	7.1

Supplement 3. Coding results of the interviews.

Open-ended questions were asked to identify what patients considered the most relevant aspects to measure after treatment.

*S = mentioned spontaneously, P = mentioned after probing, Q = mentioned during questionnaire review. *Added later after spontaneous mention in first interviews.*

Patient	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
Type	VM	CM	LVM	VM	CVM	AVM	VM	AVM	LM	LM	CLVM	AVM	CVM	LVM	
Size	5-15 cm	5-15 cm	>30 cm	5-15 cm	15-30 cm	5-15 cm	<5 cm	5-15 cm	15-30 cm	5-15 cm	>30 cm	5-15 cm	>30 cm	<5 cm	
Localization	Abdomen	Head/ neck	Head/ neck	Knee	Head/ neck	Head/ neck	Wrist	Head/ neck	Arm/trunk	Leg	Arm/trunk/ abdomen/leg	Arm	Leg	Head/ neck	Total
Treatment and outcome															
Effect on size	S	Q	S	Q	S	Q	S	S	S	S	S	S	-	Q	**93%**
Effect on pain*	S	-	-	S	-	-	S	-	-	S	S	S	P	S	**57%**
Effect on appearance	-	S	S	-	S	S	-	-	S	-	S	Q	Q	S	**64%**
Effect on physical functioning	Q	-	-	S	-	-	Q	-	-	Q	Q	-	-	-	**36%**
Adverse events*	-	S	-	-	S	-	S	S	S	S	S	-	S	-	**57%**

Supplement 4

The OVAMA Treatment Outcome questionnaire

The following questions are about the outcomes of treatment (or the wait-and-see policy) of the vascular malformation. You only have to fill in the following questions if a treatment (or the wait-and-see policy) has already been started.

1. Size of the vascular malformation

How has the size of the vascular malformation changed since the start of treatment (or the wait-and-see policy)?

Much larger	Larger	Slightly larger	No change	Slightly smaller	Smaller	Much smaller	Completely disappeared

□ I am unable to assess the size of the vascular malformation

2. Satisfaction with effect on general problems

How satisfied are you with the effect of the treatment (or the wait-and-see policy) on general problems because of the vascular malformation?

□ Very dissatisfied
□ Dissatisfied
□ Not satisfied or dissatisfied
□ Satisfied
□ Very satisfied

3. Satisfaction with effect on pain

How satisfied are you with the effect of the treatment (or the wait-and-see policy) on the pain because of the vascular malformation?

□ Very dissatisfied
□ Dissatisfied
□ Not satisfied or dissatisfied
□ Satisfied
□ Very satisfied
□ I did not have pain because of the vascular malformation

4. Satisfaction with effect on the appearance

How satisfied are you with the effect of the treatment (or the wait-and-see policy) on the appearance of the vascular malformation?

□ Very dissatisfied
□ Dissatisfied
□ Not satisfied or dissatisfied
□ Satisfied
□ Very satisfied
□ I did not have appearance complaints because of the vascular malformation

5. Satisfaction with effect on physical functioning

How satisfied are you with the effect of the treatment (or the wait-and-see policy) on the functioning of your body? For example walking, moving the head, or moving the arms.

□ Very dissatisfied
□ Dissatisfied
□ Not satisfied or dissatisfied
□ Satisfied
□ Very satisfied
□ I did not have complaints of physical functioning because of the vascular malformation

6. Side-effects

How much were you bothered by side-effects of the treatment?

□ Extremely
□ A lot
□ Moderately
□ A little bit
□ Not at all

7. Willingness to undergo treatment again

If you could choose again, would you choose the same treatment (or the same wait-and-see policy) you had?

□ Yes
□ No

If not, why not?

……

Supplement 5 - Univariate analysis of responders versus non-responders.

IQR = Inter Quartile Range.

	Responders Case number		
	Yes	**No**	***P* value**
Sex			
Male	41	15	0.92
Female	63	24	
Age in years (median, IQR)	31 (19-49)	19 (13-31)	**0.004**
VM type			
Venous	48	22	
Lymphatic	8	2	
Arteriovenous	14	1	0.77
Capillary	9	7	
Combined	25	6	
Other	0	1	
Localization			
Head and neck			
Yes	40	16	0.78
No	64	23	
Upper extremity			
Yes	25	9	0.90
No	79	30	
Lower extremity			
Yes	33	13	0.86
No	71	26	
Trunk			
Yes	23	6	0.37
No	81	33	
Tissue extension			
(Sub)cutaneous			
Yes	87	33	0.89
No	17	6	
Intramuscular			
Yes	44	19	0.49
No	60	20	
Intraosseous			
Yes	19	7	0.97
No	85	32	
Maximal diameter in cm			
<5	37	10	
5-10	24	14	0.48
10-130	28	11	
>30	13	4	
Previous therapy			
Watchful waiting	11	0	
Sclerotherapy	50	25	
Surgery	23	5	
Laser therapy	8	7	0.057
Embolization	5	2	
Compression stockings	6	0	
Radiofrequency ablation	1	0	

Part IV

Defining disease severity in peripheral vascular malformations.

Chapter 9

Clinical characteristics associated with pain in patients with peripheral Vascular Malformations

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Abstract

Objective

Vascular malformations can negatively impact the patient's quality of life. Pain is a common problem in these patients. The aim of this study was to investigate risk factors associated with pain and to assess how pain affects quality of life.

Methods

This prospective cross-sectional study was conducted in a tertiary vascular anomaly expertise center. Between June and December 2020, all patients from our local database (334 adults and 189 children) with peripheral vascular malformations were invited to complete the Outcome Measures for VAscular MAlformations (OVAMA) questionnaire to evaluate the presence, frequency, and intensity of pain. Additionally, patients were asked to complete several Patient-Reported Outcome Measurement Information System (PROMIS) scales to evaluate their quality of life. Risk factors associated with pain were identified in bivariate analysis and multivariable logistic regression. Quality of life domains were compared between patients who experienced pain and patients who did not.

Results

A total of 164 patients completed the questionnaire about pain and 133 patients completed all quality of life questionnaires. Approximately half of the patients (52%) reported pain in the past four weeks and 57% of these patients reported pain daily or several times a week. Female gender ($p=0.009$), lesions located in the upper extremity ($p<0.001$) or lower extremity ($p<0.001$), and intramuscular/intraosseous lesions ($p=0.004$) were independently associated with the presence of pain. The following quality of life domains were diminished in patients who experienced pain in comparison to patients who did not*:* *pain interference* ($p<0.001$), *physical functioning* ($p<0.001$), and *social participation* ($p<0.001$) in adults, and *pain interference* ($p=0.001$), *mobility* ($p=0.001$), and *anxiety* ($p=0.024$) in children.

Conclusion

Pain is a frequently reported complaint in patients with vascular malformations and is present in approximately half of the patients. Patients with lesions located in the upper or lower extremity, intramuscular/intraosseous lesions, and female patients are more likely to experience pain. The presence of pain negatively impacted patients' quality of life. Although VM are a benign condition and expectative management is frequently applied, our study shows that pain is a serious concern and needs to be actively assessed. Pain is a sign of various etiologies, which should be examined in order to properly treat the pain.

Article Highlights

Type of Research: Single-centre prospective cross-sectional study.

Key Findings: 52% of 164 patients with peripheral vascular malformations reported pain. Risk factors independently associated with pain included female gender, lesions located in the upper or lower extremity, and intramuscular/intraosseous lesions. The presence of pain negatively impacted the patients' quality of life.

Take Home Message: Pain is a frequently reported complaint in patients with peripheral vascular malformations that negatively impacts the quality of life. Although expectative management is often applied in this benign condition, a more thorough examination and treatment of pain are needed.

Table of Contents Summary

52% of 164 patients with peripheral vascular malformations reported pain in this prospective cross-sectional study. Risk factors independently associated with pain included female gender, lesions located in the upper or lower extremity, and intramuscular/intraosseous lesions. The presence of pain negatively impacts the patient's quality of life.

Abbreviations

CAT = Computerized adaptive test
ISSVA = International Society for the Study of Vascular Anomalies
LIC = Localized intravascular coagulopathy
NRS = Numeric rating scale
OVAMA = Outcome Measures for VAscular MAlformations
PROM = Patient-reported outcome measure
PROMIS = Patient-Reported Outcome Measurement Information System
QoL = Quality of life
VM = Vascular Malformations

Keywords: Vascular Malformations; Vascular Anomalies; Pain; Quality of Life; Patient Reported Outcome Measures

Introduction

Peripheral vascular malformations (VMs) are rare congenital vessel anomalies that can negatively impact the patient's quality of life (QoL).[1] These malformations consist of dilated and dysfunctional vessels and are classified by the International Society for the Study of Vascular Anomalies (ISSVA) according to the type of vessel involved: venous, lymphatic, capillary, arteriovenous, or a combination.[2] VMs are present at birth, grow proportionally with age, do not regress spontaneously, and may recur after treatment.[3] Therefore, VMs are considered a chronic disorder.

The clinical presentation of VMs is highly variable and symptoms vary in nature and severity, depending on the VM type, anatomical location, tissue involvement, and lesion size. VMs that appear as a mass different in colour and texture from normal tissue, may lead to a disfigurement of the face, limbs, or other body parts. Additionally, patients may experience a wide spectrum of symptoms including pain, physical impairment, bleeding, thrombosis, and psychosocial problems.[1, 2, 4]

Patients with VMs have more pain than the general population.[1] Several mechanisms may be responsible for the onset of pain, such as thrombosis (due to venous stasis), phleboliths (calcifications following thrombi), local compression, neuropathic pain, haemorrhage into adjacent structures, or ischemic pain.[5-7] The mechanism leading to pain is, however, often dependent on the VM type. While venous stasis is predominantly responsible for the onset of pain in venous malformations, arteriovenous shunting reduces capillary oxygen delivery causing ischemia and ischemic pain in arteriovenous malformations, which is a more severe condition and heralds the risk of ulceration, bleeding, and even congestive heart failure.[7] Therefore, the symptom pain should be considered as a part of a more comprehensive issue.

Previous studies have demonstrated that the incidence of pain ranged from 42-92% in patients with venous malformations.[8-10] These studies found higher rates of pain in patients with truncal or extremity lesions and intramuscular/intraosseous lesions. Two of these studies found intralesional phleboliths to be associated with pain.[8, 9] Furthermore, larger lesion size was associated with pain in one study[10], while another study did not find this association.[9] These studies, however, have predominantly reported the incidence of pain among distinct groups in venous malformations but did not investigate how pain affects the QoL. Furthermore, risk factors for pain in venous malformations have been addressed, while it is uncertain if these also apply for the other types of VMs.

VMs represent a wide clinical spectrum and to date, the relation between the clinical presentation and symptoms such as pain remains unclear. Although there exist different etiologies of pain, we believe it is relevant to better understand what the role is of pain in this patient population. Therefore, the aim of this study was to investigate pain and risk factors for pain in patients with peripheral VMs by using the novel condition-specific OVAMA (Outcome

measures for VAscular MAlformations) questionnaire.[4, 11, 12] Furthermore, we assessed how pain affects the QoL in patients with VMs.

Methods

Study design

A cross-sectional study was performed to assess pain and QoL in patients with VMs. The study was conducted at the Amsterdam University Medical Center, a tertiary vascular anomaly expertise center. This study adhered to the Declaration of Helsinki, and written informed consent was obtained digitally from all patients. The study was exempted from full ethical review by the Medical Ethics Committee, since patients were not subjected to interventions or rules of conduct.

Study procedure

Patient selection

All patients with peripheral VMs who visited the outpatient clinic between June 2012 and December 2020, identified through ICD-codes, treatment codes, and manual screening of patients visiting the outpatient clinics, were included in our VM database. The VM diagnosis was based on clinical examination and confirmed with imaging or histopathology in case of uncertainty. Patients with VMs of the central nervous system or isolated malformations in visceral organs were excluded. Data was retrospectively extracted from electronic patient files on patient age, gender, VM type according the ISSVA classification[2], lesion maximal diameter (obtained from imaging reports or measured on MRI), lesion localization, types of tissues involved, overgrowth (including leg-length discrepancy), presence of pain (documented by the clinician), phleboliths (seen on imaging), and received treatments. Lesion size (based on maximal diameter) was also registered in the following categories: 0-5 cm, 5-10 cm, 10-30 cm, and >30 cm. The lesion size categories were defined before data collection started.

Between June and December 2020, all adult patients and parents of children from our database who had an available e-mail address were sent a digital invitation for this study. They were asked to complete the questionnaires using an online PROM (patient-reported outcome measure) portal called KLIK (which translates to Mapping Quality of Life in Clinical Practice). KLIK enables patients to create an account and fill in questionnaires.[13] Parents of children 0-13 years old were instructed to help their child fill in the questionnaires. Parents of children 14-17 years old were instructed to let the child complete the questionnaires independently. The cut-off points for age were determined during the concept interviews in the OVAMA project and based on the comprehensibility of the questions by different age groups.[12]

Questionnaires

OVAMA

The OVAMA questionnaire is a condition-specific PROM for patients with VMs. In collaboration with patients, clinical and methodological experts from all over the world a core domain set for evaluating treatment in VMs was established.[4, 11] A core domain set is a collection of outcome domains recommended for measurement when evaluating treatment effect in a particular condition.[14] To measure the condition-specific core domains, the OVAMA questionnaire was

developed, which focuses on VM symptoms and appearance.[12] In this study, the OVAMA questionnaire was used to assess pain in patients with VM. The OVAMA questionnaire addresses pain by questioning the presence of pain (yes/no), pain frequency (on a 5-point textual interval scale), and pain intensity (on a 10-point numeric rating scale). All questions regarding pain referred to the last four weeks.

PROMIS

QoL was measured using PROMIS (Patient-Reported Outcome Measurement Information System[15]), which is a set of person-centered measures that evaluate physical, mental and social health in adult and children. PROMIS consist of item banks that can be administered as computerized adaptive tests (CAT). CAT is based on item response theory, and the patient receives questions that are selected from a large item bank based on their previous answers. For example, if a patient states they do not experience "*pain interference",* additional questions about during which activities *"pain interference"* occurs are skipped. A CAT aims to reduce irrelevant and redundant questions for each individual and thus shortening test length. To fully capture the QoL domains determined in the core domain set, the following PROMIS scales were identified: *'pain interference'*, *'physical functioning'*, '*anxiety*', '*depression*' and '*social participation'*. For each PROMIS scale a T-score can be calculated, which is plotted against the reference population, where the mean T-score for the general population is 50 (Table IV). T-score cutoff values for 'normal', 'mild', 'moderate', and 'severe' were based on available PROMIS validation studies.[16]

Data analyses

Baseline characteristics are presented as frequencies and percentages for categorical variables and median and interquartile range (IQR) for nonparametric continuous data. In order to detect response bias, statistical differences in baseline characteristics between responders and non-responders were explored, chi-square was used for categorical variables and Mann-Whitney U test for nonparametric continuous variables. Responders were defined as patients who completed at least one questionnaire.

Bivariate analysis was performed to compare patients who reported pain with patients who did not report pain. Chi-square was used for categorical variables and Mann-Whitney U test for nonparametric continuous variables. Logistic regression was performed to assess risk factors independently associated with pain. These variables were entered in the multivariable analysis when they yielded a p-value less than 0.20 in bivariate analysis.[17] A cut-off p-value of 0.20 assures that all pertinent and potentially predictive variables are studied.[17]

To explore statistical differences in pain frequency and pain intensity between groups, the Mann-Whitney U test was used for dichotomous variables and the Kruskal-Wallis test for nominal variables. To explore statistical differences between the subgroups of the nominal variables, post hoc Mann-Whitney U tests with Bonferroni correction were computed. A

Spearman's rank correlation was performed to correlate numerical variables with pain frequency and pain intensity and to correlate pain frequency and pain intensity with each other.

To investigate statistical differences in PROMIS T-scores between patients with and without pain, the Mann-Whitney U test was performed. A Spearman's rank correlation was performed to correlate pain frequency and pain intensity with the PROMIS scale pain interference. For all statistical analyses, $p<0.05$ was considered statistically significant. Statistical analyses were performed with SPSS Statistics, Version 26.0 (IBM Corp., Armonk, NY, USA).

Results

Of the 680 patients with a VM who were identified, 157 patients could not be contacted as contact information was not available in the hospital registration system. The remaining 334 adults and 189 children with a peripheral VM were invited by e-mail to complete the questionnaires. 31% (n=164) of the invited patients completed the OVAMA questionnaire (37% (n=125) of adults and 15% (n=29) of children) and 25% (n=133) completed all PROMIS scales. Patient baseline characteristics are listed in Table I. Several statistically significant differences in baseline characteristics were found between non-responders and responders. Responders were older of age (p=0.004), more frequently had truncal lesions (p=0.035), more frequent intraosseous lesions (p=0.006), larger lesion size (p=0.047), and fewer capillary malformations (p=0.044) (Supplemental Table I).

Patient-reported pain

Symptoms of pain were registered by clinicians in 62% (n=102) of the assessed electronic patient files. 52% (n=86) of patients indicated that they experienced pain in the past four weeks. Bivariate analysis of factors associated with patient-reported pain are displayed in Table II. When comparing the malformation types, a significant difference was found between the VM types and patient-reported pain (p=0.027). The risk ratio (RR) for each malformation type was calculated: venous 1.14, lymphatic 1.20, capillary 0.27, arteriovenous malformations 1.05, and combined type 0.88.

Multivariable logistic regression analysis showed that female gender (p=0.009), lesions located in the upper extremity (p<0.001) or lower extremity (p<0.001), and intramuscular/intraosseous lesions (p=0.004) were independently associated with pain in patients with VM (Table III).

Pain frequency

Of 86 patients who experienced pain, 19% (n=16) reported pain <1 time a week, 24% (n=21) had pain approximately one time a week, 40% (n=34) indicated to have pain several times a week, and 17% (n=15) experienced pain daily. A statistically significant difference in pain frequency was found between different lesion localizations (p<0.001). Bonferroni adjusted post hoc analyses showed significantly more frequent pain when the VM was localized in the lower extremity in comparison with trunk (p=0.009) and head/neck lesions (p=0.017). Pain frequency did not show statistically significant differences between the subgroups of the following variables: gender (p=0.84), malformation type (p=0.060), and tissue extension p=0.87). Furthermore, no statistically significant correlation was found between pain frequency and size (r=0.077, p=0.48), maximal diameter (r=0.11, p=0.33) and age (r=0.085, p=0.43). Pain frequency was statistically significant correlated with pain intensity (r=0.47, p<0.001).

Pain intensity

The median NRS-score of patients who experienced pain was 5 (IQR 3-6) out of 10. A statistically significant difference was found in pain intensity between different lesion localizations (p=0.004). Bonferroni adjusted post hoc analyses showed significantly more frequent pain

when the VM was localized in the lower extremity or in multiple localizations in comparison with head/neck lesions (p=0.045, p=0.022 respectively). Pain intensity did not show statistically significant differences between the subgroups of the following variables: gender (p=0.073), malformation type (p=0.094), and tissue extension (p=0.94). Furthermore, no statistically significant correlation was found between pain frequency and size (r=0.15, p=0.17), maximal diameter (r=0.097, p=0.38), and age (r=-0.11, p=0.30).

Quality of life

The T-scores of the PROMIS scales were calculated, median T-scores are shown in Table IV. The mean rank of the T-scores were compared between patients who experienced pain and patients who did not. Statistically significant different T-scores were found on the following scales in adults: *pain interference* (p<0.001), *physical functioning* (p<0.001), and *social participation* (p<0.001). In children, statistically significant differences in T-scores were found on the following scales: *pain interference* (p=0.001), *mobility* (p=0.001), and *anxiety* (p=0.024). All median T-scores of patients who did and did not experience pain fell within 'normal' limits. Only the median T-score of *pain interference* in adults who experienced pain was slightly above the 'normal' limit, which corresponds with mild symptoms and negatively impacts the QoL.
In adults, a high correlation was found between pain frequency and *pain interference* (r=0.60, p<0.001). Also pain intensity was highly correlated with *pain interference* (r=0.72, p<0.001). In children, no correlation was found between *pain interference* and pain frequency (r=0.00, p=1.0) or pain intensity (r=0.42, p=0.23).

Table I. Baseline characteristics of the included patients.

Categorical variables (i.e. variables having two or more categories) are presented in frequencies and percentages of the included patients. Categorical variables include dichotomous variables (i.e. variables having two categories, such as female/male). Continuous variables (i.e. variables obtained by measurement, such as age) are presented as median and IQR.

Patient Characteristics	*Case number (%)*
Male	59 (36.0%)
Age in years (median, IQR)	29 (18-50)
Children (<18 years)	39 (23.8%)
Syndrome (%)	24 (14.6%)
Klippel-Trenaunay	17 (10.4%)
Sturge-Weber syndrome	1 (0.6%)
Other	6 (3.7%)
Overgrowth	20 (12.2%)
Lesion characteristics	
Vascular malformation type	
Venous	74 (45.1%)
Lymphatic	16 (9.8%)
Capillary	14 (8.5%)
Arteriovenous	22 (13.4%)
Combined	37 (22.6%)
Unclear	1 (0.6%)
Localization	
Head and neck	63 (38.4%)
Upper extremity	36 (22.0%)
Lower extremity	59 (36.0%)
Trunk	44 (26.8%)
Tissue extension	
(sub)cutaneous	137 (83.5%)
Intramuscular	76 (46.3%)
Intraosseous	30 (18.3%)
Maximal diameter in cm (median, IQR)	8.9 (4.0-17.3)
Size groups	
<5 cm	49 (29.9%)
5-10 cm	38 (23.2%)
10-30 cm	42 (25.6%)
>30 cm	31 (18.9%)
Unclear	4 (2.4%)
Phleboliths	25 (15%)
Previous therapies	
Laser therapy	27 (16.5%)
Compression stockings	43 (26.2%)
Sclerotherapy	65 (39.6%)
Surgery	66 (40.2%)

Table II. Bivariate analysis of risk factors for patient-reported pain.

	Patient-reported pain (n=164)		
	Case number		
	No	Yes	p-value
Clinician-reported Pain			
No	50	12	**<0.001**
Yes	28	74	
Male			
No	43	62	**0.024**
Yes	35	24	
Age (median, IQR)	34.0 (17.0-55.0)	26 (19.8-39.3)	0.087
Syndrome			
No	66	74	0.80
Yes	12	12	
Overgrowth			
No	70	74	0.47
Yes	8	12	
Vascular malformation type			
Venous	30	44	
Lymphatic	6	10	
Capillary	12	2	**0.027**
Arteriovenous	10	12	
Combined	20	17	
Lesion localization			
Head and neck			
No	33	68	**<0.001**
Yes	45	18	
Upper extremity			
No	67	61	**0.021**
Yes	11	25	
Trunk			
No	55	65	0.46
Yes	23	21	
Lower extremity			
No	58	47	**0.009**
Yes	20	39	
Tissue extension			
(sub)cutaneous			
No	8	19	**0.041**
Yes	70	67	
Intramuscular			
No	54	34	**<0.001**
Yes	24	52	
Intraosseous			

No	71	63	**0.003**
Yes	7	12	
Size			
<5 cm	23	26	
5-10 cm	19	19	
10-30 cm	20	22	0.10
>30 cm	14	17	
Unclear	2	2	
Maximal diameter in cm (median, IQR)	7.0 (4.0-15.0)	9.0 (4.2-18.5)	0.60
Phleboliths			
No	71	69	0.051
Yes	7	17	
Previous treatment			
Laser therapy			
No	57	80	**0.001**
Yes	21	6	
Compression stockings			
No	67	54	**0.001**
Yes	11	32	
Sclerotherapy			
No	56	43	**0.004**
Yes	22	43	
Surgery			
No	39	59	**0.015**
Yes	39	27	

Table III. Multivariable logistic regression analysis of risk factors for patient-reported pain.
Reference categories are indicated with an asterisk (*).
In the bivariate analysis, the variables lesion localization and tissue extension were drafted as dichotomous variables, as a patient could have a VM of multiple localizations and extending into various tissues. Because the VM is usually located in one location category and, thereby, not in another location category, a negative correlation exists between the different location variables. The effect of the correlated variables on the regression model becomes less precise. Therefore, the variables were transformed into categorical variables for the logistic regression.

		Patient-reported pain		
Variable	OR	Confidence interval		p-value
Gender				
Male*	3.04	1.31	7.08	**0.010**
Female				
Age	0.99	0.98	1.02	0.61
Lesion localization				**<0.001**
Head and neck*				
Upper extremity	13.08	3.20	53.46	**<0.001**
Trunk	1.82	0.47	7.09	0.39
Lower extremity	7.56	2.69	21.27	**<0.001**
Multiple localizations	2.47	0.88	6.92	0.085
Tissue extension				
(sub)cutaneous*	2.84	1.35	6.01	**0.006**
Intramuscular and intraosseous				
Phleboliths	1.91	0.61	5.94	0.27

OR = Odds Ratio.

Table IV. Bivariate analysis of patient-reported pain and PROMIS scales.

For each PROMIS scale, a T-score can be calculated, which represents to what extent that specific QoL-related outcome is affected in comparison to the general population. The mean T-score of the reference/general population is always 50 in PROMIS scales. Subsequently, the measured T-score is plotted against the reference population and represents the deviation from the general population. For PROMIS scales, T-scores higher than 50 indicate more of the concept being measured in comparison to the general population (e.g. more *pain interference*, better *physical functioning*), and T-scores lower than 50 represent less of the concept being measured

		Patient-reported pain.				
		No		Yes		
		T-scores		*T-scores*		
	n	*Median (IQR)*	*Mean Rank*	*Median (IQR)*	*Mean Rank*	P-value
Adults						
Pain interference	112	44.0 (41.0-45.3)	31.3	55.8 (51.6-60.8)	75.4	**<0.001**
Physical functioning	120	55.8 (48.0-57.5)	74.6	48.8 (43.3-52.3)	49.4	**<0.001**
Depression	112	43.7 (40.5-51.5)	50.8	48.5 (40.6-56.4)	60.8	0.11
Anxiety	114	47.4 (42.8-53.2)	52.7	52.0 (43.8-56.5)	61.3	0.17
Social participation	111	57.3 (52.9-62.6)	68.2	52.9 (47.8-57.0)	46.1	**<0.001**
Children						
Pain interference	23	35.7 (35.7-38.9)	7.9	47.8 (43.8-53.2)	17.4	**0.001**
Mobility	25	58.1 (52.4-58.1)	17.5	46.5 (38.2-49.6)	8.2	**0.001**
Upper extremity function	23	54.7 (54.7-54.7)	13.2	54.7 (48.4-54.7)	10.4	0.13
Depression	22	38.0 (36.1-48.0)	9.9	46.0 (38.2-56.6)	13.8	0.15
Anxiety	23	38.8 (36.0-43.3)	9.2	51.1 (42.6-55.5)	15.6	**0.024**
Peer relationships	22	49.1 (46.1-56.2)	12.3	46.9 (46.1-50.5)	10.4	0.50

n = the number of patients who completed the questionnaire.

Discussion

Approximately half of the patients (52%) in this study reported to have experienced pain in the past four weeks and 57% of these patients reported pain daily or several times a week. The median pain intensity (NRS) score was 5 out of 10, which corresponds with moderate pain. A number of risk factors independently associated with pain were identified: localization in the upper or lower extremity, intramuscular/intraosseous tissue extension, and female gender. The presence of pain influenced multiple PROMIS QoL domains in adults: more *pain interference*, less *physical functioning* and less *social participation*. In children, meanwhile, the presence of pain was associated with more *pain interference*, less *mobility* and more *anxiety*. VMs are a benign condition, and especially when they are not leading to severe facial distortion or have an impact on other vital structures, expectative management is widely applied. However, the high incidence of pain in VMs and its influence on the QoL suggests that there should be a different approach to the management of pain.

Although pain might currently be assessed in the clinical setting, pain frequency and intensity should not be overlooked as it provides insight into the severity of pain. Our results show that everyday life could be highly affected by the frequent presence and intensity of pain, and pain should not be considered as something of transient nature in these patients. As pain is a sign of various etiologies (e.g. venous stasis or ischemia) it is recommended that the pain etiology is identified before therapy is initiated. Localized intravascular coagulopathy (LIC) is associated with painful thrombotic episodes and results in the formation of phleboliths.[8] Imaging and coagulation blood tests, such as D-dimer, play an important role in the identification of the etiology of pain. In this study, phleboliths were associated with pain, though not statistically significant (p=0.051). The presence of phleboliths may have been underestimated however, as not every patient received imaging.

VMs are a chronic disorder and complete elimination of the VM is generally not possible. Therefore, symptom relief is crucial in the management of these patients. Oral pain medication may be a component of treatment, although, this has not been thoroughly investigated in patients with VMs. In a retrospective study among 28 patients who received aspirin for symptom relief, 54% reported a decrease of pain.[18] Additionally, compression stockings are prescribed to relief pain in patients with extremity VMs. Compression stockings prevent dilatation of the affected veins and may avoid the formation of LIC. Despite that compression stockings are commonly used in the treatment of VMs, no high-quality evidence is available to justify its use.[19] In the current study, patients previously treated with compression stockings or sclerotherapy showed significant higher rates of pain. This may be explained by the assumption that patients who experience pain are more likely to undergo treatment to improve their symptoms. However, these results also suggest that those therapies fail to completely eliminate pain. Treatment may be accompanied by simple lifestyle adjustments to relieve pain, such as elevation of the affected limb, alternating of standing and sitting posture, and a search for pain eliciting factors.

Female gender was found to be an independent risk factor associated with pain. Several studies found that women have a different pain perception compared to men and the stereotypical gender roles may contribute to differences in pain expression, as men are less willing to report pain than woman.[20, 21] Another identified risk factor was lesions of the extremities. Rikihisa et al. also found that this was the most predictive factor for pain.[10] A possible explanation might be that painful LIC are particularly present in VM of the extremities.[22] Another possible explanation is that lesions in the upper and lower extremities cause exertional pain, as there is more movement of affected extremities when performing physical daily activities than of affected head/neck or truncal regions. The exertional pain is likely due to vascular engorgement as the vessels dilate during exercise.[23] Superficial lesions can expand easily without the oppression of other structures, while deeper (e.g. intramuscular/intraosseous) lesions might cause more stress to surrounding tissues on expansion. This may explain the high incidence of pain in patients with intramuscular or intraosseous VMs, in line with previous research.[9, 10, 24]

In this study, the PROMIS QoL domains were more affected in patients who experienced pain in comparison to patients who did not experience pain. This resulted in considerable differences in median T-scores – up to 12.3 points – between both groups. Despite these differences in median T-scores, both groups fall within what are considered normal limits, suggesting that patients with VMs who experience pain do not report a greatly decreased QoL. However, a normal median T-score may be inaccurate for this population as these PROMIS scales cannot be adjusted for age, and our patient group represents a young population.[25]
The presence of pain resulted in more *pain interference* and less *physical function/mobility*, which is imaginable as it is unpleasant to move/exercise while in pain. However, the presence of pain also resulted in less *social participation* in adults and more *anxiety* in children. The fear of pain, pain catastrophizing, or fear of the disorder aroused by pain might be attributable to the increased *anxiety* in these children.[26] These results suggest that pain is a serious concern in patients with VMs that also affects psychosocial health. In a recent meta-analysis, decreased *mental health* and increased *bodily pain* were measured in patients with VMs when compared to the US reference population, associated with a poorer QoL.[1] According to these results, we can infer that pain is a serious complaint of patients with VMs. Consequently, clinicians should actively assess pain in patients with VM to see whether interventions are needed and if taken place, whether they reduce or solve pain.

There are several strengths and limitations to the current study. Although QoL has been addressed in patients with vascular malformations, the influence of specific symptoms on QoL has not been properly investigated to our knowledge. Additionally, pain frequency and intensity were measured in this study, rather than solely pain incidence.
Coagulation blood tests are not routinely analyzed in patients with vascular malformations in our center, therefore we might have missed important causes of pain. Although, this is inherent to the study design by retrospectively viewing medical records. Further, the concept elicitation

interviews in the development of the OVAMA questionnaire were based on a heterogeneous group of Dutch patients.[12] However, we do not expect the OVAMA questionnaire will yield large differences in outcomes between different geographic groups, since the questions are focused on the presence and severity of disease-specific symptoms, which were identified with a large international study.[4, 11] Functioning and QoL implications of these symptoms are more susceptible to demographic and geographical differences.[27] Therefore, patients from different geographic regions might answer the PROMIS scales differently. However, the PROMIS scales used for QoL measurement apply to people in a variety of contexts or with a variety of diseases and are developed to minimize this possibility.[15, 28] We performed a sub-analysis on clinical characteristics between responders and non-responders, which showed that responders were significant older of age. This may have been caused by the lower response rate of children. Parents possibly did not want to burden their children with filling in the questionnaires. Furthermore, responders more frequently had intraosseous lesions, larger lesion size, and fewer capillary malformations. It might be that these factors are associated with more symptoms, and patients were, therefore, more willing to complete the questionnaires. On the contrary, non-responders possibly experienced more symptoms and, thereby, felt too much burden to complete the questionnaires. However, the exact relationship between the clinical presentation and the severity of symptoms has not been identified.

Conclusion

This study showed that pain is commonly experienced and is not transient in patients with VMs, and several risk factors for pain were identified. The QoL domains *pain interference*, *physical functioning/mobility*, *social participation* in adults, and *anxiety* in children were worse in comparison to the group without pain. Although VMs are a benign condition and expectative management is frequently applied, our study highlights that pain is a serious concern. Therefore, clinicians should be aware of the high incidence of pain in their patients. Especially in patients with the identified risk factors, pain needs to be actively assessed and treated. Pain is a sign of various etiologies, which should be examined in order to properly treat the pain. Future studies need to be conducted to detect treatment options viable for the relief of pain.

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Supplemental Table I. Responders versus non-responders.

	Responders *Case number*		
	No	Yes	p-value
Male			
No	219	105	0.53
Yes	138	59	
Age in years (median, IQR)	23.0 (12.0-42.0)	29.0 (18.0-50.0)	**0.004**
Syndrome			
No	299	140	0.59
Yes	59	24	
Overgrowth			
No	70	74	0.47
Yes	8	12	
Vascular malformation type			
Venous	151	74	
Lymphatic	34	16	
Capillary	62	14	**0.044**
Arteriovenous	42	22	
Combined	54	37	
Lesion localization			
Head and neck			
No	202	101	0.27
Yes	156	63	
Upper extremity			
No	276	128	0.81
Yes	82	36	
Trunk			
No	291	120	**0.035**
Yes	67	44	
Lower extremity			
No	235	105	0.72
Yes	123	59	
Tissue extension			
(sub)cutaneous			
No	60	27	0.45
Yes	308	137	
Intramuscular			
No	220	88	0.093
Yes	138	76	
Intraosseous			
No	323	134	**0.006**
Yes	35	30	
Size			
<5 cm	137	49	
5-10 cm	102	38	
10-30 cm	63	42	**0.047**
>30 cm	52	31	
Unclear	4	4	
Maximal diameter in cm (median, IQR)	7.0 (4.0-15.0)	9.0 (4.2-18.5)	**0.019**

Chapter 10

Appearance-related concerns and their impact on health-related quality of life in patients with peripheral vascular malformations

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Summary

Background

Peripheral vascular malformations (VMs) may lead to disfigurement of the body and face, potentially impairing aesthetic appearance. Yet, data on appearance in this population is limited. This study aimed to examine appearance-related concerns and their impact on health-related quality of life (HR-QoL) in patients with VMs.

Methods

In this cross-sectional study, 384 adults and 194 children with VMs were invited to complete the Outcome Measures for VAscular MAlformations (OVAMA) questionnaire to evaluate potential appearance-related concerns on a five-point verbal-rating scale, higher scores indicate more appearance-related concerns (e.g. colour-difference, facial-distortion, reduced self-esteem, and dissatisfaction with appearance). HR-QoL was evaluated using Patient-Reported Outcome Measurement Information System (PROMIS). Subgroups of patients reporting more appearance-related concerns were identified using univariate analysis. Associations between appearance-related concerns and various HR-QoL domains (e.g. *anxiety* and *social-participation*) were assessed.

Results

184 patients (32%) completed the questionnaires;121 patients (66%) reported that one or more appearance-outcome was severely affected (i.e.4-5 out of 5). The following factors statistically significant associated with more appearance-related concerns: capillary/combined origin, facial localization, subcutaneous tissue involvement, larger size, overgrowth, and diagnosis of a syndrome. In adults, dissatisfaction with appearance and reduced self-esteem due to the VM correlated with more *anxiety* and *depression* symptoms. Reduced self-esteem correlated with less *social-participation*. In children, bodily-distortion and being stared at were correlated with less *peer relationships*.

Conclusion

Severe appearance-related concerns were present in two-thirds of patients with VMs, impairing their mental HR-QoL. Clinicians should acknowledge appearance-related aspects, monitor psychological well-being, and offer intervention aimed at improving satisfaction with appearance.

Introduction

Peripheral vascular malformations (VMs) are congenital vessel anomalies characterized by dilated and tortuous vessels, which can be of capillary, venous, lymphatic, arteriovenous, or combined origin.[1] VMs arise during embryogenesis due to localized defects in vasculogenesis and angiogenesis, and may become visible later in life.[2]

The clinical presentation of VMs is highly variable, with signs and symptoms varying in nature and severity, depending on the VM type, anatomical location, tissue involvement, and lesion size. Symptoms include pain, swelling, physical impairment, bleeding, and thrombosis.[3-5] It is now well established that patients with VMs experience impaired health-related quality of life (HR-QoL), including worse mental health.[6, 7] Moreover, VMs differ from normal skin regarding colour, swelling, and texture and may lead to a disfigurement of the body and also of highly visible areas such as the head and neck. Therefore, appearance-related concerns may be present in this patient population.

Appearance is of great importance in the development of personality and relationships[8, 9], and negative psychosocial consequences are known to occur in patients with craniofacial abnormalities.[10] Previous studies found that children with vascular anomalies have a negative perception of how others value them.[10, 11] Furthermore, the presence of facial capillary malformations has a severely negative impact on HR-QoL, in which the emotional domain is affected mostly.[12]

In the development of the core outcome set for VMs, appearance was identified as an important aspect by 300 international experts and patients, and it was selected as a core outcome domain that should be measured when evaluating treatment effect.[4] In addition, appearance-related concerns can be an indication to initiate treatment. However, to date, no studies have addressed appearance outcomes specifically, and it is unknown which subgroups of patients report clinically relevant appearance-related concerns and which appearance aspects patients find the most cosmetically disturbing. It is relevant to recognize these appearance aspects in order to treat the appearance-related concerns properly.

Therefore, the aim of this study is to examine appearance-related concerns in patients with VMs by using the condition-specific OVAMA (Outcome measures for VAscular MAlformations) questionnaire.[4, 13, 14] Furthermore, we assessed how the appearance of VMs affects HR-QoL in these patients.

Methods

Study design

To assess appearance-related concerns and HR-QoL in patients with VMs, a cross-sectional study was performed at the Amsterdam University Medical Centres (i.e., tertiary vascular anomaly expert centre in the Netherlands). The STROBE (Strengthening the Reporting of Observational Studies of Epidemiology) guidelines for cross-sectional studies were followed.[15] The study adhered to the Declaration of Helsinki, and informed consent was obtained digitally from all patients. The study was exempted from full ethical review by the Medical Ethics Committee, since patients were not subjected to interventions or rules of conduct.

Study procedure

Patient selection

All patients with VMs who visited the outpatient clinic between June 2012 and May 2021, identified through ICD-codes, treatment codes, and manual screening of patients visiting the outpatient clinics, were included in our local VM database. The VM diagnosis was based on clinical examination and confirmed with imaging or histopathology in case of uncertainty. Patients with VMs of the central nervous system or isolated malformations in visceral organs were excluded. Data was retrospectively extracted from electronic patient files on patient age, gender, VM type according the ISSVA classification[1], lesion localization, types of tissues involved, lesion size (maximal diameter, obtained from imaging reports or measured on MRI), overgrowth (including leg-length discrepancy), the diagnosis of a syndrome, and received treatments.

Between June 2020 and May 2021, all adults and children with peripheral VMs from our database, of whom an e-mail address was available (80%), were sent a digital invitation for this study. Using an online patient-reported outcome measure (PROM) portal called KLIK (which translates to Mapping QoL in Clinical Practice) patients were asked to complete the questionnaires. KLIK enables patients to create an account and complete questionnaires.[16] Parents of children 0-13 years old were instructed to help their child fill in the questionnaires. Children 14-17 years old were asked to complete the questionnaires independently. The cut-off points for age were determined during the concept interviews in the OVAMA-project and based on the comprehensibility of the questions by different age groups.[14]

Questionnaires

OVAMA

The OVAMA-questionnaire is a condition-specific PROM for patients with VMs, which focuses on VM symptoms and appearance (supplement 1).[4, 13, 14] The OVAMA-questionnaire addresses appearance of the VM with the following items: patient-reported size, swelling, colour difference, texture difference, facial distortion, bodily distortion, being stared at, reduced self-esteem due to the appearance of the VM, and dissatisfaction with appearance. All questions refer to issues occurring in the last four weeks. The items ‘colour difference’ and

'texture difference' imply a difference compared to the normal skin. All appearance questions referred to the patient's perspective on the VM and are answered on a five-point verbal rating scale. Higher scores indicate more appearance-related concerns, scores four or five out of five are regarded as severely affected. Additionally, a comprehensive appearance score was generated by ((the sum of all 9 appearance outcomes) / 9) * 20).

PROMIS

HR-QoL was measured using PROMIS (Patient-Reported Outcome Measurement Information System[17]), which is a set of person-centred measures that evaluate physical, mental and social health in adult and children. PROMIS was used because other quality of life measures seemed not responsive to changes in HR-QoL in adults and children with peripheral VMs.[18, 19] HR-QoL domains that should be measured in patients with VMs were determined in the core domain set.[4, 13] To fully capture these HR-QoL domains, the following PROMIS scales were identified: *pain interference, physical functioning, anxiety, depression,* and *social participation*. For each PROMIS scale a T-score can be calculated, which is plotted against the reference population, where the mean T-score for the general population is 50 and the standard deviation is ten. Subsequently, the measured T-score represents the deviation from the general population. For PROMIS scales, T-scores higher than 50 indicate more of the concept being measured in comparison to the general population (e.g. more *anxiety*, more *social participation*), and T-scores lower than 50 equal less of the concept being measured.

Data analyses

Baseline characteristics are presented as frequencies and percentages for categorical variables and median and interquartile range (IQR) for nonparametric continuous data. Baseline characteristics were compared between responders and non-responders of the questionnaires in order to detect response bias, chi-square tests were used for categorical variables, and Mann-Whitney U test for nonparametric continuous variables. Responders were defined as patients who completed at least one questionnaire.

Univariate analysis was performed to compare the appearance outcomes between different subgroups of patients with VMs (e.g. gender, lesion localization). The variable age was grouped as 0-20, 21-40, 41-60, and 61-80, thereby children and adolescents were combined, and these groups reflect major developmental stages. Mann-Whitney U tests were used for dichotomous variables and the Kruskal-Wallis test for categorical nominal variables.

To investigate correlations between the various appearance outcomes and PROMIS HR-QoL scales, Spearman's rank correlations were computed. Additionally, the appearance outcomes were correlated with each other using a Spearman's rank correlation. For the correlations of the appearance outcome facial distortion, only patients with head-and-neck VMs were included (n=57). For all statistical analyses, $p<0.05$ was considered statistically significant.

Statistical analyses were performed with SPSS Statistics, Version 26.0 (IBM Corp., Armonk, NY, USA).

Results

A total of 384 adults and 194 children with peripheral VMs and valid contact information were invited by e-mail to complete the questionnaires. Of the invited patients, 32% (n=184) completed the OVAMA questionnaire; 36% (n=137) of invited adults and 24% (n=47) of invited children completed at least one PROMIS questionnaire. Patient baseline characteristics are listed in Table 1. Several statistically significant differences in baseline characteristics were found between non-responders and responders of the questionnaires. Responders were older of age (p=0.001), more frequently had intraosseous VMs (18% vs 10%, p=0.006), and fewer patients had capillary malformations (8% vs 16%, p=0.020) (Supplemental Table 1).

Appearance outcomes

Of the included patients, 66% (n=121) reported that one or more appearance-related outcomes were severely affected (i.e. scoring it four or five out of five), medical photographs of three of these patients are shown in Figure 1. 36% (n=66) of patients reported the size as large or very large, and 23% (n=41) indicated a large or very large swelling. Furthermore, 34% (n=63) found the colour of the VM very or extremely different than their normal skin, and 26% (n=48) reported that the texture of the VM was very or extremely different. 13% (n=25) described their facial features as very or extremely distorted, and 21% (n=39) expressed their bodily features as very or extremely distorted. 18% (n=33) indicated that they were being stared at a lot or all the time, and 11% (n=20) described that their self-confidence as a lot or extremely reduced. Lastly, 31% (n=56) of patients indicated that they were dissatisfied or very dissatisfied with the appearance of the VM.

Univariate analyses of factors associated with higher appearance scores, indicating more appearance-related concerns, are displayed in Table 2. Overall, the characteristics that were associated with more appearance-related concerns included: lesions of capillary of combined origin, facial localization, subcutaneous and intraosseous tissue involvement, larger lesion size, overgrowth and lesions part of an associated syndrome. Older age showed a statistically significant association with more facial distortion and dissatisfaction with appearance, meaning that with the increase of age the facial distortion worsens and patients are more dissatisfied with the appearance of the VM.

Dissatisfaction with appearance

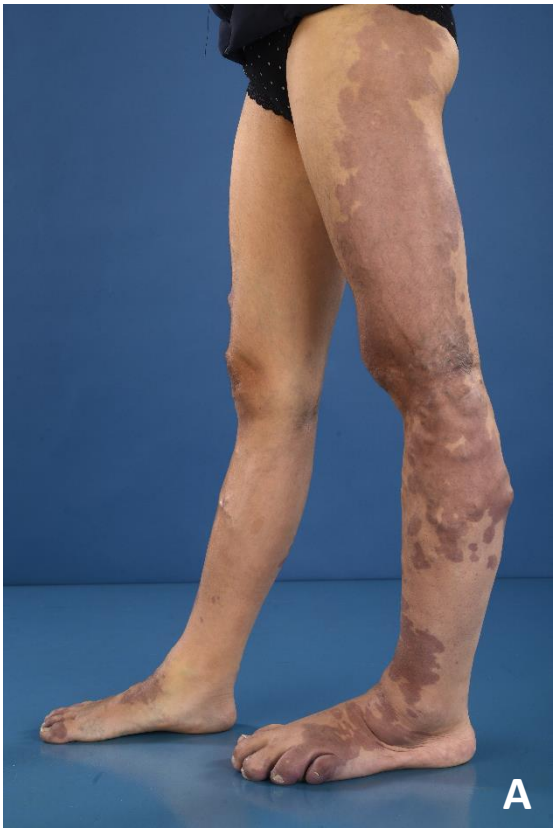
Correlations between the various appearance outcomes are displayed in supplemental Table 2. All appearance outcomes had a statistically significant correlation with dissatisfaction with appearance ($p<0.001$), meaning that if an appearance outcome was more severely affected (e.g. reporting more bodily distortion) patients were more dissatisfied with the appearance of the VM. Dissatisfaction with appearance was moderately correlated with bodily distortion (r=0.316), texture difference (r=0.352), patient-reported size (r=0.370), colour difference (r=0.442), swelling (r=0.483), and being stared at (r=0.492). A high correlation existed between

dissatisfaction with appearance and reduced self-esteem (r=0.525) and facial distortion (r=0.569).

Health-related quality of life

Correlations between the appearance outcomes and PROMIS-scales are shown in Table 3. In adults, moderate to high correlations were found between the appearance outcome reduced self-esteem and PROMIS scales *anxiety* and *depression*, meaning that patients with reduced self-esteem because of the appearance of the VM reported more *anxiety* and *depression.* Additionally, a correlation was found between dissatisfaction with appearance and *depression.* Furthermore, a moderate to high negative correlation was found between patient-reported size and the PROMIS scale *physical functioning*, meaning that a greater patient-reported size is associated with reduced *physical functioning*.

In children, a moderate to high correlation was found between bodily distortion and *pain interference,* meaning that more bodily distortion was associated with more *pain interference.* Moderate to high negative correlations were found between bodily distortion and the PROMIS scales *mobility* and *peer relationships,* meaning that more bodily distortion will lead to less *mobility* and less *peer relationships.* Further, being stared wat was negatively correlated with *peer relationships*.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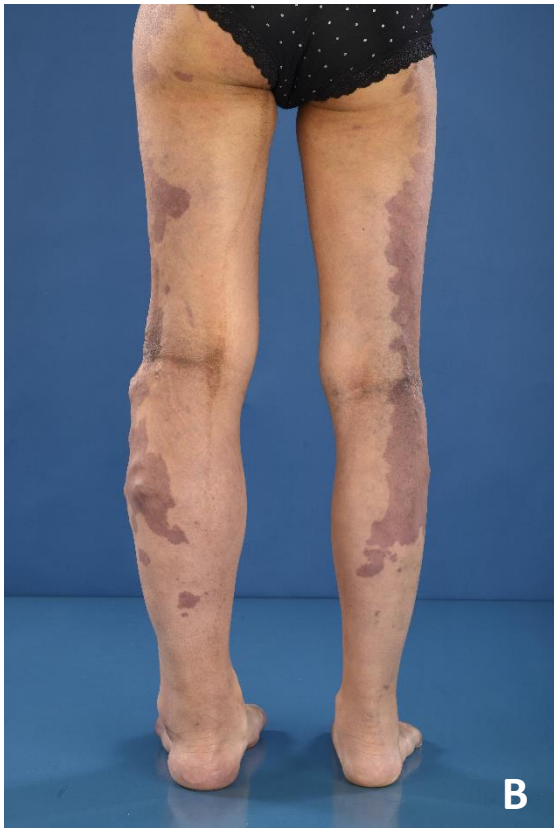
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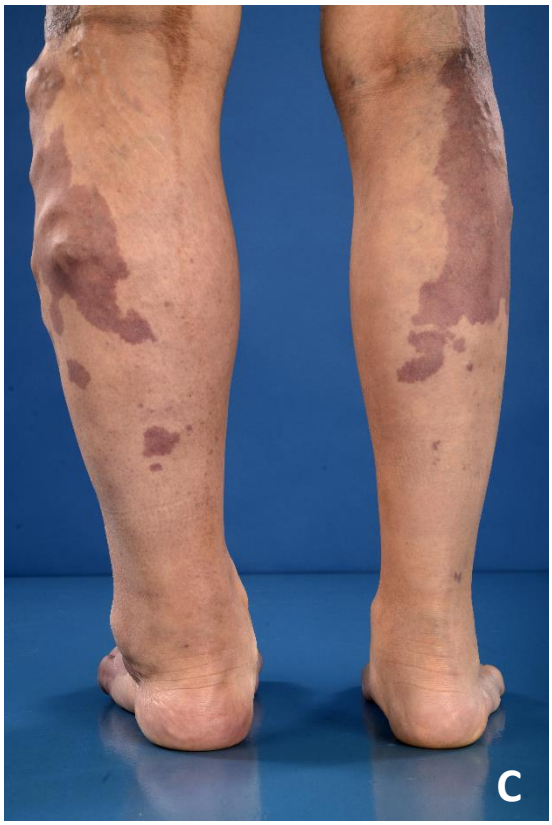
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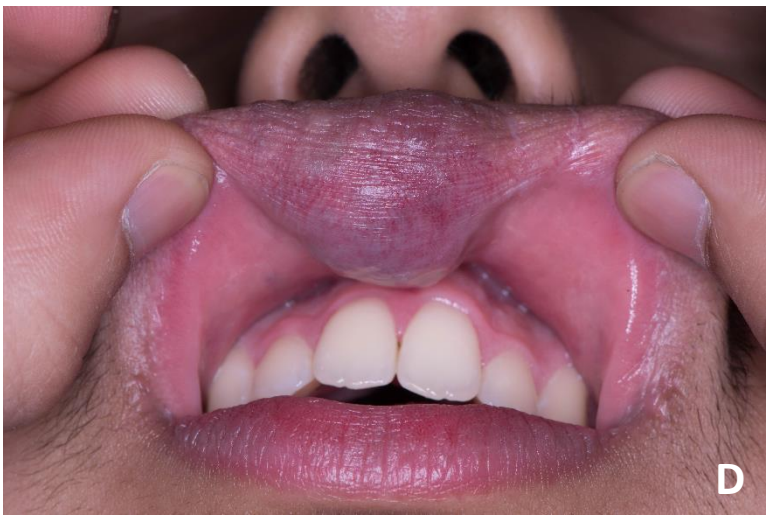
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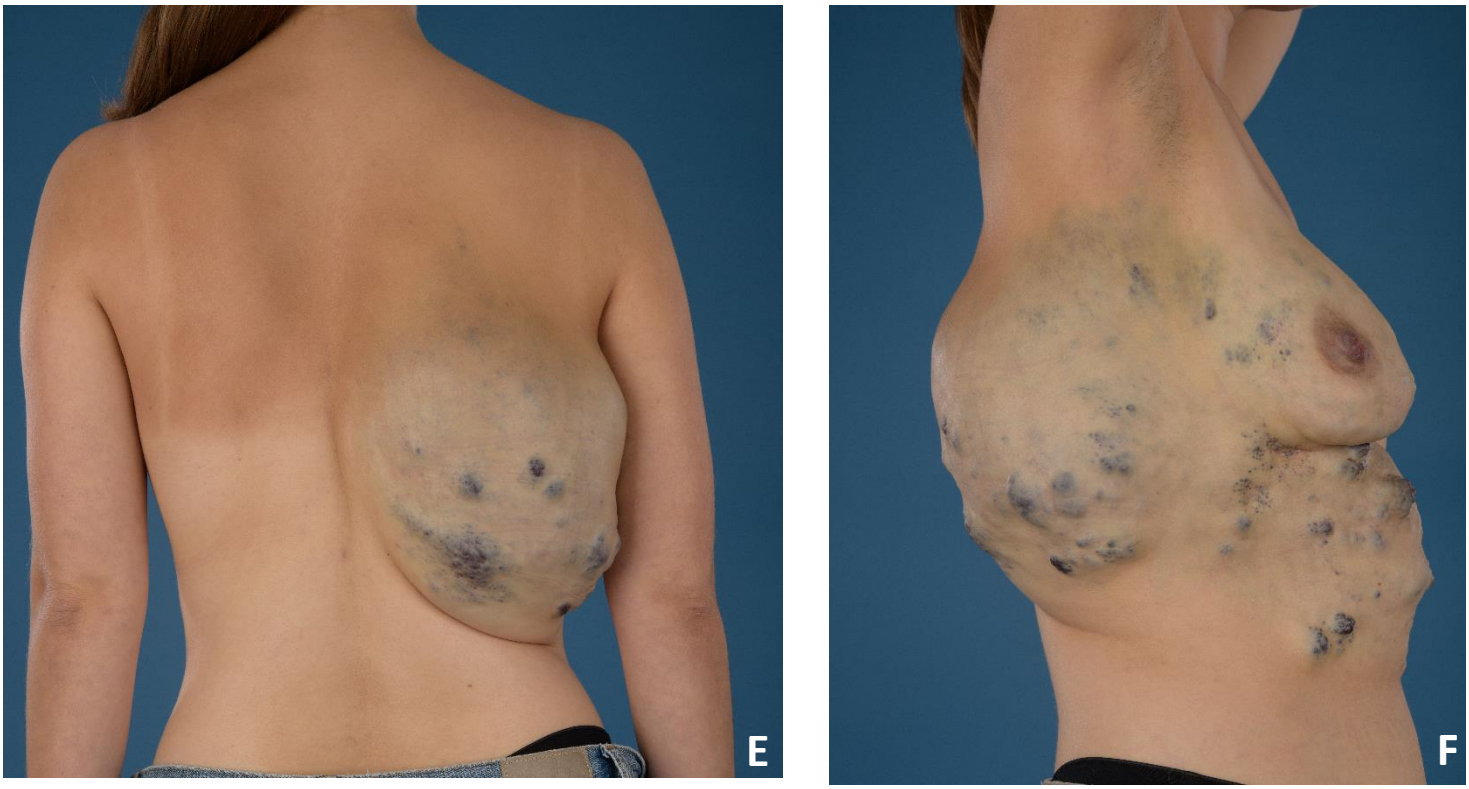

Figure 1 - Medical photographs of 3 patients with peripheral vascular malformations who expressed severe appearance-related concerns.
A, B & C: A 26-year old female with a combined capillary-venous malformation of the left leg and a capillary malformation of the right leg. Additionally, a leg-length discrepancy and overgrowth of the left leg and foot were present, and she has been diagnosed with Klippel-Trenaunay Syndrome. A: displays the side view of the left leg. B: shows the rear view of both legs. C: rear view of both lower legs with evident overgrowth of the left lower leg.
D: A 19-year old male with a small venous malformation at the inside of the upper lip. Although the VM was small, he expressed severe appearance-related concerns.
E & F: A 22-year old female with a large venous malformation of the right back, flank, and chest. D: her back with on the right side the venous malformation. E: displays the venous malformation from a lateral view.

Table 1. Baseline characteristics of the included patients (n=184)

IQR = Interquartile range.

Patient Characteristics	***n (%)***
Male	64 (35%)
Age in years (median, IQR)	29 (18-50)
Children (<18 years)	47 (26%)
Lesion characteristics	
Syndrome (%)	25 (14%)
Overgrowth	21 (11%
Vascular malformation type	
Lymphatic malformation	18 (10%)
Capillary malformation	14 (8%)
Arteriovenous malformation	23 (13%)
Combined malformation	38 (21%)
Unclear	1 (0.5%)
Localization	
Head and neck	70 (38%)
Upper extremity	43 (23%)
Lower extremity	63 (34%)
Trunk	46 (26%)
Tissue extension	
(sub)cutaneous	155 (84%)
Intramuscular	85 (46%)
Intraosseous	33 (18%)
Maximal diameter in cm (median, IQR)	7.3 (4-15)
Size groups	
<5 cm	61 (33%)
5-10 cm	33 (24%)
10-30 cm	33 (24%)
>30 cm	31 (17%)
Unclear	4 (2%)
Previous therapies	
None	38 (26%)
Laser therapy	28 (15%)
Compression stockings	48 (26%)
Sclerotherapy	66 (36%)
Surgery	71 (39%)

Table 2. Univariate analysis of risk factors for appearance-related concerns.

All appearance outcomes are on a five-point verbal rating scale, higher scores indicate more appearance-related concerns.

P<0.05 are considered statistically significant and are displayed in bold. Mdn = median, IQR = Interquartile range

	Appearance composite score		Patient-reported size		Swelling		Color difference		Texture difference		Facial distortion		Bodily Distortion		Being stared at		Reduced self-esteem		Dissatisfaction with appearance	
Patient Characteristics																				
Gender	**Mdn, IQR**	**P**	**Mdn, IQR**	**P**	**Mdn, IQR**	**P**	**Mdn, IQR**	**P**	**Mdn, IQR**	**P**	**Mdn, IQR**	**P**	**Mdn, IQR**	**P**	**Mdn, IQR**	P	**Mdn, IQR**	P	**Mdn, IQR**	P
Male	47 (33-58)	0.10	3 (2.25-4)	0.27	3 (1-3)	0.75	2 (1-4)	**0.05**	2 (1-4)	0.65	1 (1-2)	0.65	2 (1-2.75)	0.40	2 (1-3)	0.35	1 (1-2)	**0.003**	3 (2-4)	0.57
Female	51 (38-62)		3 (2.25-4)		3 (2-3)		3 (1-5)		2 (1-4)		1 (1-2)		2 (1-3)		2 (1-3)		2 (1-3)		3 (2-4)	
Age																				
0-20 years (n=57)	49 (34-57)		3 (2-4)		3 (2-4)		3 (1-4)		2 (1-4)		1 (1-2)		1 (1-3)		2 (1-3)		2 (1-2)		3 (2-4)	
21-40 years (n=69)	47 (36-59)		3 (2-4)		3 (2-3)		2 (1-4)		2 (1-3.5)		1 (1-2)		2 (1-3.5)		2 (1-3)		2 (1-3)		3 (2-4)	
41-60 years (n=38)	48 (33-62)	0.31	3 (3-3.25)	0.55	3 (2-3)	0.83	3 (1-4.25)	0.23	2.5 (1-3)	0.20	1.5 (1-3)	**0.003**	2 (1-3.25)	0.58	2 (1-3)	0.59	1 (1-2.25)	0.20	3 (2-3)	**0.05**
61-80 years (n=20)	59 (43-76)		4 (2-4)		3 (2-4)		4 (1.25-5)		3 (2-4)		1.5 (1-3.75)		1.5 (1-4)		3 (1.25-3)		2 (1-3)		4 (3-4.75)	
Lesion Characteristics																				
Syndrome																				
No	47 (33-58)	**0.003**	3 (2-4)	**<0.001**	2 (2-3)	**0.05**	2 (1-4)	**<0.001**	2 (1-3)	0.18	1 (1-2)	0.54	1 (1-3)	**<0.001**	2 (1-3)	**0.012**	2 (1-3)	0.65	3 (2-4)	0.33
Yes	62 (42-74)		4 (3-5)		3 (2-4)		5 (3-5)		3 (1-4.5)		1 (1.5)		3 (2-4.5)		3 (1.5-4)		1 (1-3)		3 (2-4)	
Overgrowth																				
No	47 (33-60)	**0.007**	3 (2-3)	**0.001**	3 (2-3)	0.083	2 (1-4)	**<0.001**	2 (1-3)	0.45	1 (1-2)	0.47	1 (1-3)	**<0.001**	2 (1-3)	**0.014**	2 (1-3)	0.75	3 (2-4)	0.26
Yes	58 (44-68)		4 (3-5)		3 (2-4)		5 (3-5)		2 (1-4)		1 (1-1.5)		4 (2-5)		3 (2-4)		2 (1-2.5)		3 (3-4)	
Vascular Malformation type																				
Venous	46 (31-53)		3 (2-4)		3 (2-4)		1.5 (1-3)		2 (1-3)		1 (1-2)		1 (1-3)		2 (1-3)		2 (1-2)		3 (2-4)	
Lymphatic	44 (37-56)		3 (2-3.25)		2 (2-3)		2 (1-3.25)		2.5 (1-4)		1 (1-1.25)		2 (1-3)		2 (1-3)		2 (1-3)		3 (2-3.25)	
Arteriovenous	47 (36-62)	**<0.001**	3 (2-4)	**<0.001**	3 (2-4)	0.089	3 (2-3)	**<0.001**	3 (1-4)	**0.045**	1 (1-2)	**0.005**	1 (1-3)	**0.034**	2 (1-3)	**<0.001**	2 (1-3)	0.56	3 (2-4)	0.12
Capillary	60 (42-71)		4 (3-5)		1 (1-3.25)		5 (3-5)		1 (1-4)		3 (1-4.25)		1 (1-3.25)		3.5 (2-5)		1.5 (1-4.25)		3 (3-4.25)	
Combined	62 (49-69)		4 (3-4)		3 (2-4)		4 (3-5)		3 (2-4)		1 (1-3.25)		2.5 (1-4)		3 (2-4)		2 (1-3)		3 (2-4)	
Localization																				
Head and neck																				
No	47 (33-62)	0.43	3 (3-4)	0.23	3 (2-4)	**0.001**	2 (1-4)	0.09	2 (1-4)	0.30	1 (1-1)	**<0.001**	2 (1-4)	**<0.001**	2 (1-3)	**0.001**	2 (1-3)	0.33	3 (2-4)	0.82
Yes	49 (38-61)		3 (2-4)		2 (1.75-3)		3 (2-4)		2 (1-3)		2 (1-4)		1 (1-2)		3 (2-4)		2 (1-3)		3 (2-4)	
Face																				
No	47 (33-60)	**0.018**	3 (2-4)	0.33	3 (2-4)	**0.032**	2 (1-4)	**0.003**	2 (1-4)	0.40	1 (1-1)	**<0.001**	2 (1-3)	**<0.001**	2 (1-3)	**0.001**	2 (1-2.25)	0.14	3 (2-4)	0.23
Yes	54 (42-64)		3 (2-4)		2 (1-3)		3 (2.75-4.25)		2 (1-3)		3 (2-4)		3 (2-4)		3 (2-4)		2 (1-3)		3 (2-4)	
Upper extremity																				
No	47 (33-60)	0.25	3 (2-4)	**0.05**	2 (2-3)	**0.04**	3 (1-4)	0.53	2 (1-3)	0.20	1 (1-2)	**0.011**	1 (1-3)	**0.002**	2 (1-3)	0.72	2 (1-3)	0.13	3 (2-4)	0.53
Yes	51 (36-64)		3 (3-4)		3 (3-4)		3 (1-4)		3 (1-4)		1 (1-1)		2 (2-4)		2 (1-3)		1 (1-2)		3 (2-4)	
Trunk																				
No	47 (36-58)	0.29	3 (2-4)	0.06	3 (2-3)	0.28	3 (1-4)	0.15	2 (1-3)	0.22	1 (1-2.5)	**0.003**	1 (1-3)	**<0.001**	2 (1-3)	0.79	2 (1-3)	0.20	3 (2-4)	0.82
Yes	55 (33-64)		3 (3-4)		3 (2-4)		3 (1-5)		2 (1-4)		1 (1-1)		3 (2-4)		2 (1-3)		1 (1-2)		3 (2-4)	
Lower extremity																				

No	47 (36-59)	0.57	3 (2-4)	**0.03**	3 (2-3)	0.09	3 (1-4)	0.90	2 (1-3)	0.68	1 (1-3)	**<0.001**	1 (1-3)	**0.008**	2 (1-3)	0.64	2 (1-3)	0.76	3 (2-4)	0.96
Yes	51 (33-64)		3 (3-4)		3 (2-4)		2 (1-5)		2 (1-4)		1 (1-1)		2 (1-4)		2 (1-3)		2 (1-3)		3 (2-4)	
Tissue involvement																				
Subcutaneous																				
No	33 (29-50)	**0.001**	3 (2-3.5)	0.18	2 (1-3)	0.13	1 (1-2)	**<0.001**	2 (1-3)	0.19	1 (1-1)	**0.024**	1 (1-3.5)	0.58	1 (1-2.5)	**0.005**	1 (1-2)	**0.046**	2 (1-3)	**0.002**
Yes	51 (38-62)		3 (3-4)		3 (2-4)		3 (1-4)		2 (1-4)		1 (1-2)		2 (1-3)		2 (1-3)		2 (1-3)		3 (2-4)	
Intramuscular																				
No	49 (38-62)	0.62	3 (2-4)	0.63	2 (2-3)	0.19	3 (1-4)	0.21	2 (1-3)	0.71	1 (1-3)	**0.019**	2 (1-3)	0.43	2 (1-3)	0.38	2 (1-3)	0.53	3 (2-4)	0.25
Yes	47 (33-62)		3 (3-4)		3 (2-3.5)		2 (1-4)		2 (1-4)		1 (1-1)		2 (1-4)		2 (1-3)		2 (1-3)		3 (2-4)	
Intraosseous																				
No	47 (33-58)	**0.001**	3 (2-4)	0.053	2 (2-3)	**0.001**	3 (1-4)	0.15	2 (1-3)	**0.002**	1 (1-2)	0.18	2 (1-3)	0.31	2 (1-3)	**<0.001**	2 (1-2)	**0.009**	3 (2-4)	0.058
Yes	58 (49-69)		3 (3-4)		3 (2.5-4)		3 (2-4.5)		3 (2-4)		1 (1-3)		2 (1-4)		3 (2-4)		3 (1-3)		3 (3-4)	
Lesion size																				
<5 cm	42 (31-52)		3 (2-3)		2 (2-3)		2 (1-3)		2 (1-3)		1 (1-2)		1 (1-2)		1 (1-3)		2 (1-2)		3 (2-3)	
5-10 cm	41 (32-53)	**<0.001**	3 (2-4)	**<0.001**	2 (1-3)	**<0.001**	2 (1-4)	**<0.001**	1 (1-3)	**<0.001**	1 (1-2)	0.32	1 (1-3)	**<0.001**	2 (1-3)	**<0.001**	1 (1-2)	0.20	3 (2-4)	**0.023**
10-30 cm	53 (38-68)		3.5 (3-4)		3 (2-4)		3 (1-4)		3 (1-4)		1 (1-3)		1.5 (1-3)		3 (1-3.75)		2 (1-3)		3 (2-4)	
>30 cm	62 (51-67)		4 (4-5)		3 (3-4)		5 (3-5)		3 (1-4)		1 (1-1)		3 (2-4)		3 (2-4)		2 (1-3)		3 (3-4)	

Table 3. Spearman's rank correlation coefficients between the PROMIS scales and the different appearance outcomes.

A positive or negative score of 0-0.3 is interpreted as low correlation, 0.3-0.5 as moderate, and >0.5 as high. Correlation coefficients in bold are statistically significant (* = $p \leq 0.05$, ** = $p \leq 0.01$).
n = the number of patients who completed the questionnaire.

	n	Appearance composite score	Patient-reported size	Swelling	Color difference	Texture difference	Facial distortion	Bodily Distortion	Being stared at	Reduced self-esteem	Dissatisfaction with appearance
Adults											
Anxiety	128	**0.239****	0.054	0.108	0.173	0.038	0.069	0.171	0.132	**0.444****	**0.257****
Depression	125	**0.222***	0.038	0.103	0.135	-0.036	0.080	0.148	0.103	**0.432****	**0.345****
Participation	124	-0.072	0.050	-0.022	-0.046	-0.104	0.053	-0.086	-0.032	**-0.230***	-0.078
Pain interference	126	0.098	**0.240****	**0.252****	-0.015	0.117	-0.142	0.131	-0.055	0.126	0.076
Physical Functioning	135	-0.158	**-0.307****	**-0.270****	-0.085	-0.109	0.037	-0.054	-0.056	-0.048	-0.124
Children											
Anxiety	26	0.223	-0.066	0.078	0.068	0.160	0.115	0.361	0.265	0.041	0.175
Depression	24	-0.053	-0.172	0.105	-0.298	-0.143	-0.227	0.331	0.040	0.042	-0.044
Pain interference	25	0.038	-0.169	0.067	0.051	0.048	-0.107	**0.430***	0.046	-0.098	-0.002
Mobility	29	-0.060	0.001	-0.135	-0.033	-0.029	0.114	**-0.490***	0.059	-0.066	-0.016
Upper extremity function	25	0.121	0.195	0.053	0.084	-0.072	-0.072	-0.356	0.082	0.178	0.046
Peer relationship	24	-0.400	-0.346	-0.345	-0.318	-0.253	-0.116	**-0.517****	**-0.505***	-0.213	-0.221

Discussion

In this study, approximately two-thirds (66%) of patients with peripheral VMs indicated that one or more appearance-related outcomes were severely affected, and one-third (31%) were dissatisfied or very dissatisfied with the appearance of their VM. The following factors associated with more appearance-related concerns: facial localization, capillary/combined origin, subcutaneous and intraosseous tissue involvement, larger lesion size, overgrowth, and the lesion being part of a syndrome. Appearance-related concerns that led to the most 'dissatisfaction with appearance' included swelling, being stared at, reduced self-esteem, and facial distortion. Another clinically relevant finding was the impact of appearance-related concerns on the HR-QoL, as dissatisfaction with appearance, a higher appearance composite score (indicating more problems with aesthetic appearance), and reduced self-esteem were associated with more *anxiety* and *depression* in adults. Furthermore, aesthetic concerns caused by the VM seemed to lead to psychosocial difficulties, i.e. reduced self-esteem seemed to worsen *social participation* in adults, and in children bodily distortion and being stared negatively affected their *peer relationships*.

Patients with capillary or combined, facial, subcutaneous, and larger VMs reported more appearance-related concerns as these lesions are generally more visible. Additionally, patients with overgrowth or associated syndromes reported more appearance-related problems. This may be because overgrowth looks distinctly different from normal skin, and larger proportions of the body are affected in these patients, which can be more noticeable. This finding is also in line with previous research that found that patients with overgrowth reported more impaired HR-QoL compared to patients without overgrowth.[20] Surprisingly, we found that intraosseous VMs were also associated with appearance-related concerns, this is probably because the included intraosseous VMs were larger in size.

Our study found that with the increase of age the facial distortion worsens and older patients were more dissatisfied with the appearance of their VM. This may be explained by the fact that VMs may evolve, as capillary malformations become darker, thicker, and more nodular over time, and arteriovenous malformations enlarge due to increased blood flow.[21-23] Patients were also more dissatisfied with their appearance when they felt they were being stared at in public. This indicates that the behaviour of others negatively affects a patient's perception of their VM.

Several studies have demonstrated that patients with VMs may have impaired mental HR-QoL.[6, 7] Our study shows that the appearance of VMs may be partly responsible for this impaired mental HR-QoL, as *anxiety* and *depression* were more likely in adults reporting appearance-related concerns (dissatisfaction with appearance, a higher appearance composite score, and reduced-self-esteem). This finding shows that the appearance of VMs profoundly affects HR-QoL. In children with VMs, *anxiety* and *depression* were less likely to occur alongside appearance-related concerns. A possible explanation could be that children are less self-conscious of their appearance in comparison to adults. During life, patients may become more

aware of the appearance of the VM as they become more aware of their appearance in general and how they are perceived by others. *Peer relationships* in children were unfavourably affected by the appearance of VMs, in particular perceived bodily distortion and being stared at seemed to affect *peer relationships.* Children may encounter stigmatization and teasing because of the appearance of the VM and may feel less accepted by peers. Discrimination and teasing early in life can impair psychological development, leading to negative self-perceptions and emotional problems that can persist through adulthood and manifest in difficulties in social situations.[24] This seems to be consistent with our findings, as reduced self-esteem due to the appearance of the VM seemed to negatively affect *social participation* in adults.

VMs are a benign condition with a lifelong disease course. In the management of VMs, clinical symptoms, such as bleeding, compression of vital structures, and pain may be more in the foreground, and appearance aspects of the VM might, therefore, be forgotten sometimes. Additionally, it can be difficult for clinicians to foresee to what extent a condition can have an aesthetic burden on a patient. However, the impact of the appearance of the VM on HR-QoL suggests that the clinician should not overlook the aesthetic appearance of the VM, and the patient's perception of their VM. In a clinical setting, appearance-related concerns should be actively assessed and patients should be asked whether their self-image is affected, particularly in patients with the identified risk factors (i.e. malformations of capillary/combined origin, facial localization, large size, and associated syndromes). Subsequently, it is important to identify the specific appearance-related concerns that may be targeted with distinct interventions. For example, laser therapy can be effective for superficial colour fading and sclerotherapy for volume decrease.[25, 26] Also, less conventional treatment methods purely focused on aesthetic improvement, such as cosmetic camouflage and medical tattooing, can therefore be deployed additionally.[27, 28] Our results suggest that appearance-related concerns worsen with age and that dissatisfaction with appearance in adulthood leads to more *depression* and *anxiety.* Therefore, one could suggest that a more aggressive therapeutic approach or psychological support could be attempted at a younger age in order to prevent difficulties with appearance and impaired HR-QoL later in life. However, currently, there is no evidence available to support this hypothesis.
Lastly, clinicians may support their patients by acknowledging that appearance-related concerns exist in patients with VMs and explain that these are common feelings among other patients and make patients aware of peer support groups.[29] Furthermore, clinicians should anticipate and monitor for signs of psychosocial impairment in their patients, possibly with HR-QoL measurement tools, and provide resources to those who might benefit from psychological intervention.

This study has several strengths and limitations. The study was able to reveal appearance-related concerns in patients with VMs, using a validated condition-specific PROM.
The sub-analysis on clinical characteristics between responders and non-responders showed several statistically significant differences; responders more frequently had intraosseous

lesions, fewer had capillary malformations, and were older of age. The older age of responders may have been caused by the lower response rate of children, as parents possibly did not want to burden their children with filling in the questionnaires. Furthermore, the study had a fairly low response rate of 32%, which might have been caused by patients who did not visit the outpatient clinic in recent years and did not feel urged to complete the questionnaires. Parents of children 0-13 years old were instructed to help their children fill in the questionnaires. Therefore, it might be that the parent's opinion unintentionally influenced the provided answers. Yet, this is unavoidable, as young children may not able to read and understand the questions independently.

Conclusion

This study showed that two-thirds of patients (66%) with VMs reported severe appearance-related concerns, and these concerns are associated with a negative impact on perceived HR-QoL. *Anxiety* and *depression* and difficulties with *social participation* and *peer relationships* occurred more often when patients had a negative perception of their appearance. The results of this study highlight the importance of paying attention to patients' perception of their aesthetic appearance. By assessing appearance-related concerns, physicians can offer interventions to potentially improve satisfaction with appearance (targeted at the specific appearance-related concern), and monitor for signs of psychosocial impairment. If necessary, they should refer patients to peer support groups or professional psychological support.

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Supplementary Materials

Supplementary Table 1. Baseline characteristics of responders versus non-responders.
IQR = Interquartile range.

	Responders *Case number or Median (IQR)*		
	No	Yes	p-value
Male			
No	153	64	0.36
Yes	241	120	
Age	24 (13-41)	29 (18-50)	**0.001**
Syndrome			
No	299	140	0.592
Yes	59	24	
Overgrowth			
No	70	74	0.47
Yes	8	12	
Vascular malformation type			
Venous	172	90	
Lymphatic	39	18	
Capillary	62	14	**0.020**
Arteriovenous	47	23	
Combined	58	38	
Lesion localization			
Head and neck			
No	233	114	0.52
Yes	161	70	
Upper extremity			
No	302	141	1.0
Yes	92	43	
Trunk			
No	321	137	0.53
Yes	73	47	
Lower extremity			
No	255	121	0.81
Yes	139	63	
Tissue extension			
(sub)cutaneous			
No	61	29	0.93
Yes	333	155	
Intramuscular			
No	232	99	0.25
Yes	162	85	
Intraosseous			
No	355	151	**0.006**
Yes	39	33	
Size			
<5 cm	157	61	
5-10 cm	108	44	
10-30 cm	70	44	0.17
>30 cm	55	31	
Unclear	4	4	
Maximal diameter (cm)	6.5 (3.1-13.6)	7.3 (4-15)	0.10

Supplementary Table 2. Spearman's rank correlation coefficients between the different appearance outcomes.

A positive or negative score of 0-0.3 is interpreted as low correlation, 0.3-0.5 as moderate, and >0.5 as high. High correlations are displayed in orange.

	Patient-reported size	Swelling	Color difference	Texture difference	Facial distortion	Bodily Distortion	Being stared at	Reduced self-esteem	Dissatisfaction with appearance
Patient-reported size		0.556	0.443	0.326	0.403	0.305	0.472	0.280	0.370
Swelling	0.556		0.371	0.524	0.265	0.379	0.408	0.345	0.483
Color difference	0.443	0.371		0.600	0.509	0.300	0.622	0.328	0.442
Texture difference	0.326	0.524	0.600		0.105	0.342	0.387	0.303	0.352
Facial distortion	0.403	0.265	0.509	0.105		-0.032	0.732	0.479	0.569
Bodily Distortion	0.305	0.379	0.300	0.342	-0.032		0.289	0.198	0.316
Being stared at	0.472	0.408	0.622	0.387	0.732	0.289		0.432	0.492
Reduced self esteem	0.280	0.345	0.328	0.303	0.479	0.198	0.432		0.525
Dissatisfaction with appearance	0.370	0.483	0.442	0.352	0.569	0.316	0.492	0.525	

Part V

General discussion

Chapter 11

General discussion and future perspectives

General discussion and future perspectives

This thesis focuses on achieving a more personalized approach to the management of vascular malformations. Personalized medicine is defined as a form of healthcare in which the individual patient characteristics of genes, symptoms, environments, lifestyle and disease prevention are considered, rather than a one-size-fits-all approach.[1] Vascular malformations are clinically heterogeneous with regard to the vascular malformation type, anatomical location, involved tissues, and lesion size. Recent discoveries expanded our knowledge about the underlying genetics of vascular malformations and the varieties of genetic mutations among patients accentuate this diversity. Consequently, a one-size-fits-all approach in the management of vascular malformations seems old-fashioned and undesirable.

Different axes of the condition were considered to reach a more personalized approach to vascular malformation management. The genotype showed to be responsible for phenotype differences, and novel techniques were explored for advancements in the field of genetics. Outcome measurement instruments were developed to measure disease burden and treatment outcome from the patient's perspective. Variations in baseline symptoms and health-related quality of life were investigated while considering underlying differences in patient and lesion characteristics.

From genotype to phenotype

In chapter 2, we attempted to systematically evaluate if the genetic mutations causative of vascular malformations result in phenotypic variability. It became clear that the underlying genetics contribute to the heterogeneity in clinical characteristics of vascular malformations, and we were able to highlight several attributable factors.

Firstly, germline mutations are present in all cells and in all tissues, therefore, vascular malformations are generally plural and present throughout the whole body, while somatic mutations result in localized affected tissue and vascular malformations confined to a certain part of the body. Secondly, distinct genetic mutations are frequently associated with a general phenotype, revealing that the various genetic mutations contribute to phenotype diversity. Thirdly, the timing during embryogenesis, strength, and cells affected by the somatic mutation may contribute to the variety of clinical features, such as lesion size and vascular malformation type.

The various genetic mutations found in peripheral vascular malformations further highlight the heterogeneity of vascular malformations and, therefore, the underlying genotype may form the basis for a more personalized approach to the management of vascular malformations in the near future.

A better understanding of the underlying genetic etiology of vascular malformations has already led to a more personalized approach to the management of vascular malformations, namely in the form of targeted therapies. Targeted therapies are the traditional example of

personalized medicine since the therapies are based on genetic profiling and are tailored to the individual patient. Molecular diagnostics of vascular malformation lesion tissue will result in a genotype profile of the vascular malformation, and subsequently, a therapy targeting the genetic mutation or hyper-activated cell signaling pathway may be administered as an individualized treatment. As targeted therapies become more readily available, the demand for molecular diagnostics to discover gene mutations in vascular malformations will also increase.

In chapter 3 of this thesis, we contribute to this personalized management of vascular malformations by investigating a less invasive approach for molecular diagnostics. The ability to perform molecular analysis on cell-free DNA collected from blood or lymph fluid out of the vascular malformation will prepare for the wide use of targeted therapies in vascular malformations. Molecular analysis of children and patients with facial, deep, or intramuscular vascular malformations in whom a tissue biopsy is undesirable or not possible can now be conveniently and minimally invasive performed with cell-free DNA. In order to keep up with the evolving landscape of genetics and targeted therapies, future studies need to optimize molecular analysis of cell-free DNA of vascular malformations.

The discovery of mutated genes in vascular malformations raises questions about how the gene defect alters endothelial cell function and ultimately leads to the development of vascular malformations. In chapter 4, we developed a research model consisting of primary cells isolated from capillary malformations, and pursued to investigate how the genetic mutations relate to changes in endothelial cell function of capillary malformations compared to healthy lesion tissue. Endothelial cells affected by somatic GNAQ mutations showed increased angiogenic sprouting capacity, which might be an explanation for the increased number of vessels in capillary malformations. Endothelial cell hyper-proliferation during vasculogenesis has been shown to result in vessel hyperfusion, which will lead to dilated and dysfunctional vessels similar to those found in vascular malformations.[2, 3]

Currently, it is unclear how other genetic mutations alter endothelial cell function and if all genetic mutations ultimately result in similar changes in endothelial cell function. Although, the phenotype differences between genetic mutations dispute this. In future research, primary cells isolated from capillary and other vascular malformations may serve as a research model to investigate differences in endothelial cell function between the various mutations. Additionally, the research model may provide a framework for investigating the effect of distinct targeted therapies on endothelial cells affected by various genetic mutations. Hence, the novel developed endothelial cell models may form the basis for further research aimed at improving a personalized approach to the management of vascular malformations.

The introduction of the ISSVA classification[4, 5] was one of the first steps to a more personalized approach to the management of vascular anomalies and made grant improvements in diagnostics and therapy.[6, 7] Now, with the unraveling of the genetic basis of vascular malformations, the current classification, based on clinical and histopathologic features, can be enhanced by incorporating the genotype.

The PIK3CA-related overgrowth spectrum (PROS) is a prime example of a classification framework based on the genotype.[8-11] The term spectrum emphasizes that there are different but related phenotypes rather than one specific phenotype. Next to vascular malformations caused by somatic PIK3CA mutations, overgrowth and macrodactyly are present in this spectrum.

In chapter 5, we reveal that patients with macrodactyly may experience progressive tissue overgrowth during adult life, despite being surgically treated in childhood.[12] Progressive growth is also present in other PROS phenotypes[8], and might be explained by the somatic PIK3CA mutation remaining present in the affected tissue and promoting growth continuously.

Vascular malformations are also known to evolve over time, as capillary malformations may become expanded, thicker and more nodular over time, and arteriovenous malformations enlarge and progress, resulting in more advanced Schobinger stages during adulthood.[13-15] Presumably, this could be partly attributable to the remaining mutated endothelial cells that stay present in the body and stimulate the growth of vascular malformations.

Genotype-enhanced future perspectives

Since the genetic discoveries in vascular malformations, a shift in the classification, diagnosis, and management took place. In future perspectives, these changes will continue to crystallize and eventually result in an ever-increasing role for the genotype, allowing for a more personalized approach to the management of vascular malformations.

Genotype-adjusted classification

The PROS spectrum is a new approach to classification which is tremendously different from the more commonly used anatomy- or disease severity based classifications. In the era of genetics and personalized medicine, research will be based on identifying the genetic origins of disorders, revealing mutations, and developing targeted therapies. In classifying these lesions also based on their common molecular signaling pathway mutations rather than different manifestations in the body and the concept of a syndrome, the classification will also implicate therapeutic relevance.

Patients with vascular malformations are clinically heterogeneous with respect to lesion characteristics, but these differences are also in disease pattern, progression, and response to treatment. These latter inter-patient differences have not yet been elucidated and might arise due to the underlying genetic mutations causing the vascular malformations.

A recent study showed that somatic KRAS mutations statistically correlated with arteriovenous malformations of the head and neck that were extensive, aggressive, or were difficult to treat and had an increased risk of recurrence after surgery, while somatic MAP2K1 mutations were associated with less severe and localized arteriovenous malformations, predominantly located at the lip instead of extensive regions of the head and neck.[16] Both KRAS and MAP2K1 regulate the same cell signaling pathway, although, MAP2K1 is located

downstream of KRAS. Possibly, somatic KRAS mutations have another, more extreme effect on the signaling pathway, resulting in more severe phenotypes. However, there is currently no evidence available to support this hypothesis.

Future studies need to further investigate differences in disease progression and response to therapy between distinct mutations, as it may influence treatment strategy. These findings emphasize the urgency of including the genotype in the classification of vascular malformations.

Genotype-adjusted diagnosis

The diagnosis of the vascular malformation type may not always be unequivocal, and a gold standard for diagnosis is currently lacking. Generally the diagnosis is based on clinical features, imaging, and histopathology. However, a study investigating the validity of the clinical diagnostic workup, usually combined with radiologic imaging, found in more than half of the cases a discrepancy with the histopathological diagnosis of peripheral vascular malformations.[17] Molecular diagnostics may aid the diagnosis of the vascular malformation type since specific mutations are generally associated with specific malformation types, and molecular analysis can now be used to identify lesions with an unclear diagnosis.

Genotype-adjusted management

Another prospect is the expanded use of targeted therapies in the field of vascular malformations to reduce lesion size and improve symptoms and health-related quality of life. Targeted therapies are based on the underlying genetic mutations in vascular malformations. Therefore, the identification of the germline or somatic mutation and the subsequent hyperactivated cell-signaling pathway is a prerequisite for the proper use of targeted therapies. Previous studies have pointed out that targeted therapies not based on the affected cell-signaling pathway do not have clinical benefits.[18, 19] As a consequence, molecular analysis will be more routinely performed to designate the appropriate targeted therapy. Targeted therapies will play a more dominant role in vascular malformation management and may be used as a stand-alone treatment, but could also be used in combination with the 'classical' treatment modalities. Targeted therapies may decrease lesion size before surgical intervention, as well as reduce the risk of recurrence following 'classical' interventions.

The mTOR-inhibitor sirolimus blocks downstream signaling and protein synthesis, resulting in angiogenic effects and its initial clinical use involved immunosuppression to prevent kidney transplant rejection.[20, 21] Other targeted therapies, such as the PIK3CA-inhibitor alpelisib, the AKT-inhibitor miransertib and the MEK-inhibitor trametinib, are predominantly used in cancer and control signaling pathways involved in cell proliferation, motility, survival and metabolism.[22, 23]

These are systemic therapies with anti-proliferative and immunomodulatory effects that may induce systemic adverse events, such as thrombocytopenia, leukopenia, anemia,

bone marrow toxicity, peripheral insulin resistance, hyperglycemia, fatigue, rash, and gastrointestinal problems.[24-27] Although, the treatment strategy must be different than in cancer because stability of the vascular malformation should be achieved rather than a maximum tolerated dose, and the treatment must be sustained for a lengthy period.

In future studies, challenges regarding the dosage and duration of targeted therapies in vascular malformations should be tackled. Guidelines are required to select patients eligible for targeted therapies, which should be based on experienced symptoms and impaired health-related quality of life so that the benefits outweigh the harm. Standardized measurement of symptoms and health-related quality of life should form the basis of patient selection.

Outcome measurement

Management of vascular malformations is challenging because of the clinical variability among patients, which has led to the use of various treatment methods. Currently, there are no evidence-based guidelines available to treat vascular malformations because of the wide diversity of methods used to evaluate treatment outcomes in clinical research, hampering the comparison of treatment results.[28-30] The Outcome measures for VAscular MAlformations (OVAMA) project was initiated to pave the way for homogeneity in outcome reporting and to measure treatment from the patient's perspective. Measurement from the patient's perspective can be performed with Patient-Reported Outcome Measures (PROMs).

With the input from patients and experts worldwide, the Core Outcome Set for peripheral vascular malformations was developed, and outcome domains that should be measured at a minimum when evaluating treatment effect were determined.[31, 32] The core outcome domains were divided into patient-reported and clinician-reported outcome domains. Subsequently, the patient-reported core domains could be further subdivided into condition-specific domains and non-condition-specific domains. The condition-specific domains included appearance, overall condition-specific symptoms, pain, bleeding, location-specific symptoms, and satisfaction with treatment and outcome. The non-condition-specific domains included overall quality of life, activities of daily living, mobility, ability to participate in work/study, confidence/self-esteem, and emotional wellbeing.

To facilitate uniform measurement of the condition-specific outcome domains established in the Core Outcome Set, in chapter 6, we described the development of the OVAMA questionnaire, a condition-specific PROM to measure symptoms and appearance in patients with vascular malformations.[33] The OVAMA questionnaire consists of all condition-specific outcome domains established in the Core Outcome Set except the outcome domain 'satisfaction with treatment and outcome' since this is only relevant at follow-up and the OVAMA questionnaire is developed to be measured prospectively, i.e., before and after treatment.

PROMs, such as the OVAMA questionnaire, can be seen as an important and fundamental tool to measure the extensiveness of the disorder as well as the effect of treatment at the individual level because they reflect the self-reported health state of the patient directly. Consequently, PROMs play a significant role in a personalized approach to the management of vascular malformations.

The development of the OVAMA questionnaires enables the measurement of the most clinically relevant symptoms in patients with vascular malformations since these were established by the vascular malformation community itself.[31, 32] Although the OVAMA questionnaire was primarily developed for clinical research, it may also be a resource in clinical practice to determine a baseline of symptoms and estimate disease burden. In this way, the OVAMA questionnaire may form the basis for treatment planning, and guide shared decision-making, i.e., balancing the clearly defined disease burden against treatment with its potential complications may help patients to make informed decisions.

The second crucial task of the OVAMA questionnaire is to measure the effect of treatment. In chapter 7, we found convincing evidence that the OVAMA questionnaire is responsive to changes in symptoms and appearance, and thereby is suited to evaluate the effect of treatment from the patient's perspective. In a landscape where treatments are generally deployed to relief symptoms and improve health-related quality of life rather than 'cure' the disorder, treatment evaluation from the patient's perspective is particularly crucial.

The constructs bleeding, leakage of fluids, and all head and neck symptoms could not be assessed for responsiveness since the majority of patients indicated that they never experienced these symptoms. Vascular malformations are a clinically heterogeneous condition, and therefore symptoms and appearance-related concerns differ significantly between patients. Although the OVAMA questionnaire currently is partially individualized since, firstly, a question is administered about whether a particular symptom is present, followed by additional questions regarding the severity and frequency of that symptom, we believe that the OVAMA questionnaire may further evolve by computer-adaptive testing using Item Response Theory (IRT) methods and the patient receives questions that are selected based on their previous answers.[34] A computer-adaptive test aims to decrease irrelevant and redundant questions for each individual, thereby shortening test length while maintaining accuracy. In this way, the OVAMA questionnaire keeps an eye on the core outcome domains while tailoring it to the individual patient.

Now, all the condition-specific outcome domains can be accurately and uniformly measured using the OVAMA questionnaire, except the condition-specific outcome domain 'satisfaction with treatment and outcome'. Chapter 8 describes the development and quality assessment of the OVAMA Treatment Outcome questionnaire that were developed to measure the outcome domain 'satisfaction with treatment and outcome' in patients with vascular malformations.

Content validity was considered adequate and construct validity was confirmed by testing predefined hypotheses on relations with the Global Rating of Change scales measuring the change in symptoms after treatment.

The findings of our and previous studies showed that satisfaction with treatment outcomes seems strongly dependent on the change in symptoms rather than on other factors such as sex, vascular malformation type, anatomical lesion location, tissue extension, size reduction measured on imaging, or the number of treatments.[35-38] This emphasizes the importance of evaluating the treatment of vascular malformations from the patient's perspective.

The OVAMA questionnaire was developed to measure changes in symptoms and appearance prospectively, i.e., before and after treatment.[33] The OVAMA Treatment Outcome questionnaire is complementary to the OVAMA questionnaire and are developed to be utilized solely at follow-up and to measure satisfaction with treatment outcome. The OVAMA Treatment Outcome questionnaire may be used separately in retrospective studies to describe outcomes in a standardized manner, however, prospective outcome measurement remains superior.

In clinical setting, treatment can now be evaluated from the patient's perspective, which is a valuable addition to the management of vascular malformations since treatments are used to improve symptoms and health-related quality of life, and many patients are subjected to multiple treatments during their life. In research, the both validated OVAMA questionnaire and OVAMA Treatment Outcome questionnaire may provide more homogeneity in outcome reporting, allowing for adequate comparison of treatments, which are crucial steps to evidence-based guidelines. Both PROMs are available at www.OVAMA.org to encourage wide use.

The non-condition-specific outcome domains present in the Core Domain Set are preferred to be measured with generic PROMs, enabling comparisons among various disorders and patient populations.[39] Preliminary results suggest that Patient-Reported Outcomes Measurement Information System (PROMIS) is able to cover all non-condition-specific outcome domains and it has shown to be reliable in measuring these outcome domains in patients with vascular malformations.

Defining disease severity

In order to move towards a more personalized approach in the management of vascular malformations, it is essential to explore which factors should be considered in this process, such as patient variables, lesion characteristics, and symptoms.

In chapters 9 and 10, we made the first steps to gain knowledge about disease severity in patients with vascular malformations. With the use of the OVAMA questionnaire to measure condition-specific symptoms and appearance, we were able to assess in depth which patients are susceptible to the symptoms of pain and appearance-related concerns and we explored the effect of these symptoms on the health-related quality of life using PROMIS scales.[40, 41] More specifically, we showed that pain predominantly affects physical well-being, appearance-related concerns affect emotional well-being, and both symptoms affect social well-being.

The clearly defined baseline of symptoms and health-related quality of life portrays the impact of the disease on the patient, which may guide shared-decision making and may serve as an indicator to initiate treatment, as was mentioned before. Shared decision-making is a communicative approach in which patients and physicians make a collaborative decision about the most preferable treatment plan based on the best available evidence, but more importantly also suits the situation and preferences of the patient.[42-44] In a world where technology is increasingly dominant, and patients have access to countless sources, the available information may be overwhelming. Therefore, it is crucial that the patient receives the appropriate information from their clinician that is tailored to the individual patient.

These chapters provide an example of the revelation of a patient profile of their most relevant symptoms, appearance-related concerns (e.g., color, swelling, size, facial distortion), and the affected health-related quality of life domains, which gives the physician direction towards fitting the information to the individual patient. Additionally, the profile of symptoms and health-related quality of life pinpoints specific treatment aims for the individual patient, and the patient may be better informed about their symptoms that may be targeted with distinct interventions. For example, laser therapy can be effective for superficial color fading, and sclerotherapy for volume decrease and pain reduction.[29, 45] Less conventional treatment methods purely focused on aesthetic improvements, such as cosmetic camouflage and medical tattooing, ought to be discussed with patients with severe appearance-related concerns.[46, 47]

Conclusively, we showed how to establish a baseline of symptoms and health-related quality of life in patients with vascular malformations. This enabled the investigation of the impact of the disease on the patient, which is a powerful tool to distinguish patients based on clinical severity. Additionally, the profile of symptoms and health-related quality of life may enhance shared decision-making in patients with vascular malformations and contributes to tailoring it to the individual patient.

Future perspectives in outcome measurement and defining disease severity

With the outcome measurement instruments developed and proposed in this thesis, a baseline of symptoms and health-related quality of life can be measured, and subsequently, the impact of the disease on the patient can be accurately determined. This is a valuable utensil in defining disease severity in patients with vascular malformations.

We believe that a model for defining disease severity may lead to a more personalized approach in the management of patients with vascular malformations. Models for defining disease severity have been made in other chronic disorders, such as inflammatory bowel disease, chronic obstructive pulmonary disease, and multiple sclerosis.[48-50] There are three main domains relevant to the evaluation of disease severity in patients with vascular malformations.

The first domain consists of the measurable disease burden, which includes the patient and lesion characteristics observed clinically, on imaging, or with other diagnostic techniques. The current domain also includes the recently discovered underlying genetics, where both the mutation type (germline or somatic) as well as the affected gene are crucial aspects. The second domain refers to the impact of the disease on the patient and includes experienced symptoms and health-related quality of life. This domain ought to be measured with the OVAMA questionnaire and PROMIS item banks. The third domain indicates the disease course and includes disease progression, number of treatments, and therapy resistance. In this domain, expectations on disease progression could be incorporated, for instance, it is known that arteriovenous malformations enlarge during life because of increased blood flow.[14, 15, 51] With the expanding knowledge on genetic mutations and their consequences on disease course, this may also contribute to the latter domain.

The findings of this thesis laid the basis for defining clinical severity in patients with vascular malformations, which is a crucial aspect of tailoring disease management to the individual patient. Multidimensional aspects of the patient and the vascular malformation are taken into account and we believe that it may serve several purposes. Together, the three domains provide an overview of all aspects of the disorder and gives an accurate estimate of the current disease severity.

The measurement of symptoms and health-related quality of life with the OVAMA questionnaire and PROMIS item banks provides an overview of the impact of the disease on the patient, which can be used to indicate treatment. In patients with minimal symptoms, treatment should be initiated with caution since complications may occur, and often multiple treatments are needed. On the contrary, patients with severe symptoms and impaired health-related quality of life necessitate a timely and more forceful approach to treatment. Furthermore, the expectations about disease progression incorporated in disease severity may indicate more aggressive treatment early in the course of the disease. The measurement and definition of disease severity is a practical tool to appoint patients who are severely affected and thus are eligible for targeted therapies since they are systemically administered and may cause systemic complications.

Repeated measurements over time of lesion characteristics, symptoms, and health-related quality of life allow for an accurate assessment of the course of the disease and monitor disease progression. Worsening of symptoms or health-related quality of life may serve as an indication to initiate treatment. The evaluation of the number of treatments, therapy resistance, and recurrence after treatment included in the domain disease course may warrant

more rigorous management, such as targeted therapies. Clinical studies investigating the disease course among various patient groups and various genotypes may expand the current knowledge on disease progression.

Currently, there still exist several challenges in patient-centered outcome measurements. Some patients may not feel urged or willing to complete PROMs. Subsequently, response rates may be low, or responders may differ significantly from non-responders, leading to biased outcome results as certain groups may be underrepresented.[52] Therefore, it is always important to investigate statistical differences between responders and non-responders and interpret if these differences might affect the results.

Additionally, completing PROMs should not burden patients unduly, and too frequent or short time intervals in the administration of PROMs need to be avoided. Offering short forms or computer-adaptive tests will reduce test length and may be less overwhelming for patients, which could be an effective strategy for improving response rates. The most convenient way to facilitate this is to offer digital questionnaires. Fortunately, there is an increasing online availability to administer PROMs, e.g., Castor, REDCap, Google Forms, and KLIK (the latter we have used in the current thesis). However, resources must be accessible to implement the PROMs in these online portals, and especially in the case of condition-specific PROMs addressing fewer patients, it may be challenging to implement the PROMs. Online PROM portals can provide instant feedback, and the results can be discussed directly with the patient, which can lead to shared decision-making, more adherence, and a strengthened self-efficacy of the patients.[53, 54] These upsides may motivate clinicians to pay attention to completed questionnaires. PROMs remain the mainstay for measuring disease burden as well as evaluating the effect of treatment at the individual level because they reflect the self-reported health state of the patient directly, and widespread use is strongly encouraged.[54-57]

The development of the OVAMA questionnaire and OVAMA Treatment Outcome questionnaire enables precise measurement of the effect of treatment on symptoms. The evaluation of treatment effectiveness and safety, historically measured by clinician-determined outcomes, can now be complemented or even replaced with patient-centered outcomes. Studies are able to investigate the effect of different treatments on the various symptoms, appearance-related concerns and assess satisfaction with treatment outcome. Consequently, knowledge will be gained about which treatment is effective in reducing specific symptoms, and the appropriate treatment method can be initiated if a patient experiences that specific symptom. More precisely, clinicians and patients are able to appoint a treatment strategy tailored to the individual patient and their symptoms and treatment goals.

To take the personalized approach to vascular malformation management one step further, treatment outcomes may be compared among different patient and lesion characteristics, including genetics. Leading to a better understanding of which treatment is effective for which patient and their specific genotype and clinical features. Hence, crucial advances can be made

to individualized treatment since the wide variety of patients are currently generally analyzed as one group, while it is recognized that different lesions and patients do not have a univocal response to treatment.

Variations in the genotype and lesion characteristics of vascular malformations result in clinically heterogeneous patients. Additionally, vascular malformations are a rare disorder for which an abundance of treatment methods are being used. All these factors contribute to the difficulty in achieving large, homogeneous patient cohorts to investigate certain treatments in clinical studies. Therefore, international collaborations for the investigation of the 'classical' treatment methods, as well as targeted therapies, are advisable and may solve this issue.

Another approach is the standardization of reported characteristics and outcome measurement in clinical studies, allowing for easy comparison and aggregation of study results in meta-analyses. In Europe, the first steps have already been taken, and the European Reference Network (ERN) for vascular anomalies (VASCA) is currently developing a registry for all patients with vascular malformations in Europe. The outcome measurement instruments developed and proposed in this thesis, the OVAMA questionnaire and PROMIS item banks, will cover the assessment of patient's symptoms and health-related quality of life and treatment evaluation.

Conclusion

This thesis made strides in the direction of a personalized approach to the management of vascular malformations through multiple facets of genetics, outcome measurement, and profiling patients based on symptoms and health-related quality of life. The thesis provided the valuable insight that the genotype is responsible for phenotypic variability and facilitated the incorporation of the genotype into the current classification and management. The development and quality assessment of outcome measurement instruments enabled the ability to measure patient-centered parameters that reflect the individual self-reported health status directly and enabled treatment evaluation from the patient's perspective uniformly. Our findings have brought new insights into the care of patients with vascular malformations and are expected to contribute to the ability to tailor treatment to the individual patient.

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Chapter 12

Summary

&

Nederlandse samenvatting

(Dutch summary)

Summary

Part I: General introduction

Vascular malformations are complex congenital lesions of the vascular or lymphatic system consisting of dilated and dysfunctional vessels that generally have a tortuous structure. The lesions portray a wide clinical spectrum with heterogeneity in involved vessel type, anatomical location, tissue extension, and lesion size. Dependent on these clinical aspects, vascular malformations may cause a variety of symptoms, including functional problems, pain, disfigurement of appearance, and psychosocial problems. These disparities between patients call for an individualized approach to their management.

This thesis addressed several aspects of a personalized approach to the management of vascular malformations. Recent discoveries have pointed out that vascular malformations are caused by somatic and germline mutations in various genes regulating growth. However, knowledge gaps remained on how these underlying mutations lead to clinical differences and how the genetic base should be incorporated into the management of vascular malformations. Secondly, patient-reported outcome measures (PROMs) reflect the self-reported health state of the patient directly and are able to measure the effect of treatment at the individual level. No measurement instruments existed to evaluate condition-specific core outcome domains. Finally, it was unknown how the specific lesion characteristics lead to certain symptoms and how this subsequently affects the health-related quality of life in the individual patient. In this thesis, these gaps in current knowledge were addressed, and the summarized results of the studies included in this thesis are presented below.

Part II : Vascular malformations and overgrowth disorders: from genotype to phenotype.

The discovery of various mutated genes uncovers that vascular malformations are even more heterogeneous than was known from clinical aspects alone. Although, it is unclear how the genetic bases precisely relate to the phenotype characteristics. In addition, the genetic discoveries should have a more prominent role in the classification and treatment of vascular malformations, resulting in a more personalized approach to their management.

In *chapter 2*, a systematic review was performed to provide an overview of known causative genetic mutations in vascular malformations and discuss associations between gene mutations and clinical phenotypes. The literature search yielded 5667 studies, of which 69 studies were included, reporting molecular analysis in a total of 4261 patients, and in 1686 (40%) patients with peripheral vascular malformations a causative mutation was detected. The study showed that the underlying genetic mutations contribute to the highly variable clinical characteristics of vascular malformations, and genotype-phenotype associations were found. In addition, some mutated genes lead to a uniform phenotype, while other mutated genes lead to more varying phenotypes. By contrast, distinct mutated genes may lead to similar phenotypes and

result in almost indistinguishable vascular malformations. Vascular malformations are currently classified according clinical and histopathology features, however, the findings of this systematic review suggest a larger role for genotype in current diagnostics and classification.

Molecular diagnostics will be increasingly performed in order to incorporate the genotype in the diagnosis, classification, and management of vascular malformations. However, collection of lesion tissue is required to perform molecular diagnostics, which is a troublesome process. In *chapter 3* a prospective case series was performed to investigate a minimally-invasive alternative for specimen collection for molecular analysis. Blood and lymph fluid was collected locally from venous, lymphatic, and combined malformations during sclerotherapy. Cell-free DNA was isolated from the collected samples and analyzed for vascular malformation-associated genes with Next-Generation Sequencing. Somatic PIK3CA and TIE2 mutations were detected in cell-free DNA of patients with venous (5/14) and lymphatic malformations (5/8). In two patients with combined malformations, somatic mutations could not be detected. We concluded that cell-free DNA obtained during sclerotherapy of venous and lymphatic malformations is an excellent alternative for tissue biopsies to perform molecular analysis. Particularly for deep-positioned or intricate located vascular malformations or other unenforceable tissue biopsies, cell-free DNA provides a solution. The findings in this study are a valuable contribution to a field in which genetics is becoming increasingly important, and where molecular diagnostics are becoming inevitable.

Currently, there is limited in-depth mechanistic insight in the pathophysiology and a lack of pre-clinical research approaches for capillary and other types vascular malformations. In *chapter 4*, we aimed to isolate and expand primary endothelial cells from capillary malformations, carrying genetic mutations, and use these to assess differences in endothelial cell function of capillary malformations compared to healthy tissue. In a prospective exploratory study of 17 adult patients with capillary malformations, we found somatic mutations in the GNAQ [p.R183Q, p.R183G or p.Q209R] or GNA11 [p.R183C] genes. We applied an endothelial-selective cell isolation protocol to culture primary endothelial cells from skin biopsies from these patients. We demonstrate that patient-derived cells can be expanded in culture, while maintaining endothelial specificity as demonstrated by Vascular Endothelial (VE)-cadherin immunostainings. In addition, we find that the angiogenic capacity of the endothelial cells from a patient with a GNAQ[p.R183G] mutation is increased compared to control endothelial cells expanded from normal skin. These proof-of-principle results reveal that primary cells isolated from capillary malformations may represent a highly valuable research model to investigate the role of endothelial somatic mutations in the aetiology of capillary malformations and could also serve as tool for pre-clinical drug testing. Future larger-scale studies are needed to investigate how mutations in the GNAQ and GNA11 genes contribute to the development of capillary malformations and associated clinical features in patients.

Vascular malformations may be accompanied by overgrowth of soft tissue or bone, and recent discoveries showed that both conditions have similar origins and are derived from mutations in the same genes. The somatic PIK3CA mutation is identified in vascular malformations, overgrowth disorders, as well as in syndromes consisting of both clinical features, which resulted in the classification of all PIK3CA-related disorders within the PIK3CA-Related Overgrowth Spectrum (PROS). In *chapter 5*, we described the long-term progression of the PIK3CA-related overgrowth disorder macrodactyly in four patients. All patients were surgically treated during childhood and showed progression of tissue overgrowth during adult life. In addition, molecular diagnosed patients showed somatic PIK3CA mutations. All patients developed severe secondary degenerative bone changes in macrodactyly affected digits, and subsequently, the continuous tissue overgrowth and degenerative bone changes led to functional problems.

Part III: Development and quality assessment of condition-specific patient-reported outcome measures in patients with peripheral vascular malformations.

In order to tailor treatment to the individual patient, a baseline of health status in symptoms and health-related quality of life should be established and patients should be able to evaluate treatment from their own perspective. PROMs are a valuable method to measure the effect of treatment at the individual level. In 2016, the OVAMA project (Outcome measures for VAscular MAlformations) was initiated to establish uniform outcome measures in clinical research on vascular malformations, involving the patients' perspective. In a previous study, a core domain set (CDS) for peripheral vascular malformations was established, i.e., a minimum set of outcome domains that should be measured when evaluating treatment effect in a certain health condition. The CDS consisted of condition-specific core outcome domains, i.e. the domains related to the condition-specific symptoms, appearance and satisfaction with treatment and outcome. However, no measurement instruments were available to measure these condition-specific core outcome domains.

Chapter 6 described a qualitative and subsequent cross-sectional study to develop a PROM for measuring symptoms and appearance in patients with peripheral vascular malformations. Fully based on the internationally developed CDS, a first draft of the PROM was made. In cognitive interviews with 14 patients, the content and comprehensibility of the first draft were extensively reviewed and adjusted accordingly into a second draft. In a subsequent cross-sectional study, the second draft was field-tested, and construct validity was evaluated by testing thirteen predefined hypotheses on known-group differences. Additionally, internal consistency (Cronbach's alpha >0.7) of groups of items was evaluated to form composite scores. Adequate content validity was ensured in the cognitive patient interviews and resulted in a PROM called the OVAMA questionnaire, consisting of six items on general problems, eight items on head and neck symptoms, and nine items on appearance-related aspects. Adequate construct validity could be established, based on ten out of thirteen confirmed hypotheses on

known-group differences. In addition, two composite scores could be made according to an adequate Cronbach's alpha for a general symptom score (0.88) and an appearance score (0.85). The development of the OVAMA questionnaire now enables treatment evaluation in vascular malformations from the patients' perspective.

In order to use the OVAMA questionnaire in a longitudinal setting and to assess the effect of treatment, the OVAMA questionnaire needs to be responsive to changes in symptoms and appearance in order to determine whether the disease status has altered since treatment. In *chapter 7*, we aimed to assess the responsiveness of the OVAMA questionnaire in patients with vascular malformations. In a prospective study, responsiveness was evaluated following the criterion approach of testing predefined hypotheses about expected relationships between the OVAMA questionnaire and Global Rating of Change scales, measuring the same constructs. The OVAMA questionnaire was considered responsive if ≥ 75% of the hypotheses were confirmed. Ninety-eight patients were recruited in a vascular anomaly center in the Netherlands, of which 63 patients completed the questionnaires at baseline and follow-up. In total, fifteen constructs of the OVAMA questionnaire were assessed for five hypotheses. Of these 75 hypotheses, 63 (84%) hypotheses were confirmed and thereby providing evidence that the OVAMA questionnaire is responsive to change. In addition to determining a baseline of symptoms and appearance, the OVAMA questionnaire can now be used to evaluate the effect of treatment from the patient's perspective.

The domain category 'satisfaction' determined in the CDS, referring to satisfaction with outcome and treatment was not included in the OVAMA questionnaire since it is only relevant at follow-up. *Chapter 8* reports the development of a PROM to measure satisfaction with treatment outcome in patients with vascular malformations. Furthermore, here we aimed to investigate relevant measurement properties of the PROM, and to assess preliminary results of satisfaction with treatment outcomes. Fully based on the internationally established CDS, a first draft of the PROM was made, called the OVAMA-Treatment Outcome scales. In cognitive interviews with 14 patients, concept validity was assessed, which led to a second draft. In a cross-sectional study, construct validity of the OVAMA-Treatment Outcome was investigated by testing nine predefined hypotheses about expected relationships with the Global Rating of Changes scales, measuring similar constructs. In univariate analysis using Kruskal-Wallis test, satisfaction with treatment outcome was compared between patients receiving different treatments. Adequate content validity was found in the patient interviews, and resulted in five items referring to satisfaction with treatment outcome and change in various symptoms, and two items referring to tolerability of treatment. In total, 104 patients completed the OVAMA Treatment Outcome and Global Rating of Change scales, and all nine hypotheses on expected relationships with the Global Rating of Change scales were confirmed, hence, construct validity was considered good. Patients treated with surgery were overall the most satisfied about treatment effect. Satisfaction with treatment outcome can now be adequately measured from the patient's perspective, and the OVAMA Treatment Outcome can be used in clinical research

to reach homogeneity in outcome reporting. These are crucial steps for evidence-based guidelines for patients with vascular malformations.

Part IV: Defining disease severity in peripheral vascular malformations.

Differences exist among patients with vascular malformations in the experienced symptoms that are present and the subsequent impact on health-related quality of life. Consequently, rationales for seeking treatment also vary among patients, and management should be adjusted to the individual patient. However, limited data were available on the relation between the clinical presentation of vascular malformations and the experienced symptoms, and it was unknown which subgroups of patients were more susceptible to certain symptoms and a decreased health-related quality of life. These are crucial steps in order to find the appropriate treatment for the individual patient.

Chapter 9 aimed to investigate pain in patients with peripheral vascular malformations, and to determine factors associated with an increased risk for pain. Additionally, the impact of pain on the health-related quality of life was explored. In a prospective cross-sectional study including 164 patients, approximately one-half of the patients (52%) reported pain in the past four weeks and 57% of these patients reported pain daily or several times a week. Female sex (P= .009), lesions located in the upper extremity (P< .001) or lower extremity (P < .001), and intramuscular/intraosseous lesions (P = .004) were independently associated with the presence of pain. The following health-related quality of life domains were diminished in patients who experienced pain in comparison with patients who did not: pain interference with daily activities (P< .001), physical functioning (P< .001), and social participation (P< .001) in adults, and pain interference (P =.001), mobility (P = .001), and anxiety (P = .020) in children. In conclusion, pain is a frequently reported complaint in patients with vascular malformations and is present in approximately half of the patients. Patients with lesions located in the upper or lower extremity, intramuscular/intraosseous lesions, and female patients are more likely to experience pain. The presence of pain negatively impacted patients' health-related quality of life. Although vascular malformations are a benign condition and expectative management is frequently applied, our study showed that pain is a serious concern and needs to be actively assessed. Pain is a sign of various etiologies and the pain etiology should be examined in order to properly treat the pain.

In *chapter 10*, a cross-sectional study was performed in 184 patients with peripheral vascular malformations to examine appearance-related concerns and their impact on health-related quality of life. In total, 121 patients (66%) reported that one or more appearance-outcome was severely affected, and the following factors statistically significant associated with more appearance-related concerns: capillary/combined origin, facial localization, subcutaneous tissue involvement, larger lesion size, overgrowth, and diagnosis of a syndrome. In adults, dissatisfaction with appearance and reduced self-esteem due to the vascular malformation correlated with more anxiety and depression symptoms. Reduced self-esteem due to the

vascular malformation correlated with less social-participation. In children, bodily-distortion and being stared at were correlated with less peer relationships, possibly due to stigma and bullying, making them feel less accepted by peers. The results of this study highlight the importance of paying attention to patients' perception of their aesthetic appearance. By assessing appearance-related concerns, physicians can offer interventions to potentially improve satisfaction with appearance and monitor for signs of psychosocial impairment. If necessary, they should refer patients to peer support groups or professional psychological support.

Part V: General discussion and future perspectives.

In *chapter 11*, the research results described in this thesis were discussed in the view of contemporary literature, and relevant future perspectives are illustrated. With the realization of this thesis, a more personalized approach to the management of vascular malformations is feasible, while including various aspects of the condition, e.g. genetic bases, clinical characteristics, symptoms, and health-related quality of life. The first steps were taken to incorporate the genotype of vascular malformations in their classification, diagnosis, and management. Additionally, we depict how these changes will continue to crystallize and eventually result in an ever-increasing role for the genotype in the management of vascular malformations.

The development and quality assessment of the OVAMA questionnaire and the OVAMA Treatment Outcomes scales allow for the assessment of the impact of the disease on the patient and enable precise measurement of the effect of treatment on symptoms. PROMs can be an important and fundamental tool to measure the extensiveness of the disorder as well as the effect of treatment at the individual level because they reflect the self-reported health state of the patient directly. Evaluation of the disease status and treatment with these newly developed measurement instruments facilitates a more personalized approach to the management of vascular malformations. The work presented in this thesis resolves the essential knowledge gaps in the field of vascular malformations and personalized medicine, with the fundamental goal of improving the care for patients with vascular malformations.

Nederlandse Samenvatting (Dutch Summary)

Deel I: Algemene introductie

Vasculaire malformaties zijn complexe aangeboren laesies van het vasculaire of lymfatische systeem bestaande uit verwijde en disfunctionele vaten die meestal een kronkelige structuur hebben. De laesies vertonen een breed klinisch spectrum met heterogeniteit in het aangedane vaattype, anatomische locatie, weefsel betrokkenheid en laesiegrootte. Afhankelijk van deze klinische karakteristieken kunnen vasculaire malformaties een verscheidenheid aan symptomen veroorzaken waaronder functionele problemen, pijn, een aangetast uiterlijk en psychosociale problemen. Deze verschillen tussen patiënten vragen om een geïndividualiseerde benadering van de behandeling.

Dit proefschrift richtte zich op verschillende aspecten van een gepersonaliseerde behandeling van vasculaire malformaties. Recente ontdekkingen hebben aangetoond dat vasculaire malformaties worden veroorzaakt door somatische en kiembaanmutaties in verschillende genen die de celgroei reguleren. Echter, bestonden er nog hiaten in de kennis over hoe deze onderliggende mutaties kunnen leiden tot klinische verschillen tussen patiënten en hoe de onderliggende genetica moet worden geïntegreerd in de behandeling van vasculaire malformaties. Ten tweede, patiënt gerapporteerde uitkomstmaten (PROMs) geven direct de zelf gerapporteerde gezondheidstoestand van de patiënt weer en ze zijn in staat om het effect van behandeling op individueel niveau te meten. Voor vasculaire malformaties bestonden er nog geen gevalideerde PROMs om de ziekte-specifieke kern-uitkomstdomeinen (CDS) te evalueren. Tot slot was het onbekend hoe de specifieke laesie karakteristieken leiden tot bepaalde symptomen en hoe dit vervolgens de kwaliteit van leven van de individuele patiënt beïnvloedt. In dit proefschrift werden deze hiaten in de huidige kennis onderzocht en de samengevatte resultaten van de studies die in dit proefschrift zijn opgenomen, worden hieronder gepresenteerd.

Deel II: Vasculaire malformaties en weefsel overgroei aandoeningen: van genotype tot fenotype.

De ontdekking van verschillende onderliggende genetische mutaties laat zien dat vasculaire malformaties nog meer heterogeniteit vertonen dan eerder bekend was op basis van alleen de klinische karakteristieken. Het is echter nog onduidelijk hoe de genetische mutaties zich precies verhouden tot de fenotype kenmerken. Daarnaast zouden de genetische ontdekkingen een prominentere rol moeten gaan spelen bij de classificatie en behandeling van vasculaire malformaties, wat eindelijk zal moeten resulteren in een meer gepersonaliseerde behandeling.

In *hoofdstuk 2* werd een systematisch literatuur onderzoek uitgevoerd om een overzicht te geven van de causatieve genetische mutaties bij vasculaire malformaties en om de correlaties tussen de genetische mutaties en klinische fenotype bloot te leggen. Het literatuuronderzoek leverde 5667 studies op, waarvan 69 studies werden geïncludeerd, waarin moleculaire analyse

werd gerapporteerd bij een totaal 4261 patiënten, en in 1686 (40%) patiënten met perifere vasculaire malformaties werd een causatieve genetische mutatie aangetoond. Het onderzoek liet zien dat de onderliggende genetische mutaties bijdragen aan de zeer variabele klinische kenmerken van vasculaire malformaties en er werden genotype-fenotype correlaties gevonden. Daarnaast liet het literatuur onderzoek zien dat sommige gemuteerde genen leiden tot een uniform fenotype, terwijl andere gemuteerde genen tot meer variërende fenotypes leiden. Daarentegen kunnen verschillende gemuteerde genen leiden tot vergelijkbare fenotypes en resulteren in bijna niet van elkaar te onderscheiden vasculaire malformaties. Vasculaire malformaties worden momenteel geclassificeerd op basis van klinische en histopathologische kenmerken, maar de bevindingen van dit systematische literatuuronderzoek suggereren een grotere rol voor het genotype in de huidige diagnostiek en classificatie.

Moleculaire diagnostiek zal in toenemende mate worden uitgevoerd om het genotype te integreren in de diagnose, classificatie en behandeling van vasculaire malformaties. Voor het uitvoeren van moleculaire diagnostiek moet er echter een weefselbiopt van de laesie worden afgenomen, wat een lastig proces is. In *hoofdstuk 3* werd een prospectieve case-serie uitgevoerd om een minimaal-invasief alternatief te onderzoeken voor het afnemen van materiaal voor moleculaire analyse. Bloed en lymfevocht werden lokaal afgenomen uit veneuze, lymfatische en gecombineerde malformaties tijdens sclerotherapie behandeling. Uit het afgenomen materiaal werd cel-vrij DNA geïsoleerd en met Next-Generation Sequencing geanalyseerd op vasculaire malformatie-geassocieerde genen. Somatische PIK3CA- en TIE2-mutaties werden gedetecteerd in cel-vrij DNA van patiënten met veneuze (5/14) en lymfatische malformaties (5/8). Bij twee patiënten met gecombineerde malformaties konden geen somatische mutaties worden gedetecteerd. We concludeerden dat cel-vrij DNA verkregen tijdens sclerotherapie behandeling van veneuze en lymfatische malformaties een uitstekend alternatief is voor weefselbiopten om moleculaire analyse uit te voeren. Met name voor diep of moeilijk gelokaliseerde vasculaire malformaties of andere onuitvoerbare weefselbiopten biedt cel-vrij DNA een oplossing. De bevindingen in deze studie zijn een waardevolle bijdrage aan een veld waarin genetica steeds belangrijker wordt en waar het uitvoeren moleculaire diagnostiek onvermijdelijk wordt.

Op dit moment is er beperkt diepgaand inzicht in de pathofysiologie en een gebrek aan preklinische onderzoek benaderingen van capillaire en andere typen vasculaire malformaties. In *hoofdstuk 4* streefden we ernaar om primaire endotheelcellen van capillaire malformaties met genetische mutaties te isoleren en te laten groeien om deze vervolgens te gebruiken om de endotheelcelfunctie van capillaire malformaties te vergelijken met gezond weefsel. In een prospectieve studie van 17 volwassen patiënten met capillaire malformaties vonden we somatische mutaties in de GNAQ [p.R183Q, p.R183G of p.Q209R] of GNA11 [p.R183C] genen. We ontwikkelden een endotheel-selectief cel-isolatie protocol om primaire endotheelcellen te kweken van huidbiopten van deze patiënten. We laten zien dat cellen afkomstig van patiënten

kunnen groeien op kweek, waarbij de endotheel specificiteit wordt behouden zoals wordt aangetoond door Vasculair Endotheel (VE)-cadherine immunokleuring. Daarnaast vonden we dat de angiogene capaciteit van de endotheelcellen van een patiënt met een GNAQ[p.R183G]-mutatie is verhoogd in vergelijking met controle-endotheelcellen van de normale huid. Deze proof-of-principle resultaten laten zien dat primaire cellen geïsoleerd uit capillaire malformaties een zeer waardevol onderzoekmodel kunnen zijn om de rol van endotheliale somatische mutaties in de etiologie van capillaire malformaties te onderzoeken en ook kunnen dienen als hulpmiddel voor het preklinisch testen van geneesmiddelen. Toekomstige grootschaligere studies zijn nodig om te onderzoeken hoe mutaties in de GNAQ en GNA11 genen leiden tot de ontwikkeling van capillaire malformaties en hoe de genetische mutaties zich uiteindelijk verhouden tot klinische kenmerken bij patiënten.

Vasculaire malformaties kunnen gepaard gaan met overgroei van weke delen of bot en recente ontdekkingen toonden aan dat beide aandoeningen een vergelijkbare oorsprong hebben en voortkomen uit mutaties in dezelfde genen. De somatische PIK3CA-mutatie is geïdentificeerd in vasculaire malformaties, weefsel overgroeistoornissen en in syndromen die bestaan uit beide klinische kenmerken, wat resulteerde in de classificatie van alle PIK3CA-gerelateerde aandoeningen binnen het PIK3CA-gerelateerde overgroeispectrum (PROS). In ***hoofdstuk 5*** beschreven we de lange termijn progressie van de PIK3CA-gerelateerde overgroeistoornis macrodactylie bij vier patiënten. Alle patiënten werden chirurgisch behandeld tijdens hun kindertijd en vertoonden progressie van weefsel overgroei tijdens hun volwassen leven. Daarnaast vertoonden moleculair gediagnosticeerde patiënten somatische PIK3CA-mutaties. Alle patiënten ontwikkelden ernstige secundaire degeneratieve botveranderingen in de door macrodactylie aangetaste ledematen en uiteindelijk leidden de voortdurende weefsel overgroei en degeneratieve botveranderingen tot functionele problemen.

Deel III: Ontwikkeling en kwaliteit validatie van ziekte-specifieke patiënt-gerapporteerde uitkomstmaten in patiënten met perifere vasculaire malformaties.

Om de behandeling op de individuele patiënt af te stemmen, moet er een uitgangswaarde van de gezondheidsstatus van symptomen en de kwaliteit van leven worden vastgesteld en moeten patiënten de behandeling vanuit hun eigen perspectief kunnen evalueren. PROMs zijn een waardevolle methode om het effect van behandeling op individueel niveau te meten. In 2016 is het OVAMA-project (Outcome measures for VAscular MAlformations) gestart om uniforme uitkomstmaten te definiëren in klinisch onderzoek naar vasculaire malformaties, waarin ook het perspectief van de patiënt wordt betrokken. In een eerdere studie werd een set van kern uitkomstdomeinen (CDS) voor perifere vasculaire malformaties bepaald, dit is een minimale set uitkomstdomeinen die gemeten zouden moeten worden bij het evalueren van het effect van een behandeling bij een specifieke aandoening. De CDS voor vasculaire malformaties bestaat uit ziekte-specifieke kern uitkomstdomeinen, welke de ziekte-specifieke symptomen, zorgen over het uiterlijk en tevredenheid met de behandeling en behandeluitkomst omvat. Echter,

waren er geen meetinstrumenten beschikbaar om deze ziekte-specifieke kern uitkomstdomeinen te meten.

Hoofdstuk 6 beschrijft een kwalitatieve en aansluitend cross-sectionele studie voor het ontwikkelen van een PROM die symptomen en zorgen over het uiterlijk meet in patiënten met perifere vasculaire malformaties. Volledig gebaseerd op de internationaal ontwikkelde set van kern uitkomstdomeinen (CDS) werd er een eerste concept van de PROM gemaakt. In cognitieve interviews met 14 patiënten werd de inhoud en begrijpelijkheid van de eerste opzet uitgebreid beoordeeld en aangepast tot een tweede opzet. In een daaropvolgende cross-sectionele studie werd de tweede versie in de praktijk getest en werd de constructvaliditeit geëvalueerd door dertien vooraf gedefinieerde hypothesen over verschillen tussen bekende groepen te testen. Daarnaast werd de interne consistentie (Cronbach's alpha >0,7) van groepen items geëvalueerd om samengestelde scores te vormen. De inhoudsvaliditeit van de PROM bleek adequaat in de cognitieve patiëntinterviews en resulteerde in een PROM genaamd de OVAMA-vragenlijst, bestaande uit zes items over algemene problemen, acht items over hoofd- en nekklachten en negen items over uiterlijke aspecten. Tevens bleek ook de constructvaliditeit adequaat, gebaseerd op tien van de dertien bevestigde hypothesen over bekende-groepsverschillen. Daarnaast konden er twee samengestelde scores worden gemaakt volgens een adequate Cronbach's alpha voor een algemene symptomen score (0,88) en een uiterlijk score (0,85). De ontwikkeling van de OVAMA vragenlijst maakt het nu mogelijk om de behandeling van vasculaire malformaties te evalueren vanuit het perspectief van de patiënt.

Om de OVAMA vragenlijst in een longitudinale setting te kunnen gebruiken en het effect van behandeling te kunnen beoordelen, moet de OVAMA vragenlijst responsief zijn voor veranderingen in symptomen en uiterlijk om te kunnen bepalen of de ziektestatus is veranderd sinds de behandeling. In *hoofdstuk 7* trachten we de responsiviteit van de OVAMA vragenlijst te beoordelen bij patiënten met vasculaire malformaties. In een prospectieve studie werd de responsiviteit geëvalueerd volgens de criterion methode door het testen van vooraf gedefinieerde hypothesen over verwachte relaties tussen de OVAMA-vragenlijst en de Global Rating of Change scales, die dezelfde constructen meten. De OVAMA-vragenlijst werd als responsief beschouwd als ≥ 75% van de hypothesen werd bevestigd. Achtennegentig patiënten uit een centrum voor vasculaire afwijkingen in Nederland werd benaderd om te participeren in de studie, waarvan 63 patiënten de vragenlijsten invulden tijdens de nulmeting en de follow-up. In totaal werden vijftien constructen van de OVAMA-vragenlijst beoordeeld voor vijf hypothesen. Van deze 75 hypothesen werden 63 (84%) hypothesen bevestigd, waarmee werd aangetoond dat de OVAMA-vragenlijst responsief is voor verandering. Naast het bepalen van een uitgangswaarde van symptomen en uiterlijk, kan de OVAMA-vragenlijst nu worden gebruikt om het effect van de behandeling vanuit het perspectief van de patiënt te evalueren.

De domeincategorie 'tevredenheid' uit de CDS, die verwijst naar tevredenheid met de behandelingsuitkomst en de behandeling op zichzelf, werd niet geïncludeerd in de OVAMA-

vragenlijst omdat deze alleen relevant is bij follow-up. *Hoofdstuk 8* rapporteert de ontwikkeling van een PROM om de tevredenheid met de uitkomst van behandeling en tevredenheid met de behandeling te meten bij patiënten met vasculaire malformaties. Tevens hadden we hier als doel om relevante meeteigenschappen van de PROM te onderzoeken en om preliminaire resultaten van tevredenheid met behandeluitkomsten te evalueren. Volledig gebaseerd op de internationaal gevestigde CDS werd een eerste concept van de PROM gemaakt, genaamd de OVAMA-Treatment Outcome scales. In cognitieve interviews met 14 patiënten werd de inhoudsvaliditeit beoordeeld, wat leidde tot een tweede concept. In een cross-sectionele studie werd de constructvaliditeit van de OVAMA-Treatment Outcome onderzocht door negen vooraf gedefinieerde hypothesen te testen over verwachte relaties met de Global Rating of Changes scales, die vergelijkbare constructen meten. In univariate analyse met behulp van de Kruskal-Wallis test werd de tevredenheid met het resultaat van de behandeling vergeleken tussen patiënten die verschillende behandelingen hadden ondergaan. Er werd voldoende inhoudsvaliditeit gevonden in de patiënten interviews, en dit resulteerde in vijf items die betrekking hadden op tevredenheid met de uitkomst van de behandeling en verandering in verschillende symptomen, en twee items die verwezen naar de verdraagzaamheid van de behandeling. In totaal vulden 104 patiënten de OVAMA-Treatment Outcome scales en de Global Rating of Change scales in, en alle negen hypothesen over verwachte relaties met de Global Rating of Change scales werden bevestigd, waarmee de constructvaliditeit adequaat bleek te zijn. Patiënten die chirurgisch werden behandeld waren over het algemeen het meest tevreden over het effect van de behandeling. Tevredenheid met de behandeluitkomst kan nu adequaat worden gemeten vanuit het perspectief van de patiënt en de OVAMA Treatment Outcome scales kunnen worden gebruikt in klinisch onderzoek om uniformiteit te bereiken in de rapportage van behandeluitkomsten. Dit zijn cruciale stappen voor evidence-based richtlijnen voor patiënten met vasculaire malformaties.

Deel IV: Het definiëren van de ernst van de ziekte bij perifere vasculaire malformaties.

Er bestaan grote verschillen tussen patiënten met vasculaire malformaties in de symptomen die aanwezig zijn en de daaropvolgende impact op de kwaliteit van leven. Vervolgens verschillen ook de redenen om behandeling te zoeken tussen patiënten en zou de behandeling moeten worden aangepast op de individuele patiënt. Er waren echter beperkte gegevens beschikbaar over de relatie tussen de klinische presentatie van vasculaire malformaties en de ervaren symptomen, en het was onbekend welke subgroepen van patiënten gevoeliger waren voor het ontwikkelen van bepaalde symptomen en een verminderde kwaliteit van leven. Dit zijn cruciale stappen om de juiste behandeling voor de individuele patiënt te vinden.

Hoofdstuk 9 was gericht op het onderzoeken van pijn bij patiënten met perifere vasculaire malformaties en het bepalen van factoren die samenhangen met een verhoogd risico op pijn. Daarnaast werd de impact van pijn op de kwaliteit van leven onderzocht. In een prospectief cross-sectioneel onderzoek met 164 patiënten rapporteerde ongeveer de helft van de

patiënten (52%) pijn in de afgelopen vier weken en 57% van deze patiënten meldde dagelijks of meerdere keren per week pijn. Het vrouwelijk geslacht (P= .009), laesies in de bovenste extremiteit (P< .001) of onderste extremiteit (P < .001), en intramusculaire/intraosseuze laesies (P = .004) waren onafhankelijk geassocieerd met de aanwezigheid van pijn. De volgende domeinen van kwaliteit van leven waren verminderd bij patiënten die pijnklachten hadden in vergelijking met patiënten die geen pijnklachten hadden: pijn interferentie met dagelijkse activiteiten (P< .001), lichamelijk functioneren (P< .001), en sociale participatie (P< .001) bij volwassenen, en pijn interferentie (P = .001), mobiliteit (P = .001), en angst (P = .020) bij kinderen. Concluderend, pijn is een frequent gerapporteerde klacht bij patiënten met vasculaire malformaties en is aanwezig bij ongeveer de helft van de patiënten. Patiënten met laesies in de bovenste of onderste extremiteit, intramusculaire/intraosseuze laesies en vrouwelijke patiënten ervaren vaker pijn. De aanwezigheid van pijn had een negatieve invloed op de kwaliteit van leven van patiënten. Hoewel vasculaire malformaties een goedaardige aandoening zijn en er vaak een expectatief beleid wordt gevolgd, toonde onze studie aan dat pijn een serieus probleem is en actief zou moet worden beoordeeld door de clinicus. Pijn is een teken van verschillende etiologieën en de etiologie van de pijn moet worden onderzocht om de pijn op de juiste manier te behandelen.

In *hoofdstuk 10* werd een cross-sectionele studie uitgevoerd bij 184 patiënten met perifere vasculaire malformaties om de zorgen over het uiterlijk en de impact op de kwaliteit van leven te onderzoeken. In totaal rapporteerden 121 patiënten (66%) dat één of meer uiterlijke kenmerken ernstig was aangetast, en de volgende factoren waren statistisch significant geassocieerd met meer uiterlijke kenmerken: capillaire/gecombineerde oorsprong, lokalisatie in het gelaat, betrokkenheid van subcutaan weefsel, grotere laesie, overgroei van weke delen of bot en de diagnose van een geassocieerd syndroom. Bij volwassenen hielden ontevredenheid over het uiterlijk en een verminderd zelfvertrouwen als gevolg van het uiterlijk van de vasculaire malformatie verband met meer angst- en depressiesymptomen. Tevens correleerde een verminderd zelfvertrouwen als gevolg van de vasculaire malformatie met minder sociale participatie. Bij kinderen waren lichamelijke vervorming en worden aangestaard gecorreleerd met minder relaties met leeftijdsgenoten, mogelijk als gevolg van stigmatisering en pesten, waardoor ze zich minder geaccepteerd voelden door leeftijdsgenoten. De resultaten van dit onderzoek benadrukken het belang van aandacht voor de perceptie van het esthetische uiterlijk van patiënten. Door de zorgen over het uiterlijk te beoordelen, kunnen artsen interventies aanbieden om de tevredenheid met het uiterlijk mogelijk te verbeteren en controleren op tekenen van psychosociale achteruitgang. Indien nodig moeten ze patiënten doorverwijzen naar lotgenotengroepen of professionele psychologische ondersteuning.

Deel V: Algemene discussie en toekomstperspectieven.

In *hoofdstuk 11* werden de onderzoeksresultaten die zijn beschreven in dit proefschrift besproken in het licht van de hedendaagse literatuur en werden relevante toekomstperspectieven geïllustreerd. Met de realisatie van dit proefschrift is een meer

gepersonaliseerde benadering van de behandeling van vasculaire malformaties haalbaar, waarbij verschillende aspecten van de aandoening worden meegenomen, zoals de genetische basis, klinische kenmerken, symptomen en de kwaliteit van leven. De eerste stappen werden gezet om het genotype van vasculaire malformaties te integreren in de classificatie, diagnose en behandeling van vasculaire malformaties. Daarnaast laten we zien hoe deze veranderingen zich verder zullen uitkristalliseren en uiteindelijk zullen resulteren in een steeds grotere rol voor het genotype in de behandeling van vasculaire malformaties.

De ontwikkeling en kwaliteitsvalidatie van de OVAMA-vragenlijst en de OVAMA-Treatment Outcome scales maken het mogelijk om de impact van de ziekte op de patiënt te beoordelen en het effect van de behandeling op de symptomen nauwkeurig te meten. PROMs kunnen een belangrijk en fundamenteel instrument zijn om de ziektelast van de aandoening en het effect van de behandeling op individueel niveau te meten, omdat ze de zelf gerapporteerde gezondheidstoestand van de patiënt rechtstreeks weerspiegelen. Evaluatie van de ziektestatus en behandeling met deze nieuw ontwikkelde meetinstrumenten vergemakkelijkt een meer gepersonaliseerde aanpak van de behandeling van vasculaire malformaties. Het in dit proefschrift gepresenteerde werk lost essentiële kennislacunes op het gebied van vasculaire malformaties en gepersonaliseerde geneeskunde op, met als fundamenteel doel het verbeteren van de zorg voor patiënten met vasculaire malformaties.

Appendices

PhD Portfolio

Name PhD student: Merle Louise Elisabeth Stor
PhD period: June 2020 – December 2022
Promotor: Prof. dr. C.M.A.M. van der Horst
Copromotores: Dr. S.E.R. Horbach
Dr. M.M. Lokhorst

PhD training	Year	Workload (ECTS)
Courses		
Practical Biostatistics	2020	1.5
Basic course Legislation and Organisation for Clinical Reseachers (eBROK)	2020	1.5
Research data management	2020	1.0
Scientific writing	2021	1.5
Seminars, workshops and master classes		
Weekly department seminars	2020-2023	2.0
Weekly department research meetings	2020-2023	2.0
Monthly department research workshops	2020-2023	1.0
Plastic, reconstructive and hand surgery department seminars (every 3 months)	2020-2023	1.0
Presentations		
"Liquid biopsy van cel-vrij DNA afgenomen uit vasculaire malformaties voor het uitvoeren van moleculaire diagnostiek". Afdeling Pathologie Amsterdam UMC, Amsterdam, The Netherlands (oral)	2020	0.5
"Symptomen, uiterlijke klachten en de kwaliteit van leven van patiënten met vasculaire malformaties, hoe kunnen we het meten?" Aangeboren Vaatafwijkingen Team Amsterdam UMC, Amsterdam, The Netherlands (oral)	2020	0.5
"De ontwikkeling van een patiënten registratie bij zeldzame aangeboren afwijkingen." Vasculaire Malformaties expertisemeeting, Breukelen, The Netherlands (oral)	2022	0.5

"The development and validation of patient-reported outcome measures for measuring symptoms, appearance, and quality of life in patient with vascular malformations." Pre-congress day, International Society for the Study of Vascular Anomalies (ISSVA) World Congress 2022, Vancouver, Canada (oral)	2022	2.0
"Appearance-related concerns in patients with peripheral vascular malformations." International Society for the Study of Vascular Anomalies (ISSVA) World Congress 2022, Vancouver, Canada (poster)	2022	0.5
"Clinical characteristics associated with pain in patients with peripheral vascular malformations." International Society for the Study of Vascular Anomalies (ISSVA) World Congress 2022, Vancouver, Canada (oral)	2022	0.5
"Cell-free DNA obtained during sclerotherapy as a novel method for molecular analysis of venous and lymphatic malformations." International Society for the Study of Vascular Anomalies (ISSVA) World Congress 2022, Vancouver, Canada (oral)	2022	0.5
"The output and achievements of the Outcome measures of Vascular Malformations (OVAMA) project." The CHORD COUSIN Collaboration (C3) meeting (Virtual), New York, USA (oral)	2022	0.5
"Appearance-related concerns and their impact on health-related quality of life in patients with peripheral vascular malformations." British Association of Plastic, Reconstructive and Aesthetic Surgeons (BAPRAS) x Nederlandse Vereniging voor Plastische Chirurgie (NVPC) Combined Congress, Amsterdam, The Netherlands	2023	0.5

(Inter)national conferences

23nd International Workshop of the International Society for the Study of Vascular Anomalies (ISSVA), 2020 (virtual) Vancouver, Canada	2020	0.5
CS-COUSIN COMFA Joint Conference (virtual) London, United Kingdom	2021	0.5
Debates & Updates Meeting of the International Society for the Study of Vascular Anomalies (ISSVA), 2021 (virtual), Boston, USA	2021	0.5
Nederlandse Vereniging voor Plastische Chirurgie (NVPC) dagen najaarsvergadering, Amsterdam, The Netherlands	2021	0.25
Landelijk expertisenetwerk congres Vasculaire Malformaties, Breukelen, The Netherlands	2022	0.25
International Society for the Study of Vascular Anomalies (ISSVA) World Congress 2022, Vancouver, Canada	2022	0.5
International Society for the Study of Vascular Anomalies (ISSVA) World Congress 2022, Pre-congress day, Vancouver, Canada	2022	0.25
British Association of Plastic, Reconstructive and Aesthetic Surgeons (BAPRAS) x Nederlandse Vereniging voor Plastische Chirurgie (NVPC) Combined Congress, Amsterdam, The Netherlands	2023	0.25

Teaching	Year	Workload (ECTS)
Lecturing		
Organiser and coordinator of the Plastic, Reconstructive, and Hand Surgery course for the bachelor of Medicine of the University of Amsterdam	2021	9
Giving a lecture on Vascular Malformations for the Plastic, Reconstructive, and Hand Surgery course for the bachelor of Medicine of the University of Amsterdam	2021	0.25
Giving a workshop about Shared Decision Making for the Plastic, Reconstructive, and Hand Surgery course for the bachelor of Medicine of the University of Amsterdam	2021	0.25
Organiser and coordinator of the Plastic, Reconstructive, and Hand Surgery course for the bachelor of Medicine of the University of Amsterdam	2022	9
Tutoring		
Eline Tan, research student	2020-2021	2.0
Sagheer Javaid, research student	2021	0.5
Anne-Ruth Eits, research student	2022	2.0
Thomas Douwes, research student	2022-2023	1.0
Florine Binnendijk, research student	2023	1.0
Other		
Outcome measures for vascular malformations Steering group	2020-2023	1.0
Development of a database of patients with vascular malformations in the Amsterdam UMC	2020-2023	1.0
Article on liquid biopsy for the molecular analysis of vascular malformations for HEVAS Magazine.	2021	0.5
Interview on appearance-related concerns in patients with vascular malformations for HEVAS Magazine.	2022	0.5

Parameters of esteem	Year
Grants	
HEVAS-SKTN Grant	2021
Amsterdams Universiteitsfonds	2022