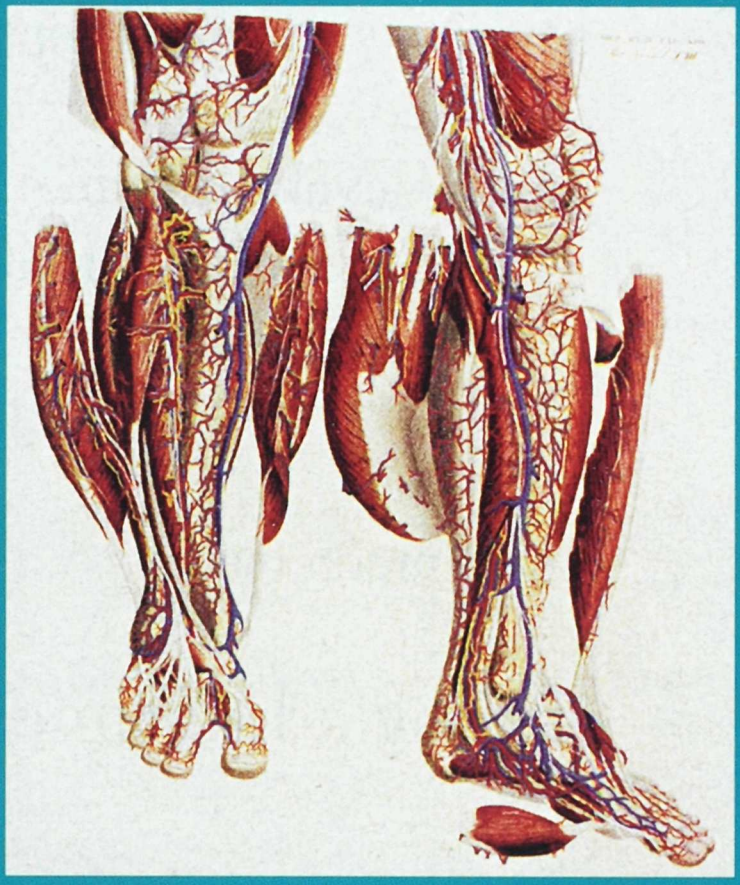
Progress toward understanding vascular malformations



Corstiaan C. Breugem

Assertions

Belonging to the PhD thesis of Corstiaan C. Breugem

Current information about vascular malformations indicates that almost áll peripheral vascular malformations are venous malformations, although it is even possible that all of these vascular malformations are primarily neural malformations.

The currently universally accepted biological classification of vascular anomalies introduced in 1982, is an oversimplification of the complexity of these anomalies, and increased knowledge about the pathogenesis suggests that this classification system needs revision.

The fruits of genetic linkage analysis will extend beyond strawberries and port-wine.

One day, someone with a venous malformation located on the lower extremity, might win the Amsterdam marathon.

When we look at how often vascular anomalies occur and when we look at the complicated construction, it is not surprising that vascular anomalies occur, but it is surprising that they don't occur more often. (adapted from H. Reid)

With vascular malformations it happens more commonly that 'too little' is operated than 'too much'.

If you don't understand it, make it more complex.

All our knowledge has its origin in its perceptions. (Leonardo da Vinci)

Balance is the challenge.

It's your attitude at the beginning of a task more than anything else that will determine your success or failure.

To put the 'finishing touch' to your PhD thesis with a newborn baby does not conjoin with the optimal preparation for a triathlon.

Progress toward understanding vascular malformations

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Progress toward understanding vascular malformations

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Faculteit der Geneeskunde

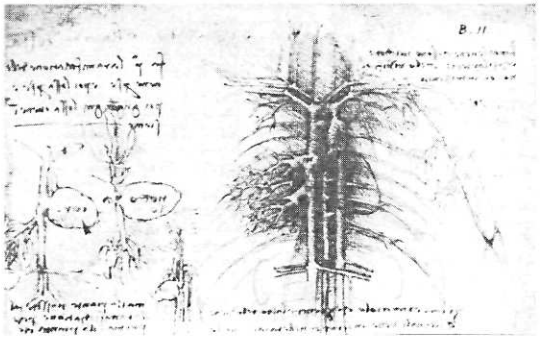
For my dearest Darinka,
Sophia,
and my loving parents.

It is not the critic who counts;
not the man who points out how the strong man stumbled or where the doer of the deeds could have done better.
The credit belongs to the man who is actually in the arena,
whose face is marred by the dirt and sweat and blood;
who strives valiantly; who errs again and again;
because there is no effort without error and shortcoming;
who does actually try to do the deed who knows the great enthusiasm;
the great devotion and spends himself in a worthy cause, who at the worst if he fails, at least fails while daring greatly.
Far better it is to dare mighty things,
to win glorious triumphs, even though checkered by failure,
than to rank with those poor spirits who neither enjoy nor suffer much,
because they live in the great twilight that knows neither victory or defeat.

Theodore Roosevelt, April 1899

Contents

Introduction and outline of this thesis



Leonardo da Vinci (ca. 1508)

Introduction

More than two decades ago Mulliken and Glowacki proposed a biological classification system to create some clarity in classifying vascular anomalies (figure 1). According to this classification, vascular lesions are differentiated into vascular tumors ór vascular malformations (1). This classification system is currently the most accepted framework to classify vascular anomalies and was also accepted by the International Society for the Study of Vascular Anomalies (ISSVA) (2). Due to syndrome names and different nosology, the terminology describing vascular anomalies has been a source of confusion in the medical literature. Recently Hand et al has proven that this nosologic confusion is also widespread in standard genetic textbooks, and it is expected that many physicians still describe vascular malformations as being "hemangiomas, cavernous hemangiomas or venous angiomas " (3).

One of every three newborns has a vascular birthmark, but most of these fade away or remain small (4). A small proportion of babies are born with vascular anomalies that require referral to a center specializing in these anomalies. Vascular malformations should be differentiated from the much more common hemangiomas. Vascular tumors are divided into hemangiomas (by far the largest group), tufted angiomas, kaposiform hemangiomas and other rare tumors (5). Hemangiomas are the most common tumors during infancy. By the age of one year, approximately 8-10% of Caucasian children have at least one hemangioma (5-7). Most hemangiomas arise in the neonatal period, but subcutaneous and visceral hemangiomas may not manifest itself until the second or third months. Hemangiomas are further characterized by a proliferative phase, a stable phase and an involutary phase. By the age of 7 years 70% of hemangiomas are fully involuted, while at the age of nine years 90% have involuted. Histologically hemangiomas are composed of tightly packed sinusoidal channels, lined by rapidly dividing endothelial cells (5). Hemangiomas very seldom require treatment, but bleeding hemangiomas and lesions influencing function (eg. obstructing vision or breathing) may require intervention.

Vascular malformations are congenital lesions and are characterized by their structural defects. The endothelial cells have a normal turnover throughout their natural history (5). They are divided anatomically into capillary, venous, arterial or lymphatic malformations; or combinations. According to the flow in the lesion they can further be divided into high- or low-flow lesions. Any lesion with an arterial component is classified as a high-flow lesion. Trauma, hormonal change or sepsis may exacerbate progression of these malformations. Data on the prevalence and incidence is rare since many inconspicuous small capillary malformations are never presented and reported.

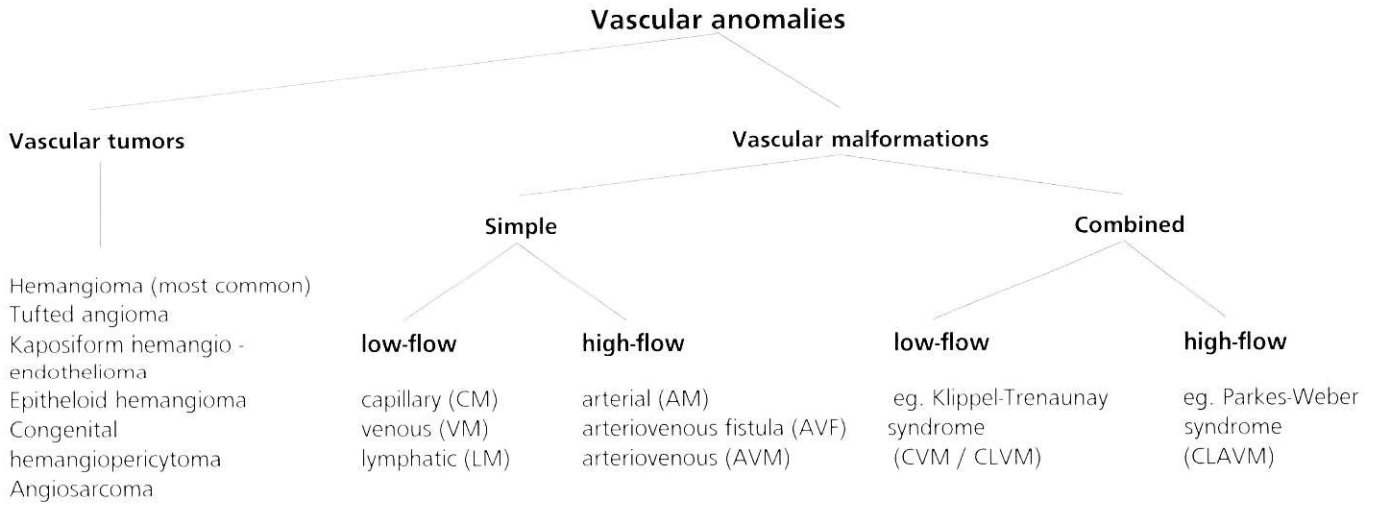


Figure 1: Classification of vascular anomalies (1)

In general the incidence is estimated to be between 0.3 and 2.1% with capillary malformations representing the majority of lesions (7-9). The distribution of vascular malformations appears to be variable in different parts of the world (7,10-12). With the biological classification suggested by Mulliken and Glowacki is possible to make a clinical diagnosis in 90% of cases; in the other 10% some form of radiological intervention is needed for the diagnosis (5,8). Often patience is needed and with time (natural history) will it be possible to make a diagnosis in those patients where it was not possible to make a diagnosis at the first visit.

It is once that the diagnosis is made that the real problem arises. What is the best treatment for these lesions? In this day and age where our 'outside' is so important patients/parents present much earlier for advice and treatment. Internet is available to many people in the world, and many patients/parents are willing to spend hours looking for advice. It is possible for parents presenting with a young child inquiring about treatment options, to leave your outpatient clinic disappointed because treatment options are few, often unsatisfactory and with an uncertain outcome. When these patients inquire about the prognosis, we often have to inform them about the unpredictability of these lesions. Some patients / parents travel from clinic to clinic in the hope that some remedy or treatment cure will be offered. Often the patients/parents are explained that many muscles are involved by the vascular malformation (and sometimes bone), and that complete cure from the lesions will only be acquired with devastating mutilation of the affected part. Only then will these patients accept that no complete remedy is available.

Vascular malformations can present with a wide spectrum of involvement and subsequent symptoms. Capillary malformations are often satisfactorily treated with pulsed-dye laser treatment, but treatment results of other deeper located vascular malformations are often unsatisfactory, with intervention often resulting in recurrences (2,13-17). After surgical intervention, lesions often reoccurred in a more aggressive way than before surgical therapy. For that reason, treatment options have been very conservative, with patients often instructed to "live with it". Momentarily two words dominate the rules of therapeutic management of all types of vascular malformations: a multidisciplinary approach and modesty (18).
Only by defining the etiology of the pathologic process will it be possible to understand the progression of these malformations, subsequently diagnose these lesions correctly, and to ultimately treat these patients effectively. Before the pathogenesis of the abnormal vascular system is understood, we must at least understand the embryology involved in the development of the normal vascular system. Medical students and (hopefully) physicians will often open an anatomy book to study the vascular system, relying on the consistency of the vascular

determinants. it is only once we realize that many blood vessels (with their proper diameter) are formed even before the heart of the embryo starts beating, that we really appreciate the real complexity of the embryology of the vascular system (19). Even when we look at embryology textbooks we start realizing how much is speculation (19). With molecular and genetic studies we are now able to study some of the mechanisms that control the development of the vascular system. Our quest to treat cancer has stimulated our will to understand the vascular system (angiogenesis), and since the 1970's our knowledge about that growth of capillary blood vessels has exponentially increased. Long term in vitro culture of capillary endothelial cells and the discovery that certain proteins are mitogenic for these endothelial cells has greatly increased our knowledge of the vascular system (20).

Aims and outline of this thesis:

Although the classification system proposed by Mulliken and Glowacki has provided much clarity at a time when there was a lot of confusion in the medical literature, this classification may oversimplify the ontogeny of these vascular anomalies. The medial telangiectatic nevus (nevus simplex or stork bites) is a vascular malformation that usually clinically disappears (21). Natural involution is thus involved in these lesions. Kaposiform hemangio-endotheliomas clinically have a phase characterized by proliferation, while the histological characteristics are those of a lymphatic malformation. The classification system further states that hemangiomas are neoplasmas and in such thus distinct from true structural deficits. Large face hemangiomas have been associated with the so-called PHACE syndrome (22). Here the hemangiomas are associated with **p**osterior fossa arterial anomalies of the cerebrovasculature, (**h**emangiomas), **a**rterial abnormalities, **c**oarctation of the aorta, and **e**ye abnormalities. There are other syndromes characterized by dysmorphology of hemangiomas, including those related to midline sternal cleft defects, usually associated with midline abdominal raphe (23,24). It is also known that sometimes there are patients in which clinical or histological characteristics of both vascular malformations and hemangiomas are seen (25). At present the biological classification system suggested by Mulliken and Glowacki is the best we have, and should not be discarded before a better classification system is universally accepted.

The first step to understand vascular malformations is to better understand the genesis of the normal vascular system. Next we should describe the molecular pathology involved in vascular malformations; this will hopefully lead us to categorize vascular malformations more accurately,

and subsequently treat them effectively. For that reason **Chapter 2** is devoted to what our current state of affair is with regard to our understanding of vascular malformations. This comprehensive review not only describes our present understanding of the normal vascular system, but also describes the best known molecular mechanisms for the different vascular malformations.

The most common vascular malformations are capillary malformations (port-wine stains). These malformations are currently also the best treatable with pulsed-dye laser (PDL). The unpredictability of this treatment medium has made us search for the underlying pathology. Only by defining the pathologic processes involved will we be able to understand the reasons for the difference in response and in the long term introduce better treatment options, or even better, be able to prevent these lesions from developing. In the past certain genes have been identified to be causative for inherited vascular malformations eg. mucocutaneous venous malformations (TIE-2 gene) (26). Although a familial tendency has been described in the medical literature, a locus for capillary malformations had never been mapped (27). **Chapter 3** is devoted to mapping a locus for an autosomal dominant disorder in a three-generation family that manifested itself with multiple cutaneous capillary malformations. It is likely that the genes involved in the familial cases of capillary malformations are also involved in the sporadic cases. In **Chapter 4** we have summarized the present understanding of the pathology involved in capillary malformations. Although we do not want to describe the influence of PDL in capillary malformation treatment, it is inevitable to include some information about PDL in a discussion about capillary malformations. In this chapter we want to compare the histology of the normal skin to pathology involved in capillary malformations, and provide an overview of our current molecular understanding of capillary malformations.

Although a molecular description will provide us with a nice diagnosis, the most important question is whether treatment is possible. To answer that question an accurate description of the tissues involved is necessary. As mentioned earlier, 90% of vascular anomalies are diagnosed clinically, but sometimes radiology can be necessary to diagnose these anomalies. Although ultrasonography is cheap and easily available, it is notoriously operator dependent, but is currently the simplest method to make a definite diagnosis (28). In **Chapter 5** we provide an overview as to why MRI is currently the best modality to investigate vascular anomalies, to see the extent of involvement or when intervention is anticipated. Although MRI is currently the best modality that we have to study vascular malformations, it also has some limitations which will also be discussed. We further suggest a diagnostic flow-chart developed on the basis of MRI features, designed to help determine the composition of a vascular birthmark.

Historically surgical treatment options for vascular malformations have been poor (2,13-17). In an attempt to investigate why vascular malformations may respond so badly to surgical intervention, the MRI characteristics of a group of patients who presented at our vascular anomalies clinic is described in **Chapter 6**. Although previous studies have described the characteristic differences between high- and low-flow vascular malformations, there is little detailed information available delineating the vascular malformation (29). This study is performed to provide more detailed information regarding the extent of local tissue involvement, and subsequently describe associated features of the tissue adjacent to the vascular malformation in the lower extremity.

In a retrospective study on 356 hemangiomas and 224 vascular malformations, Boyd et al found that hemangiomas are not often associated with osseous involvement, while vascular malformations in general are often associated with osseous changes (30). This study includes patients with bone hypertrophy/hypotrophy without visible bone involvement (31% of patients had bone changes). There is also no differentiation between patients having upper- or lower extremity involvement, since only the "extremities" are differentiated from the "head and neck" group. Since that article there have been some reports on osseous involvement associated with vascular malformations, but little attention is given to the clinical symptoms and signs of patients with vascular malformations and associated bone involvement (31,32). In an attempt to assess the osseous involvement in the lower extremity, we conducted a study on all the patients that presented at our vascular anomalies clinic over the last ten years. **Chapter 7** focuses on the clinical and radiological characteristics of the patients with vascular malformations of the lower extremity with osseous involvement.

Once a diagnosis is made and radiological visualization has excluded any therapeutic intervention, unfortunately many patients are sent home without a curative solution. These patients are often told to "live-with-it" and if necessary elastic stockings and analgesia are prescribed. In the medical literature capillary malformations have received attention on what the psychological impact of the malformation may be (33,34). Currently most quality of life studies are focused on capillary malformations presumable because they are the most common vascular malformation; they occur often in the face and are often treatable with pulsed-dye laser. Very little is known of the psychological impact vascular malformations located on other parts of the human body have on patients. In an attempt to clarify some of these questions, we conducted a study in **Chapter 8** to investigate what the influence of vascular malformations located on the lower extremity on everyday living would be.

In **Chapter 9** we provided the summary and conclusions of this thesis and provided some ideas and directions for further research.

Summary of main questions of thesis

- What is our present understanding of how the normal peripheral vasculature develops, and how is this knowledge applicable to help us better understand vascular malformations?
- Is it possible to find a locus for capillary malformations?
- What is our current understanding of the pathology involved in capillary malformations?
- How can MRI be utilized to describe the composition of vascular birthmarks?
- Can MRI characteristics of vascular malformations of the lower extremity help us to better understand these lesions?
- What are the clinical characteristics of patients with vascular malformations of the lower extremity with associated osseous involvement?
- Do patients with vascular malformations of the lower extremity have a worse quality of life compared to patients without vascular malformations?

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Progress toward understanding vascular malformations

Adapted from Gray's Anatomy

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Plastic and Reconstructive Surgery 107(6):1509, 2001

Introduction

In the past the study of vascular malformations has been hampered in several ways. Especially the use of eponyms and other confusing nomenclatures and lengthy descriptions by the different authors has made proper classification and diagnosis of vascular birthmarks difficult (1-6). Furthermore, at least a basic understanding of normal embryogenesis of the vascular system was in fact mandatory for adequate definition of vascular malformations and subsequent correct diagnosis. The recent progress in this respect through a more sophisticated nomenclature and molecular studies has changed this. The universally accepted biological classification of vascular anomalies by Mulliken and Glowacki introduced in 1982, was refined in 1996 (1,2). With this biological classification it is possible to diagnose vascular anomalies in two major groups: vascular tumors (majority are hemangiomas) and vascular malformations.

In addition to the amended classification there has been an extensive improvement in our knowledge of the molecular background of vascular embryogenesis (7-12). Surgeons involved in treating patients with vascular malformations should be aware of the advances in developmetal biology, which allows better understanding of mechanisms of vascular malformations. They should be able to inform patients about these advances and stimulate possible participation in research. The purpose of this review is to (1) provide a short review of the literature on some current concepts in molecular genetics as it applies to vascular anomalies, giving special attention to the present knowledge of the complex embryology of the vascular system. We further (2) summarize certain genes responsible for the vascular malformation, and (3) give surgeons an opportunity to appreciate the recent advances in understanding the mechanism of the development of vascular malformations. The developmental background of hemangiomas will not be discussed here.

Molecular genetics in general

The entire human genome contains 3 billion nucleotides (base pairs of DNA), that together encode for 100,000 different genes (13). These genes are packed together in 23 pairs of chromosomes, in such way that every human somatic cell nucleus contains the entire human genome. Every individual gene can produce one (sometimes more) transcription products, i.e. proteins that exhibit structural, regulatory or enzymatic activity. A single gene may cause different disorders, a single disorder may be caused by mutations in different genes, and a single gene

may have different functions with time, for instance acting prenatally as a developmental gene and postnatally as a tumour suppressor gene (13-16).

Developmental processes are regulated by networks of signal transduction pathways that relay and integrate information from outside the cell, through a receptor in the cell membrane, to the nucleus to regulate the expression of target genes. The extracellular signals are usually "ligands", such as diffusable growth factors or extracellular proteins, which bind to membrane receptors (Figure 1). There is also a propagation of information from the nucleus outward to alter structures in the cytoplasm, to modify the cell's responsiveness to signals from the outside and to affect the activities of neighbouring or distant cells (13,15,17). The overal effect of signaling pathways and their regulation of gene expression is to control cell proliferation, migration, differentiation, and programmed cell death (apoptosis). The coordinated control of groups of cells is fundamental in the formation of complex structures (13-19).

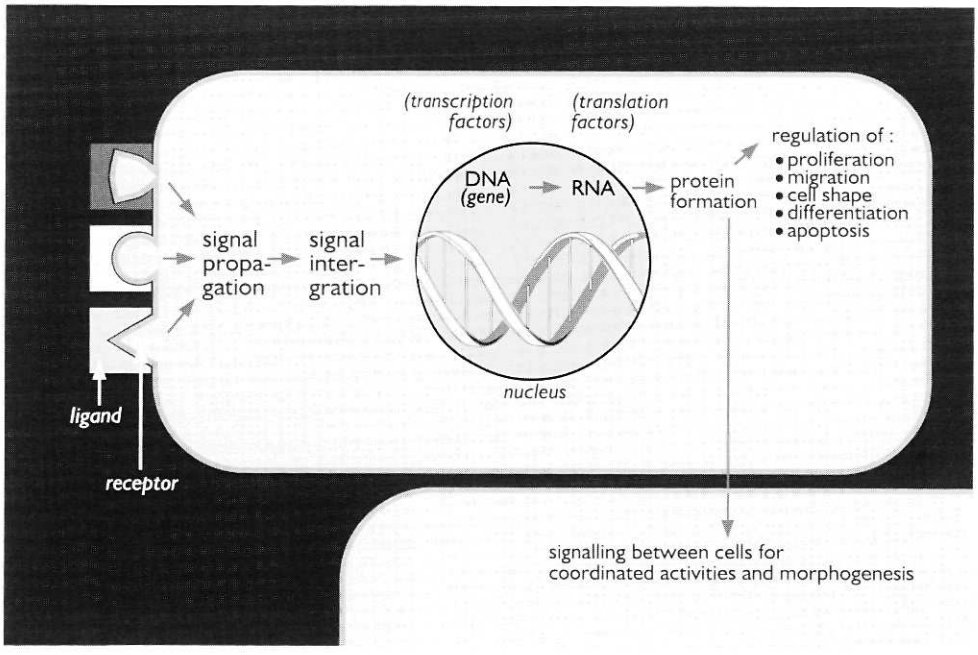

Figure 1:
General molecular aspects. Developmental processes are regulated by networks of signal transduction pathways that relay and integrate information from outside the cell, through a receptor membrane, to the nucleus to regulate the expression of target genes.

Vascular development: pathways and genes

The increased knowledge of vascular development has especially become possible through long-term in vitro culture of endothelial cells, the study of proteins that are mitogenic for endothelial cells, and manipulations of the mouse genetic make-up, all leading to a better understanding of growth of capillary and endothelial cells (7-12,20-54). The major data regarding vascular development, and the molecular mechanisms involved will be reviewed here; for more detailed reviews the reader is referred to specialized literature (7-12,22,30).

The vascular system is build by two processes: vasculogenesis and angiogenesis. In vasculogenesis a primitive vascular plexus is established from endothelial precursors. The vascular plexus is connected to the developing heart tube, and after onset of the heart beat, the vascular channels are perfused with blood and the primary circulation is established by the end of the third week of development (7,9,21). In angiogenesis new vessels arise from these preexisting vessels by migration and proliferation of endothelial cells.

Vasculogenesis

At the end of the second week of embryonic development the primitive vascular plexus is formed (21). The mesodermal precursors of endothelial and blood cells differentiate into solid clumps of epitheloid cells and isolated masses called " blood islands" (20,21). The inner cells differentiate into haematopoietic precursors, and the outer cells of these clusters flatten to form primitive endothelial cells. Lumen formation of the primitive capillaries may result from endothelial vacuolization (intracellular lumen formation), or from continuation of a pre-existing lumen through joining of distal endothelial cells (intercellular lumen formation) (21). Peri-endothelial support cells are then recruited to encase these endothelial cells. In capillary vessels these cells are pericytes, in larger vessels it consists of smooth muscle cells and in the heart they are myocardial cells (22-25)(Figure 2).

Angiogenesis

Once the primary plexus is formed, more endothelial cells are generated, which can form new vessels. Angiogenesis occurs through two different mechanisms: sprouting and non-sprouting. Small blood vessel can be formed by sprouting (budding) from pre-existing, larger vessels (7,21). In intussusception (non-sprouting angiogenesis) the pre-exiting vessels are split by transcapillary

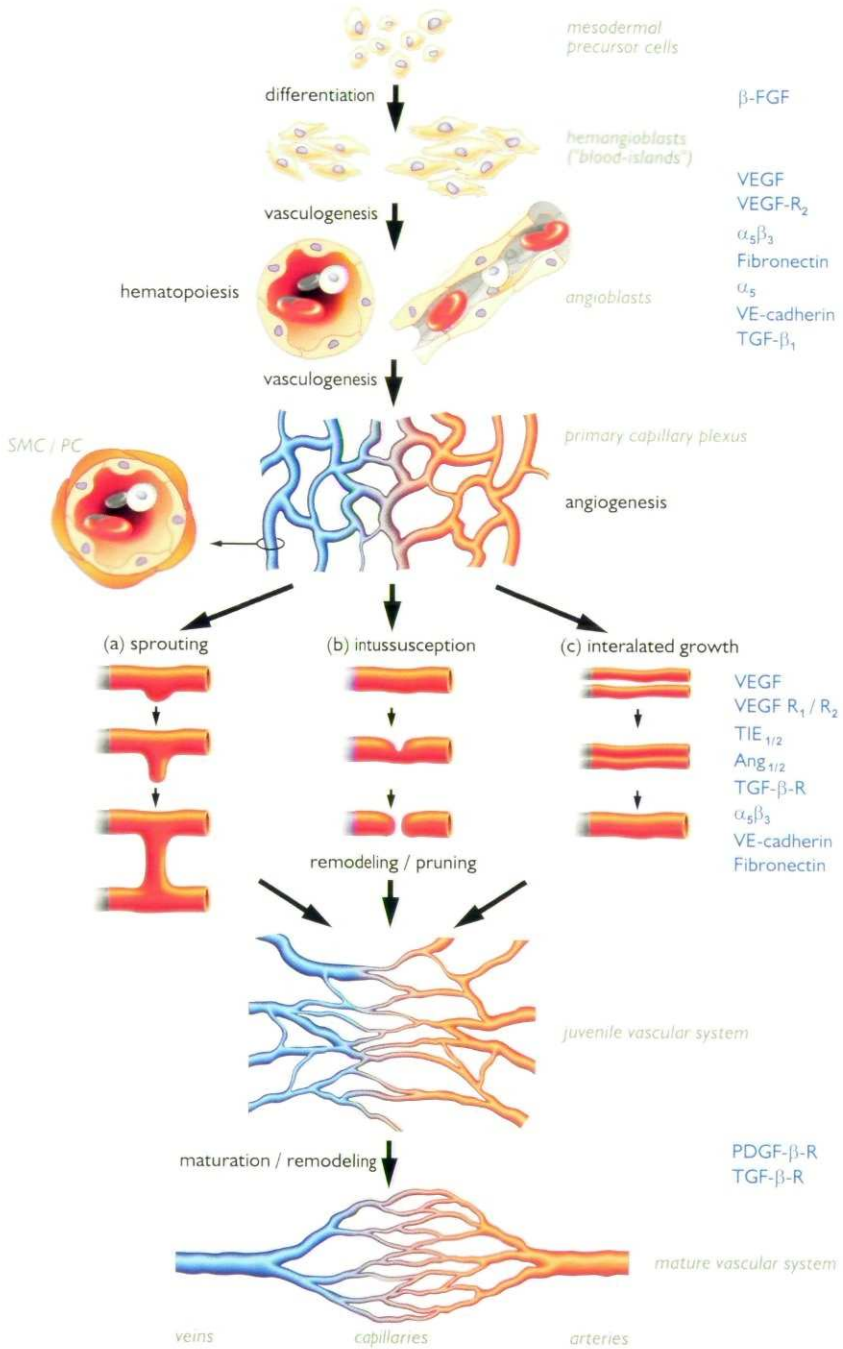

Figure 2:
Summary of our present understanding of some important phases involved in vascular development.

pillars or invagination by surrounding pericytes and extracellular matrix (26,27). A possible third mechanism involves the intercalated growth of blood vessels, allowing merging of pre-existing capillaries to increase diameter and length (26). The surrounding basement membrane of the endothelial cells is locally degraded, with the endothelial cells located under this area changing shape and invading the surrounding stroma. Once the endothelial cells cease to proliferate, they tightly adhere to each other to form the lumen of a new capillary vessel.

Remodeling and maturation

The process of remodelling is common for both angiogenesis and vasculogenesis (9-11). During this poorly understood event new vessels are formed while others regress, and vessel lumen diameter and thickness change to suit the local tissue needs (28). Wang et al has proven that the pathological and functional difference between arteries and veins is not only determined by physiological differences such as direction of flow, blood pressure and oxygenation (29). During the earliest stages of angiogenesis arterial and venous endothelial cells are molecularly distinct and are thus in part also determined genetically. The transmembrane ligand Ephrin-B2 is an example of a marker located on arterial endothelial cells from early vasculogenesis This ligand is not located on venous endothelial cells. In the same way the receptor for Ephrin-B2 (Eph-B4) marks veins and not arteries, and Ephrin-B2 knockout mice have a defective remodeling proces of mainly the veins into properly formed branched structures. However, there is also some disruption of remodeling of arteries, suggesting reciprocal interactions between arterial and venous endothelial cells.

Lymphatic system

In contrast to the vast literature on blood vessel development, very little attention is given to the development of the lymphatic vasculature (11,30). The lymphatic system begins to develop at the end of the fifth week (21). Lymphatic vessels develop as endothelial outgrowths from the venous system. First six lymphatic sacs are formed (21). These lymph sacs sprout from large central veins. The primary lymph sacs form new sprouts which then grow out to the periphery of the embryo, whereas the main lymphatic ducts form by union of the duct primordia (30).
The lymphatic capillaries consist of single layer endothelial cells, without surrounding pericytes, and without valves. These lymphatic capillaries merge into collecting lymphatic vessels. These vessels consist of a thin endothelial lining, surrounding by an incomplete layer of smooth muscle

cells, with valves. Finally, lymph is delivered by either the thoracic or right lymphatic duct to the venous system. Their wall contains tunica intima, media and adventitia, with the media containing bundles of smooth muscle cells.

Genes involved in vascular development

Endothelial cell fate is determined by the combined effects of a large number of positive and negative signals simultaneously transduced by numerous receptors. Many molecules have been defined that regulate vessel growth in vivo and in vitro. Table II summarizes the better-studied molecules involved in early vascular development. In the following section only the best known factors will be reviewed.

The formation and remodeling of blood vessels is in general controled by paracrine signals: many are protein ligands that bind and modulate transmembrane receptor tyrosine kinases (RTK's) (7,9,22,31). Although negative regulators such as angiostatin, endostatin and thrombospondin are known, positive regulators are much better documented (32-34). Members of the VEGF acting as a positive regulator, have the most compelling evidence (27,30,35). Other well-described positive regulators are fibronectin, α5-integrin, VE cadherin, TGF-ß1 (26,30,31).

Basic fibroblast growth factor (b-FGF)

In vitro findings suggest that basic fibroblast growth factor (bFGF) may participate in angioblast differentiation in the formation of "blood islands" (8,31).

Vascular endothelial growth factor (VEGF)

VEGF acts in a paracrine manner: it is produced by endoderm, but the receptors are mesodermally derived angioblasts. Two VEGF receptors, VEGF-R1 (flt-1) and VEGF-R2 (flk-1), are both expressed by angioblasts and endothelial cells (36,37). If embryo's (both human and mouse), lack VEGF-R2 they die at embryonic day 8.5 (E8.5), due to defects in the development of both endothelial and hematopoietic cells (38). There is nearly a complete lack of vascular structures with an absent dorsal aorta and many smaller vessels. Vegf-r1 knockout mice also die at E8.5, but in contrast have normal endothelial and hematopietic progenitors, with endothelial cells undergoing migration and proliferation, but not assembling into tubes or functional blood vessels (39). Mice made deficient for vegf die at E 11-12 with a delayed differentiation of endothelial cells and a defective sprouting process of the capillary vessels (40,41). Further studies implicate that vegf exerts a dose-dependent effect early in the formation of the vasculature inducing EC proliferation,

promoting cell migration and inhibiting apoptosis (8,40). Recently, Gerber et al indicated that vegf plays an important part in longitudinal bone growth by stimulating angiogenesis into the epiphyseal growth plate (42). They indicated that vegf-mediated capillary invasion is an essential signal that regulates growth plate morphogenesis and triggers cartilage remodeling. Thus, vegf is also an essential coordinator of chondrocyte programmed cell death, chondroclast function, ECM remodeling, angiogenesis and bone formation is in the growth plate (42).

Due to the lack of specific markers, studies of the development of the lymphatic vasculature have been hampered. The vegf-receptor-3 (flt-4) has been identified as a possible lymphatic marker (43). Vegf-c also stimulates migration and proliferation of endothelial cells, but with a lower potency than vegf-a (26). Mice overexpressing vegf-c in the skin specifically develop hyperplasia of lymphatic vessels (44). Since vegf-c binds to vegf-r2 and vegf-r3, the precise mechanisms of the effect on lymphangiogenesis and not on endothelial cell function remains uncertain. Vegf-r3 expression is found in the developing embryo in especially the perimetanephric, axillary and jugular areas. These venous strutures are flt-4 positive from around day 11.5 of embryonic development. Lymphatic endothelium is adult mice is expressed in these areas (43). Deficiency of vegf-c as well as flt-4 results in early embryonic lethality of which the precise cause still needs to be determined (31).

Tie receptor family and angiopoietin

Mice made deficient for another vascular endothelial cell selective RTK's, tie-1 and tie-2, also undergo abnormal angiogenesis and remodeling of the vascular system (45,46). Each receptor has a distinct role. Sato et al have shown that embryos deficient in tie-1 had an abnormal endothelial cell differentiation and failed to develop structural integrity of vascular endothelial cells, thus resulting in edema and subsequent extensive, localized haemorrhage. The homozygous mutant embryos died at day E 14.5 (45). Tie-2 function is involved in angiogenic processess of endothelial cells (sprouting and branching and/or remodeling). Endothelial cells were present in normal numbers in knockout mice, but the vessels were immature, lacking organization into small and large vessels. The vessels also lacked an intimate encapsulation by peri-endothelial support cells. All tie-2 homozygous mutant embryos died at E10.5 (45). The ligand binding to tie-1 is still unknown, but tie-2 receptor is modulated by Angiopoietin-1 (ang-1) ánd ang-2, both binding with similar affinity (47). Ang-1 promotes tyrosine phosphorylation of tie-2, playing a role in regulating the assembly of non-endothelial components of the vascular wall. Suri et al observed that mice deficient for ang-1 produce vessels with a deficiency of smooth muscle cells and pericyte precursors (48). The clinical picture of ang-1 deficient-mice is similar to tie-2 deficient mice; i.e. the vascular plexus is not fully remodeled into small and large caliber vessels. Ang-2

presents a negative signal to tie-2; inhibiting ang-1 induced kinase activation of tie-2 receptor. This is very remarkable given the high homology between ang-1 and ang-2. The negative ang-2 signal causes vessel structures to become loosened, reducing endothelial cells contacts with matrix and dissociating peri-endothelial support (22). This loosening appears to render the endothelial cells more accessible and responsive toward the angiogenic inducers such as vegf. Maisonpierre et al suggest that although ang-1 may provide a maturation or stabilization signal through tie-2 that can be blocked by ang-2, such inhibition may result in continued remodeling or even the initiation of vascular sprouting in the context of simultaneous vegf exposure but may result in vessel regression in the absence of vegf (47).

Platelet derived growth factor (PDGF)

Angiopoietin-1 released from mesenchymal cells binds to the tie-2 receptor on endothelial cells and then releases or activates a releasing signal for mesenchymal cells (10). Most data indicates this recruiting signal to be PDGF-BB if pericytes are involved (49). This recruitment may also involve PDGF-AA or heparin binding epidermal growth factor (HB-EGF) when smooth muscle is involved (9). Pdgf has been proposed to be involved during two aspects of vessel formation: the autocrine stimulation of endothelial cells and the paracrine recruitment of mural cell precursors to the vessel wall (8,45). Both the pdgf-b and pdgf-ß receptor null mice reveal similar phenotypes (50). The defective development of vascular smooth muscle cell lineage was particularly striking in the kidney (51). The absence of pericytes was associated with capillary aneursym's. PDGF-BB is not critical for endothelial tube formation, but appears to be important for structural integrity of endothelial tubes through an effect on recruitment of pericytes (30). It is suggested that once mesenchymal cells are recruited to the forming vessel, PDGF expression must be suppressed so that the cells can differentiate into mural cells (8,45).

Transforming growth factor-beta (TGF-β)

When the mesenchyme cell makes contact with the developing vessel, transforming growth factor-beta (TGF-β1) is activated (52-54). This causes mesenchyme cells to differentiate into pericytes and smooth muscle cells, inhibition of endothelial and smooth muscle cell proliferation and migration, as well as alterations in the accumulation of the extracellular matrix (figure 3). It also induces apoptotic endothelial cell death. Analysis of deficient tgf-ß mice revealed no morphological abnormalities at E 8.5, but at E9.5 there were obvious abnormalities ranging from delayed vasculogenesis to total absence of vessels, and 50% of homozygous deficient embryos died around day 9.5 (54).

Coagulation factors and integrin

Although there are many more proteins and molecules affecting vascular development, there have been some interesting observations about the nature of the involvement of coagulation factors. The phenotype of tissue factor-null embryos, a protein involved in initiation of coagulation, exhibits many similarites to mice made deficient of tie-2 receptor/angiopoietin-1: all lacked vascular remodeling (55). This procoagulant receptor is somehow involved in recruiting perivascular cells; whereafter tgf-b expression is triggered that inhibits endothelial cell proliferation in vitro. During vascular development, endothelial cells are required to migrate (7,10). The endothelial cells need to proliferate to change size and to migrate towards the angiogenic stimulus (26). Cell migration requires proteolysis of the extracellular matrix. Proteinases are not required for mural cell migration, but they play a significant role during smooth muscle cell migration (56). During the migration process, cell adhesion molecules such as integrins, vascular endothelial cadherin and fibronectin are involved (24,31). For instance the function of integrin $\alpha v\beta 3$ appears to be critical for formation and/or maintenance of newly fomed vessels (57,58). Vascular endothelial cadherin is expressed early during vasculogenesis at inter-endothelial junctions, probably playing a role during lumen formation (35).

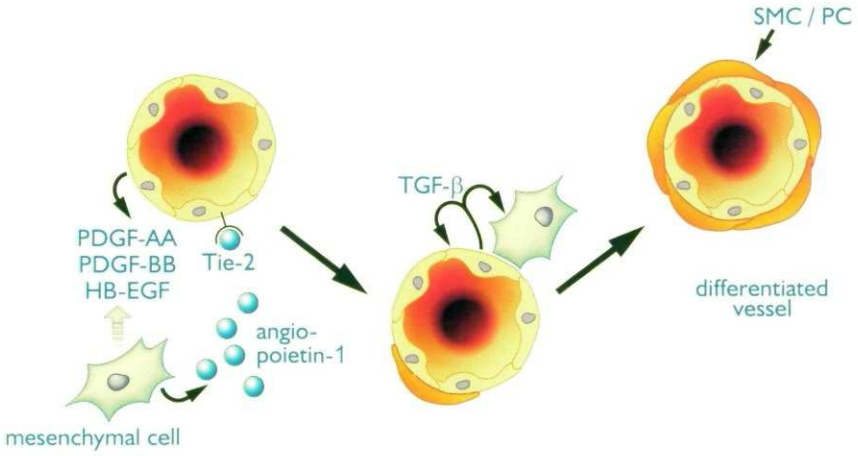

Figure 3:
Model of peri-endothelial support cells recruited to encave the endothelial cells.

Other factors involved in vascular development

During maturation and remodeling of blood vessels both intra- and extraluminal factors are involved (7,11). Although the circulation does not initially influence the development, forces generated by the circulation may later affect the vascular system. Shear stress can induce

Protein/molecule /receptor	Function	Deficient mice/other animals	Reference
FGF	Participation in angioblast differentiation from mesoderm (formation of " blood islands")	No overt abnormalities	8,31
VEGF-R2 (Flk-1)	Endothelial cell (EC) birth, migration and proliferation	Defect in endothelial and haemtopoietic cells (near complete lack of vasculature) ‣ death at E 8.5	38
VEGF-R1 (Flt-1)	Tube formation and endothelial cell-cell interactions	No functional blood vessels or tubes (but with normal EC) ‣ death at E 8.5-9.5	39
VEGF	Dose-dependent effect during vasculogenesis, largely restricted effect on receptors, allowing EC to proliferate, migrate, and assemble in tubes	Deficient EC differentiation and defective sprouting ‣ death at E 11-12.	40,41
av ß3	Cell adhesion molecule, involved in attachment of cells and substrate, may also affect proteins involved in matrix degradation	Abnormal lumen patency of dorsal aortae, and aberrant pattern of adjacent vascular plexus	57,58
TIE-1	EC differentiation and vessel integrity	Abnormal EC differentiation ‣ decreased vessel integrity ‣ hemorrhage → death E 14.5	45
TIE-2	Assembly of non-endothelial vessel wall components	Abnormal organization of vessels (normal EC), with abnormal encapsulation of peri-endothelial cells ‣ death E 10.5	45, 113
Ang-1	(activating ligand) Recruitment of SMC and pericyte precursors	Abnormal recruitment of SMC and pericyte precursors, and abnormal organization in large and small vessels ‣ death E 12.5	48
Ang-2	(inhibitory ligand) Contact between EC and matrix	Loosenening of matrix contact and support cell interactions	47
PDGF-BB	Paracrine recruitment of mural cell precursors to vessel wall (PDGF derived from EC)	Decreased SMC / PC recruitment and proliferation ‣ vessel dilatation ‣ hemorrhage → death	50,51
TGF-b	Stimulates mesenchymal cells to differentiate into PC and SMC; inhibits EC and SMC proliferation and migration, and stimulates matrix accumulation	50% of deficient mice die in utero; analysis at E 9.5 shows abn ranging from delayed vasculogenesis to total absence of vessels; and defects in haematopoiesis	52,53,54
TF	Recruitment of perivascular cells	Abnormal recruitment of SMC / PC leading to defective vascular remodeling → death E 8.5	55

Table A (left page):
Summary of the most important factors known to be involved in vascular development (see text for meaning of abbreviations used).

endothelial cell-cell modification, as well as cell-extracellular-matrix junctions and upregulating growth factor like PDGF-BB (7,45,59). This could then activate the PDGF-BB receptor expressed on perivascular mesenchymal cells, pericytes and smooth muscle cells, leading to their attachment to endothelium. This in turn activates TGF-β, which may induce alterations in the extracellular matrix that stabilize the phenotype of endothelial cells as well as inhibiting their proliferation. It has also been proven that non-perfused blood vessels regress (7). A classic example is the bunch of sprouts initially invading the avascular cornea, with only one artery and vein persisting. There have been numerous studies demonstrating the role of hypoxic regulation of VEGF in retinal vascularization (26,60). A local physiologic hypoxia created by the rapidly differentiating neural elements upregulates VEGF from astrocytes. Once the retinal tissue is vascularized and the oxygen demand is met, the astrocyte production of VEGF is reduced (61). Hypoxia upregulated by VEGF is caused at least in part by an increased transcription mediated by hypoxia-induced factor-1 (HIF-1) (62). The deprivation of oxygen and nutrients such as glucose may regulate gene expression in an overlapping pattern (63).
Although in vitro many factors are proven to be involved in vascular development, its functions in vivo are usually only speculative. The finding that loss of even a single allele may well result in embryonic lethality, shows that there must be a careful balance of positive and negative regulation. Most likely, a delicate interaction of factors is involved (41).

Clinical implications

The best described applications of the above mentioned factors as they apply to vascular malformations are reviewed in the next section (Table III). The possibility of hereditary influence is also taken into account.

Capillary malformations

Port-wine stains

The pathogenesis of the ectatic capillaries seen in port-wine stains (PWS) or capillary malformations is thought to be at least part of an abnormal neural regulation proces (64). It is suggested that

the defect lies in the maturation of the cutaneous sympathetic innervation causing vasodilatation. Shelley and Livingood were the first to publish data on familial multiple nevi flammei in 1949 (65). Since then there has been a few descriptions of families with multiple port-wine stains indicating a possible autosomal dominant inheritance with a variable expression (66-69). Of 280 consecutive capillary malformation patients applying for laser treatment at our unit, 55 patients (19.6%) had family members with the same anomaly (70). Some authors have observed familial occurrence of medial telangiectatic nevi, but because the medial telangiectatic nevus is such a common finding, genetic contribution to its cause is rendered difficult (67,69,71,72). Although reports are thus suggesting an autosomal dominant mode of inheritance in some cases of capillary malformations, usually these lesions are not considered to be inherited (2,6,73).

Telangiectasias

It has been proven that hereditary haemorrhagic telangiectasia (HHT) or Rendu-Osler-Weber syndrome, shows systemic vascular dysplasia (74-78). The clinical diagnosis is based on the presence of at least two of the following characteristics: recurrent epistaxis, telangiectases elsewhere than the nasal mucosa, visceral involvement and a positive family history (75-77). The term HHT stems from the recurrent haemorrhage from vascular lesions, especially in the nasal mucosa and gastrointestinal tract, and from the presence of dermal, mucosal, and visceral telangiectasia (76,78,80). Complications are derived from lesions located in especially the lung and brain (78,79). The disorder can cause severe morbidity with a 10 – 15 % mortality rate (77). Inheritance is autosomal dominant, and penetrance is age-dependant, being 97% by the age of 40 years.
Linkage studies have identified two distinct loci for HHT. McDonald et al mapped an HHT-gene to markers on chromosome 9q33-34 in two large unrelated multi-generation family's (78). This locus was named HHT1. McAllister et al identified endoglin as the HHT1 gene and established HHT as the first human disorder defined by a mutation in a member of the TGF-b receptor complex (81). Different kinds of mutations were identified, all leading to a stop codon. Endoglin is a glycoprotein expressed at high levels in membranes of human vascular endothelial cells both of capillaries, arterioles and venules (82,83). Endoglin is the most abundant protein to bind TGF-b on endothelial cells (85). In vivo TGF-b is a potent stimulant of angiogenesis and a mediator of vascular remodeling as it controls extracellular matrix production by endothelial cells, smooth muscle cells and pericytes (81). McAllister et al favours the hypothesis that HHT1 acts on cellular level as a recessive gene, requiring in addition inactivation of the normal allele as a prerequisite for formation of the capillary vascular malformation. This theory is supported by localisation of the vascular lesions to discrete regions within affected tissue, without evidence of abnormal vessel formation outside the lesions. Endothelial cells lacking endoglin probably respond

poorly to TGF-b and form abnormal vessels in respons to stimuli such as trauma (80).
Further linkage studies demonstrated that HHT is a genetically heterogeneous disorder with other families being unlinked to 9q (75,76,81,84). These families were reported to have less pulmonary arteriovenous malformations. Vincent et al mapped a second (HHT2) locus to chromosome 12q11-14 in two family's (86). A physical map over the candidate interval demonstrated activin receptor-like kinase 1 (ALK 1) as a candidate gene (87). ALK 1 is a receptor for ligands of the TGF-beta family. The variable reaction of cells to activin and TGF-beta may be modulated by tissue-specific expression of this receptor. Piantanida et al has identified a family where linkage studies could not include the HHT1 or HHT2 loci (88). In the family they studied the liver was more commonly involved.

Ataxia-telangiectasia (AT or Louis-Bar syndrome) is an autosomal recessive disorder characterized by ocular and cutaneous telangiectasias, cerebellar ataxia, and recurrent respiratory tract infections (4,89). As many as one third of patients develop cancer, the majority being lymphoid tumors (90). The progressive ataxia usually appears during the second to third year, the ocular and cutaneous lesions occur somewhat later at 3-6 year of age. Death usually follows in the second decade of life from the sinopulmonary infections or lymphoreticular malignancy (91).
The causative gene "ATM" is located at chromosome 11q23 and cloned in 1995 (92). ATM is a large protein-kinase that is one of the primary sensors of DNA damage (breakage). By an as yet undetermined mechanism, the ATM protein appears to cause abnormalities in cell cycle control (93). ATM then directly phosphorylates the tumor suppressor gene"p53" and interacts with many other molecules. Most research has been focused on the cerebellar degeneration and less attention has been paid to the genesis of the telangiectasia.

Angiokeratomas (capillary-lymphatic malformations)

Several recessive genes are known to be causative in enzymatic deficiency disorders presenting with cutaneous vascular papules. The best known examples are autosomal recessive fucosidosis and X-linked recessive Fabry disease (4). Fabry's disease is a rare error in glycosphingolipid catabolism resulting from mutations of the lysosomal enzyme alpha-galactosidase A (Alpha-Gal-A) gene located at Xq22.1 (94). The disorder is characterized biochemically by an abnormal glycolipid attached to sphingosine and deposited in cytoplasm of vascular endothelium and pericytes, renal epithelium, and cardiac muscle fibres (4). The male-restricted disease presents during childhood or early teens with intense burning pains in fingers and toes. Shortly afterwards vesicles appear over hips, buttocks and perineum. The cutaneous lesions are minute bloodfilled and purple or bluish-red papules, often with overlying keratosis. They do not disappear with

pressure. Most men die in their early forties, as a result of renal failure or hypertensive cardiovascular disease (95). Different mutations have been reported, permitting reliable carrier identification and prenatal diagnosis and facilitating identification of candidates for enzyme replacement therapy (94).

Fucosidosis is another disorder presenting with diffuse angiokeratomas, and is characterized by an absence or deficiency of the lysosomal enzyme alpha-L-fucosidose (96). Glycosaminoglycans and glycolipids accumulate in the tissues of affected individuals, who present with mental retardation, spasticity, and skeletal dysplasia (97). The multiple angiokeratomas are primarily located on the trunk and upper legs. Several patients have rapid neurological deterioration, leading to decerebration and death before the age of 5 years (98). Many different mutations in FUCA1, the autosomal recessive gene involved, has been found (98,99).

Venous malformations

Extracranial venous malformations

Most venous malformations occur sporadically and present as a solitary lesion (4). Rarely familial occurrences with multiple lesions are described (100-102). Clinical manifestations in these families were multiple cutaneous and mucosal venous malformations. Boon et al mapped the locus for an autosomal dominant form to chromosome 9p (100,101). Another good example of a venous malformation inherited in an autosomal dominant pattern is the blue rubber bleb naevus syndrome (BRBN) (103,104). Here cutaneous vascular lesions are associated with gastrointestinal bleeding. Gallione et al suggested that the gene causing BRBN syndrome may be identical to the gene causing the autosomal dominant venous malformations with isolated cutaneous lesions (101). In the chromosome 9p21 region some candidate genes are located, especially like the interferon gene cluster and MTS1 (p16) tumor supressor gene. Interferon a2a is known to be anti-angiogenic and currently used in the treatment of hemangiomas (105). The MTS1 gene is proposed to be involved in tumors like melanoma, acute lymphoblastic leukemia, glioma, and non-small cell lung carcinoma (106-109). Petty et al reported on the concurrence of malignancies in patients with hereditary cutaneous venous malformations (106). Maffucci syndrome is a sporadic entity characterized by multiple venous malformations and enchondromas (4,110,111). This syndrome has its onset usually in childhood or adolescence. The benign skeletal lesions often degenerate to a malignancy.

Blei et al described families demonstrating autosomal dominant segregation of childhood hemangiomas and venous malformations in the same family's (73). This was surprising as these vascular anomalies have different pathological characteristics (1,4). Possibly both lesions involve the deregulation of a common regulatory pathway (73). Walter et al established linkage to chromosome 5q in some of these family's (112). Three genes involved in angiogenesis map to this region: FGFR-4, PDGFR-b and fms-related tyrosine kinase-4. Further studies may demonstrate whether any of these genes are involved.

Other families have been found where cutaneous and mucosal venous malformations were inherited in an autosomal dominant way (100,113). Here the Tie-2 receptor tyrosine kinase has a specific kinase-activating mutation, resulting in an increase in receptor autophosphorylation and altering downstream signaling (113). Recently Korpelainen et al have proven that the mutant receptor activates STAT1 signaling, which is normally not activated by wild-type TIE-2 (114). Homozygous inactivation of the Tie-2 receptor are lethal in mice but no phenotype is apparent in heterozygous mice (115). The mutation is thought to cause an abnormal interaction between EC and SMC, leading to dilated venous channels surrounded by few smooth muscle cells. Normally, veins that increase in diameter are lined by an increasing number of endothelial cells in a monolayer, and an increasing number of smooth muscle cells in multiple layers (10). In contrast, in a venous malformation the number of smooth muscle cells does not increase with increasing luminal diameter and the luminal size may become much larger compared to normal vessels (116). Such defects in vascular remodeling may result from different mechanisms: Vikkula described the activating mutation of the receptor, Suri et al the absence of the ligand, and Sato et al described a deficient TIE2 receptor as cause for venous malformations (45,50,113).

Glomangiomas are characterized by undifferentiated smooth-muscle cells, the so-called glomus cells, that surround convoluted venous channels (117). Glomangiomas can be solitary or multiple (118). Solitary types are most common (±90%), and diagnosed by classic symptoms, (1) excruciating pain out of proportion to size, (2) very localized tenderness, (3) temperature sensitivity (cold precipitates the pain), (4) a positive Love sign (ability to localize pain to a pinpoint spot) and (5) a positive Hildreth's sign (painful symptoms abolished by proximal tourniquet). Solitary lesions have not been reported to be hereditary, but multiple tumors are often hereditary. Multiple glomus tumors (MGT) are regularly painless, frequently localized on the upper limbs, only partially compressible, and usually not found on the mucosa (117). Familial aggregation has been described repeatedly indicating autosomal dominant inheritance (117-119). Mutations in the TIE-2 gene have not been found, and a locus was identified on chromosome 1p21-22 called VMGLOM

VASCULAR ANOMALY	HISTOLOGIC CHARACTERISTICS	PROTEIN INVOLVED AND LOCALIZATION
Capillary malformation	Ectatic capillaries with decreased perivascular neural density	?
Hereditary Hemorrhagic Telangiectasis (HHT1)	Dilated arterioles and venules (lung AVM)	Endoglin, # 9q
HHT 2	(no AVM's in lungs)	Activin receptor -like kinase 1, #12q
Ataxia-telangiectasia (AT)	Ocular and cutaneous telangiectasis	ATM, # 11q
Autosomal recessive fucosidosis	Glycolipids and glycosamino- glycans accumulate in tissues	Alpha-L-fucosidosis, # 1p34.1 - 1p36.1
X-linked recessive Fabry disease	Abnormal glycolipid attached to sphingosine and deposited in cytoplasm of vascular endothelium and pericytes	Alpha-galactosidase, # Xq22.1
Cutaneo-mucosal venous malformation	Dilated venous channels, flat endothelium, scattered smooth muscle cells	TIE-2, # 9p
Glomangiomas	Undifferentiated smooth muscle cells (glomus cells), surrounding convoluted venous channels	VMGLOM, # 1p21-22
Cerebral venous malformation	Vascular spaces are lined by single layer of endothelium	Gene unknown, # 7q, 7p, 3q
Lymphatic malformations	Dilated lymphatic channels	Gene unknown, # 5q34 - 35
Klippel-Trenaunay Syndrome	Capillary/ venous malformation with associated overgrowth	Gene unknown, possibly # 5 or #11

Table B :
Histological and molecular characteristics of some vascular anomalies. (# = chromosome)

(117). Since cutaneous vascular malformations and glomangiomas are similar, it is possible that their ethio-pathogenic mechanisms are also similar. Therefore genes interacting with the TIE-2 signaling pathway are possible candidates for VMGLOM.

Intracranial venous malformations

Cerebral cavernous malformations (CCM) may involve any part of the central nervous system and are generally well-demarcated (120). The prevalence is suggested to be 0.5% (121,122). Most cases of CCM are classified as sporadic, but an autosomal dominant inherited form occurs. (123,124). Here affected members have multiple vascular lesions. Linkage to 7q21-22 (CCM1) was proven in families of Hispanic descent, but no known angiogenic factors are mapped to this region (125-127). Multilocus analysis in non-Hispanic Caucasian kindreds with familial CCM demonstrated linkage to three loci: CCM1 (in 40%), CCM2 (in 20%) (7p13-15), and CCM3 (in 40%) (3q25.2-27) (127). There are candidate genes, one being the preproendothelin-1 (ppET-1) gene (128). The expression of endothelin (ET-1), a major player in the development of vascular tissue, is regulated by ppET-1 (129). Recently, a member of the RAS family of GTPases (located on chromosome 7q) was found to be mutated in CCM1 families, which suggests that the RAP1A signal transduction pathway is involved in the pathogenesis of CCM (130).

Lymphatic malformations

Lymphatic malformations (LM) are composed of dilated lymphatic channels (131). They are filled with a proteinaceous fluid, and do not have connections to the normal lymphatic system. Lesions can be both macrocystic and microcystic, thoracic lesions usually being macrocystic, and (the more common) cervicofacial lesions microcystic (4,12). By analyzing a two-generation family, an autosomal dominant type of congenital hereditary lymphedema has been mapped to chromosome 5 (132). The candidate region contains a gene that encodes the vascular endothelial growth factor R-3 gene. Cervicofacial LM further occurs in trisomy 13, 18, and 21, and Turner syndrome (12,133,134). Vikkula et al have suggested that sporadic cases of LM are caused by de novo dominant somatic mutations, and that germline mutations are lethal (12).

Arterial malformations

Isolated cases of arterial anomalies have been described only rarely (135). Most arterial anomalies are asymptomatic and are discovered coincidentally or until a coexistent arterial disorder becomes evident (4). According to our knowledge no familial cases are reported in literature.

Complex/combined lesions

Klippel-Trenaunay syndrome (KTS) is known by the triad: cutaneous capillary malformations, congenital varicose veins, and hypertrophy of bone and soft tissue of the affected limb (4,136). Frequently, the triad is accompanied by lymphatic abnormalities (4,73). The etiology of this peripheral angiodysplasia is not clear. Some familial cases have been described, but in general KTS is sporadic in appearance (4,137-139). Aelvoet et al investigated the genetics in 86 KTS patients and found two familial cases (140). In both families the phenotype skipped a generation, possibly pointing to multifactorial inheritance and reduced penetrance (12). Some families in this study also had an increased occurence of capillary malformations (140). It is possible that CM's, in at least these families, is caused by the same gene as the fully expressed KTS phenotype (12). Ceballos-Quintal et al described a KTS patient, whose mother had severe varicositas and a large "capillary hemangioma" on her back, and whose grandmother had developed varicositas at a young age (141). The authors suggest that the KTS is inherited in a autosomal dominant way, and that the milder phenotype in the mother and grandmother might be explained by the variable expressivity in this family (141). A chromosome translocation in a KTS patient with multiple dysmorphias and a developmental delay have suggested localisation at chromosome 5q or 11p, but much work still has to be done to understand the molecular mechanisms of these rare anomalies (142).

Other less common vascular syndromes, such as Bannayan and Riley-Smith syndrome suggests an autosomal dominat inheritance (143). Proteus syndrome is recognised by the unique constellation of partial gigantism of the hands and feet, asymmetric overgrowth, lipomas and capillary-venous malformations, but with no clear evidence that this syndrome is heritable (4,144,145). The concept of somatic mazaicism has been suggested, but more studies are necessary to confirm this.

Conclusions

Although our knowledge of the embryogenesis of the vascular system has increased dramatically in the last two decades, there are still many unanswered questions, mainly due to the complexity of the vascular system. However, the exact mechanism for normal and abnormal vascular development will eventually be understood through identification of the causative genes and elucidation of the developmental pathways.

Familial vascular abnormalities represent only a minority of the vast number of total cases. As it is

likely that genes implicated in familial cases are also involved in sporadic cases, thus making studying of these rare familial cases very helpful. Surgeons involved in treatment of patients with vascular malformations should be aware of the present knowledge, because of the implications for treatment in the (possible near) future, and because of genetic counseling of patients and their relatives. Surgeons further have access to affected tissues obligatory for further fruitful studies. In applicable sitiations sugeons should further stimulate participation in research projects.

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More on vascular malformations

Comment by M.M. Cohen,

Plast. Reconstr. Surg. 109(7):2591, 2002.

Sir:

The recent article by Breugem et al. is an outstanding review of the progress in the field of vascular malformations, including the relevant molecular biology. The authors are to be congratulated on their extensive and well-balanced coverage.

The purpose of this letter is to update three errors and one omission in their review to keep them separate from an otherwise excellent article. Topics discussed include the (1) erroneous estimate of the number of genes in the human genome; (2) erroneous conceptions of Klippel-Trenaunay syndrome, particularly inheritance; (3) oversimplified view that the vascular syndrome of Bannayan and Riley "suggests autosomal dominant inheritance," when its molecular basis is, in fact, well established; and (4) omission of genetic and syndromic lymphedema, including two molecularly characterized disorders. Of the 18 molecular articles cited by me in this letter, nine were available by 1999, six were available by 2000, and only three were from 2001 but appeared before Breugem et al. corrected their proofs.

The human genome has about 35,000 genes. Breugem et al.state "The entire genome contains 3 billion nucleotides (base pairs of DNA) that together encode 100,000 different genes." The reference for their statement was from 1993, the yesteryear of interpreting the human genome (1,2). Venter et al. indicated that only about 1.1 percent of the human genome is spanned by exons (3). Modern estimates, available from many sources are on the order of 35,000 genes, give or take a few thousand (3-6). However, earlier estimates anticipated today's thinking. In 1967, Muller calculated that the mammalian genome would contain a maximum of 30,000 genes, and in 1970, Crow and Kimura estimated that there were 30,000 human gene loci (7,8).

Klippel-Trenaunay syndrome defined. Klippel-Trenaunay syndrome* consists of (1) combined vascular malformations of the capillary, venous, and lymphatic types **, (2) varicosities of unusual distribution, in particular, the lateral venous anomaly observed during infancy or childhood; and (3) limb enlargement (9,10). Male and female individuals are equally affected. The lower limb is involved in almost 95 percent of patients, the upper limb accounting for about 5 percent of patients. Approximately 15 percent have combined upper and lower limb involvement. Uncommonly, patients may have trunk involvement only (10). Well over 1500 cases have been recorded, and these occur sporadically (11-15). Servelle alone documented 768 surgically treated patients (16).

Challenging four commonly held assumptions: The following four conceptions frequently found in the literature on Klippel-Trenaunay syndrome can be seriously challenged: (1) addition of arteriovenous fistulas and renaming the disorder Klippel-Trenaunay-Weber syndrome (16-21) (2) overlap with Sturge-Weber syndrome (22-27), (3) the presence of a bleeding diathesis of the Kasabach-Merritt type (14), and (4) familial aggregation (13,18,28-35). These conceptions were extensively discussed by Cohen (10). However, only presumed familial instances and proper diagnosis will be discussed here.

Familial aggregation: Careful scrutiny of published "familial cases" of Klippel-Trenaunay syndrome indicates one or more of the following problems: (1) inadequate documentation of cases; (2) overinterpretation of minor manifestations in relatives, including "nevus flammeus," hemangiomas, and varicosities,*** all of which occur commonly in the general population; and (3) defining Klippel-Trenaunay syndrome as a capillary malformation with or without "hemihypertrophy" with no mention of lymphatic malformations, lateral venous anomaly, lymphatic vesicles, or venous flares within the capillary malformation or macrodactyly. Thus, it is either unlikely or uncertain that these cases represent Klippel-Trenaunay syndrome. Only the affected brother and sister described by Lindenauer are well-documented examples of Klippel-Trenaunay syndrome within a family (13).

Diagnosis: The combination of cutaneous capillary malformations and hemihyperplasia only is not sufficient to establish a diagnosis of Klippel-Trenaunay syndrome. Documenting the lymphatic and venous manifestations of Klippel-Trenaunay syndrome more thoroughly is essential in the future, including magnetic resonance imaging with gadolinium to distinguish lymphatic from venous malformations. Careful study (magnetic resonance venogram or phlebography/ venography) should also document the lateral venous anomaly and any abnormalities that may be present in the deep veins of the leg (10).

Most presumed familial instances have been published by dermatologists and geneticists who have not examined patients or presumably affected relatives with the techniques described above, commonly used by surgeons. *If there are a few instances of familial aggregation, future documentation must be much more thorough to prove this (10).*

Diagnosis of Bannayan-Riley Ruvalcaba syndrome. Bannayan-Riley-Ruvalcaba syndrome is an autosomal dominant disorder consisting of macrocephaly, vascular malformations, lipomas, hamartomatous polyps of the distal ileum and colon, pigmented macules on the shaft of the penis, and Hashimoto thyroiditis (36,37). Ruvalcaba et al. reported two male patients with macrocephaly, intestinal polyposis, and pigmented spotting of the penis (38). Bannayan described the combination of macrocephaly, subcutaneous lipomas, visceral lipomas, and vascular malformations (39). Riley and Smith observed the association of macrocephaly,

pseudopapilledema, and vascular malformations (40). Cohen and Dvir et al. suggested lumping these three earlier recognized syndromes as a single entity, and Cohen suggested combining the names of the first authors of the three original reports to keep learning hurdles to a minimum: "Bannayan-Riley-Ruvalcaba syndrome" 36,41). Gorlin et al.(37) then demonstrated all three phenotypes in a single family through four generations. *PTEN* mutations have been found in both Bannayan-Riley-Ruvalcaba syndrome and Cowden syndrome (42-46), and phenotypic overlap has been described (47-50). Marsh et al. demonstrated that the *PTEN* mutational spectrum also involves overlap, suggesting a single genetic entity they called the "*PTEN* hamartoma-tumor syndrome" (46).

PTEN molecular biology and mutations. PTEN is a member of the protein tyrosine phosphatase (PTPase) superfamily defined by an invariant signature motif $Cys(X)_5Arg$; within this catalytic domain, cysteine is a catalyst and arginine plays an important role in binding the phosphoryl group of the substrate. *PTEN* is a tumor suppressor gene that maps to 10q23. Its name is derived from phosphatase (P) and the cytoskeletal protein tensin (TEN). For proper functioning, the phosphatase domain must be intact (51,52).

PTEN mutations have been identified in 60 percent of patients with Bannayan-Riley-Ruvalcaba syndrome ($n = 32$), in 81 percent of patients with Cowden syndrome ($n = 37$), and in 91 percent of patients with phenotypic overlap ($n = 11$) (45,46). Mutations are of the missense, nonsense, deletion, insertion, and splice-site types (43,46). They are scattered along the length of *PTEN*, except for exons 1,4, and 9. A hot spot for mutations is the core phosphatase-containing exon 5.

Genetic and syndromic lymphedema. With respect to lymphedema, Breugem et al. state, "By analyzing a two-generation family, an autosomal dominant type of congenital hereditary lymphedema has been mapped to chromosome 5 (53). The candidate region contains a gene that encodes the vascular endothelial growth factor R-3 gene. Cervicofacial lymphatic malformations further occur in trisomy 13, 18, 21, and in Turner syndrome." Actually, however, much more is known about lymphedema, both at the clinical and molecular levels. Several examples follow.

Lymphedema is etiologically heterogeneous and may be seen in a number of genetic disorders and in several chromosomal syndromes. In Turner syndrome, the hands and feet have transient lymphedema during infancy, and recurrent lymphedema of the extremities may be observed in some patients. *It is not just a matter of a webbed neck ("cervicofacial lymphatic malformation,"* vide supra). Neonatal lymphedema similar to that found in Turner syndrome has been associated with a Y;16 translocation (54). In Noonan syndrome, features may include generalized lymphedema, peripheral lymphedema, pulmonary lymphangiectasia, intestinal lymphangiectasia, fetal hydrops, and "cystic hygroma." Hennekam syndrome is characterized by lower extremity

Table 1:
Genetic and molecular basis of three disorders with lymphedema

Genetic and Syndromic Lymphedema	Cardinal features	Inheritance	Gene Map Location	Gene	Mutation	References
Milroy congenital primary lymphedema	Lymphedema, mostly of lower extremities	Autosomal dominant Evidence of locus heterogenity and possible oligogenic inheritance in some families	5q34-q35	VEGFR3 (FLT4)	Gly857Arg Arg1041Pro His1035Arg Leu1044Pro Pro1114Leu	55,56,57
Lymphedema distichiasis syndrome	Lymphedema, primarily of extremities, and double rows of eyelashes	Autosomal dominant (MPH1)	16q24.3 4 bp	FOXC2	297C→G	58
Cholestasis-lymphedema syndrome	Neonatal cholestasis and lymphedema affecting mainly lower extremities but also hands, scrotum, and periorbital soft tissues	Autosomal recessive	15q	?	?	59

lymphedema and intestinal lymphangiectasia. Inheritance is autosomal recessive, but the gene map location and the specific gene involved have not been determined at this writing. The molecular bases have been established for Milroy congenital primary lymphedema and lymphedema-distichiasis syndrome. Furthermore, the gene map location for cholestasis-lymphedema syndrome has been determined. These three disorders are summarized inTable 1. Histologic features consist of hypoplasia or aplasia of lymphatic channels.

Mutations that cause autosomal dominant Milroy congenital primary lymphedema demonstrate haploinsufficiency, thus inactivating VEGFR3 tyrosine kinase (55,56). There is also evidence of locus heterogeneity, and it is possible that some families may have oligogenic inheritance (57). Mutations in *FOXC2*, a forkhead family transcription factor, cause the autosomal dominantly inherited lymphedema-distichiasis syndrome. A nonsense mutation and a frameshift mutation have been identified (58). The autosomal recessive cholestasis-lymphedema syndrome has been mapped to 15q, but to date, the responsible gene has not been identified (59).

* The original article of Klippel and Trenaunay (9) shows that "Trenaunay," not"Trénaunay," is correct; there is no accent é, although many articles have added the accent.

** Because Klippel-Trenaunay syndrome has combined capillary, lymphatic, and venous malformations, lymphatic vesicles appear on the surface of the cutaneous capillary malformation and ooze lymph fluid. The lateral venous anomaly may have protrusions known as venous flares on the surface of the cutaneous capillary malformation (10).

*** Varicosities in Klippel-Trenaunay syndrome have early onset in infancy or childhood, with more extensive distribution than classic varicose veins in the general population.

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Reply

"The times , they are changing"
Bob Dylan, 1967

We thank Dr. Cohen for his thoughtful comments to our review article (1). As we mentioned in our introduction: "The purpose of this review is to provide a *short* review of the literature on some current concepts in molecular genetics. . .." Therefore, our main objective was to produce a compact article with optimal readability. Our aim was to trigger further interest in the readers in gaining information about the developmental and molecular background of vascular malformations and to provide easy access to more comprehensive articles.

With regard to the number of genes, we fully agree that the estimate was high. However, at the time the article was written this estimate was still used in several textbooks (2). The articles on the human genome referred to by Dr. Cohen were published well after our article was submitted (3,4). We are all in a constant battle to remain up-to-date with our literature. In a meta-analysis performed by Olkin, he concluded that in the 40,000 medical–scientific journals each year, more than one million articles are published (5). On a daily basis new information is provided; certainly, this also happens regarding the human genome. Dr. Cohen comments that the "current" estimate on the human genome is about 35,000. However, more recently Katsanis et al. provided good evidence that the human genome is considerably larger (nearly 30 percent) than this estimate (6). Time will tell which number will be right.

The *PTEN* gene is another good example on how fast facts are changing in the molecular world. Zhou et al. proved not only that germline mutations in the *PTEN* tumor suppressor gene are seen in 60 percent of Bannayan-Riley-Ruvalcaba syndrome patients, but also that mutations are associated with patients having genuine Proteus syndrome and those having a clinical phenotype closely resembling Proteus syndrome (7).

In a short review it is impossible to include a summary of all available information. This is the only reason we omitted all further data regarding (syndromic) lymphedema from our review, despite the deep involvement of one of us (R.C.M.H.) in both clinical delineation and molecular dissection of disorders that display lymphedema, through an International Lymphedema Network, including the American National Lymphology Network. Dr. Cohen rightfully points out the importance of disorders with lymphedema. His remarks have urged us to plan a separate review article for submission to a plastic surgery journal.

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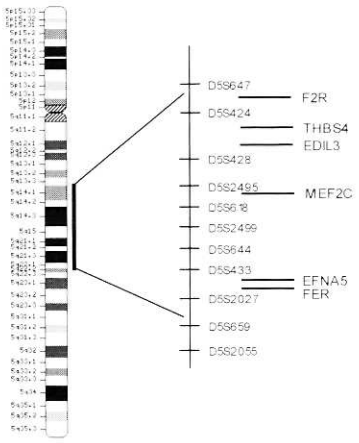
A locus for hereditary capillary malformations mapped on chromosome 5q



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Abstract

Capillary malformations (port-wine stains) are the most common vascular malformations occurring in 0.3% of live births. Most capillary malformations occur sporadically and present as a solitary lesion. Capillary malformations can also occur as a component of well described syndromes. Familial occurrence of multiple capillary malformations has been described in the literature, suggesting autosomal dominant inheritance with variable expression in this subgroup. A hereditary basis underlying the development of solitary capillary malformations has not been found but may well be possible. We have mapped a locus for an autosomal dominant disorder in a three-generation family that manifested itself with multiple cutaneous capillary malformations to chromosome 5q13-22. This locus spans 48 cM between the markers D5S647 and D5S659 and harbours several candidate genes. By defining the gene(s) responsible for capillary malformations, we will gain more insight in the pathogenesis of this disorder. It is likely that genes implicated in these familial cases may be involved in the more sporadic cases.

Introduction

Capillary malformations (port-wine stains) are the most common vascular malformations occurring in 0.3% of live births (OMIM 163000)(18). The majority of capillary malformations (CMs) are located at the head and neck, with 85 % occurring in a unilateral distribution that follows a dermatome (34). Most CMs occur sporadically and present as a solitary lesion (28). CMs can also occur as a component of well described syndromes, such as the Klippel-Trenaunay syndrome (OMIM14900) and Sturge-Weber syndrome (OMIM 185300) (15). With advancing age capillary malformations undergo progressive ectasia resulting in typical red-to-purple lesions (3,29). The psychological burden associated with CM is high and often warrants intervention, such as pulsed dye laser (PDL) (21). The efficacy of PDL in treating patients with CMs is very variable, in part due to the depth and the diameter of the ectatic blood vessel (20). For accurate diagnosis and subsequent optimal treatment, knowledge of the pathogenesis is of paramount importance.

Familial occurrence of multiple CM was first described in 1949 by Shelley and Livingood (32). Since then a limited number of similar families were published, suggesting autosomal dominant inheritance with variable expression (reviewed in ref. 7). A hereditary basis underlying the development of solitary CM has not been suggested. Recent studies have provided first clues for the molecular basis of vascular malformations. Mutations in the *TEK* gene, located at chromosome 9p21, can be the cause of familial mucocutaneous venous malformations composed of thin-walled vascular channels surrounded by deficient smooth muscle cells (OMIM 600195) (5,35). Autosomal dominant venous malformations with accompanying glomus cells (OMIM 138000) were linked to chromosome 1p21-p22(4,17). Genetic linkage of inherited central nervous system vascular malformations has been established to three chromosomal loci; 3q25-27, 7p13-15, and 7q21-22 (*CCM1* gene) (OMIM 6032851, 603284, 604214). (8,15). Hereditary hemorrhagic telangiectasia (HHT, OMIM 187300) has also been assigned to at least two genes. *ENG* (endoglin), a TGF-beta binding protein of endothelial cells, at chromosome 9q34.1 and *ACVRL1*, an activin receptor-like kinase, at chromosome 12q (25,36). Four kindreds with autosomal dominant hemangiomas (OMIM 602089) associated with vascular malformations were used to show linkage to 5q31-q33 (6,37). The localizations of genes involved in lymph vessels may also be important, as the lymphatic system develops in part from the venous system (7). Linkage studies have mapped the lymphedema-distichiasis syndrome (OMIM 153400) at 16q24.3 (24). Later on it was proven that mutations in the *FOXC2* gene cause not only this entity, but may also cause several other lymphedema syndromes (9,11). Congenital hereditary lymphedema Milroy type (OMIM 153100) was localized at chromosome 5q35.3, and proven to be caused by mutations in

the *FLT4* gene (10,19). Here we report on the results of a whole genome linkage screen of a large family in which capillary malformations occurred in an autosomal mode of inheritance, and discuss some of the candidate genes within the linked region.

Methods

After oral consent, DNA was isolated from blood samples. Whole genome linkage analysis was performed using the Linkage Mapping set MD10 (PE-biosystems). The 17 members used for genome screening are indicated with * in figure 1. This set contains 400 markers spread over the genome with an average distance of 10cM. Markers were amplified by multiplexed PCRs and fragments were analysed on an ABI310 (PE-biosystems), using genescan and genotyper software. Additional markers in the 5q14-22 (D5S2495, D5S2499, D5S2055 and D5S659) region were Cy5 labeled by PCR and analysed on an ALF sequencer (Pharmacia). 2-Point LOD scores were calculated using the MLINK program of the LINKAGE package, assuming autosomal dominant inheritance with complete penetrance.

Results

Clinical findings

The pedigree is shown in Figure 1; all family members were examined by the same person (CCB). The proband (Fig 1: III 3) applied for pulsed dye laser treatment at our hospital. She was a 6-year-old girl with a CM on the upper part of her left leg (figure 2). The diameter of this lesion was 31 x 27 cm. She had several smaller CMs on both her feet and left arm ranging in size from 1 x 1 cm to 2 x 3 cm. The mother of the proband (II-7) had several CMs on her right arm, inside her left lower arm, and on her right lower leg. Dimensions varied between 1 x 1 cm and 3.2 x 4 cm. The proband had one affected sister (III 4), who had large CM (± 30 x 30 cm) on her lateral right upper leg. Several smaller CM were seen all over her body ranging in size from 1 x 1 cm to 7,5 x 4 cm. The grandmother of the proband (I 2) has deceased but was reported to have had multiple small CMs on her thorax. Three aunts and two uncles of the proband had multiple CMs. One uncle (II-3) had a large CMs on the antero-lateral side of his left upper leg (30 x 30 cm) and a smaller CM on the anterior side of his left lower leg (10 x 5 cm). When he was nine years old he had an overgrowth of this leg (9 cm leg length difference) which was subsequently treated with an epiphysodesis. This has resulted in a small leg length difference (0.5 cm) as an adult, but

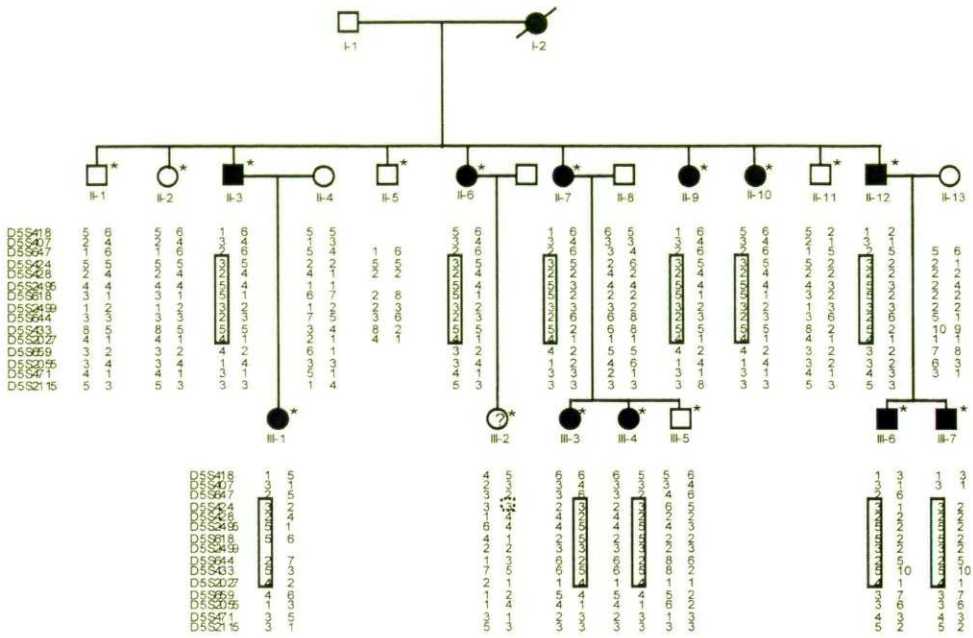

Figure 1:
Pedigree of present family with multiple cutaneous capillary malformations. The family members included in the original whole genome screen are marked by an *asterisk*. The segregating haplotype is indicated by the *boxed regions*. A recombination between D5S647 and D5S424 in III-3 determines the centromiric border and recombinations between D5S2027 and D5S659 in II-6 and II-12 determine the telomeric border. The affection status of individual III-2 is uncertain. When she is considered affected, the linked region will be reduced to the region between D5S647 and D5S428. On the other hand, when she is not affected, the linking region is between D5S424 and D5S659 (see discussion).

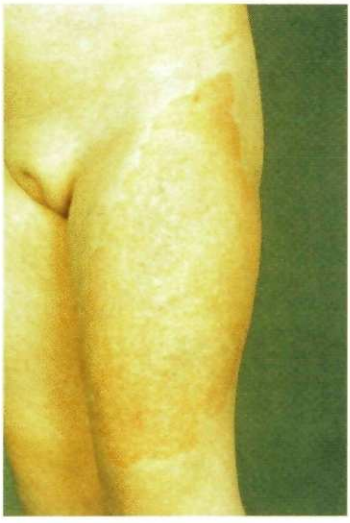

Figure 2:
A photograph of the CM located on upper left leg of the proband.

the difference in leg circumference remained unchanged. He had no other symptoms indicative of Klippel-Trenaunay syndrome. His daughter (III-1) had multiple CMs located all over her body. She also had a hemangioma on the back of her head. An aunt of the proband (II 6) had one daughter (III 2) that had a hemangioma on her forehead, but no signs of capillary malformations. The two boys (III 6, III 7) both had multiple CMs, III 6 also having a salmon patch ("angel's kiss") on his forehead as an infant.

Molecular findings

Seventeen family members (all persons excluding 3 spouses (II-4, II-8 and II-13)) were included in a whole genome screen. Highly significant linkage was found only for D5S644 (LOD score 4.2) and D5S433 (LOD score 3.9), D5S618 (LODscore 3.9) and D5S424 (LOD score 4.2). Additional markers in this region (D5S2495, D5S2499 and D5S2027) were analysed and haplotypes were constructed (LODscores are given in table 1). Cosegregation with CMs is seen between markers D5S647 and D5S659. The borders of this haplotype are determined by a crossover between D4S647 and D5S424 in III-3 (proximal border) and crossovers between D5S2027 and D5S659 in II-6 and II-12 (distal border) (Fig. 1). The linking region on 5q13-22 spans 48 cM according to the

Locus	cM	Z at q=0	Zmax
D5S418	58.55	-14.9	0.2 at q=0.3
D5S407	64.67	-1.1	2.0 at q=0.5
D5S647	74.07	-0.8	2.3 at q=0.5
D5S424	81.95	4.2	4.2 at q=0.0
D5S428	95.40	0.0	0.0 at q=0.0
D5S2495	97.21	3.9	3.9 at q=0.0
D5S618	99.42	3.9	3.9 at q=0.0
D5S2499	102.62	2.1	2.1 at q=0.0
D5S644	104.76	4.2	4.2 at q=0.0
D5S433	111.97	3.9	3.9 at q=0.0
D5S2027	119.50	1.5	1.5 at q=0.0
D5S659	122.01	-5.4	1.3 at q=0.15
D5S2055	125.91	-5.7	1.3 at q=0.15
D5S471	129.83	-6.7	0.7 at q=0.15
D5S2115	138.64	-7.0	0.4 at q=0.2

Table 1:
Two-point LODscores (Z) for the 5q14-22 markers at recombination fraction (q) 0 and the maximum LODscores (Zmax) and the recombination fraction (qmax) at which this is found.

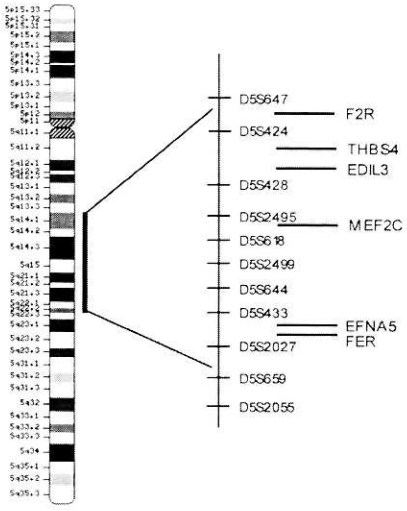

Figure 3:
Schematic presentation of chromosome 5 showing the position of the linking region and the location of candidate genes.

Marshfield map (http://www.marshfieldclinic.org/research/genetics) and contains many candidate genes, including *F2R, EDIL3, THBS4, MEF2C, FER* and *EFNA5* (NCBI database; http://www.ncbi.nlm.nih.gov) (figure 3).

Discussion

We present a family in which several members had capillary malformations of variable severity. The inheritance mode fits best an autosomal dominant pattern of inheritance. We localize the gene causative for the vascular malformations in a candidate interval of 48cM at chromosome 5q13-22. This localization has not been described in other families with similar vascular malformations. This locus is further distinct from previously described loci for orther vascular anomalies, like venous malformations, ataxia-telangiectasia (OMIM 208900), Fabry disease (OMIM 301500) and Rendu-Osler-Weber syndrome (OMIM 187300) (4,5,7,25,36).

Most sporadically occurring CMs are solitary lesions, but the affected family members in our study all had multiple CMs. The affected family described by Boon and co-workers to have autosomal dominantly inherited venous malformations also had multiple lesions, while most venous malformations are sporadic and isolated (5). The exact significance of this finding in

hereditary cases is still unclear but the Knudson two-hit hypothesis has been mentioned as a possible explanation (5,22).
In the present study two family members also had a hemangioma. Blei et al identified six kindreds in which hemangiomas were segregated in an autosomal dominant trait (6). In these families several members had associated vascular malformations. Walter et al showed linkage to 5q31-q33 using four of these families for a genome wide linkage screen (37). Despite hemangiomas and vascular malformations having distinct clinical characteristics, it is possible that the development of both may involve the deregulation of a common regulatory pathway (6). On the other hand, the co-occurrence can be explained as sheer coincidence. Therefore, the affection status of individual II-2 is considered unknown in this study, as she presented only with a hemangioma and did not have any CMs. The locus decreases to the region between D5S647 and D5S428 if hemangiomas and capillary malformations would arise from the same gene defect and, thus, II-2 would be affected, or decreases to the region between D5S424 and D5S659 if they are two independent trait and II-2 would be unaffected. At present vascular tumors (hemangiomas) and vascular malformations are still classified as two separate categories and maybe after molecular investigation of more families with hemangiomas and vascular malformations we will see that this distinction is not as black and white as be always assumed (28).

CMs are congenital lesions located in the upper reticular and papillary dermis (3). Several studies have failed to demonstrate a difference between endothelial cells of capillary malformations and normal vessels (12,20,23,29). Pericytes were found to be predominantly located in the inner part of the vessel wall (31). In general the celmetabolism of these pericytes did not look more active than normal. Two studies have postulated that a decreased innervation may be responsible for the progressive ectasia of the capillary malformation (30,33). In one study the capillary malformations lacked not only sympathetic innervation, but also sensory innervation (30). In combination with the absence of anomalies in endothelial cells and pericytes it is likely that a defective genesis of vascular innervation is a major cause of capillary malformations. The 5q linkage area is at present still quite large. Several candidate genes implicated in neurogenesis are located in this area. These include the *FER* and *THBS4* genes shown to be involved with neurite outgrowth and the *EFNA5* protein involved in axon guidance (1,2,38). The *MEF2C* gene may be involved in myogenesis and neurogenesis (26,27). In addition, *EDIL3* and *F2R* are involved in vasculogenesis (14,16).

Although a hereditary basis for CMs is suggested, they clearly represent a minority of the total cases of CMs. In analogy to other genes it is possible that genes implicated in familial cases will also be involved through somatic mutations in the more common sporadic cases. It has been

proven that the vascular endothelium and the surrounding support cells reciprocally influence each other, and it is likely that any disruption in the cellular physiology of either cell type can result in dysfunction. Thus, multiple genes might be implicated in CMs. Further refinement of the present linkage region and subsequent mutation analysis should allow detection of a causative gene.

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Capillary malformations: beyond port-wine and strawberries, toward a neural malformation ?

Mascagni (ca. 1785)

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Introduction

Capillary malformations (port-wine stains) are the most common vascular malformations occurring in 0.3% to 2.1% of newborns (1-3). The majority of capillary malformations (CM) are found on head and neck region, with 85% occurring in a unilateral, dermatomal distribution (4,5). The V2 dermatome is most commonly involved (57%); while in lesions where more than one dermatome is involved, the V2 is involved with V1 and / or V3 in 90% of cases (4). CM may also be part of a complex vascular anomaly e.g. Sturge-Weber- or Klippel-Trenaunay syndrome, but this paper will focus only on the non-syndromal capillary malformations (2,6). The mainstay of treating CM is flashlamp-pumped pulsed dye laser treatment (PDL), but a significant minority of these patients will achieve a suboptimal response. (7,8).

For an accurate diagnosis and subsequent optimal treatment, the pathogenesis has to be known. We want to provide an overview of our current understanding of the pathogenesis of capillary malformations. We first summarize some relevant issues of normal skin anatomy, and then compare this to the pathology involved in capillary malformations. In the subsequent discussion some theories about the etiology will be mentioned, and although this discussion will not focus on pulse dye laser *per se*, it is inevitable to include some information in a discussion about capillary malformations. We further summarize the most important parts of the increased molecular knowledge applicable to capillary malformations.

Normal skin anatomy

Findings by Johnson et al suggest that the major vascular organization of the dermis is defined in the first trimester of pregnancy (9). By 14 weeks of development the reticular dermis is distinguishable from the papillary dermis (10). At this stage the dermis is also invaded by the downward invagination of developing epidermal appendages.

The cutaneous microcirculation is organized in two horizontal plexuses (11-13) (Figure 1). The most superficial plexus is located 1 – 1.5 mm below the skin surface and is located at the junction of the papillary and reticular dermis and is called the subepidermal plexus (papillary plexus). The deep plexus is located at the dermis-subcutaneous border and is called the deep dermal plexus (reticular plexus). Pearl and Johnson have shown that a subcutaneous vascular plexus is located between the dense and loose adipose subcutaneous tissue and appears to be the homologue to the panniculus carnosus in lower mammals (14). The subcutaneous vascular

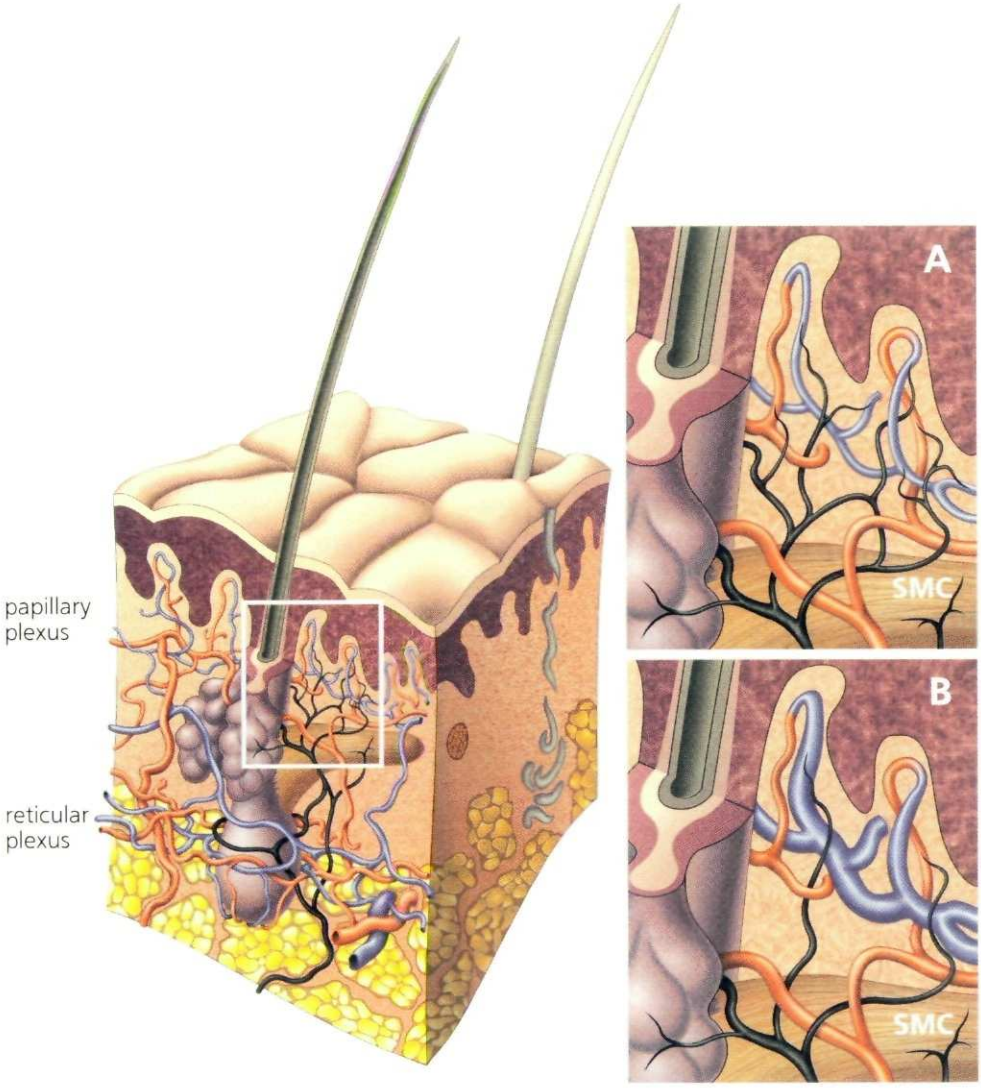

Figure 1:
Section of normal skin on the left defining the papillary (subepidermal) vascular plexus and the reticular (deep dermal) plexus. (red = arteries, blue = veins, black = nerves) On the right side of the picture is an enlargement of part of the skin with (A), demonstrating the normal skin, and (B) the skin in capillary malformations. (SMC = smooth muscle cell = arrector pili muscle of hair follicle). In (B) the decreased nervous innervation to the vasculature, with normal nervous innervation to the SMC is demonstrated. The dilated vasculature in response to the decreased innervation is also demonstrated in the capillary malformation (B).

network is best developed in the face, while the least numerous subcutaneous network is located in the lower extremities (14). The subcutaneous vascular plexus is divided into a deep, middle and superficial vascular plexus (15,16), but this discussion will only focus on the dermal circulation. The reticular plexus is formed by perforating vessels from the underlying muscle and subcutaneous tissue, and gives rise to arterioles and venules to form the papillary plexus (11). The reticular plexus also gives separate lateral tributaries to the sweat glands and hair bulbs and may pass deeply to supply adipose tissue. There seem to be some direct connections between the ascending arterioles and descending venules (arteriovenous fistulas). The papillary plexus receives branches from al three plexuses of the subcutaneous vessel system (15). The capillary loop arises from terminal arterioles from the papillary plexus and has an ascending limb, an intrapapillary loop (with a hairpin turn) and a descending loop that connects to the postcapillary venules (11,12). *Each* dermal papilla has its own single capillary loop. The endothelial tube at the crest and the intrapapillary descending limb is 1–1.5μm wider than the ascending limb. The characteristic of an arterial capillary is the homogenous appearing basement membrane material in the vessel wall. The character of the descending limb changes at the border of dermal papilla. The endothelial tube becomes wider and the basement membrane material in the wall loses its homogenous appearance and develops multilayers (multilaminated basal lamina layer is characteristic of venous vessels) before it connects to the papillary plexus. Bridged fenestrations are normally found in capillaries involved in rapid exchange of molecules between the vascular system and tissues e.g. choroid plexus of the brain and renal glomeruli. In healthy skin bridged fenestrations are limited to venous capillary loops adjacent to the eccrine sweat glands and dermal papilla of the hair.

Some of the differences between the vessels of the upper dermis and the lower dermis are summarized in Table A (11,13,17). The deeper dermal arterioles have elastic tissue in their wall, while several layers of smooth muscle cells also arround them. Some of the ultrastructural differences between the vessel components of the microcirculation are summarized in Table B (11-13,17-21). The terminal arterioles have a single smooth muscle cell layer, while the larger ascending arterioles have two distinct smooth muscle cell layers *over* a discontinuous elastic layer (18). The wings (the most lateral parts) of the smooth muscle cells in the terminal arterioles

Table A:
Some differences between the two dermal vascular plexuses

	Papillary plexus	**Reticular plexus**
Vessel diameter	25 μm	50 μm
Wall thickness	4 -5 μm	10 - 16 μm
SMC / PC layer	1 – 2	4 – 5
Position of bundles collagen	periphery of vessel	subendothelial position

Table B:
Some ultrastructural differences between the vessel components of the microcirculation (SMC = smooth muscle cells, PC = pericytes, EC = endothelial cells)

	Larger arteriole	Terminal arteriole	Arterial capillary	Venous capillary	Postcapillary venules	Larger venules
Vessel diameter	17 -26 µm	17-12 µm	10-12µm	10-12 µm	12-35 µm	35 - 50 µm
Wall thickness	?	?	2 - 3 µm	2 - 3 µm	3.5 - 5 µm	?
Basement membrane	Homogenous	homogenous	homogenous	Multilayer	multilayer	multilayer
Bridged fenestrations	-	-	-	+ (limited to eccrine sweat glands + dermal papilla of hair)	-	-
Elastin layer	+ (between EC and SMC)	+ (at periphery of vessel)	-	-	-	-
SMC or PC	SMC	SMC	PC	PC	PC	PC
SMC / PC layers	2	1	1	1	2-3	More PC (number not stated)
SMC / PC wings		Totally surround and even overlap	Average of 80% of EC encircled, never overlap	Average 80% encircled, never overlap	Average 80% encircled, never overlap	PC's more randomly placed
SMC / PC interdigitations on EC	++	++	+++	+++	+++	++
Tropomyosin and alpha-actin in SMC/PC	+++	+++	+	+	+++	+++

often overlap and totally surround the endothelial cells.

Smooth muscle cell/ endothelial cell interdigitations do occur, but less than in the case of pericytes and endothelial cells. As the diameter of the arteriole decrease from 26ìm, the elastic fibers gain a more peripheral position in the vessel wall. At 15ìm diameter the elastic fibers have disappeared and only a thin sheath divides the wall and the surrounding veil cell. At a diameter of 10 - 12ìm this elastic sheath disappears; this is the beginning of the capillary bed. Smooth muscle cells are not found below the 15ìm diameter level. Here the endothelial cells are surrounded by pericytes. Most vessels in the papillary plexus are postcapillary venules. Pericytes form tight junctions with endothelial cells through breaks in the basement membrane. Pericytes differ from smooth muscle cells by being thinner, lacking dense bodies, and having fewer myofilaments, but they do have proteins e.g. tropomyosin and alpha-actin for contraction (11,19-21). The basic structure of the postcapillary venule in the papillary plexus is similar to the larger venules at the reticular plexus, with the exception of vessel diameter and number of endothelial cells. The postcapillary venules have 2 - 3 layers of pericytes surrounding them, while venous capillaries have one layer of pericytes. At the border of the dermis and the subcutaneous tissue are valve containing veins. These veins have smooth muscle cells, elastic fibers and a homogenous basement membrane, but they do not have an internal elastic lamina (which are characteristic features of arteries).

The skin has a rich nervous supply. On reaching the dermis, nerve fasiciculi branch extensively to form a deep reticular plexus (22,23). This plexus serves the dermis, including most hair follicles (arrector pili), sweat glands and larger arterioles. From this plexus many fasiciculi pass to ramify in the papillary plexus at the border of the papillary and reticular dermis. Branches pass more superficially from this plexus terminating on reaching the basal epidermis, or in relation to encapsulated receptors. Sensory nerves are derived from the neural crest (posterior root ganglia), while the motor fibres are derived from cells of the sympathetic ganglia. The guidance of the developing axon by for example neurotrophins, has been a subject of considerable differences in opinions (22,24-27). In the development of peripheral nerves, axonal outgrowths precedes migration of Schwann cells. These two soon form a functional unit of migration, the nerve fibre, whose progress is guided by local conditions. It is likely that axonal growth and guidance depend on a fine balance of cell surface and extracellular matrix molecules (28). One of the most accepted theories is that growth cones are guided by some form of attraction called the neurotropism or chemotropism hypothesis (29). For example in the developing epithelium of the mouse face, studies have indicated that a chemoattractant originates at the epithelium that lures sensory afferents of the trigeminal system (30).

Capillary malformations (port-wine stain) anatomy

It has been suggested that CM are congenital lesions confined to the upper dermis (31-34). With advancing age capillary malformations undergo progressive ectasia resulting in the typical red-to-purple lesions (31,35). Ectasia of vessels was found to begin at age 10, with histologic findings in younger children revealing a normal vasculature with regard to number and size of blood vessels (36).

By staining endothelium with the PAL-E antibody the pathology of capillary malformations has been attributed to represent capillaries and / or medium sized venules or small veins (35). Braverman et. al. studied a capillary malformation in a 60-year old male by light and electron microscopy and by 3-dimensional reconstruction from photomicrographs of 1-im sections (32). The vascular dilatations were found to represent only post-capillary venules. In the papillary dermis vessels consist of terminal arterioles, arterial and venous capillaries and postcapillary venules, with the postcapillary venules being the majority of the vessels in this layer. Electron microscopy by Schneider et al revealed ectatic venules, in the subepidermal plexus and the first third of the reticular layer, while only a few patients had their subepidermal capillaries widened slightly (37).

Barsky et al have shown that CM consists of increased number of ectatic vessels. The walls of these vessels are thin, while the lining endothelium cells are flat (31). Several studies have proven that the endothelial cells lining the ectatic channels function normally (35,38). Immunofluorescence studies of the three major constituents of the vessel wall, fibronectin, factor VIII and collagenous basement membrane-type IV collagen, did not show abnormalities in the vessel wall (39). It has been proven in a three-dimensional histological reconstruction of capillary malformations, that multiple clusters of small diameter (10 - 50mm) blood vessels occur (40). Smoller and Rosen showed that the density of the pericapillary neurons is abnormally low (41). Only 17% ± 10% (mean ± SD) of vessels studied in capillary malformations were associated with nerves. In normal skin 75% ± 11% of vessels coursed 0.03mm of a nerve ($p<0.005$). They further found no difference in vessel numbers in CM and normal skin. A significant reduction in the response of CM to both vasoconstrictor and vasodilator stimuli has been demonstrated suggesting a reduction in autonomic control (42). It has been demonstrated that CM show occasional nerve fibres and that there is a deficit in both symphathetic and sensory innervation only in connection to the dilated vessels (43).

Histology has revealed that thickening of the post-capillary venule wall is a constant feature (37). This was already visible in a six-year old child, but was more obvious in adults. The thickening was caused by laminated basement membrane, amorphous material and collagen fibrils. Immunohistological studies revealed an increased deposition of basement membrane components

such as type IV collagen, laminin and fibronectin (44). This is in contrast to studies performed by Finley et al (31). Electron microscopy by Schneider et al has shown that the pericytes were located in the inner part of the wall. Functionally they did not look more active than normal (37). A striking finding by electron microscopy was that bridged fenestrations were observed in nearly all patients examined.

Discussion

Although our knowledge about CM has substantially increased in the last two decades, there are still many unanswered questions. A combination of molecular and histopathological information will be needed to ultimately identify the involved pathology and the subsequent etiology. With our current quest to understand pathological conditions like cancer and diabetes mellitus, molecular research in the mechanisms of angiogenesis and vasculogenesis has provided us with useful information about the etiology of vascular malformations (review ref. 45). By defining the regional pathology involved, the relational anatomy of all the associated structures (such as nerves and muscles) becomes important. It is possible that not only vessels are involved in the pathology of vascular malformations, but that the surrounding structures maybe involved as well. In capillary malformations the decreased nerve innervation is a good example.

The treatment of CM has significantly improved with the flashlamp-pumped pulsed tunable dye laser (PDL) (47). By observing the results of PDL, information is gained that could inform us about the pathogenesis of CM. The optimal parameters for laser treatment of CM have been derived theoretically by making use of computer models (48-50). It is clear that for a certain combination of wavelength, pulse duration and radiant exposure, only a limited range of blood vessel size can be injured optimally (49,50). PDL with a wavelength of 585 nm and a pulse duration of 450 is can provide optimal thermal damage with minimal energy. In normal skin PDL with 7 J/cm^2 penetrates the skin approximately 1.5 mm in depth, only reaching the most superficial vessels (51). Higher radiant exposure eg. 10 J/cm^2 can penetrate to a depth of 2.5 mm. These penetration values are in normal skin, and is expected that this is less for capillary malformations. Larger vessels require more fluences because a larger area is heated and the absorption of light in blood prevents the blood in the centre of the vessel lumen to be involved in the heating process (8). Smaller blood vessels require higher fluences as well because the amount of energy lost by conduction of heat becomes a much greater fraction of the absorbed energy. This theory has been confirmed by histochemically by Hohenleutner et al (52). Thus the depth and the diameter of the vessels in capillary malformations will influence the response to PDL, this without

even considering other factors such as melanin content and the blood flow (8). By using the appropriate wavelength and supplying enough energy within the thermal relaxation time of the target chromophores (oxyhaemoglobin) it is possible to specifically damage the vasculature and the surrounding perivascular tissue (47). Despite findings from our institute indicating that children do not need less PDL sessions, it is often suggested in the literature that children have fewer PDL treatments than adults and that better treatment results are achieved with children (8, 53,54). It has been shown that lesions located in the centrofacial region respond less favorably to PDL (clearing 70.7%) than other head and neck capillary malformations (clearing 82.3%) (55,56). Lesions located in the V 2 dermatome in adults and children respond less favorably than other lesions on the head and neck. Centrofacial regions were defined as lesions located on the medial part of the cheek and the upper cutaneous lip and nose. Lesions located on the periorbital region, the neck and temple responded best to the PDL.

The slight differences in skin thickness observed between different parts of the body, are not sufficient to explain the differences in response on treatment with PDL. Structural characteristics such as the orientation of the dermis, the density of the vessels, nerves, adnexae and fibrous proteins all likely play a role in the response pattern (55). The central part of the face is characterized by embryonic fusion planes and close-set rigid sebaceous follicles embedded in a dense fibrous stroma which may also influence the result (55,57). The angiosome concept (discussed later) may shed some light on this topic.

Fiskerstrand et al examined 30 biopsies of patients with capillary malformations before PDL treatment (58). In 16 patients with a good response the vessels were significantly more superficially located. Poor responders had significantly smaller vessels. They further suggested that vessel diameter mainly determines the color of the capillary malformation. Pink lesions have the smallest diameter vessels and purple lesions the largest. Vessel depth was partly correlated with the color with red lesions being more superficially located than purple and pink lesions. It seems that pink capillary malformations have a poor response to PDL due to the small vessel size and deep location, while red lesions have a better response due to the superficial lesions (58,59). This is in contrast to results from Kane et al (60). They stated that flat, pink capillary malformations cleared the quickest, while red-purple, nodular lesions cleared the slowest. It is thus unclear if clinical characteristics have any predictive value (7). Making use of reflectance spectra for visible light from normal skin and capillary malformation skin, it has been indicated that the redness seen in capillary malformations depends on both the concentration of dermal blood as well as how it is distributed (61). By using a videomicroscope Motley et al, and later Eubanks et al, identified that when lesions had ectasia isolated to the vertical capillary loops (type 1 lesion), the response to treatment was better than when lesions also had ectasia in the horizontal superficial

ring pattern (type 2 lesion) (62,63). Type 3 lesions are a combination of type 1 and 2 and are likely to respond poorly to PDL. By using high-resolution ultrasound Troilius et al have recently shown that the depth of capillary malformations only correlates to some extend to the response of the capillary malformation has to PDL treatment (64).
Overgrowth is often associated with certain forms of CM and may indicate that those lesions have a component outside the dermis. Skeletal overgrowth may occur particularly in the maxillary region (65). There seems to be a correlation between the diameter of the subcutaneous vessels and the diameter of the subdermal vessels (14). Pearl et al have indicated that with the subcutaenous vascular plexus seen in the head and neck area, the larger arteries and concomitant veins were seen every 1 cm, while in the lower extremity they were seen every 8 - 12 cm. This compares to the locations of the most and the least concentration of CM.

Findings have suggested that the pathogenesis of CM is at least partly described by an abnormal neural regulation process (41). It has been postulated that the lack of sympathetic nerves to regulate blood flow is the cause of the progressive vascular ectasia. It has been suggested that the pathology lies in maturation of the cutaneous sympathetic innervation. CM lack not only sympathetic innervation, but also sensory innervation (43). Sensory neuron peptides produce, transport and release neuropeptides at the peripheral site. Substance P is an example of a neuropeptide known to stimulate smooth muscle cell growth. It is thus possible for CM to be a disease related to trophic effects of peripheral nerves. The dilated vessels in the dermis were found to have defective innervation, while other structures in the skin like sweat glands and hair follicles showed normal density of nerve fibers (figure 1). The nerve bundles were often seen to pass the ectatic channels without giving off any branches (43).
The lack of innervation of capillary malformation vessels can result in gradual ectasia of these vessels in response to increased perfusion. This may explain the progress of the disease from childhood to adult age (8). The larger venules have pericytes that are more randomly placed, with the pericytes not interdigitating with as many endothelial cells as in the post-capillary venule. These venules also have valves at the border with the subcutaneous tissue, which could contribute to the vessels becoming ectatic. It seems that capillary malformations are confined to vessels surrounded by pericytes and not smooth muscle cells, but further studies should investigate this. Historical histological studies performed by Miescher and Schnyder have shown that in young persons the vessels in CM had a lake-like distribution in the upper dermis, but that with increasing age the ectasias also involved the deeper dermal plexus and subcutaneous vessels (36,46). Normally bridged fenestrations occur only in venous capillaries. They are thin plates in the shape of discs that occur within the plasma membrane of venous capillary endothelial cells. They represent

areas where molecular exchanges are taking place at an accelerated rate between the circulation and the immediate surrounding tissues. In normal skin they are found in the venous capillaries surrounding sweat glands and hair follicles. There is little literature available about the role these bridged fenestrations play in CM. They are probably caused by increased passage of vesicles through the endothelial cells (34). Increased pressure on the walls by stasis may play a role. The consequence of bridged fenestrations is an increased permeability with perivascular deposition of amorphous exudates, which might influence the PDL result.

It has been suggested that the developing vascular plexus is normally followed by accompanying nerves, based on observations made in the forelimb of quail embryos by Taylor et al (69). Both nerves and blood vessels seem to undergo a highly stereotypic sequence of development. The close spatial relationship between nerves and blood vessels either suggest a high degree of developmental interdependence or shared patterning mechanisms. It is still not clear whether peripheral nerves use the blood vessels as a substrate for path finding in the limb bud or whether neural crest cells guide the developing vascular tree (69,70). The endothelial cells and axonal growth cues are guided by differential adhesivity cues provided by the extracellular matrix (ECM) of the enviroment which they invade, and both may respond in a chemotactic or trophic manner to cues provided by diffusible factors (71,72). The blood vessels and nerves appear to be inhibited to invade the same particular region, including the presumptive dermis (71,73). In the quail forelimb blood vessels are present at day 2,5 (corresponds to 3,5 weeks of human gestation) and that nerves are visible at day 4 (corresponds to 4,5 weeks in humans) (69). The definitive neurovascular anatomical pattern was established by day 7,5 (corresponding to 8 weeks in the human embryo). Martin et al suggest that nerves do not use blood vessels as pathways along which they develop (71). They suggested that nerves and blood vessels follow the same route during embryology, but that they respond independently to the same mescenchymal cues. Recently they made the interesting observation that normal wound healing is impaired in the absence of nerves (74). The mechanisms of invasion by endothelial cells (blood vessels) and axons (peripheral nerves) are similar: both have filopodia, while obstructions in front of the development are dissolved by secreting proteases (71).

Taylor and Palmer introduced the angiosome concept over a decade ago (66). They considered the human body to be composed anatomically of three-dimensional blocks of tissue supplied by particular source arteries. Most of these anatomic territories span between skin and bone, but some are completely submerged beneath the skin surface like the vertebral angiosome in the head and neck (67). In the extremities these angiosomes are arranged in longitudinal sectors,

but in the head and neck the angiosomes are often irregular and sometimes convoluted (68). The 13 angiosomes of the head and neck are supplied by branches of the internal carotid, external carotid and subclavian arteries. Blood supply to the skin follows the connective tissue network. Where tissues are fixed and rigid, the vessels travel within / close to these skin areas, emerging from around the fixed skin margins to subsequently radiate long distances in skin areas where the tissues are mobile (67). In the head and neck the skin of the face is attached in a circle around the skull base, zygomatic arches, orbits and the root of the nose. At the alar base, the anterior border of the masseter muscle and along the lower border of the mandible, the skin is tethered (67). At these fixed skin margins the main skin perforators pierce the fascia from their source arteries. They then radiate in the mobile skin. In the neck the main perforators emerge from source arteries where the skin is attached along the anterior and posterior borders of the sternocleidomastoid muscle, over the anterior border of the trapezius muscle and along the hyoid bone above the clavicula and the sternum below. When we look at the areas previously described as areas not responding so well to PDL treatment like the centrofacial area or the angle of the jaw, these areas seem to correspond well to the areas where the skin is fixed and where the skin perforators are located. This explanation is not applicable everywhere (eg. periorbital and neck) but it should be interesting to describe the areas not responding well to treatment to the angiosomes and to study whether any correlation can be identified.

The hair follicles and sweat glands in CM have a normal vascularity and nervous innervation (sympathetic cholinergic) when compared to the ectatic vessels (43). It has been proven that the reticular vascular plexus in the dermis gives separate lateral tributaries to the sweat glands and hair bulbs (11). By 14 weeks of development the dermis is invaded by the downward invagination of developing epidermal appendages (9). At this stage the two dermal plexusses are also distinguishable. Due to economical reasons the hair follicles have been studied in more detail in sheep than in humans. The primary elements of vascularization at the bursae pili start by day 78 of prenatal development (75). They receive their vascularization from the subepidermal vascularization. El-Bab et al have shown that in sheep the hair papillae only start to receive their vascularizing vessels by the 104'th day of gestation (75). There are separate vessels from the subepidermal vascular plexus supplying the hair papillae.
In humans most hairfollicles have an arrector pili muscle, excluding the follicles on the face (23). Hairfollicles also have sensory innervation; free nerve endings around the hairfollicles. In CM there is decreased innervation to the pericytes in the postcapillary venules, while the innervation of the smooth muscles of the hairfollicels are not affected. Skin appendages eg. sweat glands and sebaceous glands are ectodermal in origin and are innervated by cholinergic sympathetic

fibers, while dermal blood vessels are mesodermal from origin and innervated by sympathetic adrenergic fibres (23). It is possible that the peri-endothelial support cells release growth factors to attract the axons that differ between these groups. It is likely that by gaining information about the difference in innervation of pericytes and smooth muscle cells, that more information about CM will also be gained. It seems that the pathologic process involved in capillary malformations occurs before the invagination of the ectodermal appendages (week 14), because the innervation of structures (eg. smooth muscle cell of hairfollicles) developing after this time seem normal. There seems to be a "window of opportunity" for the capillary malformations to occur if the developing nervous system is susceptible (by genetic factors?) to infections, lack of certain vitamins, mechanical or other factors. With more than 80% of capillary malformations occurring on the face, it seems that there is a specific period when the developing neurons in the face are more susceptible resulting in an "abnormal" development.

The first description of familial multiple nevi flammei was presented in 1949 by Shelley and Livingood (76). Since then there have been a few descriptions of families with multiple capillary malformations, suggesting a possible autosomal dominant inheritance with variable expression (45). Although a hereditary basis underlaying the development of capillary malformations is suggested, they clearly represent a minority of the total cases of capillary malformations seen. It is likely that genes implicated in these familial cases may be involved somatically in the more common sporadic cases. Recently a locus for an autosomal dominant disorder in a three-generation family has been mapped to chromosome 5q13-22 (77). It has been proven that the vascular endothelium and the surrounding support cell reciprocally influence each other, and it is likely that any disruption in the cellular physiology of either cell type can result in dysfunction (78). Although the candidate interval (5q13-22) at present is large, further studies and refinement of this region and mutation analysis of the described genes will determine if any of these loci are responsible for the phenotype.

Recent papers have informed us about the role pericytes play in the control of the developing vasculature (78,79). There seems to be a delicate reciprocal interaction between pericytes and endothelial cells. The endothelial cell further has several synthetic properties which are important in the endothelium's interaction with vasoactive amines of mast cells and nerves (80). A defective endothelial function may result in a defective neurite outgrowth. Although we have seen that endothelial cells are histologically normal in CM's, we know very little about their protein synthesizing function which may in theory influence pericyte development. During vasculogenesis endothelial cells are derived from mesoderm. Embryonic data suggest that these initial enothelial

tubes may be responsible for the subsequent development of the peri-endothelial support cells (78). The exact mechanisms by which endothelial cells recruit pericytes during vessel formation is unclear. Since there appears to be a tight control between the number of endothelial cells and mural cells, it is likely that multiple sites of control exist (78,79). Pericytes have more points of contact on endothelial cells than smooth muscle cells with the subsequent chance of problems arising at the pericyte site being bigger (11). Potential regulators include soluble factors acting in a paracrine and/or autocrine fashion, gap junctions, adhesion molecules, mechanical forces secondary to blood flow and blood pressure, as well as homotypic (enothelial cell - endothelial cell, mural cell - mural cell) and heterotypic (enothelial cell-mural cell) cell interactions. The anatomy of the smooth muscle cell and pericytes in relation to the endothelial is such, that decreased innervation in both will cause dilatation much faster on the venous side than on the arterial side.

Endothelial cells recruit mesenchymal cells via the elaboration of factors such as platelet-derived growth factor (PDGF), heparin-binding epidermal growth factor (HB-EGF) or basic fibroblast growth factor (bFGF) (78,80) (figure 2). The mural cell precursors migrate to the endothelial cells where they make contact with each other. Interactions between these cells lead to activation of transforming growth factor-beta 1 (TGF-b 1) (80). TGF-b 1 induces the mesenchymal cells to express pericyte markers and inhibits endothelial cell proliferation. Growing endothelial cells synthesize PDGF and HB-EGF which are potent stimulators of pericyte proliferation (83). TGF-b 1 is located in our candidate gene region (5q13-22), and pericyte growth has been shown to be inhibited by TGF-b (83). The tyrosine kinase receptor, TIE-2 binds the growth factor angiopoietin-1 and is specifically expressed in vascular endothelial cells (84). Angiopoietin-1 mutant embryos also have abnormal vasculature architecture due to the failure of endothelial cells to recruit pericyte and smooth muscle cell precursors to the developing vessel wall (85). Other possible genes located in the 5q13-22 region are the transcription factor myocyte enhancer factor-2 C (MEF2C), FER gene and the EFNA5 protein. In a study performed by Lin et al targeted deletions of the mouse MEF2C gene resulted in severe vascular abnormalities (86). Endothelial cells were present and able to differentiate but failed to organize normally in a vascular plexus. Peri-endothelial support cells failed to differentiate in the mutant embryos. The vascular defects in MEF2C mutant embryos resemble those in mouse mutants lacking VEGF and Flt-1 and suggest that MEF2C is required either directly or indirectly for VEGF signaling during vasculogenesis. Drake et al have proven that the deletion of the MEF2C gene resulted in defects that were accompanied by a reduction in angiopoietin-1 and VEGF mRNA production, indicating that MEF2C is required for expression of important endothelial-directed cytokines (87).

Although many factors have been identified that influence the nerve growth cone growth, it is still unclear how the growth direction is specified (69). Contact guidance mechanisms have to operate parallel with neurotropism, and physical cues in the pathway will probably also play a role (23). There seem to be a variety of attractive and repulsive molecules expressed within the extracellular tissues as substrate bound or diffusible gradients (24-27). The possible role of contact inhibition in developmental processes has also been investigated. Culture experiments have indicated that when chick peripheral sensory neurons are confronted, their mobility is inhibited and that their growth cone will collapse (23,88). F-actin accumulates in the lamellae and at the sites of contact with the target cells (28). It has been suggested that the increased F-actin concentration may be responsible for the attractive guidance. During development both motorneuron and sensory axons choose the correct peripheral nerves from the onset (88,90). It also appears that the motorneuron axons influence the pathfinding of the sensory axons (89). It is suggested that a target-derived growth factor causes axons to grow towards the source of the factor (91). In experiments in which muscle-less limbs were created, muscle nerves did not form (92). Martin et al have also proven that the early removal of the skin results in the absence of the cutaneous nerves that normally supply the denuded region (71).

Several neurotrophic factors have been identified that influence the turnover of vertebrate neurons and only some will be mentioned (24-27). Nerve growth factor (NGF) has been identified to have an in vitro and in vivo influence on nerve cell growth (93). Antibodies to NGF caused the death of neuronal subsets at times when they reached their peripheral targets, and added NGF rescued the neurons that would otherwise die. Several other neurotrophins have been identified of which neurotrophin-3 and NT-4/5 have been identified by molecular cloning (94,95). NT-3 has been shown to be essential for the normal development of atria, ventricles and cardiac outflow tracts, suggesting the wider function of this neurotrophin (96). Other growth factors to influence the growth and survival of neurons include the *fibroblast growth factors* (FGF) (97). There is increasing evidence that the neurite outgrowth also depends on the presence of electric fields, originating in the neural tube itself and in the skin. McCaig et al have proven that in vitro the growth cone behavior is severely influenced by the presence of electric fields (98). It is subsequently possible that neurotrophins and endogenous electric fields also interact in vivio. The EFNA 5 protein is also located in the candidate area (5q13-22) and it has been suggested that this protein may be involved in axon guidance (99). Arregui et al have further indicated that the FER protein is involved in neurite outgrowth (100). Also located in our candidate region on chromosome 5 are neurogenin (Ngn) and NeuroD. Both are able to activate neurogenesis, but it is suggested that Ngn expression precedes that of NeuroD (review, 101).

It is possible that peri-endothelial support cells release mediators / growth factors that causes

axons to grow towards these pericytes. A defective release could subsequently cause a defective nervous innervation. Further studies on CM's should also be directed at the innervation of the dermal vasculature. Although histological studies have demonstrated that the ectatic part involved in CM is confined to the venular part, we know little about the real innervation of the arteriolar part and further research will have to clarify this aspect.

Conclusions

Since the pathology involved in CM seems to be located in the post-capillary venules and small venules, it seems that our definition of port-wine stains being capillary malformations is wrong. One important pathological characteristic detected in "capillary malformations" so far is the decreased neural innervation. It thus seems legible to describe these lesions as a neural malformation as well. Maybe the description of port-wine stains being a venular / neural malformation is the best description to date. It is even possible that all the vessel deformation is secondary to the neurological pathology, and that port-wine stains are pure neural malformations with vascular dilatation being secondary. Only time will tell. Since the biological classification of vascular malformations by Mulliken and Glowacki has been of enormous value to bring at least some clarity in these issues, we suggest continuing using this system until a new universally accepted classification will arise from further histological and molecular studies. With the common developments of the vascular system, nervous system and lymphatic system it seems logical that future investigations on capillary malformations will concentrate on the "genesis" as a whole and not only on vasculogenesis and angiogenesis.

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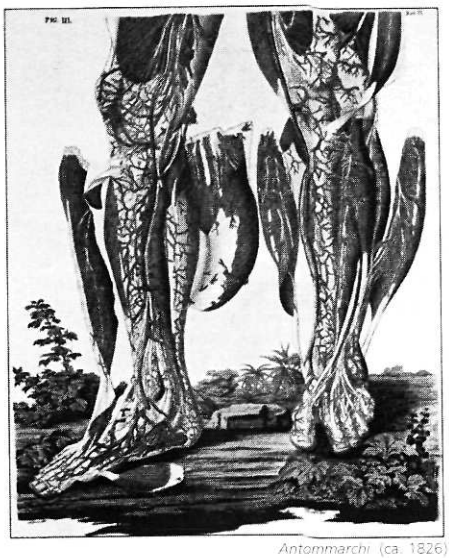
Use of MRI for the Evaluation of Vascular Malformations of the Lower Extremity



Antommarchi (ca. 1826)

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Plastic and Reconstructive Surgery 108(4):570,2001

Introduction

With the biological classification of Glowacki and Mulliken it is possible to diagnose congenital vascular anomalies as being either hemangiomas or vascular malformations (1). Nearly 90% of lesions can be differentiated by history and clinical evaluation (2). Vascular malformations are the result of developmental errors of vascular morphogenesis. By definition vascular malformations are always present at birth, but often they present clinically later in life (1,2). They present with a wide range of abnormalities; from small and insignificant capillary nevi to large and hemodynamically important arteriovenous fistulae (2,3). Clinically lesions grow commensurately with the child. Trauma, sepsis or hormonal changes can exacerbate progression of the lesion (1,2). Vascular malformations are anatomically subdivided according to the predominant channel anomaly into either capillary, arterial, venous and lymphatic, or combinations (1-3). They can be further subdivided into high- or low-flow malformations. Any lesion that has an arterial component is considered a high-flow malformation.

Once the diagnosis of a vascular malformation is made it is of paramount importance to define not only the flow characteristics but also the full range of extension since the prognosis and appropriate treatment vary substantially for each type of anomaly. The two most useful non-invasive imaging techniques for assessing vascular malformations are Magnetic Resunance Imaging (MRI) and ultrasonography (4-16). MRI is a non-invasive method and at this moment it is the best single modality to demonstrate detailed information regarding the involved anatomic structures, extent and flow characteristics of vascular malformations (4-6,8,11). This information is vital to plan possible imaged-guided procedures or surgery. Although surgeons involved in treating patients with vascular malformations will have their requested MRI interpreted by a radiologist, it is imperative for these surgeons to also be able to understand and interpret the described MRI features before intervention is anticipated. The aim of this review is to give surgeons involved in treating patients with vascular malformations an opportunity to gain some background on MR images when assessing vascular malformations. Although MRI is a powerful modality for assessing vascular malformations, we will also discuss some of the limitations of MR imaging. We further suggest a diagnostic flow chart based on MRI features designed to help determine the composition of a vascular birthmark when intervention is anticipated. For the more thorough reviews on especially the radiographic features the reader is referred elsewhere (11-14).

Low-flow vascular malformations

The diagnosis of venous malformations is strongly suspected by clinical characteristics: they are blue and are easily compressible and increase in size when venous pressure increases (2,13). Many venous malformations cause pain. Most of the venous malformations consist of spongy masses of sinusoidal spaces having variable communication with adjacent veins. The most common symptomatic vascular malformations of the extremities are venous (2,11). Venous malformations may further also consist of dysplasias of large and small venous channels. Lymphatic malformations are like venous malformations low-flow lesions. Lymphatic components of the malformation may contain cystic structures of various sizes varying from macrocystic to microcystic (2,11,13,19). Thoracic lesions are usually being macrocystic, and (the more common) cervicofacial lesions microcystic (14). Lymphatic malformations often have a rubbery or cystic component, but unlike venous malformations they cannot be manually compressed. Often the overlying skin contains small lymphatic vesicles or capillary malformations or both (14). Superficial vesicles are sometimes seen on the skin representing extensions of deeper laying lymphatic malformations (2).

Clinical features can often differentiate between high- and low flow lesions, but with ambiguous lesions, low-flow lesions can consistently be distinguished from high-flow lesions on the basis of MR findings (4-14) (Table A). Low-flow lesions are characterized on MRI by a serpentine pattern with internal striations and septations. Pathologically these findings correlate with fibrofatty septa between endothelium-lined vascular channels, or intervening muscle fiber remnants (4). The high signal intensity seen on the long TR/TE spin-echo (SE) sequences, is attributed to stagnant blood flow in these abnormally, dilated venous spaces. The hyperintens meshwork of low-flow malformations is also called "venous lakes". These features though characteristic, are not pathognomonic of low-flow malformations, also occurring in rare tumors such as angiosarcomas or myxoid tumors (17,18).

Table A: MRI characteristics of vascular malformations

	Low-flow anomalies	High-flow anomalies
T 1-weighted Image	1. Increased signal intensity when compared to fat and skeletal muscle	1. Signal voids 2. Small amount of tissue matrix
T 2-weighted Image	1. Decreased signal intensity when compared to fat 2. Increased signal intensity when compared with with skeletal muscle	1. Signal voids 2. Small amount of tissue matrix

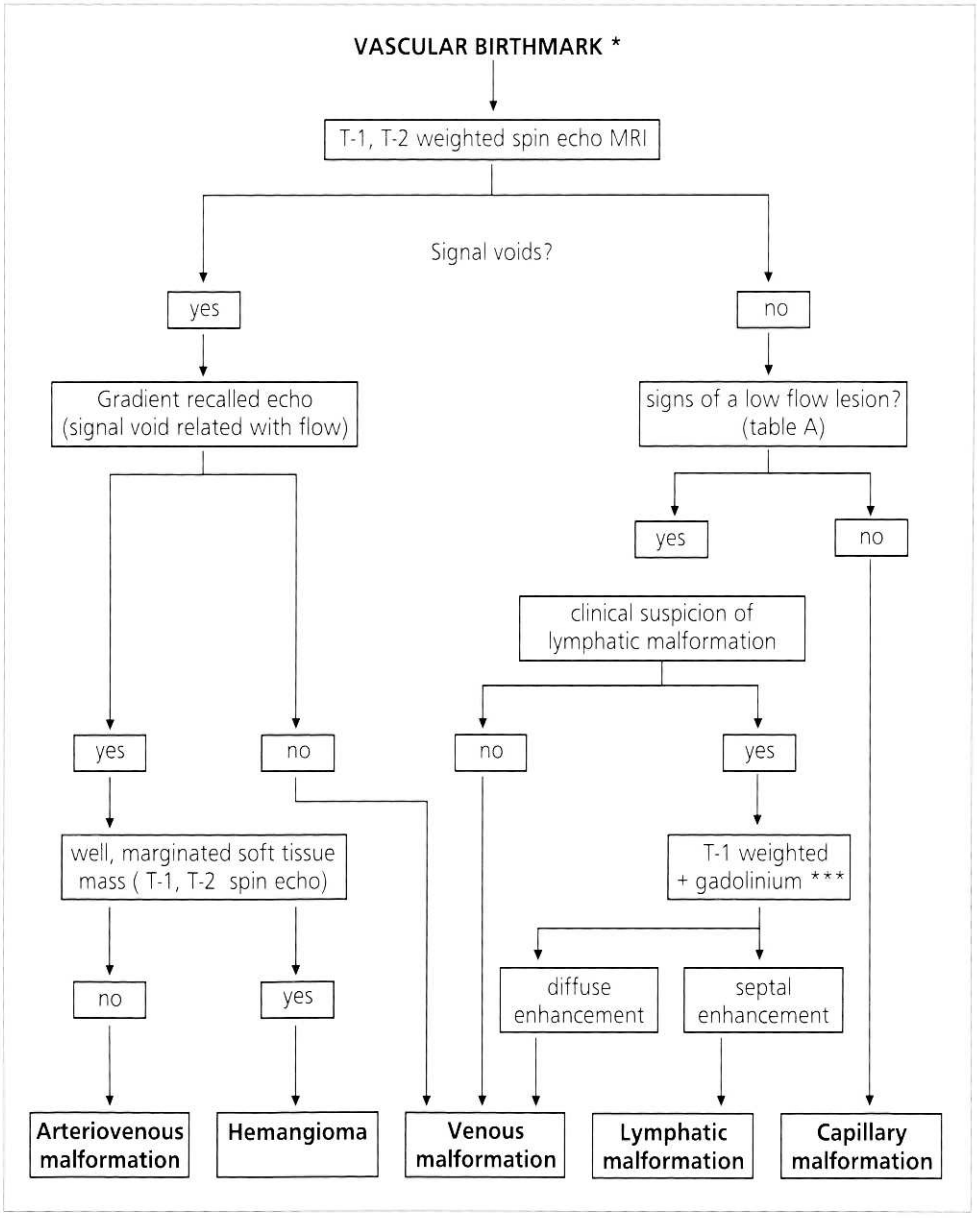

Figure 1:
Suggested decision tree to help determine the composition of vascular birthmarks based on MRI characteristics. * Hemangiomas with a clear history of rapid enlargement followed by involution are not included. ** Capillary malformations have only minor skin thickening on MRI. *** Contrast medium plays an important role in the evaluation of vascular malformations. Some authers suggest giving contrast to all vascular malformations. We only administer a contrast medium when there is a good indication as mentioned in the text i.e. clinical suspicion for a lymphatic malformation.

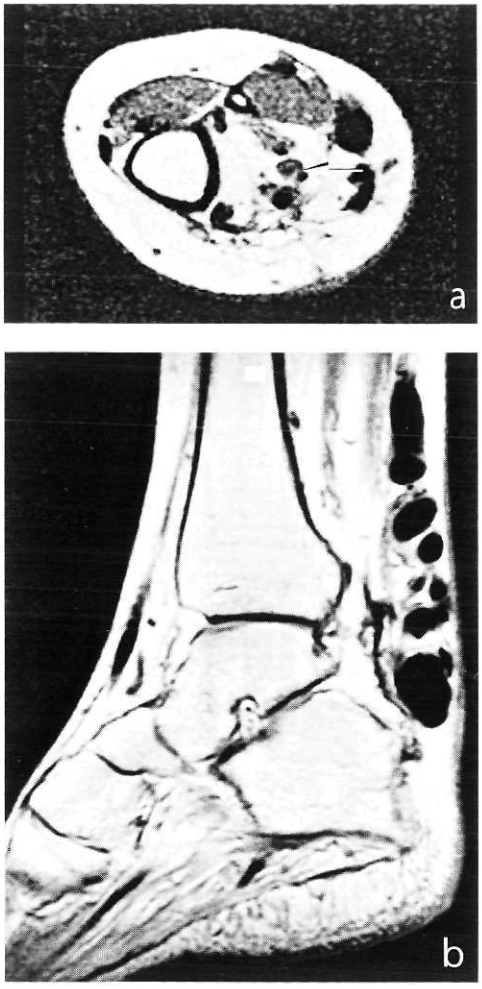

Figure 2.
A T2-weighted transverse image 5-cm above the ankle joint taken of a female patient with a pulsatile mass located just above her ankle on the dorsal side of the leg. Figure 2(a) is an axial image taken 5 cm above the ankle joint, with the arrow demonstrating a signal void. The high-flow malformation is located primarily in the posterior compartment of the leg. This compartment is largely replaced by fibrofatty tissue/muscle atrophy. Figure 2(b) is a sagittal image demonstrating large signal voids.

With administration of a contrast medium eg.gadolinium, septal enhancement may be visualized in lymphatic malformations, defining the septations or cysts of macrocystic lymphatic malformations (11). Microcystic lymphatic malformations may appear quite homogeneously in signal intensity. The contents of the lymphatic cyst are not enhanced with the administration of intravenous gadolinium. Fluid levels seen with macrocystic lymphatic malformations suggest hemorrhage or proteinaceous contents (19,20). T-2 weighted MRI of venous malformations shows a hyperintense mass, that enhances diffusely with intravenous contrast. Fat suppression on the T-2 weighted image is recommended so that venous malformations can be separated from the high signal of subcutaneous fat (4-6). Signal voids in venous malformations on spin-echo sequences, have been explained to possibly be thrombosed vessels, phleboliths, linear, fibrous striations cut in cross section or small AVF's (4,6,10,11). Contrast administration results in variable enhancement and is important to document veins with extremely slow flow that may not be seen on MR venography (14). The addition of gradient-recall-echo (GRE) sequences in imaging vascular malformations, may show high signal intensity within areas of void on SE images, indicating that these areas are flow related (10,11). A drawback of GRE is that the difference between high-flow and low-flow lesions is not so well described on GRE images as seen on spin-echo sequence (4). A previous, recent X-ray or CT scan may also suffice in diagnosing a phlebolith. The presence of

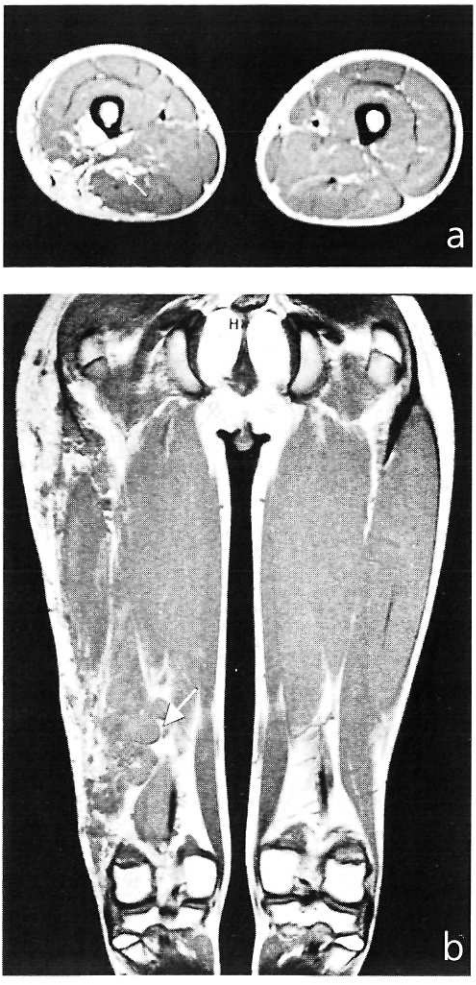

Figure 3.
A 14-year-old boy was referred with pain in his right upper leg. He had a lateral venous anomaly that was resected four years earlier, but now he has increasing pain in this leg. Figure 3(a) is a T-2 weighted axial image 10-cm above the knee showing a hyperintense image in the subcutaneous tissue, but also infiltrating the muscle and the femur. The white arrow shows a fluid level (in the quadriceps muscles), sometimes seen in a slow-flow malformation. Figure 3(b) is a coronal image indicating the wide area of infiltration. Above the knee some intra- muscular venous convolutes are clearly visible (arrow).

phleboliths has been described as being pathognomonic of venous malformations (21).

High-flow vascular malformations

Most arterio-venous malformations become symptomatic during puberty, except for the extremely high-flow lesions, where cardiac overload may present in infancy (14). Clinical features may include local hyperthermia, pulsations, thrill and a bruit. Extremity high-flow lesions are often associated with overgrowth. Flow voids seen in high-flow lesions are attributed to a "time-of-flight phenomena" or "turbulence-related dephasing" (6). Additional characteristics used to diagnose high-flow lesions are the visible feeding arteries and draining veins (hypertrophic high-flow vessels), the small amount of tissue matrix and absence of venous lakes in comparison to low-flow vascular malformations (4). High-flow lesions are known to have a variety of MRI findings, including some with hyperintense components on T2-weighted imaging sequences, focal accumulations of fatty tissue, hypertrophy of muscle and bone changes which include sclerosis or lytic defects (14). Hemangiomas consistently have high-flow signal voids within the lesion seen on spin-echo sequences during their proliferative phase (5,7). Proliferating-phase hemangiomas are distinguished from high-flow vascular lesions in that the later has no parenchymal component (13,14,18).

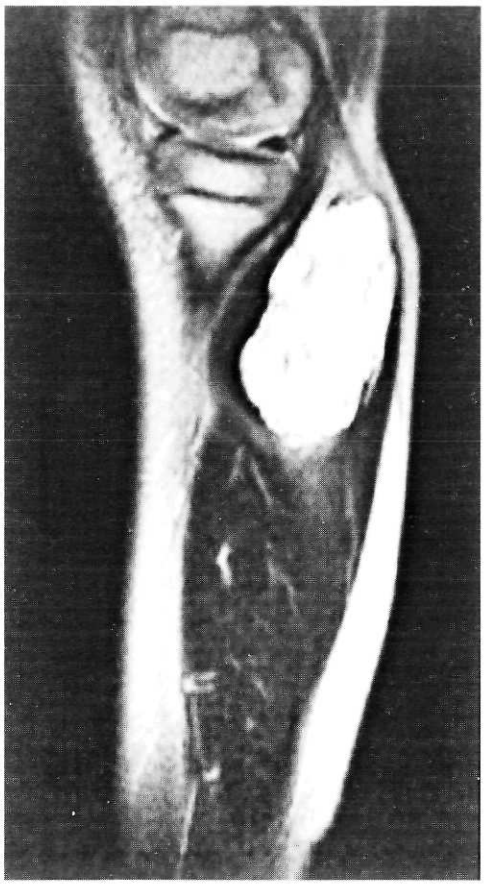

Figure 5.
A three-year-old boy was referred with a localized swelling just underneath his left knee joint. According to his parents it had rapidly increased in size in the first eighteen months of his life, thereafter the swelling had been stable. A sagittal T2 weighted image showed signal voids and a well marginated enhancing parenchymal mass located in the proximal head of the medial gastrocnemius muscle. The diagnosis of an involuting hemangioma was confirmed. Contrast injection is often needed to help differentiate between hemangiomas and malignant tumors.

Hemangiomas are usually focal, well-marginated soft tissue masses of low T-1 weighted and high T-2 weighted signal intensity. During the involuted phase, hemangiomas have signs of a low-flow lesion (5,8). Involuting hemangiomas retain their specific MRI appearances until they are replaced by fat. Congenital fibrosaroma is an important differential diagnosis in infant's (11). It is most common in the lower limbs and is also characterized by rapid growth (22). This lesion shows less homogeneous signal characteristics after contrast administration, while hemangiomas show diffuse enhancement with contrast (11). MRI does not distinguish between benign and malignant forms but can differentiate necrosis from solid tissue which can then guide the biopsy to make a definite diagnosis (22). It is best to always perform a biopsy of any atypical lesions when the clinical features, imaging results, or both are equivocal.

Imaging of endothelial lesions

The value of the MRI as initial diagnostic investigation in vascular malformations was already proposed by Pearce et al. in 1988 (23). We do not feel that MRI warrants to be the initial investigation in each patient. If only a diagnosis is needed and no immediate intervention is anticipated like so often in children, then gray-scale ultrasonography coupled with color Doppler flow imaging has the advantage of providing a

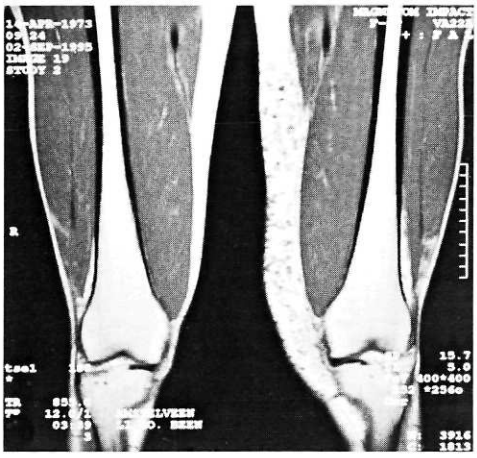

Figure 4.
A twenty-year old male presented with a swelling on the medial side of his left upper leg extending down to his knee. Although being visible for years, he only recently developed severe complaints of pain. Figure 5 shows a T-1 weighted spin echo (TR 850 / TE 12) coronal image of a low-flow malformation located subcutaneously with no muscle or intra-articular infiltration.

rapid, relatively inexpensive and non-invasive assessment of the vascular lesions (15,16). If the lesion is clinically a vascular malformation and intervention is anticipated, MRI is our initial diagnostic investigation. In that case it is of paramount importance to have an accurate estimate of infiltration. Although ultrasonogrophy is notoriously operator dependent, other limitations of ultrasonography include the small field of view, restricted depth of penetration, especially with high-frequency transducers; difficulty in depicting flat superficial lesions; and detecting tiny vessels with low flow (15). Ultrasonography does not ónly have an important role in diagnosing vascular malformations, but some feel it also has an important place in assessing lesions during follow-up and also in inventional radiology (13-16).

MRI is a non-invasive and non-ionizing method, with up-to-date no harmful side effects described (6). T1-weighted images are obtained to delineate the anatomy, while T2-weighted sequences were obtained to demonstrate the flow characteristics/pathology. For effective evaluation sequences are obtained in at least the axial and coronal planes, incorporating both extremities for comparison. Although there is no signal modality providing more information about the flow characteristics and internal structure as MRI, there are some limitations (4,5,7-9,17). MRI is a relatively expensive imaging modality and requires sedation in infants. It is further not possible to accurately visualize capillary malformations with MRI, except for the minor skin thickening (6,9). Sometimes increases in subcutaneous thickness or prominent veins are seen with capillary malformations (11). Although the different MRI characteristics of venous- and lymphatic malformations have been mentioned before, they can occur together. The administration of a contrast medium is important to separate the two components. Although MRI can diagnose high-flow lesions by the presence of dilated feeding arteries and draining veins, it cannot differentiate arteries from veins (4,7,8). If embolization is considered, angiography is necessary to delineate the nidus and supporting vessels in high-flow

Lesion	MRI				Ultrasound
	T1-weighted	**T1 contrast Enhanced**	**T2 weighted**	**Gradient Echo**	
High-flow					
Proliferating hemangioma	Flow voids Low signal soft tissue mass	Uniform intense enhancement	Flow voids High signal mass	High-flow vessels within and around soft tissue mass	Discrete soft tissue mass containing high-flow vessels
Arterio-venous malformation	Flow voids No focal mass, but soft tissue thickening	Diffuse enhancement	Flow voids Variable signal	High-flow vessels Abnormal tissue	High-flow vessels with softtissue thickening
Low-flow					
Involuted hemangioma	High signal mass (fat)	No	Decreased signal (fat)	No high-flow vessels	No flow voids Echogenic mass
Venous malformation	sointense to muscle on T1; ± high signal (trombi)	Diffuse or inhomogeneous enhancement	High signal mass Signal voids Phleboliths	Signal voids and no high flow vessels	Soft tissue mass of variable echogenicity
Lymphatic malformation	Low signal mass	Rim or no enhancement	High signal mass	No high flow vessels Fluid/fluid levels	Cystic (macrocystic) Echogenic (microcystic)

Table B:
Magnetic resonance imaging and ultrasonography features of vascular anomalies
(*adapted from* Burrows PE, Laor T, Paltiel H et al. Diagnostic imaging in the evaluation of vascular birthmarks. Dermatol. Clin.16:455-488,1998

lesions (5,24,25). The angiographic characteristics of high-flow lesions are dilated and lengthened afferent arteries, with early opafication of the enlarged efferent veins (21). Angiography entails an invasive intervention requiring use of ionizing radiation and contrast material with the associated sequella (26-29). In low-flow malformations no additional information is obtained with angiography. In the literature the results of MR angiography (MRA) are promising to delineate the nidus, and in the future MRA will undoubtfully have an important place (6,12). MRA can also be used to differentiate between the draining veins and feeding arteries. Currently in our center angiography is still performed more commonly then MRA. Angiography is only performed when a radiological intervention is suspected, and then often as a combination of angiography/embolization. Intracortical invasion of the vascular malformation is not well evaluated on MRI and when intraosseous vascular malformations and secondary bone changes are evaluated, it is better to perform a computed tomography scan (30). A disadvantage of a CT scan is that lesion size is often underestimated, and that it is impossible to determine flow characteristics.

In conclusion the treatment of vascular malformations still remains a great challenge to treat in modern medicine, even to the most experienced clinicians. Attributing to this problem is the extreme rarity of these vascular lesions (2,3). In our unit MRI has become the golden standard when confronted with a vascular malformation that warrants accurate evaluation for possible therapeutic intervention. Although no single imaging technique answers to all our questions, the exact reason for requesting an investigation should determine the choice of investigation requested. We suggest a diagnostic flowchart based on MRI features designed to help determine the composition of a vascular birthmark when intervention is anticipated.

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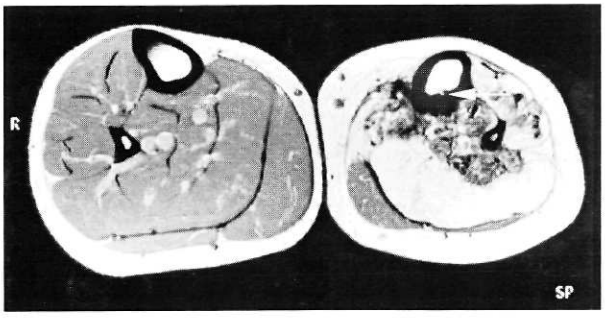
MRI Findings of Vascular Malformations of the Lower Extremity



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Abstract

Vascular malformations are congenital lesions resulting from a defect during embryogenesis. Magnetic Resonance Imaging (MRI) is a very effective method to demonstrate detailed information regarding involved structures, extent and flow characteristics of vascular malformations. In previous MRI studies most of the emphasize is laid on the difference between high- and low-flow lesions, while little detailed information is available about the extent of local tissue involvement. These additional characteristics may influence the approach in treating these malformations and improve understanding of the pathogenesis.

We retrospectively reviewed MRI's of 40 patients with vascular malformations of the lower extremity. Thirty-four patients had low-flow lesions, and 6 high-flow lesions. Of the low flow lesions 23 patients (67,6%) had muscle infiltration, with four of the six (66,6%) high flow lesions having muscle infiltration. Nine of the 11 males (81,8%) with low-flow lesions had associated muscle infiltration, in comparison to 14 of the 23 females (60,8%) with low-flow lesions ($p = 0,206$). Eighty percent of the vascular malformations located on the thigh with muscle involvement, had involvement of the anterior muscle group, while 86,6% of the patients with a vascular malformation located on the leg and with associated muscle involvement, had at least the posterior muscle group involved ($p=0,0049$). Ten patients (25%) of the whole group had bone infiltration. Low flow lesions often had multifocal lesions (20,5%); while associated muscle atrophy was visible in 10 low flow lesions and in two high flow lesions. In low flow lesions with muscle infiltration ($n = 23$), 43% ($n = 10$) had associated surrounding muscle atrophy ($p = 0,009$). Hypertrophy of the subcutaneous tissue was visible in 11 low-flow patients (32,4%).

The high amount of muscle and bone involvement in vascular malformations of the lower extremity is emphasized with this study. Of particular interest was the difference in affected muscle groups. The angiosome concept is used to explain this preponderance, and we feel the angiosome concept could also be used when assessing possible intervention. The surrounding muscle atrophy and multifocal nature of these anomalies are further important considerations when assessing the possibility of intervention.

Introduction

By performing a thorough medical history and clinical evaluation nearly 90% of vascular anomalies can be differentiated into either hemangiomas or vascular malformations (1,2). According to the predominant channel anomaly vascular malformations are anatomically subdivided into capillary, arterial, venous and lymphatic, or combinations (1-3). Based on flow characteristics vascular malformations can further be categorized in high- or low-flow malformations. If the type of flow is characterized and intervention is anticipated, the complete extent of the vascular malformation must be known before any form of treatment is initiated (2-8). In the past conventional radiography, Doppler ultrasonography, arteriography, venography and later contrast-enhanced computed tomography have been used as methods of investigating vascular malformations (2,4,6,9,10). Although some of these still have an important role in assessing vascular malformations, at this moment MRI is the most important single modality for assessing vascular malformations when intervention is anticipated (11,12). MRI is a non-invasive method to demonstrate detailed information regarding the involved structures, extent and flow characteristics of vascular malformations (11). Many studies have demonstrated the superiority of MRI in differentiating between high- and low flow vascular malformations (7,8,12-23). In most of these studies the difference between high- and low flow lesions is clearly described, but little detailed information is available that accurately delineate the vascular malformation. These additional characteristics could influence our approach in treating these malformations, and improve our understanding of the pathogenesis. The reasons for performing this retrospective study of vascular malformations of the lower extremity were to (1) provide more detailed information regarding the extent of local tissue involvement and (2), describe associated features of the tissue adjacent to vascular malformations of the lower extremity.

Patients and methods

Between January 1996 and July 1998 54 patients with vascular malformations of the lower extremity were referred to the congenital vascular anomalies team in our institution. Patients were referred to our hospital by specialists, and were referred for diagnostic purposes and/or possible treatment considerations. From this group, 40 patients had a MRI made either in this institution or were referred with a MRI.

These 40 MRI's were analyzed by a radiologist without clinical knowledge of the patients. The

following parameters were considered: (1) flow characteristics, (2) muscle infiltration, (3) bone infiltration, (4) intra-articular extension, (5) hyper- or hypotrophy of the muscle adjacent to the malformation, (6) hyper- or hypotrophy of the subcutaneous tissue, and (7) lesion focality being either uni- or multifocal.

Low-flow lesion characteristics were described as hyperintense signal intensities when compared with fat on T2-weighted images and predominantly decreased signal intensity when compared with fat on T1-weighted images. Low-flow malformations further have a hyperintense signal intensity compared with skeletal muscle on both T1- and T2 weighted images. High-flow lesions were diagnosed when (flow-related) signal voids were seen on the T2-weighted images (TABLE A). Contrast medium (for example gadolinium) was not used consistently in the past and the use is subsequently not included in this study.

The results were analyzed by descriptive statistical analysis using the Statistical Package for the Social Sciences (SPSS 8.0) for Windows, or StatXact3 (24). Proportions between groups were compared using (Fisher's) exact tests. Influence of age between groups was compared by using the Wilcoxin-Ranktest. If the p values were less than 0.05 they were considered to be statistically significant.

Table A: MRI characteristics of vascular malformations

	Low-flow anomalies	High-flow anomalies
T 1-weighted Image	1. Increased signal intensity when compared to fat and skeletal muscle	1. Signal voids 2. Small amount of tissue matrix
T 2-weighted Image	1. Decreased signal intensity when compared to fat 2. Increased signal intensity when compared with with skeletal muscle	1. Signal voids 2. Small amount of tissue matrix

Results

Of the 40 patients were MRI's were performed, 27 were female and 13 male. The mean age of patients when the MRI was performed was 20 years and ranged from 3 to 58 years (figure 1). Thirty-four patients had MRI characteristics indicating low-flow lesions, while six patients had flow-related signal voids indicating a high-flow lesion. All six high flow lesions were located on the leg, while the low flow lesions were evenly distributed over the whole of the lower extremity.

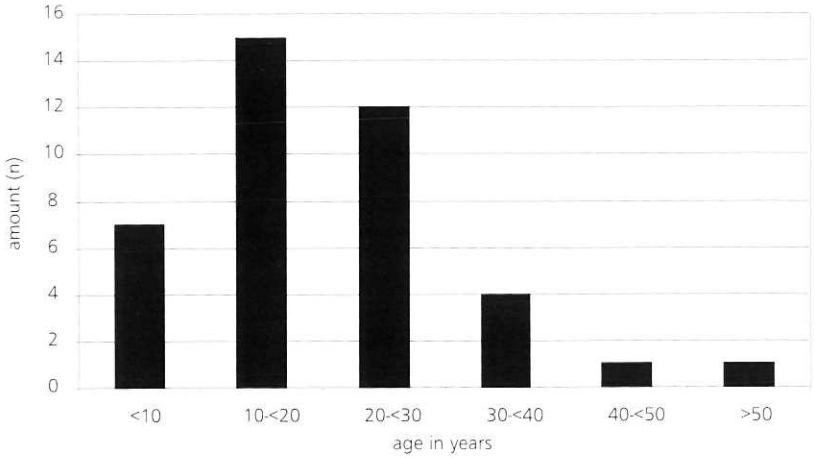


Figure 1:
Age groups when MRI was performed.

	Low	High
Total	34	6
Age (mean) *	19.4	24.5
male	11 (32.4%)	2 (33.3%)
female	23 (67.6%)	4 (66.6%)
Infiltration		
Muscle	23 (67.6%)	4 (66.6%)
Bone	7 (20.5%)	3 (50.0%)
Associated		
Muscle atrophy	10 (29.4%)	2 (33.3%)
Subcutaneous hypertrophy	11 (32.4%)	1 (16.7%)
Multifocal lesion	7 (20.5%)	1 (16.7%)

Table B:
MRI findings of low-flow and high-flow vascular malformations with regard to frequency, * mean age in years when the MRI was performed, gender distribution, muscle - or bone infiltration, and associated lesions. All percentages given are for the group as a whole, i.e. either low- or high-flow lesions.

Eleven of the 13 males (84.6%) and 23 of the 27 female patients (85.1%) had low-flow malformations (TABLE B).

Twenty-seven patients had muscle infiltration, of which 23 were low flow lesions. Of the low flow lesions three patients had infiltration in only one muscle, 6 patients had two or three muscles involved, while 14 patients had more than three muscles involved. Of the group as a whole, 11 of the 13 males (84.6%) and 16 of the 27 females (59.6%) had muscle infiltration (p

			gender		
Flow			male	female	Total
low flow	muscle involvement	no muscle involved	2	9	11
		one muscle	2	1	3
		2-3 muscles	2	4	6
		more than 3 muscles	5	9	14
	Total		11	23	34
high flow	muscle involvement	no muscle involved	0	2	2
		one muscle	1	0	1
		more than 3 muscles	1	2	3
	Total		2	4	6

Table C::
Comparing the low- and high-flow lesions and gender with associated muscle infiltration.

= 0.105) (TABLE C). Nine of the 11 males (81.8%) with low-flow lesions had associated muscle infiltration, in comparison to 14 of the 23 females (60.8%) with low-flow lesions (p = 0.206). Of the 14 patients with vascular malformations restricted to the thigh, ten had muscle involvement. The different affected muscle groups are depicted in figure 2. Of the 15 patients with restricted involvement of only the leg, 12 patients had muscle involvement. Of these 12 patients, only two patients did not have involvement of the posterior muscle group. The affected muscle groups of the leg are depicted in figure 3. This difference between affected muscle groups in the low flow lesions was statistically significant different (p = 0.0049). Of the 8 patients with the vascular malformation located over both the thigh and the leg, three patients had muscle involvement. All three patients had the posterior muscle group involved in the leg. In the thigh one patient had no muscle involvement, the other the posterior muscle group and the third patient had involvement of the anterior muscle group. Of the 15 patients with a vascular malformation located in a muscle in the leg, 13 patients (86.6%) had involvement of at least the posterior muscle group. Of the 12 patients with a vascular malformation located in the thigh, 9 patients (75%) had involvement of the anterior muscle group.

Of the 40 MRI's performed, 10 patients (25%) had intra-osseous involvement (TABLE D). The mean age of the patients with bone infiltration was 24.9 years (median 20 years) in comparison to the 18,4 years (median 18 years) of the patients with no bone involvement (p = 0.43). Of the seven low-flow lesions with bone involvement, five were located on the distal half of the femur. All low flow lesions had muscle infiltration (p=0.043) with five patients having more than three muscles involved (p=0.03). Two of the three high-flow lesions with bone involvement had more

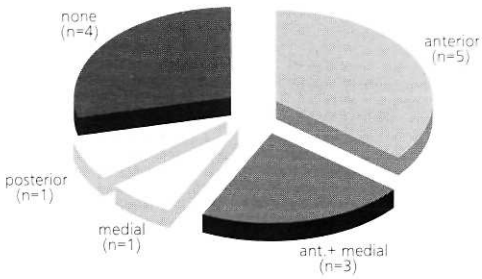


Figure 2:
The affected muscle groups when the vascular malformations were restricted to the thigh

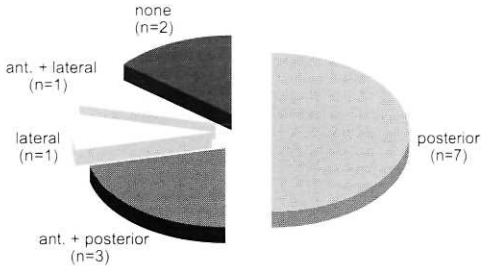


Figure 3:
The affected muscle groups when the vascular malformations were restricted to the leg

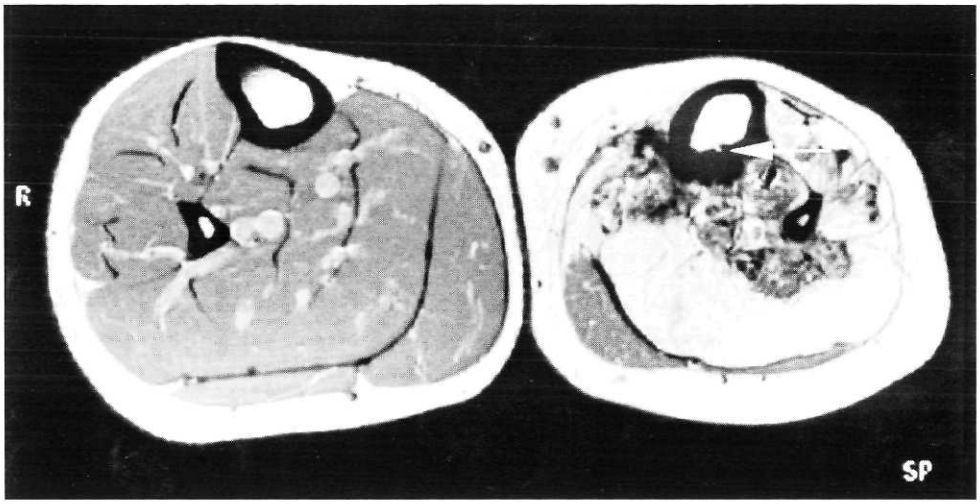
than three muscles involved. The one high flow lesion without muscular involvement had subcutaneous involvement of the lower leg, also affecting the calcaneus. Three patients had intra-articular extension; all being low-flow lesions and all affecting the knee joint.
Eight patients had a multifocal involvement. Seven being low-flow lesions, with all of these lesions having associated muscle infiltration ($p = 0.176$). These patients all had more than three muscles involved (p 0.07), with six of the seven low flow lesions affecting females ($p=0.27$). Three patients also having associated bone infiltration. The multifocal lesions were evenly distributed between the thigh and the leg.

Adjacent to the vascular malformation, muscle atrophy was visible in 12 patients, 10 of these having low flow malformations. Of the 23 low-flow malformations with muscle infiltration, 43%

			Bone involvement		
Flow			no	yes	Total
low flow	muscle involvement	no muscle involved	11	0	11
		one muscle	2	1	3
		2-3 muscles	5	1	6
		more than 3 muscles	9	5	14
	Total		27	7	34
high flow	muscle involvement	no muscle involved	1	1	2
		one muscle	1	0	1
		more than 3 muscles	1	2	3
	Total		3	3	6

Table D:
Intra-osseous involvement compared with muscle infiltration

Figure 4:
An axial T-2 weighted spin echo image 10 cm below the knee. The left leg of the patient shows a hyperintense mass in the soleus muscle, with both medial and lateral gastrocnemius muscles being atrophic. The peroneus and anterior tibial muscle are still identified, but the rest of the muscle groups have been largely replaced with fatty tissue. The left leg is much thinner then the right, but has subcutaneous hypertrophy in comparison to the right lower leg. There are no signal-voids visible. The low-flow malformation also infiltrates the periosteum of the left tibia (arrow).



(n = 10) had associated muscle atrophy (p=0.009). Seven of these 10 patients had three or more muscles infiltrated (p=0.07). Five of these vascular malformations were located on the thigh, four on the leg and one on both the leg and the thigh. Six of these low flow lesions had associated intra-osseous infiltration (p=0.01). The mean age of the patients with associated muscle atrophy was 24.6 years. The mean age of patients with no associated muscle atrophy was 18 years (p = 0.49). There where two low flow lesions with associated muscle hypertrophy of the surrounding muscles, both were located on the lower extremity.

Twelve malformations had associated hypertrophy of the subcutaneous tissue in the same leg. Eleven of these lesions were low-flow lesions, with 5 of these having associated muscle infiltration with no signs of subcutaneous involvement. Three female and one male patients had involved genitalia, all having low-flow lesions. The one male with an affected scrotum, also had intraperitoneal involvement. There was one female were both legs were affected. She had low-flow malformations located subcutaneously, with associated subcutaneous hypertrophy.

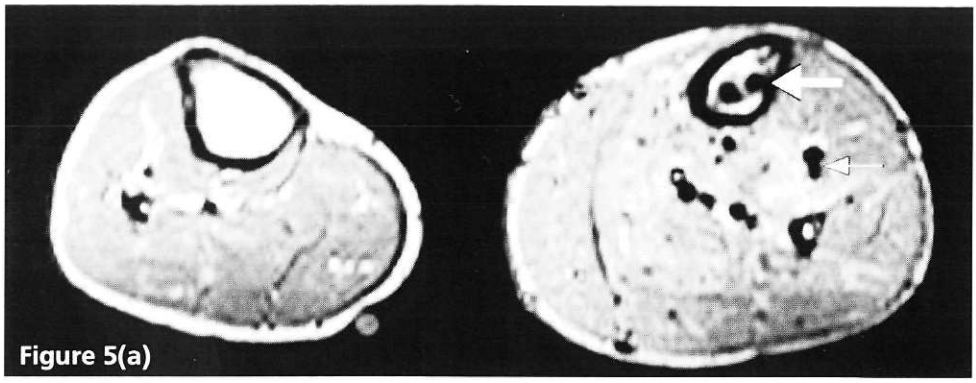


Figure 5(a)

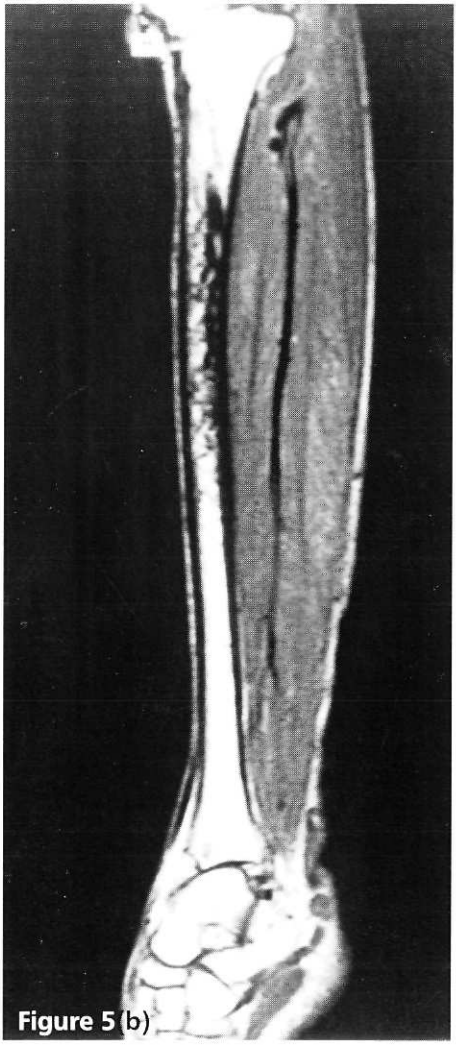


Figure 5(b)

Figure 5:
Figure 5(a) Transverse T-2 weighted MR image about 7 cm under the knee joint showing muscle hypertrophy in the affected extremity. In the muscle (thin arrow) and in the tibia (thick white arrow) are signal voids indicating a high-flow malformation. Figure 5(b) is a sagittal T-1 weighted MR image with depiction of the intra-osseous infiltration.

Discussion

Although history and clinical features can normally distinguish in over 90% of cases between hemangiomas and vascular malformations, special imaging may be necessary when vascular lesions have an ambiguous appearance (2,3,8). Even when the diagnosis is made, the lesion composition, flow and extent of the vascular malformation should be evaluated to define possible intervention. MRI is an excellent modality for elucidation of the nature and extent of infiltration of vascular malformations (7,8, 12,16,17). Vascular malformations in general have a 1:1, female: male ratio, with venous malformations being twice as common in females compared to male's (2). Venous malformations

are also the most common symptomatic vascular malformations affecting the lower extremities (2,12). With our descriptive study no definite diagnosis was made, but the female preponderance of 2:1 seen in our group seems to confirm the literature.

Although the amount of high flow lesions in our group is small (n=6), it was interesting to note that all these lesions were located on the leg, and in 66% (n=4) the distal third of the leg. In all six high flow lesions at least the dorsal side of the leg was involved. Low flow lesions were evenly distributed over the lower extremity.

Sixty-seven percent (23/34) of the low-flow vascular malformations in our study, and four of the six high-flow lesions (66%), had muscle involvement. Despite the relatively high incidence of male associated muscle infiltration (84,6%) in comparison to female muscle infiltration (59,6%), it was not statistically significant (p= 0,105). For any intervention to be successful the whole vascular malformation has to be obliterated radiologically and/or excised. It is imperative to calculate pre-operatively the amount of functional loss if the whole muscle (group) is excised. With our study we have seen that if muscle is involved, that in 86,9% (20 of the 23) of patients two or more muscles are involved, and that in 60% of cases (14/23) more than three muscles are involved. A particular interesting finding of this study was the difference in affected muscle groups between the thigh and the leg as depicted in figures 2 and 3 (p=0,0049). The difference in muscle infiltration between low flow lesions restricted to the thigh and the leg could be explained by the angiosome concept. An angiosome defines the three-dimensional vascular territories supplied by source arteries and veins to each tissue layer between the skin and bone. Tayler et al have proven in a recent study that the muscles of the anterior compartment of the leg were supplied by only one angiosome, while the other muscle groups of the leg received branches from two or more angiosomes (25). They have also proven that the zones between angiosomes represented by anastomotic vessels, occurs usually within tissue, especially muscles, not between them. In the leg multiple angiosomes supply the posterior muscles, in theory thus resulting in an increased possibility for the development of vascular malformations during embryogenesis. The angiosome concept is not so well described for the thigh, but our analysis suggest that here the anterior muscle group is supplied by multiple angiosomes, and that the posterior muscle group of the thigh is supplied by less angiosomes, thus resulting in less vascular malformations in this area. Historically the results of surgical management of vascular malformations, especially venous, have been disappointing (2-4,8). It is possible that the fear of function loss associated with complete excision, has resulted in many incomplete resections in the past. With incomplete resection, the residual vascular malformation may react in an aggressive

way due to a change in blood hemodynamics, often resulting in more symptoms than preoperatively (7,10). The lack of respect for facial anatomic planes seen in angiosomes and in theory also in vascular malformations is also important preoperative information.

Another possible explanation for the disappointing results of surgical intervention in the past could be our lack to realize the importance of intra-osseous involvement. In our study 25% had osseous involvement. Nine of the ten patients with bone involvement had associated muscle infiltration; seven of these nine patients had four or more muscles invaded by the vascular malformation. It is thus unlikely that sole surgical intervention will be successful in treating these patients. Most of the bone infiltration occurred around the distal half of the femur and the proximal half of the tibia, with only one high flow lesion infiltrating the calcaneus. The association of clinical features and the preponderance of bone involvement around the distal femoral epiphysis and the proximal tibial epiphysis will have to be evaluated further. Infiltrative growth is not a characteristic of vascular malformations and with the mean age of patients with intra-osseous infiltration not differing from patients without bone involvement (p=0,43), it is unlikely that intra-osseous involvement is caused by progression of the malformation.

Seven of our patients (20,5%) with low-flow lesions had a propensity for discontinuous multifocal involvement. This multifocal nature of low-flow malformations has been mentioned before by Rak et al (10). This multifocal nature is important information when planning treatment of vascular malformations. Especially patients with low flow lesions where more than three muscles are invaded by the vascular malformation, preoperative assessment for multifocal lesions is of paramount importance.

No associated muscle atrophy was found in lesions were no muscle was infiltrated. The high amount of associated muscle atrophy in low-flow malformations with muscle infiltration in our group (43%, $p = < 0,05$) was an interesting finding. We did not have any previous MRI's of the patients to compare them with, so we don't know if this atrophy developed with time. The mean age when MRI was performed on these 10 patients was not statistically different from the other patients (p=0,49). It is unlikely that this atrophy develops with time, but it may progress with time. Pre-operative it is thus imperative to calculate not only the functional loss caused by excision, but it is also important to assess the functional state of the remaining muscles.

Conclusions

Vascular malformations can present in a wide range of abnormalities, and often several investigations are necessary before an intervention is performed. MRI is an excellent method when confronted with a vascular malformation that warrants evaluation for possible therapeutic intervention. Our knowledge of the genesis of vascular malformations is still consisting of many " signal voids ", and maybe after molecular description these deficiencies in our knowledge will be cleared.

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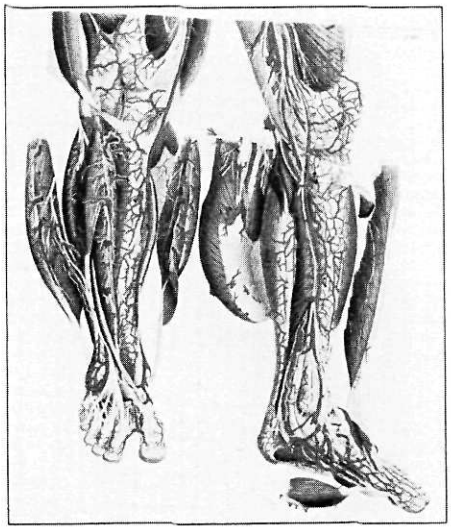
Vascular malformations of the lower extremity with osseous involvement



Mascagni (ca. 1785)

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Abstract

Vascular malformations are rare, congenital lesions which often have associated skeletal changes. Over a period of ten years, 90 patients at our clinic had their vascular anomaly of the lower extremity, examined by either CT-scan or MRI. Of these, 18 patients (20%) had bone involvement. A questionnaire was send to these 18 patients (8 male, 10 female) to evaluate their initial age of presentation, initial symptoms and current complaints. Radiological imaging revealed 15 low-flow lesions and three high-flow lesions. The mean age at presentation to a physician was 6 years of age. Pain was the most common complaint. Disparity in leg length of 2 cm or more was observed in ten patients. Of the 16 patients with muscle infiltration, 13 patients had four or more muscles involved, indicating that treatment by resection alone would require radical surgery.

Introduction

Although most vascular malformations are treated in specialised centres, it is important for surgeons in general hospitals to be able to describe them correctly. Descriptions are often confusing with terms like venous angioma's, cavernous angioma's, hemangioma's or cavernous hemangioma's being used (1). According to the International Society for the Study of Vascular Anomalies, vascular anomalies are classified into either vascular tumours (mostly hemangiomas) or vascular malformations (1-3).

Hemangiomas are common tumours and are distinguished by endothelial proliferation, characterised by a phase of rapid postnatal growth followed by slow involution. These lesions are rarely treated surgically unless for a recalcitrant ulceration or bleeding, or if they cause a functional deficit such as dyspnoea, or obstruction of the upper eyelid (2,3). Involution is nearly always complete by age ten years (1).

Vascular malformations have a different origin. They are rare congenital lesions caused by a defect during vascular embryogenesis. By definition these lesions are always present at birth, but sometimes only become clinically evident later in life (3). According to the flow in the lesion they can be categorised in either high- or low flow lesions. Any lesion with an arterial component is a high-flow lesion. Vascular malformations can be anatomically divided into either capillary-, venous, lymphatic or arterial- malformations, or combinations of the above. Several syndromes have been described of which Klippel-Trenaunay Syndrome (KTS) and Parkes-Weber Syndrome (PWS) are well known (1). KTS is characterised by capillary malformations, venous anomalies with bone and soft tissue hypertrophy of one or more limbs (1). Often there is an associated lymphatic malformation. PWS is known by the same characteristics except that PWS has arteriovenous malformations (high-flow lesions). Vascular malformations usually grow proportionally with the child, but sudden progression can be seen secondary to trauma, thrombosis, sepsis, hormonal changes or surgical intervention (3). The clinical symptoms of these lesions can vary considerably, ranging from small inconspicuous capillary malformations (port-wine stains), to large arteriovenous malformations causing an overflow congestive heart failure. Vascular malformations are always present at birth although they may not be evident (3). Most of these malformations present at an early age. Late presentation is particularly a feature of an arteriovenous malformation. Venous malformations (figure 1) are often visible early on in life either as a small blue patch or a soft blue mass (3).

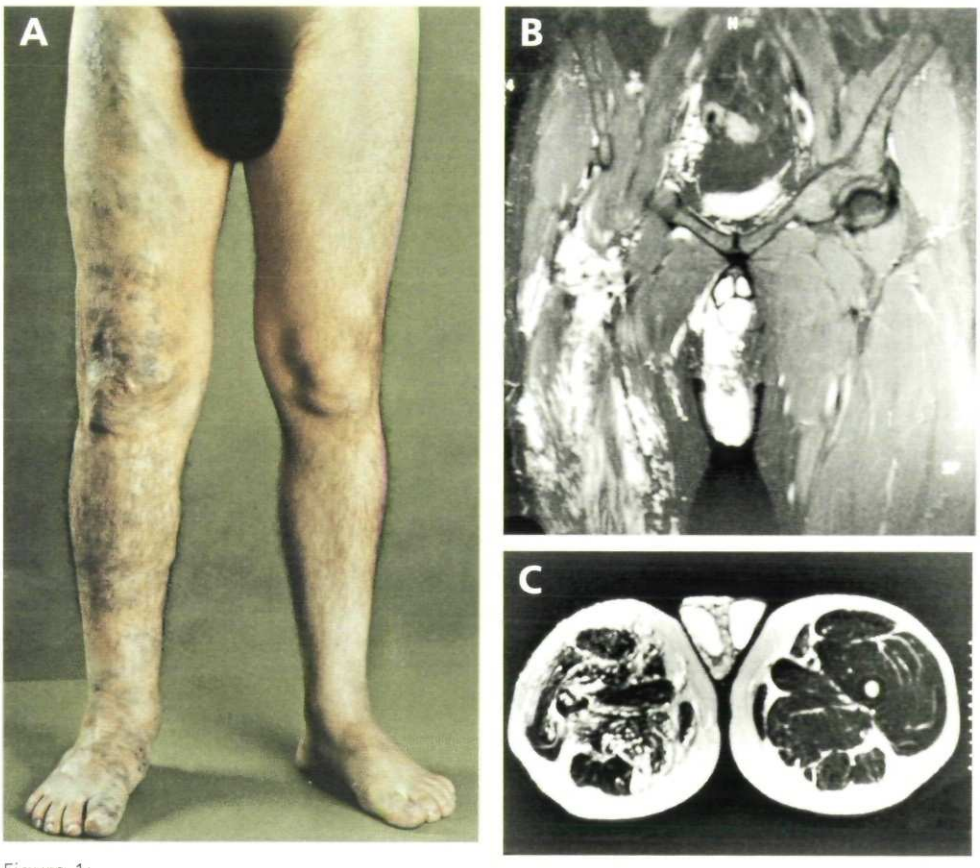

Figure 1:
Figure 1(a) - Photograph showing diffuse involvement of a venous malformation in the whole right leg. Figure 1(b) – The MR coronal STIR image shows the vascular malformation as areas of high signal intensity. The pathology can be appreciated in the right upper leg (left side of picture), extending towards the knee (not shown), in the scotum and in the pelvis. Note the asymmetry of vessels next to the bladder. Figure 1(c) – The MR axial T2 weighted image shows infiltration into subcutaneous fat and in all muscles of the right leg. The femoral bone is deformed due to compression and infiltration of vessels. There is also infiltration in the scrotum.

Skeletal alterations are commonly associated with vascular malformations while they are rarely seen in conjunction with hemangiomas (4). There are only a few studies which have investigated the clinical symptoms and signs of vascular malformations with associated osseous involvement (3-8). We have therefore reviewed the symptoms and the incidence of osseous involvement in patients with vascular malformations of the lower limb presenting at our clinic over the past ten years.

Patients and methods

Between January 1990 and December 1999, 149 patients (65 men and 84 women) with vascular malformations on the lower limb were referred to our special vascular anomalies clinic. For diagnostic and / or treatment considerations 90 patients had their vascular anomaly evaluated by either CT-scan or MRI. Flow characteristics, and muscle- and bone involvement were retrospectively evaluated in a similar way as reported previously (9). Eighteen had bone involvement. A questionnaire was send to these in order to evaluate their initial presenting symptoms. We wished to determine at what age they became aware of the vascular malformation, when a medical opinion was sought and what their initial symptoms were. The patient could choose from different categories as mentioned in table I. More than one answer was possible. The radiological involvement of the 18 patients was reviewed by a radiologist (M.M.) who did not have any clinical information about the patient.

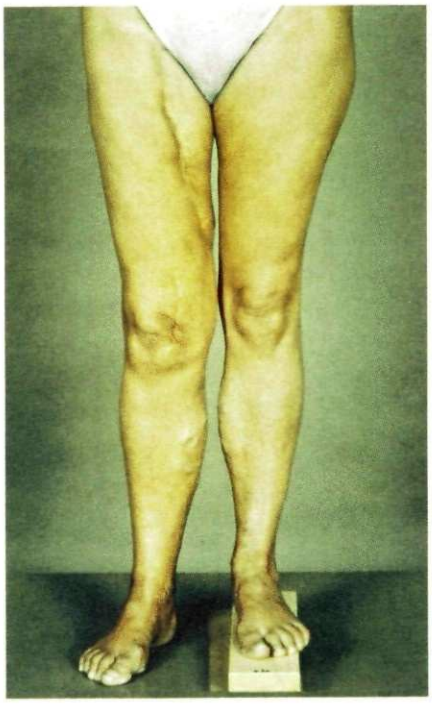

Figure 2:
Photograph showing hypertrophy of the right leg

Table I:
Initial and current complaints of patients with a vascular malformation of the lower limb and bony involvement.

Complaints	Number of patients
Initial	
a. pain	6
b. cosmetic reasons	3
c. pain and cosmetic reasons	4
d. length difference between the legs	6
e. bleeding/fluid discharge from skin	2
f. other (non-healing of fracture)	1
Current	
a. pain	9
b. cosmetic reasons	8
c. tiredness in the legs	7
d. bleeding / ulcer	2
e. no complaints	2
f. other	1

Table II:
Bone involvement was diagnosed in 18 patients (n). Division of different vascular groups and gender. (F = female, M = male, VM = venous malformations, KTS = Klippel-Trenaunay Syndrome, PWS = Parkes-Weber Syndrome, AVM = arteriovenous malformations)

Low flow lesions (n =15)		**High flow lesions** (n = 3)	
VM (n=11)	F (n=6) M (n=5)	PWS (n=2)	F n=1) M(n=1)
KTS (n=4)	F (n=2) M (n=2)	AVM (n=1)	F (n=1)

Results

There were eight men and ten woman patients. Imaging revealed 15 low-flow lesions and three high-flow lesions. The different groups are summarised in Table II.

The mean age of patients at the time of the questionnaire was 32 years (range 10 to 61 years). Three patients failed to return their questionnaire, one of whom had died. It was possible to retrieve all necessary information from the patients' medical file. The mean age that family became aware of the vascular anomaly was one year of age (range: at birth to 36 years). Patients with high flow lesions became aware of their lesion at a mean age of six years while the

Table III:
Leg length discrepancy in ten patients with vascular malformations and bony involvement.

	Hypertrophy	Hypotrophy
Total number of patients	8	2
Type of vascular malformation		
KTS	4	
PWS	2	
AVM	1	
VM	1	2
Amount of disparity		
> 5 cm		2
> 2 cm to < 5cm	3	2
2cm	3	
Mean age (years) at which disparity became visble to family	3.5	2

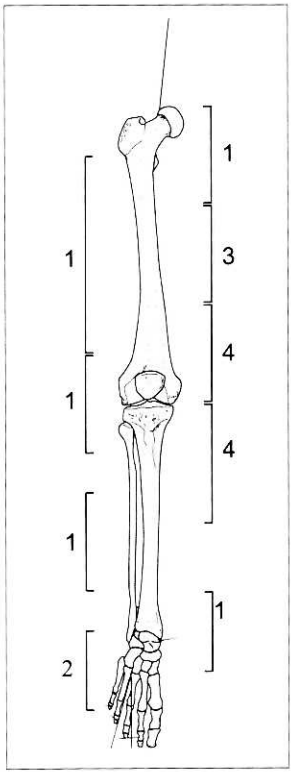

Figure 3:
Diagram showing the number of patients with areas of bony involvement

low flow lesions were visible from a mean age of one year. The age when patients presented to the doctor ranged from birth to 36 years (mean 6 years of age). The mean age of presentation at a medical clinic was the same for low-flow and high-flow lesions. Due to pain symptoms in her leg one patient presented at her General Practioner at the age of three years. No diagnosis was made and she presented again at age 17 with a femoral fracture after minor trauma. Cutaneous capillary lesions and subcutaneous varicose veins had been visible for years.

Ten patients had a leg-length discrepancy; eight had a hypertrophy of the affected leg while 2 patients had hypotrophy of the affected leg. The different groups are specified in Table III. Three of the eight patients with hypertrophy presented initially with pain and not because of the hypertrophy. These three included one high-flow anomaly, one KTS and one venous malformation. The two patients with hypotrophy both had venous malformations. The hypotrophy consisted of 2.5 cm and 4 cm and became visible at a mean age of 2 years. The different symptoms patients of our whole group presented with are reviewed in Table I.

The different areas of bone involvement are shown in figure 3. Twelve patients had reactive bone changes, while six patients had intraosseous extension of the malformation. Reactive bone changes included cortical thickening or depression. Intra-osseous extensions were visualised in all three high flow lesions, two venous malformations and one KTS. Seven patients had intra-articular extension diagnosed with MRI. Two patients had involvement of the calcaneus (one high- and one low flow lesion) and had no muscle involvement. Of the 16 patients with muscle infiltration, 13 patients had four or more associated muscle involved. One patient had only one involved muscle, while two patients had three involved muscles.

Discussion

In our study of patients with vascular malformations of the lower limb, 20% had bony involvement. We are fully aware that this is a select group since all had lesions for which MRI was indicated. This is not a true reflection of vascular malformations of the lower limb in general. The size of the group is too small for statistical analysis. With our study we have proven that patients with vascular malformations and associated osseous involvement often present at a late stage. All, however, had cutaneous lesions. Pain is the most common presenting symptom. Leg-length discrepancy of 2 cm or more was detected in more than 50% of patients. Patients with bony involvement often have associated multiple muscular involvement. For surgery to be curative a severely mutilating intervention would have to be undertaken.

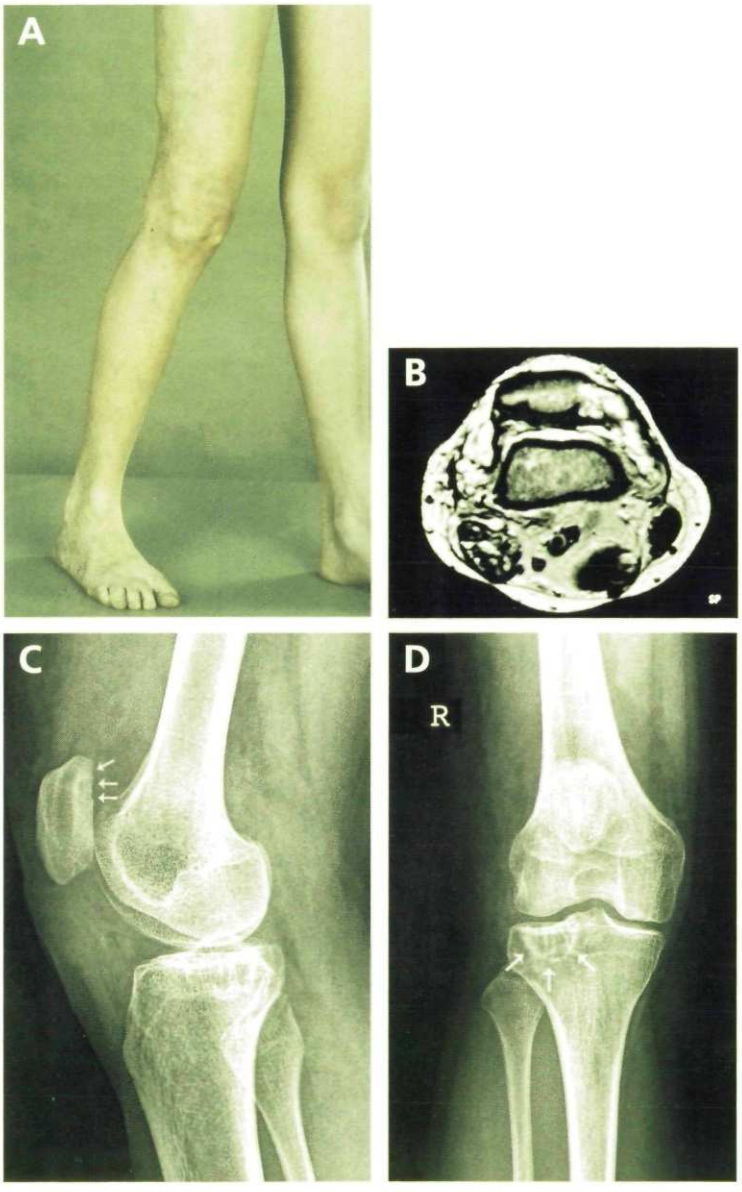

Figure 4:
Figure 4(a) - Photograph showing lateral deviation of the right lower leg due to hypertrophy. There is vascular malformation from the thigh to the foot. Figure 4(b) – MR axial T2 weighted sequences at the level of the knee joint. The vascular malformation is seen as dilated vessels, infiltrating in the subcutaneous fat, muscles, knee joint and patella and patellar retinaculum. Figure 4(c) – Radiograph showing soft-tissue involvement and destruction on the underside of the patella and, figure 4(d) involvement of the proximal tibia.

The mean age when these lesions were noticed was one year, but patients presented much later for medical attention. If patients have no cutaneous lesions, the asymptomatic intra-osseous lesion is often discovered by coincidence when imaging studies are undertaken for other reasons (7).
Pain was the most common presenting feature. This is mainly related to episodes of thrombosis or hematomas, while persistent venous hypertension and muscle involvement would more likely result in tiredness of the leg. Of the six patients with an intra-osseous lesions, *none* initially complained of pain, while 10 of the 12 patients with reactive bone changes initially complained of pain. At review, three of the six patients with intraosseous lesions complained of pain. Of our 18 patients, 14 specifically complained of intermittent episodes of pain in their knee joint. Specific symptoms from the knee were anticipated in the seven patients with intra-articular extension, and MRI confirmed ligamentous involvement in the other seven patients. Although hemarthrosis was never a presenting symptom in our group it is evident that repetitive hemarthrosis will have disastrous consequences. Episodes of effusions and knee hemarthrosis may cause a flexion contracture, leg muscle atrophy, equinous deformity of the foot, progressive ankylosis of the knee joint and early osteoarthritis of the knee joint (2,3). In a study by Enjolras et al, 15 of the 17 patients with pure venous malformations of the limbs had a chronic localised intravascular coagulation disorder. This coagulopathy caused episodes of thrombosis or bleeding leading to hemarthrosis. This condition became worse after surgical intervention (5). If surgery is considered, treatment with low-molecular-weight heparin is advised to minimise haemorrhage and thrombo-embolic complications during and after surgery.

Discrepancy in leg length was a presenting symptom in six patients, while at the time of the questionnaire ten patients (55%) had a leg length discrepancy of 2 cm or more. This became evident in the hypertrophy group at a mean age of 3.5 years, but was seen at two years in the group with hypotrophy. The resultant secondary scoliosis is rarely noted before the child starts walking, resulting for presentation at a later age (1). All 4 KTS and the three high-flow lesions had limb overgrowth while just one venous malformation had a hypertrophy of 2 cm. Hypotrophy was seen in only two other venous malformations. This confirms previous findings that bone of patients with venous malformations involving skeletal bones are usually normal or hypoplastic (3-5,10). The mild limb length discrepancy seen in 70% of Klippel-Trenaunay syndrome patients does not seem to progress after the age of 10 years (11).
Boyd et al have indicated that skeletal alterations are commonly associated with vascular malformations while they are seen in less than 1% of hemangioma's (4). MRI is, at present, the most effective way of demonstrating involvement of the surrounding structures and to define the type of flow (9). Despite some reports indicating that vascular malformations with associated

osseous involvement are rare, our findings seem to indicate the opposite (12). Twenty percent (18/90) of our group of patients with vascular malformations of the lower extremity had bony involvement. Most bony vascular malformations described in the medical literature are in the craniofacial bones and the bodies of vertebrae (6-8). In a study of 108 patients, Wenger and Wold found 84 (77%) bony vascular malformations in either the craniofacial bones or the spine (6). The characteristic radiological appearance of vascular malformations of the skull and spine is often a well-circumscribed zone of rarefaction which may have a honeycombed appearance. It may also have a polka-dot appearance in the spine and a sunburst-like appearance in the skull (7). Involvement of long bones may lack these features and present a diagnostic challenge (6,8).

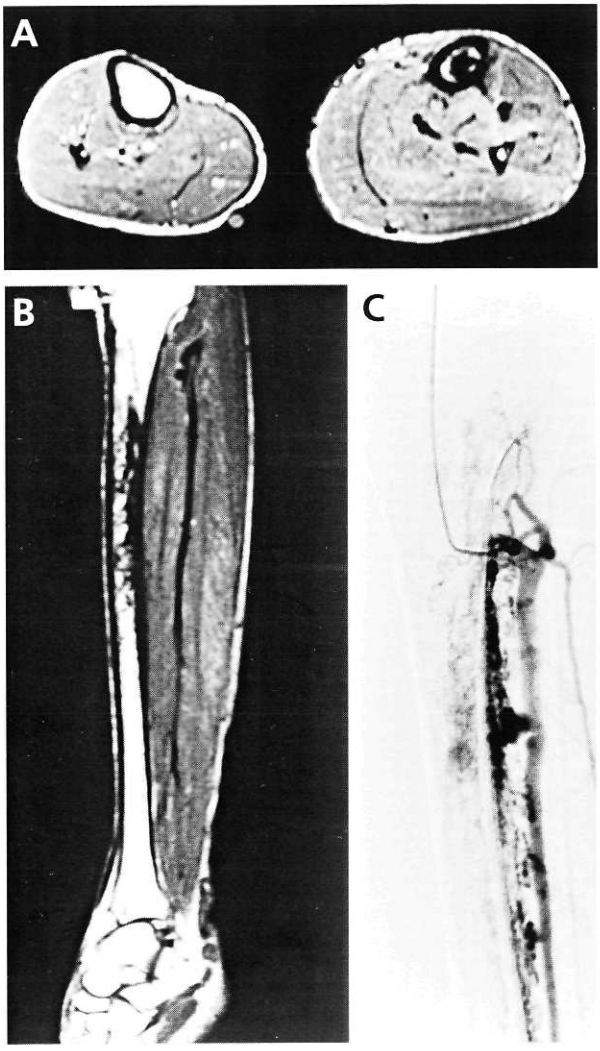

Figure 5:
Figure 5(a) - MR axial proton density weighted image of both lower legs. There is a marked hypertrophy of the left leg (right side of picture), with signal voids, representing a high flow malformation in the muscles and in the tibia. Figure 5(b) – MR sagittal T1 weighted image showing the osseous involvement of the tibia. Figure 5(c) - Angiography of the same high flow lesion, infiltrating muscles and bone.

There have been only a few reports of intraosseous vascular malformations affecting long bones (6-8,12,14-21). In a study by Boyd et al of the 158 patients with vascular malformations in the limbs, 50 (31%) had bony changes. Most of the patients had hyper- or hypotrophy. The exact amount of osseous involvement is not stated and the patients were not divided into upper and lower limbs. The combined vascular malformations on the extremity had aspects typical of both lymphatic [distortion (23%), hypertrophy (81%)] and venous [(hypoplasia (19%), demineralisation (8%)] changes. Intraosseous and lytic changes were characteristic of high-flow lesions. The distinctive thickened irregular trabecular pattern (well-defined lesions with a lattice-like trabecular pattern on plain x-ray) and presence of a high signal intensity on MRI are the most helpful diagnostic imaging features for making the diagnosis of an intra-osseous vascular malformation in long bones (6). It is important to remember that the periosteal reaction may mimic osteosarcomas and chondrosarcomas (12). Phleboliths are characteristic for venous malformations and not for all vascular malformations. Massive intraosseous involvement results in decreased bone density and an increased risk of fractures.

Medical literature states that vascular malformations with associated bone involvement most often involves the diaphysis of long bones (12,14-21). As shown in figure 3, we could not confirm this since the distal half of the femur and the proximal half of the tibia were most often involved. In many patients without hypertrophy the distal femur or proximal tibia were affected indicating that the theory that increased vascularity in this growthplate area results in hypertrophy seems unlikely (4).

While a full discussion on the treatment of these malformations falls outside the scope of this present study, it should be emphasised that each case should be treated on its own merrits. It is stated that two words dominate the rules of therapeutic management of all types of vascular malformations: a multidisciplinary approach and modesty (22). Options for treatment include symptomatic treatment with compression stockings and analgesia, surgical resection and/or intralesional transarterial embolisation and ultimately amputation (23). Often patients with bony involvement also have muscular involvement making treatment more complicated. Surgical treatment is often associated with profuse bleeding, incomplete resection and local recurrence (7,23). The difference in treatment between high- and low-flow lesion is crucial (23-27). Arterio-venous malformations could be excised and/or embolized (22,23,27). Embolisation alone as treatment modality of intraosseous arteriovenous malformations of the limbs is associated with a fairly high risk of recurrence (27). Definitive solutions have been achieved in cases treated with a combination of surgery and interventional radiological techniques. The management in the growing child with limb-length inequality requires careful assessment, sequential limb-length evaluations and careful formation of a treatment plan. Treatment should be by a multidisciplinary

team since certain 'simple' procedures like the ligation of varicose veins in KTS could have disastrous consequences (23,25). Treatment should be conservative and intervention should only be initiated when symptoms develop, except when prophylaxis against progression is feasible or when complications arise (23). In general, elastic support is supplied for the venous hypertension. Orthopaedic procedures should be prophylactic to control bony overgrowth. It has been suggested that the vascular malformation should be treated before bony involvement (10). Patients with high-flow lesions and hypertrophy (often PWS) who require correction of excess leg length, are often made worse by the classic epiphysiodesis (10). If the symptoms of the patients are severe and the surrounding soft tissue allows resection, some authors recommend *en bloc* resection of the vascular malformation, including the affected bone (12,15,20). Reconstruction consists of skin expansion / local flaps in the simple cases, and free flaps in the more complicated cases. If the malformation is cosmetically and functionally acceptable, a conservative approach is often suggested (22). In our study, 70% of the patients had four or more muscles affected, indicating that resection would result in a mutilating intervention. Here analgesia and compression stocking are the mainstay of treatment.

Because of the rarity of vascular malformations, experience in diagnosis and treatment by most clinicians is limited. This may lead to misdiagnosis and a poor outcome. Vascular malformations are generally treated in specialised centres by a multidisciplinary team. This study proves that vascular malformations are probably more commonly associated with bone involvement than has been realised hitherto.

Acknowledgement:

We thank all members of the vascular anomalies team for their valuable work.

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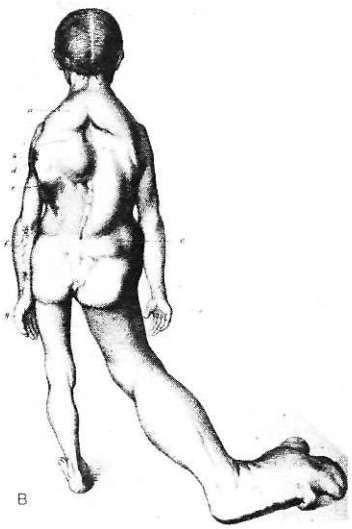
Quality of life in patients with vascular malformations of the lower extremity



Patient of H. Friedberg, 1867

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Submitted for publication

Abstract

With the exception of capillary malformations (port-wine stains), the adverse psychosocial effects of vascular malformations has not received much attention in the medical literature. We, therefore, studied health related quality of life of patients with vascular malformations (VM) located primarily on the lower extremity. Their quality of life was compared with the quality of life of a general population sample and associations with demographic, clinical, and therapy characteristics were studied.

VM patients presented to us over a ten year period were included. Patient's self-assessment of quality of life was measured by the MOS Short Form Health Survey Questionnaire (SF-36™), encompassing eight dimensions: physical functioning, role-functioning physical, bodily pain, general health perceptions, vitality, social functioning, role-functioning emotional, and mental health .

Eighty-one patients (33 male, 48 female,) aged 14 to 61 years, completed the SF-36. Ten patients (12%) had high-flow lesions, while 71 (88%) of patients had low flow lesions. Twenty-three patients (28%) had hypertrophy of the lower extremity, while 11 patients (14%) had hypotrophy. Sixty-nine patients (85%) had the vascular malformation located only on the lower extremity. Six (7%) also had their upper extremity (hand) involved, and 20 patients (24.7%) had > 10% TBSA affected. Eight patients (10%) needed special shoes. Fifty-one patients (63%) had a previously performed a MRI, of which 62.7% (32/51) had muscle involvement. Fifty-one patients (63%) had been operated on. Of the 34 patients wearing elastic compression stockings, 25 patients (74%) indicated that they were satisfied with the stockings.

Compared to a general population sample our VM patients reported impaired vitality and higher levels of pain, while no differences were seen regarding the other dimensions of quality of life. Demographic, clinical, and therapy characteristics could explain quality of life only to a limited extent: explained variability of the SF-36 dimesions ranged from 0% for mental health to 34.5% for role functioning physical.

Since our study sample was small, larger numbers of patients should be studied as well as other potential determinants of quality of life. In addition, health measures other than the SF36 should be explored, such as disease targeted indices.

Introduction

Vascular malformations are congenital lesions although they often only become clinically visible later in life (1,2). These lesions can be divided anatomically into venous, capillary, arterial, lymphatic and/or combinations, and on basis of rheology into either high- or low-flow lesions (1-3). Any lesion with an arterial component is considered as a high flow lesion. Clinical features may be very diverse, ranging form small and insignificant capillary malformations to arteriovenous malformations causing heart failure, or amputations of the lower extremity (1).

Several syndromes have been described of which the Klippel-Trenaunay syndrome (KTS) is one of the best known (1). KTS is characterized by capillary malformations, venous anomalies with bone, soft tissue hypertrophy of one or more limbs and sometimes an associated lymphatic malformation. Parkes-Weber syndrome (PWS) is defined by the same characteristics except that this syndrome has arteriovenous malformations (high-flow lesions). The etiology of vascular malformations is poorly understood, but with recent improved radiological and molecular techniques progress has been made in understanding these lesions (4).

Treatment options are few and results are often unsatisfactory (1-3, 5-9). Often surgical resections were performed, with the lesions only returning in a worse way than before. In for example the lower extremity, patients sometimes have multiple muscles affected, and/or associated bone involvement (10). Symptoms can be multiple with complaints frequently occurring from thrombophlebitis in venous malformations, leakage from lymphatic malformations or ulcers from arteriovenous malformations. Because surgical results are often unsatisfactory patients are told that they *"have to live with it"*. Especially in the extremities discomfort / pain is commonly treated with elastic compression stockings and analgesia (1,3,5).

In comparison to the well described physical morbidity associated with vascular malformations, little has been published about the associated adverse psychosocial effects of these malformations. Explanations may be that the incidence of vascular malformations is low (0.5 – 2.1%), and these anomalies are not life threatening (1-3, 11,12).

With the exception of one study about KTS (13), all studies on psychosocial effects in patients of vascular malformations done so far, focused on patients with capillary malformations in the head- and neck area (port-wine stains) (14-19). This can be explained by the fact that capillary malformations are the most common vascular malformation and since they mostly occur in the

head and neck area, can be a severe psychological burden to the patient. Moreover, these lesions can be fairly successfully treated by pulsed dye laser. Some studies have demonstrated that patients with capillary malformations suffer considerable psychosocial disabilities, which they often suppress, while other studies have demonstrated that these patients have no significant problem behavior (14-19). Interpretation of the results of the study in KTS patients (13) is difficult since it did not differentiate between those with upper- *or* lower extremity involvement. Moreover, subjects were members of an organization for patients with KTS and it was not known whether a subject was a patient with KTS *or* a family member.

The aims of our study, therefore, were: (1) to assess the quality of life in patients with vascular malformations located primarily on the lower extremity (2), to compare their quality of life to a general Dutch population sample, and (3) to identify the association between demographic, clinical and therapy characteristics and quality of life.

Patients and methods

Patients

Consecutive patients, aged 14 years and older, with vascular malformations located primarily on the lower extremity who presented at our special vascular anomalies clinics between January 1990 and December 1999 were included in this study. Patients with small demarcated capillary malformations were seen at the plastic surgery- or dermatology out-patients clinics and were subsequently not included in this study.

The following major categories of data were analyzed: demographic, clinical, therapy, and quality of life characteristics. These characteristics were collected from the participants by way of a mailed questionnaire.

Clinical characteristics comprised of: gender, flow characteristics, hypertrophy/hypotrophy, leg length discrepancy, involvement of only the extremity or further extension outside the extremity, uni- or multifocal lesions, joint or hand involvement, genitalia involvement, TBSA affected and muscle or bone involvement on MRI. Therapy characteristics included prescription of elastic stockings, special orthopedic shoes and a history of surgical interventions of the lower extremity. It was also assessed whether patients were satisfied with their elastic compression stockings or

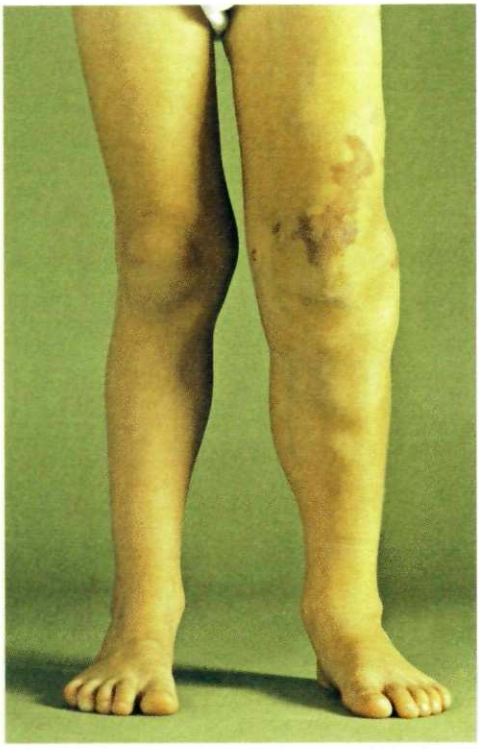

Figure 1:
Clinical picture of a patient with a vascular malformation located in her left leg.

not, and experienced a lot of, a little or no benefit from the surgical interventions.

Hypertrophy/hypotrophy was defined as an increase/decrease in length of the affected extremity in comparison to the non-affected extremity. The amount of hypertrophy/hypotrophy was classified in 4 categories: (1) normal, (2) < 2 cm, (3) 2-5 cm, (4) > 5 cm. Total body surface area (TBSA) was defined as all affected visible skin. The patients were requested to give an indication of the extent of the vascular malformation on a presented drawing. The Lund and Browder chart was used to assess the size of the vascular malformation (20). From this drawing it is possible to see if the vascular malformation extends proximally to the groin, if the lesion extends to/over a joint, if there are multiple lesions, if the genitalia are involved, and if the upper extremity (i.e. hand) is involved. MRI's were performed when an intervention was anticipated, when the diagnosis was unclear or when involvement was assessed to give advice about a possible prognosis. These MRI's were retrospectively analyzed by a radiologist without clinical knowledge of the patients. The following parameters were considered: flow characteristics (low or high), muscle infiltration (no, 1 muscle, 2-3 muscles, >3 muscles) and bone involvement.

Orthopedic shoes were always prescribed in conjunction with the department of rehabilitation and were only prescribed for patients with a leg length discrepancy of ≥ 2 cm or severely deformed feet. Patients that were treated with an "inlay" in the shoe were not included in this group. Elastic compression stockings were prescribed if the patient had recurrent episodes of thrombophlebitis, and / or edema of the lower extremity and / or skin irritation at the lower extremity.

Patient's self-assessment of quality of life was measured by the Multiple Outcomes Study (MOS) Short Form Health Survey Questionnaire (SF-36™) (21,22). The SF-36 is a generic multidimensional instrument consisting of eight multi-item scales representing: 1. physical functioning (PF, extent to which health limits physical activities such as self-care, walking, climbing stairs); 2. social functioning (SF, extent to which physical health or emotional problems interfere with normal social activities); 3. role-functioning physical (RP, extent to which physical health interferes with work or other daily activities); 4. role-functioning emotional (RE, extent to which emotional problems interfere with work or other daily activities); 5. mental health (MH, general mental health including depression, anxiety, behavioral-emotional control, general positive affect); 6. vitality (VT, feeling energetic and full of pep versus tired and worn out); 7. bodily pain (BP, intensity of pain and effect of pain on normal work, both inside and outside the home); 8. general health perceptions (GH, personal evaluations of current health, health outlook and resistance to illness). SF-36 scores of our patients with vascular malformations were compared to the SF-36 scores of a general Dutch population sample (n= 1742 , age range = 16-96 , 56 % male) (21). SF-36 scores were transformed to a 0-100 scale, a higher score indicating a better quality of life state.

Statistical analysis

Differences between the mean SF-36 scores of the vascular malformation (VM) patients with those of the reference population were tested with Student's t-test. Univariate associations between demographic, clinical, and therapy characteristics on the one hand, and SF-36 scores on the other hand were assessed by Student's t-test, one way analysis of variance, or Pearson's correlation coefficient or their non-parametric equivalents when appropriate. All significant characteristics (set at $P \leq 0.20$) identified from univariate analysis were studied with multiple linear regression (with a stepwise forward selection strategy), using the F-statistics with P=0.05 on the criterion level for selection. To search for violations of necessary assumptions in multiple regression, normal plots of the residuals of the regression models were produced. Furthermore the influence of outliers (Cook's distances) and possible presence of co-linearity (Tolerance/

Variance Inflation Factor statistics) were assessed. P values less than 0.05 were considered to be statistically significant.

Results of the multivariate regression analysis are expressed in regression coefficients, and partial and total explained variability, R^2, of the SF-36 scores. Regression coefficients indicate the change in SF36 score with one unit change of the explanatory variable. Partial R^2 is the percentage of the variation of the SF-36 score that is explained by the single explanatory variable adjusted for the influence of the other explanatory variables. Total R^2 is the total percentage of the variation of the SF-36 score that is explained by the explanatory variables together. All analyses were done with SPSS for Windows 11.0 (SPSS Inc., Chicago IL, USA).

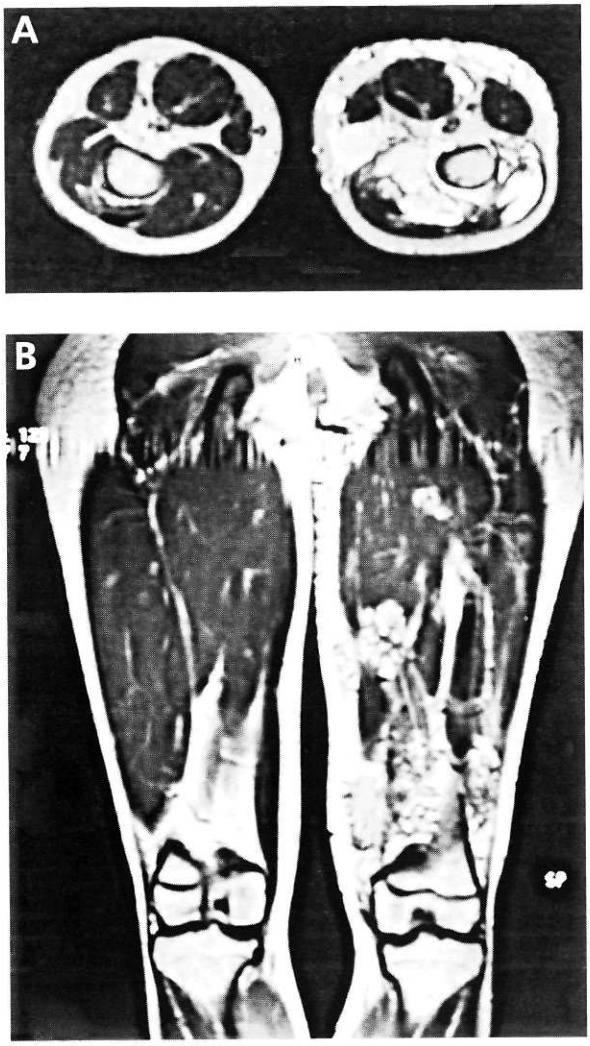

Figure 2:
MR image of a patient in our study group with a vascular malformation located on her left thigh. In figure *2*(a) is a T-2 weighted coronal image through the thigh, while *2*(b) is a transverse view. Both images showing hyperintense lesions on the T-2 weighted image, without signal voids, indicative of a low-flow lesion. Note the diffuse intramuscular involvement.

Table A: Demographic, clinical and therapy characteristics

Age in years (mean (SD) range)		30.4 (10.5) 14-61	
		N	(%)
Sex	- male	33	(41%)
	- female	48	(59%)
Flow	- high	10	(12%)
	- low	71	(88%)
Hypertrophy	- normal	47	(58%)
	- hypertrophy	23	(28%)
	- hypotrophy	11	(14%)
Leg length difference (cm)	- 0	47	(58%)
	- <2	22	(27%)
	- ≥2	12	(15%)
Vascular malform.	- leg only	69	(85%)
	- leg & other extremit ies	12	(15%)
Joint involvement	- yes	58	(72%)
	- no	23	(28%)
Hand involved	- yes	6	(7%)
	- no	75	(93%)
Multiple lesions	- yes	7	(9%)
	- no	74	(91%)
Genitalia involved	- yes	3	(4%)
	- no	78	(96%)
TBSA	- ≤ 10%	61	(75%)
	- > 10%	20	(25%)
* Muscle involvement	- yes	32	(63%)
	- no	19	(37%)
* Bone involvement	- yes	12	(24%)
	- no	39	(76%)
Orthopedic shoes	- yes	8	(10%)
	- no	73	(90%)
Elastic stockings	- yes	34	(42%)
	- no	46	(58%)
Operations leg	- yes	51	(63%)
	- no	30	(37%)

* Characteristics of 51 patients with an available MRI.

Results

Patient characteristics

Of the 149 patients presenting at our clinic during the described time, 113 patients were older than 14 years of age and thus eligible for this study. Eleven of the 113 questionnaires were not returned, either because the patient had deceased or the patient had moved and the new address was unknown. Of the remaining 102 questionnaires, 82 were returned (80%). One of the remaining 82 patients was not included in the study because of an amputation of the leg.

Of the 81 patients, 33 patients (41%) were male and 48 female (59%). Mean age at the time of the questionnaire was 30.4 years (SD: 10.5, range: 14-61) and did not differ between males and females. Clinical and therapy characteristics of the patients are shown in Table A.
Out of the 81 patients, 10 had high flow- and 71 had low flow lesions. Of these latter patients, 44 had venous malformations, 12 had KTS, and 10 had lymphatic malformations (6 pure lymphatic- and 4 venous-lymphatic malformations). Eleven patients had hypotrophy, of which 8 had venous malformations. Hand involvement was present in 6 patients (1 venous, 4 KTS and 1 lymphatic malformation). With respect to TBSA the majority (N=61) had 10% or less body surface area affected (16 patients <1%, 34 patients between 1-5%, 11 patients between 5-10%). Fifty-one patients (63%) had a previously performed MRI at our institution, of which 32 had muscle involvement. Six patients had 1 muscle involved, 7 patients had 2 or 3 muscles involved, while 19 patients had 4 or more muscles involved. Bone involvement was observed in 12 patients. Signs of muscle and bone involvement characteristics have been described elsewhere (23).

Out of the 8 patients (10%) needing special shoes, 3 patients indicated that they had no hypertrophy, but deformed feet. Of the other 5 patients, 4 had hypertrophy and 1 hypotrophy of the affected extremity. Two of these patients had a leg length discrepancy of < 2 cm, 2 patients had a discrepancy between 2 - 5 cm, and 1 patient had a leg length discrepancy of > 5 cm.

Thirty-four patients were wearing elastic compression stockings, of which 25 patients (74%) were satisfied wearing these stockings. The two groups of patients either wearing elastic compression stockings or not, were evenly distributed between the different types of vascular malformations and also with regard to those patients with muscle and bone involvement on MRI.

Fifty-one patients (63%) had undergone one or more surgical interventions of the affected leg in the past. Seventeen patients had had 1 operation, 9 patients 2 operations, and 25 patients 3 or

Table B: Mean (SD) SF-36 scores of Dutch patients with vascular malformations of the leg and a Dutch general population sample.

Group	N	PF[b]	RP[b]	BP[a,b]	GH[b]	VT[a,b]	SF[b]	RE[b]	MH[b]
Vasc. malf. population	81	83.2 (18.7)	73.1 (40.7)	68.9 (25.6)	67.0 (26.6)	62.7 (18.0)	79.7 (22.5)	78.2 (37.0)	73.6 (17.6)
Dutch general population sample	1742	83.2 (22.6)	76.6 (36.1)	75.0 (23.3)	70.9 (20.6)	68.6 (19.3)	84.2 (22.3)	82.5 (32.8)	76.9 (17.4)

[a]: P<0.05, AVA patients compared to the Dutch reference population.
[b]: PF=physical functioning, RP=role-functioning physical, BP=bodily pain, VT=vitality, GH=general health perceptions, SF=social functioning, RE=role-functioning emotional, MH=mental health.

more operations. Nineteen patients indicated that they experienced no benefit from the operation, while another 19 reported little benefit.

Comparison of quality of life between the vascular malformations patients group and a general population sample

Mean SF-36 scores of the vascular malformation patients and a general Dutch population sample are displayed in Table B (24). Only for the dimensions bodily pain and vitality statistically significant differences were observed between both groups; vascular malformation patients reporting lower levels of quality of life than the general population sample.

Associations between demographic, clinical, and therapy characteristics and quality of life

In Table C univariate associations between selected demographic, clinical, and therapy characteristics and the SF-36 scores are shown. Of the demographic variables studied, higher age was statistically significantly associated with lower physical functioning ($r = -0.24$, $P = 0.03$) and role functioning-physical ($r = -0.26$, $P = 0.02$).
Regarding clinical characteristics involvement of the hand was associated with worse physical functioning, role functioning physical, vitality and social functioning. Patients with vascular malformations on more extremities than only the affected leg, reported lower levels of general health perceptions than patients with only one leg affected. Total body surface area involvement of more than 10% was also associated with worse general health perceptions as well as with worse role functioning-physical. Patients with muscle or bone involvement did not report different quality of life scores than patients without involvement. None of the other selected clinical characteristics were statistically significantly associated with quality of life dimensions.

Of the selected therapy characteristics, patients wearing orthopedic shoes reported lower levels of quality of life on most SF36 dimensions: physical functioning, role functioning physical, bodily pain, general health perceptions, vitality, and social functioning. Patients who had undergone operations of the affected leg perceived both their role-functioning physical and role-functioning emotional as worse than patients who had not undergone operations. In addition, they also reported higher levels of pain than patients who had not been operated.

Table C.
Mean SF-36 scores by demographic, clinical and therapy characteristics. Only characteristics significant at the P≤0.20 level are shown. [a]:P<0.05; Abbreviations as in Table B.

		PF	RP	BP	GH	VT	SF	RE	MH
Sex							84.4		
	- male						76.6		
	- female								
Hypertrophy						64.5			
	- normal					57.0			
	- hypertrophy					67.3			
	- hypotrophy								
Length diff. (cm's)									74.8
	- 0								76.2
	- < 2								64.7
	- > 2								
Vasc. malform.		84.7	76.5	70.5	69.4[a]	63.8	81.3	81.6	
	- leg only	74.2	54.2	60.1	53.2	56.3	70.8	58.3	
	- leg + other extremities								
Joint involv. malf.			69.3						
	- yes		82.6						
	- no								
TBSA		86.0	80.0[a]	71.2	71.0[a]	64.5			
	≤ 10%	74.4	52.5	62.1	55.0	57.3			
	> 10%								
Hand involved		64.2[a]	16.7[a]	53.2	46.8	47.5[a]	60.4[a]		60.7
	- yes	84.7	77.7	70.2	68.6	63.9	81.3		74.7
	- no								
Multiple lesions				57.4				38.1	
	- yes			70.0				82.0	
	- no								
Orthopedic shoes		57.5[a]	25.0[a]	48.8[a]	48.1[a]	45.6[a]	62.5[a]		63.5
	- yes	86.0	78.5	71.1	69.1	64.6	81.6		74.8
	- no								
Elastic stockings		78.1	58.3	60.1			74.6		
	- yes	86.5	83.2	74.7			82.9		
	- no								
Operations leg			65.5[a]	64.3[a]				72.5[a]	
	- yes		85.8	76.9				87.8	
	- no								

After introduction of the most important univariate associations ($P \leq 0.20$) in multivariate regression analysis, many of the above described associations disappeared (Table D). However, wearing orthopedic shoes remained statistically significantly ($P < 0.05$) associated with poorer quality of life scores, mainly in the physically oriented dimensions. For example, mean physical functioning scores of patients wearing orthopedic shoes were 27.5 points lower than *corresponding scores of* patients not wearing orthopedic shoes. On the multivariate level, wearing elastic stockings and having undergone operations of the affected leg were associated with poorer role functioning physical and higher levels of pain. Higher age only remained statistically significant associated with lower physical functioning: each increase in age of 1 year was associated with a 0.37 point lower physical functioning. Or in other words, compared to a patient who is 10 years younger of age, a 10 year older patient on average reports a 3.7 points lower physical functioning. Gender was not associated with any of the SF36 scores. Regarding the clinical characteristics, TBSA, hand involvement, and multiple lesions, were associated with only one SF36 dimension.

The collectively explained variability (R^2) of the SF36 scores was small, ranging between 0% and 34.5% (Table D), with the highest explained proportions found for the physically oriented dimensions.

Discussion

Our study suggests that patients with vascular malformations located primarily on the lower extremity do not have a considerable impaired quality of life when compared to a general Dutch population sample. Our study population group reported more bodily pain and worse vitality than a general Dutch population sample (24). Regarding other dimensions of health related quality of life as assessed with the SF36, no differences were observed between both groups. Because the mean age of our VM patients (30.4 years) is lower than that of the reference population (47.6 years), and since SF36-scores are negatively associated with age (24,25), this might have resulted in a slight underestimation of the difference in quality of life scores.

This is the first study on health related quality of life in patients with vascular malformations of the lower extremity. Because no other studies have been performed on the quality of life of vascular malformation patients, the significance of our results can not be put in perspective in comparison to other vascular malformation populations. As already mentioned before, comparison of our results with the results of a previous study on psychological aspects of KTS is not possible

Table D: Forward stepwise regression models[a] to explain quality of life dimensions in AVA patients (Regression coefficients[b] (95% confidence limits) (partial explained variance, partial R^{2} [c]) and total explained variance (total R^{2} [c])).

[a]: No violations of necessary assumptions in multiple regression analysis could be detected (examination of residuals, detection of outliers, measures of collinearity).[b]: Regression coefficients indicate the change in SF36 score with one unit change of the explanatory variable. [c]: R^{2} is the percentage of the total variation of the SF-36 score that is explained by the explanatory variables together. Partial R^{2} is the percentage variation of the SF-36 score that is explained by the single explanatory variable adjusted for the influence of the other explanatory variables. Abbreviations as in Table B.

	PF	RP	BP	GH	VT	SF	RE	MH
Age, *1 yr older*	-0.37 (-0.72, -0.19) (3.4%)							
TBSA, *>10% vs. ≥10%*				-16.0 (-29.2, -2.7) (5.6%)				
Hand involved, *Yes vs. no*		-47.5 (-78.7, -16.3) (14.7%)						
Multiple lesions, *Yes vs. no*							-43.9 (-71.5,-16.3) (10.2%)	
Orthopedic shoes, *Yes vs. no*	-27.5 (-39.8, -15.3) (19.9%)	-40.1 (-66.9, -13.3) (6.7%)	-23.2 (-40.6, -5.7) (5.2 %)		-19.1 (-31.9, -6.3) (9.0%)	-19.2 (-35.7, -2.7) (5.3%)		
Elastic stockings, *Yes vs. no*		-17.7 (-32.9, -2.5) (3.9%)	-12.8 (-23.4, -2.2) (6.9%)					
Operations leg, *Yes vs. no*		-25.4 (-41.1, -9.7) (9.2%)	-12.7 (-23.5, -1.8) (4.8%)					
Total R^{2}	23.3%	34.5%	16.9%	5.6%	9.0%	5.3%	10.2%	0%

(13). Namely it only consisted of KTS patients (60 adults and 37 children), and did not differentiate between patients with upper or lower extremity involvement. Moreover, it is not clear whether the information was supplied by the KTS patient or his/her family member. Additionally, quality of life measures other than the SF-36 were used and the assessed physical quality of life aspects were not clearly specified.

The total explained variation in health related quality of life of the vascular malformation patients by the selected characteristics was small. Since the number of patients studied was small, insufficient statistical power to detect significant associations might have attributed. In addition, it suggests that quality of life is influenced by many more factors than the selected ones in the present study. It is reasonable to assume that the patients' quality of life is a result of a complex interaction of disease outcome, personal traits, coping behavior, social support, and the quality of care received. Moreover, disease-specific health indices may potentially be more sensitive than the SF36, since they assess health outcomes that are more directly related to the underlying disease process. Interestingly, a small impact of demographic and clinical characteristics on quality of life has also been found in other chronic disease populations, for example in stroke, obstructive pulmonary disease, end-stage renal disease, and heart disease (26-29).
To our surprise, patients with hyper- or hypotrophy did not report impaired physical functioning, not even patients with a leg length discrepancy of e" 2cm. Since our group of patients with severe leg length discrepancy (> 5 cm) is small (n = 2), it is possible that a larger group might indicate this to also be an important parameter. We realize that the leg length discrepancy was evaluated subjectively from a questionnaire and, therefore, the obtained information may not be fully reliable. On the other hand, we feel that most patients are aware of any eventual leg length difference and that if the patient has a leg length discrepancy, that it must have been evaluated sometime during their many hospital visits. Nevertheless, it is suggested that in possible further studies a more accurate approach should be followed. Our study had 12 patients with a leg length discrepancy of e" 2 cm and only 5 of those had special orthopedic shoes, while 7 were subsequently treated satisfactorily with an "inlay". Although only 10% of our patients were wearing special orthopedic shoes, wearing orthopaedic shoes appeared to be independently associated with impaired physical quality of life dimensions. This finding needs to be established in future studies among greater numbers of patients before indications for these special shoes can be defined.

Although many of the patients wearing elastic stockings were satisfied (74%) with the elastic compression stockings effect, patients wearing elastic stockings still reported lower quality of

life than patients not wearing elastic stockings. This may be explained by the fact that they are wearing stockings because of their poor quality of life. On the other hand, we hypothesize that reported quality of life scores would have been worse if they had not worn elastic stockings. This should be explored in a future study, assessing the quality of life of patients before and after the start of the use of elastic stockings.

Against our expectation, also no associations were observed between flow characteristics and quality of life. The same holds true for muscle and bone involvement. Again lack of sufficient statistical power due to the small sample size may have attributed. It may be expected that in a larger sample with differentiation for periosteum involvement bone involvement may appear to be associated with quality of life. In practice patients often complain of superficial thrombophlebitis giving them pain, and in future studies this parameter should also be evaluated.

Conclusions

This is the first study on quality of life of patients with vascular malformation located primarily on the lower extremity. In view of our findings it can be concluded that vascular malformation patients perceive impaired vitality and higher levels of bodily pain compared to a general population sample. Demographic, clinical, and therapy characteristics can explain quality of life only to a limited extent. Since our study sample was small, larger numbers of patients should be studied as well as other potential determinants of quality of life. In addition, health measures other than the SF36 should be explored, such as disease targeted indices.

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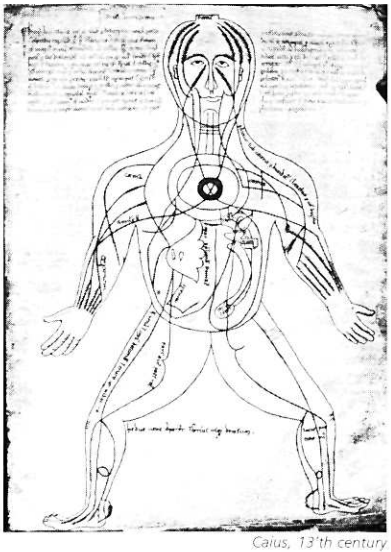
Summary, conclusions and future perspectives



Caius, 13'th century

Summary, conclusions and future perspectives

Vascular malformations have been, and are still among the most poorly understood entities in medicine. The Latin appellation for capillary malformations (port-wine stain) is *nevus flammeus* and it connotes the superstition that the mother was frightened by fire during pregnancy (1). Since medieval times there has been considerable stigma surrounding these anomalies. In the Middle Ages the newborn babies' skin abnormality was sometimes seen as a sign of the mother's wrongdoing (sin) during the pregnancy (1). Montaigne (1533-1592), a skeptical essayist, had no doubts about the origin of these congenital stains: "*We know by experience that woman impart the marks of their fancy to the bodies of children they carry in their womb*" (2). Until a classification of Mulliken and Glowacki was introduced in 1982, terms like hemangioma's, venous angioma's, cavernous angioma's, or cavernous hemangioma's were used interchangeably for lesions that were in fact vascular malformations. Since the biological classification was introduced, there has at least been a way to differentiate vascular anomalies into two groups: vascular tumors (mostly hemangiomas), and vascular malformations. Since 1982 there have been some fundamental improvements in our understanding of these anomalies. With recent improvements in especially our molecular understanding increasing rapidly, this thesis is an attempt to summarize some important improvements of recent years. This thesis is not a comprehensive review of our knowledge applicable to vascular malformations, and the interested reader is referred to specialized books.

It is obvious that some information supplied in **chapter 2** is already out-dated. It was also not possible to include all the available information in an overview as was proven by the commentary of Michael Cohen in *Plastic and Reconstructive Surgery ®* (3). With treatment options for cancer stimulating research in vascular development (angiogenesis), new information is found regularly. In January 2003 Medline had ± 20 000 "hits" for TGF-beta, and nearly 8 000 for VEGF. Despite this abundance of information we want to summarize some fundamental improvements in our understanding with regard to vascular malformations. Only some factors will be discussed, and referral to the Internet is suggested for a complete and more recent update.

After capillary malformations, venous malformations are the most common vascular malformations. The incidence is unknown, but is estimated to be between 1/5000 and 1/10 000 newborn babies (4). In chapter 2 the mutation found at R849W (chromosome 9) in familial venous malformations was described, but a different mutation, Y897S, has also been found in the same kinase domain (5). The signalling cascade of the TIE-2 receptor is complex, and the exact explanation of the phenotype caused by the mutant TIE-2 receptor is not fully explained. It

is suggested that the mutation causes changes in control of the endothelial cell cycle leading to a relative deficiency of smooth muscle cells (4). It is still unclear how the mutation exactly results in the phenotypic effects and more research is being performed.
Glomuvenous malformations (GVM) are venous malformations with"glomus cells" around the convoluted venous-like channels and are subgroup of venous malformations (4). Usually it is possible to differentiate clinically between these lesions and venous malformations (6). GVM are raised, bluish-purple, have a cobblestone surface and are very painful on palpation. In contrast to other venous malformations, GVM are rarely encountered on mucous membranes and are nearly never intramuscular. Venous malformations are blue lesions, encountered on the mucosa and intramuscular. In contrast to the nodular appearance of GVM, venous malformations are often flat. Hyperkarotosis is another way to differentiate between GVM and VM. This is found with GVM, while it is not seen in venous malformations. Postural emptying is also not possible with GVM, while this is well known for venous malformations. Genetic studies have supported the clinical and histological difference seen between venous malformations and glomuvenous malformations. It is suggested that 78% of GVM are inherited, while only 2% of venous malformations are inherited. The VMGLOM has been identified as a locus on chromosome 1 as mentioned in chapter 2. Characterization of additional families with inherited GVM enabled Irrthum et al to narrow the region to single 1.48-Mpb YAC (7) There are no known genes for vasculogenesis is this region, and it is suspected that the mutated gene could be novel factor regulating vasculogenesis / angiogenesis. It should be interesting whether this gene works in conjunction with the TIE2 gene or if it acts in TIE2 signalling, because VM have a relative deficiency of smooth muscle cells and GVM have a variable abundance of modified smooth muscle cells (3).

Chapter 3 is devoted to mapping the locus for an autosomal dominant disorder in a three-generation family that manifested itself with multiple cutaneous capillary malformations. It is possible that the multifocal nature of these lesions might be a clue to a familial predisposition. We have mapped a locus for an autosomal dominant disorder in this family to chromosome 5q13-22. This was the first time that a locus was demonstrated in capillary malformations. Since this publication one other study has demonstrated a locus in the same area, and it is expected that with time the gene(s) will be found (8). Our described locus spans 49cM between the markers D5S647 and D5S659 and includes several candidate genes. As indicated in the next chapter, abnormal neural development is possibly the major cause of the capillary malformation. Several genes involved in neurogenesis are located in this area. These include the FER gene shown to be involved in neurite outgrowth, and EFNA5 protein involved in axon guidance. Further refinement of the present linkage region and subsequent mutation analysis should allow

detection of the causative gene. Recent studies are indicating that the clear-cut differences we make between hemangiomas and capillary malformations by using the classification suggested by Mulliken and Glowacki, may not be so clear-cut as we think (9).

In **Chapter 4** we have summarized the present understanding of the pathology involved in capillary malformations. It is clear form this summary that there is still a considerable amount of information to be gained from thorough histological (electron microscopy) studies. It seems that the pathology of the capillary malformations is located in the post-capillary venules and small venules, and that our definition of port-wine stains being capillary malformations is wrong. One important pathological characteristic detected in "capillary malformations" so far is the decreased neural innervation. It thus seems legible to describe these lesions as a neural malformation as well. It is even possible that all vessel deformation is secondary to the neurological pathology, and that capillary malformations are pure neural malformations with vascular dilatation being secondary. Further studies on capillary malformations should concentrate not only on the vasculature, but should also include studying the innervation of the dermal vasculature. Although histological studies have demonstrated that the ectatic part involved in capillary malformation is confined to the venular part, we know little about the real innervation of the arteriolar part and further research will have to clarify this aspect. Angiogenesis is also an essential process of nerve regeneration. Endoneural vasculature provides access for hematogenous macrophages and oxygen / nutrients to enter the damaged nerve, essential for the outgrowth of neurites (10). It is surprising how little we know about the influence that VEGF has on neural development. The role of VEGF in angiogenesis of the nervous system is unquestionable, and the concept that VEGF also has direct neural effects is gaining more experimental support (10). With the common developments of the vascular system, nervous system and lymphatic system it seems logical that future investigations on capillary malformations will concentrate on the "genesis" as a whole and not only on vasculo- and angiogenesis.

Although a thorough molecular description will provide us with a nice diagnosis, the most important question is whether treatment is possible. To answer that question an accurate description of the tissues involved is necessary. In **Chapter 5** we provide an overview as to why MRI is currently the best modality to investigate vascular anomalies if intervention is anticipated, but this chapter also discusses some of the limitations of MRI. Although clinical characteristics can often differentiate between different types of vascular malformations, MRI is often needed to delineate the involvement of vascular malformation. Chapter 5 also summarizes some differences in high- and low-flow lesions concerning MRI and ultrasonography. Although no single imaging technique answers all our questions, the exact reason for requesting an investigation

should determine the choice of investigation. A decision-tree to help determine the composition of vascular birthmarks on the basis of MRI characteristics is further suggested. Even with contrast enhancement given to differentiate between lymphatic- and venous malformations, it is sometimes difficult to differentiate between these lesions. Clinical characteristics are important since venous and lymphatic lesions can sometimes occur together, making an attempt to differentiate between these anomalies by only looking at the MRI very difficult.

Historically surgical treatment options for vascular malformations have been poor. In the past the MR characteristics differentiation between high- and low-flow lesions have been well described, but there is little detailed information available that accurately delineates the vascular malformation. In **Chapter 6** we not only define the MRI characteristics of high- and low-flow lesions, but further provide more detailed information regarding the extent of local involvement and describe associated features of the tissue adjacent to the vascular malformation in the lower extremity. We retrospectively reviewed 40 MRI's of 34 patients with low-flow lesions and 6 patients with high-flow lesions. Of the 34 low-flow lesions 23 (67.6%) had muscle involvement, while 4 high-flow lesions had muscle involvement. When the different muscle compartments were compared, it was seen that 80% of the lesions in thigh involved at least the anterior compartment, while 86.6% of lesions located at the leg had at least the posterior compartment involved. The angiosome concept is used to explain this phenomenon. The zones between angiosomes (anastomotic vessels = choke vessels) are not between muscles, but within them. It is possible that in the past only resection of the visible vascular anomaly was performed. The resection probably ended at the end of the muscle – subsequently not at the end of the angiosome. It is expected that the residual vascular malformation reacted in an aggressive way because of the change in blood dynamics. This study further indicates that 61% of patients with muscle involvement had more than three muscles involved. Twenty-five percent of patients also had bone involvement. It is thus unlikely that surgical intervention alone will be curative when treating these patients. This observation is further enhanced by the 20% of patients having multifocal lesions. A substantial amount of patients with associated muscle involvement also had associated muscle atrophy, again indicating that even if it is possible to resect the vascular malformations, it is imperative to look at the remaining muscles (although assessing the precise function of these remaining muscles is difficult). Our study also included 11 low-flow lesions with subcutaneous hypertrophy. Five of these lesions had no associated subcutaneous vascular malformations. It is possible that this aberrant fat is associated with the vascular malformation, much like some pure capillary malformations are associated with limb hypertrophy. Further studies are needed to clarify these observations. The angiosome concept should also be tested at other locations e.g. head and neck, and the upper extremity/hand.

Previous studies have characterized the radiological skeletal alterations seen with vascular malformations, but there is a paucity of information with regard to the clinical characteristics of patients with vascular malformations with associated osseous involvement. **Chapter 7** describes the clinical characteristics of 18 patients with osseous involvement associated with vascular malformations on the lower extremity. Despite some reports indicating that vascular malformations with associated osseous involvement are rare, the contrary seems true with our study. Twenty percent (18/90) of our group of patients with vascular malformations of the lower extremity had osseous involvement, of which 15 patients had low-flow lesions, and 3 had high-flow lesions. As seen in table C and D in chapter 7, pain was the most common reason for presenting to a physician, together with a disparity in leg length. Of the six patients with an intra-osseous lesion, *none* initially complained of pain, while 10 of the 12 patients with reactive bone changes initially complained of pain. This pain could possibly be explained by tension caused by periosteal lifting. Only 50% of the patients with symptoms of knee hemarthrosis had intra-osseous extension. Although the other patients all had ligamentous involvement in the knee, this was often very subtle on the MRI. Chapter 7 focuses only on the 18 patients with osseous involvement visible on the MRI, and no mention or comparison is made of the symptoms of the patients without osseous involvement. Further studies should identify if any difference exists.

Once a diagnosis is made and some radiological visualization has excluded any therapeutic intervention, unfortunately many patients are send home without a satisfactory curative treatment solution. These patients are often told to "live-with-it" and for example, often elastic stockings and analgesia are prescribed for lesions located on the extremities. With the exception of capillary malformations (port-wine stains), the adverse psychosocial effects of vascular malformations has not received much attention in the medical literature. This in comparison to the well described physical morbidity. Due to this paucity of information we conducted a quality of life study with the MOS Short Form Health Survey Questionnaire (SF-36™) in **Chapter 8** in patients with vascular malformations located primarily on the lower extremity who presented to us over a ten year period. Several possible predictors were also examined. This first study on health related quality of life in patients with vascular malformations of the lower extremity suggests that patients with vascular malformations located primarily on the lower extremity do not have a considerable decreased quality of life when compared to the general Dutch population. Our study population group report more bodily pain and worse vitality than a general Dutch population sample. Because the mean age of our VM patients is lower than that of the reference population, and since SF36-scores are negatively associated with age, this might have resulted in a slight underestimation of the difference in quality of life scores. Regarding other dimensions of health related quality of

life as assessed with the SF36, no differences were observed between both groups. The total explained variation in the quality of life of patients with vascular malformations on the lower extremity by the selected characteristics is small. Influence of patients wearing orthopedic shoes, elastic compression stockings, and those with hand and lower extremity involvement can explain some of the decrease in quality of life. Since the number of patients studied was small, insufficient statistical power to detect significant associations might have attributed. In addition, it suggests that quality of life is influenced by many more factors than the selected ones in the present study, but because of the heterogeneity of these anomalies it seems that it will be difficult to describe characteristics specific for these anomalies. It is reasonable to assume that the patients' quality of life is a result of a complex interaction of disease outcome, personal traits, coping behavior, social support, and the quality of care received. More studies are needed to investigate the quality of life in patients with vascular malformations. When we look at our study, it is possible that patients with for example predominantly hand involvement will have a worse quality of life than patients with predominantly lower extremity involvement. Further studies should be conducted to clarify this question.

Management of patients with vascular malformations encompasses the whole spectrum of care of patients. Most centers treating these patients have teams consisting of a spectrum of medical specialties. Although most vascular malformations are not life-threatening, it is also most often not a curable disease. Two words dominate the rules of therapeutic management of all types of vascular malformations: a multidisciplinary approach and modesty. Education is essential and patients should be educated in the natural history of the malformation and of the limitations of the current treatment methods.

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Samenvatting, conclusies en toekomst perspectieven

Vasculaire malformaties zijn afwijkingen die, zowel in het verleden als in het heden, nog steeds niet goed begrepen worden. De Latijnse benaming voor capillaire malformaties (wijnvlekken) is *naevus flammeus*, omdat men vroeger geloofde dat de moeder tijdens de zwangerschap geschrokken zou zijn van vuur (1). Sinds mensenheugenis worden deze afwijkingen omringd door stigma's. In de Middeleeuwen werden deze huidafwijkingen van pasgeboren baby's soms gezien als een teken van verkeerd (zondig) gedrag gedurende de zwangerschap (1). Montaigne (1533-1592), een sceptisch schrijver, had geen enkele twijfel over de oorsprong van deze aangeboren vlekken: *"We weten uit ervaring dat vrouwen deze tekens van hun verbeeldingskracht meegeven aan de lichamen van de kinderen die ze in hun baarmoeder dragen" (2).* Totdat de classificatie van Mulliken en Glowacki werd geïntroduceerd in 1982 werden termen als hemangiomen, veneuze angiomen, caverneuze angiomen of caverneuze hemangiomen afwisselend gebruikt voor laesies die in feite vasculaire malformaties zijn. Nadat deze biologische classificatie was geïntroduceerd, was er tenminste een manier beschikbaar om vaatafwijkingen in twee groepen te verdelen: vasculaire tumoren (meestal hemangiomen) en vasculaire malformaties. Sinds 1982 zijn er fundamentele verbeteringen gekomen in ons begrip van deze afwijkingen. Mede gebaseerd op de recente snelle ontwikkeling van onze kennis over moleculaire processen, vormt dit proefschrift een poging om enige belangrijke verbeteringen van inzicht van de afgelopen jaren te beschrijven.

Het is duidelijk dat een deel van de informatie in **hoofdstuk 2** nu alweer verouderd is. Het was ook niet mogelijk om alle beschikbare informatie te verwerken in een overzicht, zoals ook werd aangegeven in het commentaar van Michael Cohen in *Plastic and Reconstructive Surgery* ® (3). Mede dankzij oncologisch onderzoek naar vaat ontwikkeling (angiogenese) komt voortdurend nieuwe informatie beschikbaar. In januari 2003 gaf Medline op Internet ongeveer 20.000 hits voor de zoekterm TGF-beta en bijna 8.000 bij de term VEGF. Ondanks deze overvloed aan informatie willen we toch een poging ondernemen tot het beschrijven van enkele fundamentele verbeteringen in ons begrip van vasculaire malformaties. Slechts enkele aandachtspunten kunnen hier worden besproken. Voor uitgebreidere, en ook meer recente informatie, wordt verwezen naar relevante Internet adressen.

Na capillaire malformaties, zijn veneuze malformaties de meest voorkomende vasculaire afwijkingen. De frequentie van voorkomen is onbekend, maar wordt geschat tussen 1:5000 en 1:10.000 bij pasgeboren baby's (4). In hoofdstuk 2 wordt de mutatie beschreven in R849W (chromosoom 9) bij een familiaire veneuze malformatie. Sindsdien is er nog een andere mutatie

gevonden, Y8976S, die ook in hetzelfde kinase domein voorkomt (5). Zowel de werking van de signaal cascade van de TIE-2 receptor, als de oorsprong van het bijbehorende fenotype zijn nog niet duidelijk. Er wordt verondersteld dat de mutaties veranderingen veroorzaken in de controle van de endotheelcelcyclus, die vervolgens kunnen leiden tot een relatief tekort aan gladde spiercellen (4). Momenteel word hier meer onderzoek naar verricht.

Glomuveneuze malformaties (GVM) zijn veneuze malformaties waarbij "glomus cellen" betrokken zijn die rondom de gedilateerde venen aanwezig zijn. GVM is in feite een subgroep van de veneuze malformaties (VM) (4). Meestal is het onderscheid tussen deze afwijkingen en veneuze malformaties klinisch te maken (6). GVM zijn verheffingen in de huid, blauw purper van kleur met een knobbelig oppervlak. Bij palpatie zijn ze erg pijnlijk. In tegenstelling tot andere veneuze malformaties worden GVM zelden aangetroffen op mucosa en zijn ze bijna nooit intramusculair aanwezig. Veneuze malformaties zijn blauw gekleurde laesies, die zowel voorkomen op mucosa als intramusculair. In tegenstelling tot het knobbelige voorkomen van de GVM, zijn veneuze malformaties meestal vlak. Hyperperkerotose is een ander kenmerk voor differentiatie tussen GVM en VM. Dit verschijnsel komt alleen voor bij de GVM en niet bij veneuze malformaties. Differentiatie is ook mogelijk door middel van houdingsafhankelijk onderzoek. Bij dit onderzoek laat de GVM geen verschil zien en de VM wel.

Genetische studies ondersteunen de klinische en histologische verschillen tussen de beide afwijkingen. Er wordt aangenomen dat 78% van de GVM erfelijk zijn, terwijl dit slechts 2% is bij de VM. Het betrokken gen "VMGLOM" is geidentificeerd als een locus op chromosoom 1 zoals al aangegeven in hoofdstuk 2. Aanvullend onderzoek bij extra families met familiaire GVM stelden Irrthum et. al. in staat om het gebied op chromosoom 1 te vernauwen tot 1.48-Mpb YAC (7). Er zijn geen bekende genen gerelateerd met vasculogenese in dit gebied en verondersteld wordt dat het gemuteerde gen een nieuwe factor kan zijn die het proces van vasculogenese regelt. Het zou interessant zijn om te onderzoeken of dit gen met TIE-2 samenwerkt òf dat het een signaalfunctie heeft voor TIE –2, te meer omdat VM een relatief tekort aan gladde spiercellen heeft en GVM een variabel overschot vertoont aan gemodificeerde gladde spiercellen (3).

Hoofdstuk 3 is gewijd aan het in beeld brengen van de locus van een autosomale dominante afwijking in een familie bestaande uit drie generaties. Deze afwijking manifesteert zich als een multipele cutane capillaire malformatie. Het is mogelijk dat de multifocale aard van deze laesie een aanwijzing zou kunnen zijn voor de familiaire predispositie. We hebben de locus voor deze autosomale dominante afwijking in deze familie vastgesteld op chromosoom 5q13-22. Dit was

de eerste keer dat een locus werd gevonden voor capillaire malformaties. Na publicatie heeft een andere studie een locus gevonden in hetzelfde gebied en het is te verwachten dat in de loop van de tijd de bijbehorende gen(en) zullen worden aangetoond. (8). Onze beschreven locus omvat 49cM tussen de markers D5S647 en D5S659 en bevat verschillende kandidaat-genen. Zoals in hoofdstuk 4 wordt aangegeven, is de abnormale neuronale ontwikkeling mogelijk de voornaamste oorzaak van capillaire malformaties. Verschillende genen die betrokken zijn bij neurogenese bevinden zich in dit gebied. Dit gebied bevat het FER-gen waarvan is aangetoond dat het betrokken is bij het zenuw groeiproces, en het EFNA5-proteïne ook van invloed op axon differentiatie. Verdere verkleining binnen het huidige gebied op 5q en de daarop volgende mutatie-analyse zou ons in staat moeten stellen om het juiste gen te kunnen bepalen. Recente studies geven echter aan dat de expliciete verschillen die we maken tussen hemangiomen en capillaire malformaties, door gebruik te maken van de classificatie van Mulliken en Glowacki, wel eens niet zo eenduidig zouden kunnen zijn als we nu veronderstellen (9).

In **hoofdstuk 4** hebben we de huidige stand van zaken uiteengezet betreffende de pathologie van capillaire malformaties. In deze samenvatting wordt duidelijk dat er nog steeds aanzienlijke hoeveelheden informatie kunnen worden gewonnen uit grondig histologisch (elektronen microscopie) onderzoek. Het lijkt erop dat de pathologie van capillaire malformaties zich bevindt in de post capillaire venules en de daarop aansluitende kleine venules en dat onze definitie van "wijnvlekken" als zijnde een capillaire malformatie, onjuist is. Een belangrijk pathologisch kenmerk bij capillaire malformaties is de verminderde neuronale innervatie. Het lijkt dus aannemelijk deze laesies ook als neuronale malformaties te beschrijven. Het is zelfs mogelijk dat alle vasculaire afwijkingen secundair zijn aan neurologische pathologie en dat capillaire malformaties zuivere neuronale malformaties zijn met secundaire vasculaire dilatatie. Verder onderzoek van capillaire malformaties zou zich niet alleen op de vaatopbouw moeten richten, maar ook op de innervatie van de vaten. Hoewel histologisch onderzoek heeft aangetoond dat het gedilateerde gedeelte van de capillaire malformatie zich beperkt tot het venulaire gedeelte, weten we nog erg weinig van de werkelijke innervatie van het arteriolaire deel en moet verder onderzoek hierin nog meer duidelijkheid verschaffen. Angiogenese is ook een essentieel proces bij de regeneratie van zenuwen. Endoneuronale vaten geven toegang voor hematogene macrofagen en zuurstof/ voedingsstoffen om de beschadigde zenuw te bereiken. Dit proces is essentieel voor de ontwikkelen van neuronen (10). Het is verbazingwekkend hoe weinig we weten over de invloed van VEGF op de ontwikkeling van zenuwen. De rol van VEGF in de angiogenese van het zenuwstelsel is onweerlegbaar, en het concept dat VEGF ook direct neuronale effecten heeft, krijgt steeds meer steun vanuit relevante experimenten (10). Met de gezamenlijke ontwikkelingen

van het vasculaire systeem, het lymfatische systeem en het zenuwstelsel lijkt het logisch dat toekomstig onderzoek naar capillaire malformaties zich zal concentreren op de "genese" als geheel en niet slechts op de vasculo- en angiogenese.

Hoewel een volledige moleculaire beschrijving ons zal helpen bij het stellen van een duidelijke diagnose is de belangrijkste vraag natuurlijk welke behandeling mogelijk is. Om deze vraag goed te kunnen beantwoorden is een beschrijving van het betrokken weefsel noodzakelijk. **Hoofdstuk 5** geeft een overzicht waarom MRI op dit moment de beste mogelijkheden geeft tot het bestuderen van vaatmalformaties in het bijzonder als een interventie wordt overwogen. Echter, in dit hoofdstuk wordt ook gewezen op de beperkingen van de MRI. Hoewel klinische kenmerken vaak differentiëren tussen de verschillende typen vasculaire malformaties mogelijk maken, is MRI vaak onmisbaar bij het definiëren van de ernst van de afwijking. Hoofdstuk 5 geeft ook een overzicht van enkele verschillen tussen "high-flow"en "low-flow" laesies bij de MRI en bij echografie. Geen enkele beeldvormende techniek kan antwoord geven op al onze vragen, waardoor de keuze van techniek bepaald wordt door de onderzoeksvraag. Een beslisboom, die kan helpen een onderscheid te maken tussen de verschillende vasculaire malformaties op basis van MRI kenmerken, wordt gepresenteerd. Zelfs met behulp van contrast middelen kan het erg moeilijk zijn onderscheid te maken tussen lymfatische- en veneuze malformaties. Klinische bevindingen blijven belangrijk, vooral omdat veneuze- en lymfatische laesies soms samen voorkomen, waardoor differentiatie alleen door MRI heel moeilijk blijkt te zijn.

Historisch gezien zijn de mogelijkheden om vasculaire malformaties chirurgisch te behandelen gering. In het verleden is differentiatie op basis van high- en low- flow laesies op de MRI goed beschreven, maar er is weinig gedetailleerde informatie beschikbaar die duidelijk de vaatmalformatie vaststelt. In **hoofdstuk 6** definiëren we niet alleen de MRI kenmerken van high- en low-flow laesies, maar geven we bovendien gedetailleerde informatie betreffende de omvang van de lokale uitbreiding en beschrijven kenmerke van het aangrenzende weefsel op de onderste extremiteiten. We hebben daartoe 40 MRI's van 34 patiënten met low-flow laesies en 6 patiënten met high-flow laesies bestudeerd. Bij 23 van de 34 patiënten met low-flow laesies waren ook de spieren aangetast, terwijl dit bij de high-flow laesies 4 maal optrad. Als de verschillende spiercompartimenten worden vergeleken, blijkt dat bij 80 % van de laesies in het boven been het anterior compartiment was aangetast. Het posterior compartiment was bij 86.6% van de laesies in het been betrokken. De "angiosoom hypothese" wordt ook toegepast om dit fenomeen te verklaren. De zones van de angiosomen lopen niet gelijk met de begrenzing van de spieren, maar er middenin. Het is mogelijk dat in het verleden slechts resectie van de zichtbare vaatafwijkingen werd uitgevoerd. De resectie werd waarschijnlijk beëindigd aan het

einde van de spier en niet aan het einde van het angiosoom. Vermoedelijk reageert het restant van de afwijking ten gevolge van de hemodynamische veranderingen op een agressieve manier onder invloed van de veranderde bloed dynamica. Deze studie maakt verder duidelijk dat bij 61 % van de patiënten met intramusculaire uitbreiding er meer dan drie spieren betrokken waren. Vijfentwintig procent van de patiënten vertoonde ook intraossale uitbreiding. Het is dus onwaarschijnlijk dat chirurgische interventie op zich zelf kan leiden tot genezing van deze patiënten. Deze observatie wordt verder versterkt door het feit dat 20 % van de patiënten multifocale laesies hebben. Een aanzienlijk aantal van de patiënten met intramusculaire uitbreiding vertoonden bovendien locale spieratrofie. Dit wijst er tevens op dat zelfs als het mogelijk is om de vasculaire malformatie te excideren, ook de resterende spieren onderzocht moeten worden (hoewel het moeilijk is om de exacte functies van de resterende spieren te beoordelen). Onze studie omvatte ook 11 low-flow laesies met subcutane hypertrofie. Vijf van deze laesies hadden geen geassocieerde subcutane vasculaire malformaties. Het is mogelijk dat het afwijkende vetweefsel ook is geassocieerd met de vasculaire malformatie, zoals ook wel zuivere capillaire malformaties zijn geassocieerd met hypertrofie van de extremiteiten. Verder onderzoek is nodig om deze observaties te verduidelijken. De angiosoom hypothese moet tevens nog uitgetest worden voor andere locaties zoals hoofd, nek en de bovenste extremiteiten.

Eerdere studies hebben de radiologische geconstateerde skeletafwijkingen, die in samenhang met vasculaire malformaties worden gezien al beschreven. Er is weinig bekend over klinische kenmerken van patiënten met vasculaire malformaties en ossale uitbreiding. **Hoofdstuk 7** beschrijft de klinische kenmerken van 18 patiënten met botaantasting in samenhang met vasculaire malformaties van de onderste extremiteiten. Ondanks het feit dat enkele onderzoeken aangeven dat vasculaire malformaties met geassocieerde botaantasting zeldzaam zijn, lijkt het omgekeerde de uitkomst van onze studie te zijn. Twintig procent (18/90) van onze groep patiënten met vasculaire malformaties van de onderste extremiteit vertoonden ook botaantasting. Hiervan hadden 15 patiënten low-flow laesies en 3 high-flow laesies. Zoals wordt getoond in tabel C en D in hoofdstuk 7, was pijn de meest voorkomende reden om een arts te consulteren, veelal vergezeld van een klacht over de ongelijke beenlengte. Van de zes patiënten met ossale uitbreiding klaagde er aanvankelijk geen over pijn, terwijl 10 van de 12 patiënten met periosteale betrokkenheid al direct pijn aangaven. Deze pijn kan mogelijk verklaard worden uit de spanning die optreedt ten gevolge van de druk van de malformatie op het periosteum. Slechts 50 % van de patiënten met symptomen van hemarthrose in de knie vertoonden intra-ossale uitbreidingen. Hoewel alle andere patiënten aantasting van de ligamenten in de knie hadden, was dit op de MRI nauwelijks zichtbaar. Hoofdstuk 7 richt zich op de 18 patiënten die zichtbare botaantasting

hadden op de MRI. Er wordt niet ingegaan op de symptomen van patiënten zonder botaantasting. Verder onderzoek moet aangeven in hoeverre er werkelijke verschillen bestaan.

Wanneer de diagnose is gesteld en de radiologische visualisatie therapeutische interventie heeft uitgesloten, worden de patiënten vaak naar huis gestuurd zonder een curatieve behandeling. Deze patiënten wordt dan vaak verteld om er maar mee te leren leven en bijvoorbeeld gebruik te maken van pijnstillers en elastische kousen om hun laesies van de onderste extremiteiten te behandelen. Met uitzondering van capillaire malformaties hebben de negatieve psychosociale gevolgen van vasculaire malformaties weinig aandacht gekregen in de medische literatuur. Dit in tegenstelling met de aandacht voor de fysieke morbiditeit. Hiertoe hebben we een studie naar de kwaliteit van leven opgezet met behulp van de MOS Short Form Health Quetionnaire (SF-36). In **hoofdstuk 8** worden patiënten met vasculaire malformaties van de onderste extremiteit beschreven die zich in de afgelopen tien jaar bij onze kliniek hebben gepresenteerd. Verschillende mogelijke voorspellende factoren zijn onderzocht. Deze eerste studie naar de kwaliteit van leven van patiënten met vasculaire malformaties van de onderste extremiteit suggereert dat patiënten met deze afwijkingen geen aanzienlijk verlaagde kwaliteit van leven aangeven als we dat vergelijken met de algemene beleving van de Nederlandse bevolking. Onze populatie gaf echter wel aan meer lichamelijke pijn en een verminderde vitaliteit te hebben dan de Nederlandse bevolking. Omdat de gemiddelde leeftijd van onze populatie lager is dan die van de referentie groep èn omdat de SF-36 score negatief geassocieerd is met leeftijd, zou dit kunnen hebben geleid tot een geringe onderschatting van het verschil van de scores van de kwaliteit van leven. Ten aanzien van andere aspecten van gezondheid in dit kwaliteit van leven onderzoek vinden we geen verschillen tussen beide groepen. De totale geconstateerde variatie in de kwaliteit van leven van deze patiënten met vasculaire malformaties aan de onderste extremiteit is dus gering. De invloed van patiënten met orthopedische schoenen of elastische kousen en patiënten met hand- of andere vasculaire malformaties kan een deel van de vermindering in de kwaliteit van leven verklaren. Wegens het geringe aantal ondervraagde patiënten, is er onvoldoende statistische onderbouwing om significante verbanden met de verschijnselen aan te tonen. Bovendien wordt gesuggereerd dat de kwaliteit van leven nog door veel meer factoren wordt beïnvloed dan de onderzochte factoren in dit onderzoek. De variatie van deze afwijkingen maakt het erg moeilijk om specifieke kenmerken van deze afwijkingen te beschrijven. Het lijkt aannemelijk dat de kwaliteit van leven van deze patiënten het resultaat is van een complexe interactie tussen de individuele symptomen, de persoonlijkheid, de copingsmechanismen, de sociale ondersteuning en de kwaliteit van de ontvangen zorg. Verder onderzoek naar de kwaliteit van leven bij deze patiënten is nodig.

Het behandelen van patiënten met vasculaire malformaties omvat het gehele spectrum van de patiëntenzorg. De meeste behandelcentra beschikken over gespecialiseerde teams waarin een aantal relevante medische disciplines zijn vertegenwoordigd.

Hoewel de meeste vasculaire malformaties niet levensbedreigend zijn, zijn ze meestal ook niet te genezen. Twee woorden bepalen het beleid dat moet worden toegepast bij de behandeling van alle vasculaire malformaties: een multi-disciplinaire aanpak en bescheidenheid. Daarnaast is het belangrijk dat patiënten worden voorgelicht over het natuurlijke beloop van de afwijking en tevens over de beperkingen van de huidige behandelmogelijkheden.

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Addenda

Writing a thesis is a group effort and involves many people, although only one name appears on the front cover. Although it is impossible to be complete, I want to thank some people in particular.

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This thesis would not have been possible without the assistance of patients and families of patients with vascular malformations.

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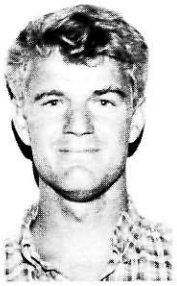
The birth of my daughter in March of this year was again a confirmation to me of how beautiful the human body is designed by God. Although we are beginning to understand something about the incredibly complicated construction of the human body, this study on vascular anomalies has indicated to me that our knowledge is still only reaching the tip of the iceberg.

In 1925 M.R. Reid stated the following assertion which is still applicable today:

> *" In view of the common development on each side of the vascular tree, and in view of the enormous constructive and destructive changes necessary before the final pattern of the vascular tree is reached, it is a marvel not that abnormal congenital communications occasionally, or rarely, occur, but that they do not occur more often" .*

I hope that this thesis will provide an impetus for further scientific research.



Corstiaan Cornelis Breugem was born in Bleiswijk, the Netherlands on the 23rd of July 1970. At the age of six years his family emigrated to Paarl, Western Cape, South Africa. There he finished high school at Boys' High School, Paarl, in December 1988. He did his medical training at Tygerberg Hospital, the University of Stellenbosch and graduated in December 1994. In 1995 he worked as a House Officer at Windhoek General Hospital, Windhoek, Namibia. For the greater part of 1996 he worked as a Senior House Officer in the Accident and Emergency department of Barnet Hospital in London, the United Kingdom. The last two months of 1996 he did research on craniosynostosis at the department of Plastic Surgery of Tygerberg Hospital, South Africa. During this time he met "a Dutch girl called Darinka". From the beginning of 1997 he worked in the general surgery department of Livingstone Hospital, Port Elizabeth, South Africa. In August 1997 he followed his heart to the Netherlands and he started work in the department of general surgery in Amersfoort, the Netherlands. From May 1998 he worked as a Senior House Officer at the department of Plastic, Reconstructive and Handsurgery at the Academic Medical Center, University of Amsterdam. During that time much of the work presented in this thesis was initiated. In 2000 he started with his general surgery training at the Lucas Hospital in Apeldoorn. From October 2002 he continued his residency training in Plastic, Reconstructive and Handsurgery at the Academic Medical Center of the University of the Amsterdam. In October 1998 he married Darinka, and in March 2003 their daughter Sophia was born.

The important thing is not to stop questioning.
Albert Einstein

Table Mountain - South Africa