INFANTILE HAEMANGIOMA

PATHOGENESIS, EVALUATION, AND THERAPY

Sherief Janmohamed

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CHAPTER 1

Educational paper: Pathogenesis of infantile haemangioma, an update 2014 (part I)

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ABSTRACT

Infantile haemangioma (IH) is the most frequent childhood tumour. Although it is benign and self-limiting, severe complications can arise due to localisation and fast tumour growth. Management and therapy of IH has changed greatly after 2008 with propranolol. However, the pathogenesis remains elusive. This update provides an overview of all possible mechanisms currently considered. We discuss the possibility that several mechanisms act together, although local hypoxia seems to be important. Clinically, in about half of the cases, an IH is preceded by an anaemic macula (local ischaemia) or a so called precursor lesion. Laboratory findings indicate stabilisation and an increased transcription activity of hypoxia inducible factor 1α (HIF1α), leading to up-regulation of its downstream target genes (such as VEGF, vascular endothelial growth factor), which normally occurs in cases of hypoxia. *Conclusion:* Three main hypotheses have been proposed, namely 1) the theory of tissue hypoxia, 2) the theory of embolization of placental endothelial cells, and 3) the theory of increased angiogenic and vasculogenic activity.

- This update provides an overview of all possible mechanisms currently considered in the pathogenesis of infantile haemangioma.
- We discuss the possibility that several mechanisms act together, although (local) hypoxia seems to be crucial.

LIST OF ABBREVIATIONS

$BNIP_3$	BCL_2/adenovirus E_1B kD-interacting protein (BNIP) family member 3
CA-IX	Carbon Anhydrase IX
ERK	Extracellular signal-Related Kinase
GLUT-1	Glucose Transporter 1
HIF1α	Hypoxia Inducible Factor 1α
HIF2α	Hypoxia Inducible Factor 2α
IDO	Indoleamine 2,3 dioxygenase
IGF	Insuline-like growth factor
IH	Infantile Haemangioma
MAPK	Mitogen-Activated Protein Kinase
MMP-9	Matrix Metallopeptidase 9
mTOR	Mammalian Target Of Rapamycin
$mTORC_1$	mTOR Complex 1
pAKT	Phosphorylated v-akt murine thymoma viral oncogene homolog 1
pS6	Phosphorylated S6 protein
ROP	Retinopathy of prematurity
SDF1α	Stromal cell-derived factor 1α
TNF-α	Tumour Necrosis Factor α
VEGF	Vascular Endothelial Growth Factor
VEGF-A	Vascular Endothelial Growth Factor A
VEGFR	Vascular Endothelial Growth Factor Receptor

INTRODUCTION

Reported incidences of infantile haemangioma (IH) vary greatly, but in any case it is the most frequent childhood tumour, with incidences of 5–10% up to 20% in prematurely born infants[1-3]. These tumours occur predominantly in the Caucasian population[4]. IHs follow a typical course: they arise within the first few days to weeks after birth and most IH grow exponentially for up to 6 to 9 months. Hereafter, regression follows, by approximately 10% per year[5]. Thus, most IHs have gone at the age of 10 years but a scar can remain[6]. Although IHs are benign and self-limiting, severe complications may arise due to localisation and accelerated tumour growth[7]. Figures 1–7 show several different IHs. Despite extensive literature (especially after 2008), the pathogenesis is still not clear[8]. This update provides an overview of mechanisms that are currently being considered, and some aspects of these theories are discussed.

In a proliferative IH, rapidly growing endothelial cells form blood vessels. Increased apoptosis of endothelial cells in the involution phase leads to regression of blood vessels. Eventually, the thick multilaminated basement membrane surrounding the endothelial layer is replaced by adipocytes in fibrous tissue[9]. Furthermore, a considerable increase in the number of mast cells during the involution phase may alter the balance of angiogenic factors, thus promoting regression[10]. The empirically based current therapy aims to induce/accelerate the natural involution process[9]. Systemic propranolol induces quick therapeutic responses and has made corticosteroids and all other treatment options obsolete[11,12]. However, propranolol has potential side effects and sometimes must be used for 1–2 years (own experience). As topical use has limitations as well, we are still searching for a better alternative. Propranolol and corticosteroids both act on factors induced by HIF1α as a result of local hypoxia[12,13], supporting a crucial role of local hypoxia in IH. A better understanding of the pathogenesis may improve targeted therapy options in the management of IH[14].

Characteristics of proliferating versus involuting IHs[15]

IHs consist of multipotent stem cells (CD133+), immature endothelial cells (CD31+), pericytes (SMA+), dendritic cells (factor XIIIa+) and mesenchymal cells (with adipogenic potential). During the proliferative phase, endothelial and interstitial cells express a marker of proliferation, namely the antibody MIB 1. Furthermore, CD31+ endothelial cells are clonal and express a particular phenotype: IDO, LYVE-1, merosin, CCR6, GLUT-1, antigen Lewis Y (Ley), antigen FcRγII, and CD15. This phenotype changes over time with the maturation of endothelial cells (see figure 8). However, GLUT-1 stays positive and therefore can discriminate between IHs and other vascular malformations[16,17]. During involution, endothelial cells express caspaces, which are known markers of apoptosis.

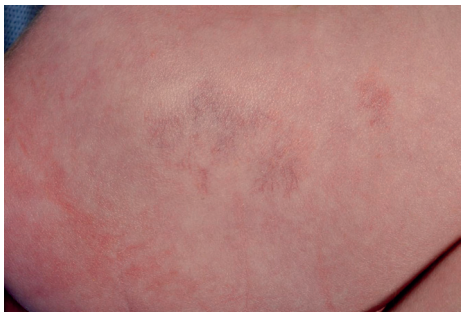


Figure 1 – Precursor lesion before development of an infantile haemangioma

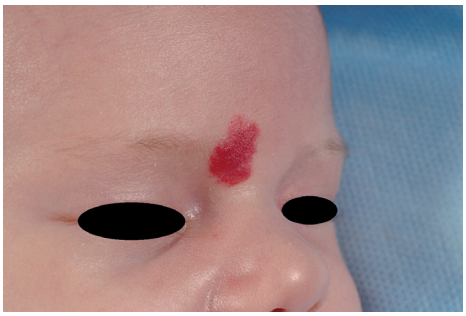


Figure 2 – Infantile haemangioma in the active (proliferative) phase

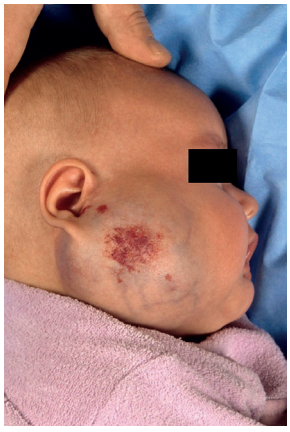


Figure 3 – Infantile haemangioma with both deep swelling and a superficial component

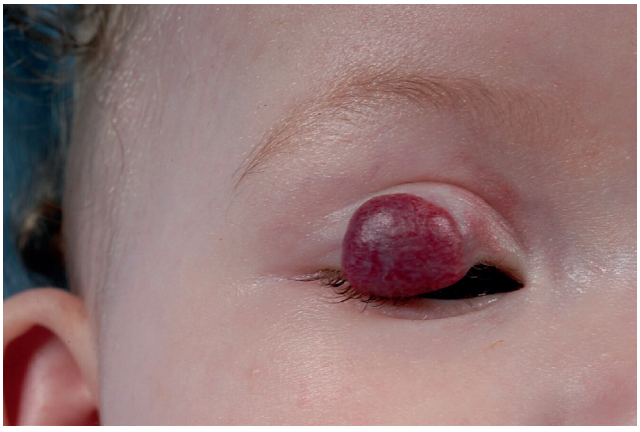
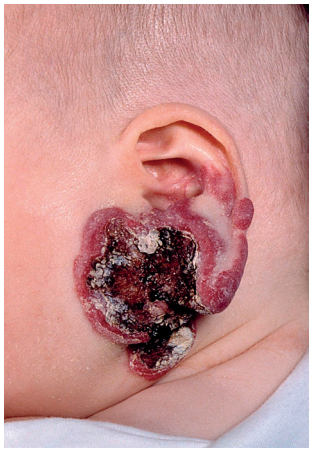


Figure 4 – Alarming infantile haemangioma: risk for eye abnormalities



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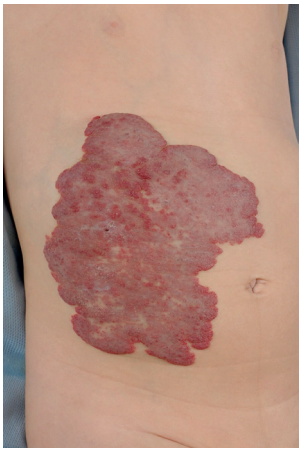

Figure 6 – Infantile haemangioma in the involutive phase

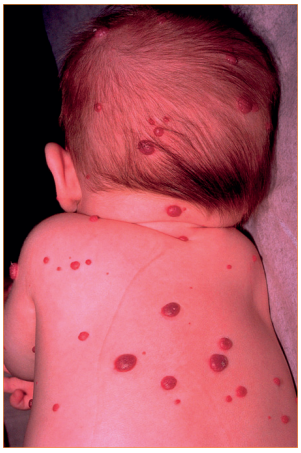

Figure 7 – Haemangiomatosis

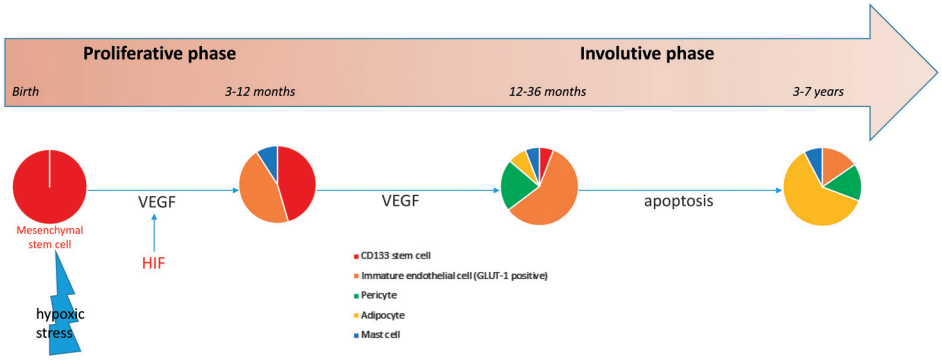

Figure 8 – Pathophysiological mechanisms at a cellular level in the course of infantile haemangiomas: a stem cell (CD 133), under hypoxic conditions resulting in the activation of the HIF pathway and overexpression of VEGF, multiplicates, and differentiates into endothelial progenitor cells (CD31+), mesenchymal cell precursors of adipocytes and pericytes. Based on figure 6 of the article of Léauté-Labrèze C, Prey S, and Ezzedine K. J Eur Acad Dermatol Venereol. 2011 Nov;25(11):1245–53

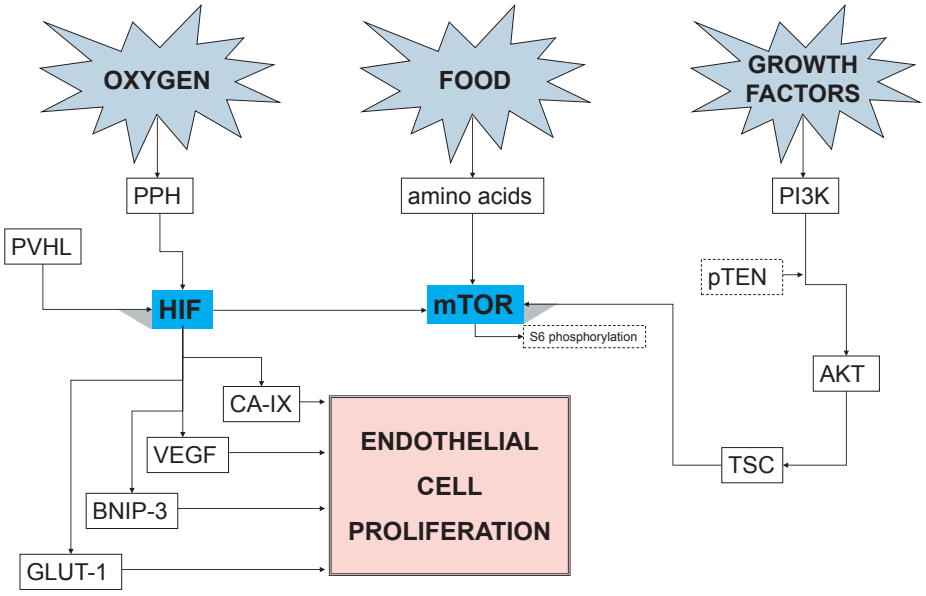

Figure 9 – Simplification of the interaction between the hypoxia inducible factor (HIF) pathway, the mammalian Target Of Rapamycin (mTOR) pathway, and several factors, resulting from local hypoxia into endothelial cell proliferation (proliferative infantile haemangioma). Explanation: hypoxia triggers stabilisation at the protein level of the transcription factor hypoxia-inducible factor (HIF)1α. HIF1α in turn stimulates transcription of downstream target genes such as those encoding BCL2/adenovirus E1B kD-interacting protein family member 3 (BNIP3), carbon anhydrase (CA)-IX, glucose transporter (GLUT)-1, phosphorylated protein kinase B (pAKT), phosphorylated S6 protein (pS6) and vascular endothelial growth factor (VEGF). These target genes can be regulated either directly by HIF1α or by hypoxia-induced regulation of mammalian target of rapamycin complex (mTORC)1 signalling. mTORC1 is a key player in the mTOR pathway, a protein complex with a central role in regulating cellular metabolism, driven by growth factors and nutrients as well as hypoxia.

Simultaneously there is an increase in expression of markers of maturation and activation of endothelial cells such as HLA-DR and ICAM-1 (CD54). Mesenchymal cells differentiate into adipocytes at this stage. Moreover, in a recent report it was concluded that apoptosis is prevented in proliferative IHs by an up-regulated autocrine VEGF/VEGFR2 signalling loop. VEGF also activates the survival-promoting PI3K/Akt pathway. Activation of Akt in turn stimulates the expression of anti-apoptotic proteins, such as Bcl-2. Thus the up-regulated autocrine VEGF loop promotes IH-derived endothelial cells survival via regulation of the PI3K/Akt/Bcl-2 pathway[18].

Hypotheses

Many mechanisms have been considered to explain the development of IHs. Three competing hypotheses are currently being considered, which are, however, not mutually exclusive[19]:

1. Tissue hypoxia.
2. Embolization of placental endothelial cells.
3. Increased angiogenic and vasculogenic activity.

Tissue hypoxia seems to be the most powerful inducer of angiogenesis (and vasculogenesis). A relation was found between placental hypoxia and IHs[20]. Also the relationship between low birth weight and IH and the association between ROP and IH points to hypoxia[21].

As a less important hypothesis, genetic involvement has been proposed[22].

Hypoxia

Local hypoxia may be involved in the pathogenesis of IH[23-26]. In 50% of cases the skin is blanched (precursor lesion) at the site where an IH will eventually develop, supporting the idea that local ischaemia is important. A hypoxic environment triggers stabilisation at the protein level of the transcription factor HIF1α[27]. HIF1α in turn stimulates transcription of downstream target genes such as BNIP3, CA-IX, GLUT-1, pAKT, pS6, and VEGF[28]. These target genes might be regulated either directly by HIF1α signalling or by hypoxia-induced regulation of mTORC1 signalling[29]. mTORC1 is a key player in the mTOR-pathway, a protein complex with a central role in regulating cellular metabolism, driven by growth factors, nutrients as well as hypoxia (figure 9). Deregulation of the mTOR-pathway may lead to disorganized growth[30]. As macrophages secrete pro-angiogenic molecules such as TNF-α and interleukin-1, they are also thought to be involved in the evolution of IHs[9,25,31]. Of all theories proposed, the hypoxia-theory seems attractive given the anaemic macula (precursor lesion) often seen and the endothelial cell origin of IHs (cells typically growing under hypoxic conditions)[32]. It is known that the target genes (VEGF, GLUT-1, etcetera) can also be stimulated by hypoxia via HIF2α (alone or in combination with

HIF1α), with the same result[33-35]. HIF-1 is a heterodimer of two proteins: HIF1α and HIF1β. HIF2α forms a functional heterodimer with HIF1β resulting in the HIF2 complex, which activates transcription from the same DNA recognition sites as HIF-1. This activation is stimulated under hypoxic conditions[33-35]. Circulating bone marrow-derived endothelial progenitor cells form new blood vessels in ischemic tissues using mediators regulated by HIF1α. Mobilization is enhanced by VEGF, MMP9 and oestrogen, whereas homing is secondary to localized expression of SDF1α[25].

Placental origin

The placental theory is attractive because it would explain the programmed life cycle of IH. IH might represent "benign metastases" originating from the placenta or other cells that proliferate in areas of low oxygen tension, such as the "end artery, vascular dead end" sites occurring in embryonic fusion planes[36]. Therefore placental embolization is thought to play a causative role[37,38]. Chorionic villus sampling has been associated with an increased incidence of IHs[39]. GLUT-1 is strongly expressed in IHs but not in other vascular malformations; GLUT-1 is also expressed in the placenta. Furthermore, IHs and the placenta also express other molecular markers such as merosin, laminin, Lewis Y antigen, FcγR2, IDO, and IGF-2[40,41]. It has also been noted that placenta and IH have high levels of genetic similarity when compared with other vascular tumours and normal structures[42]. Therefore it has been hypothesized that IH precursor cells originate from the placenta, although subsequent molecular genetic investigations revealed no evidence for maternal-foetal microchimerism[19]. This however does not rule out the possibility of the placental origin of IH tissue because the placenta is predominantly foetal in origin.

Increased angiogenic and vasculogenic activity

Vasculogenesis vs angiogenesis[43]

Both vasculogenesis and angiogenesis have been proposed as mechanisms contributing to the neovascularization in IH. Vasculogenesis is the de novo formation of blood vessels from stem cells. It was long believed that this occurs in foetal life only. Angiogenesis on the other hand is the growth of new blood vessels from pre-existing vessels, which includes migration of endothelial cells.

The group of Greenberger found in 2008 that mesenchymal cells, isolated from proliferative IHs using CD133-coated magnetic beads, are capable of differentiating into endothelial cells, pericytes (perivascular cells), and adipogenic lineages[43]. When implanted into immune-deficient mice, these IH-derived stem cells formed GLUT-1 positive vessels. Greenberger and colleagues therefore concluded that vasculogenesis is an important mechanism underlying IH genesis. Khan and colleagues found evidence that CD133-selected IH-derived stem cells recapitulate human IH in a murine in vivo model[44]. This

did not work with IH-derived endothelial cells. Clonal IH-derived stem cells produced human GLUT-1-positive micro vessels and after a while also human adipocytes. These results demonstrate that IH-derived stem cells are the cellular precursors of IHs. Similar results were found by Xu et al[45].

In the proliferative phase, the blood vessels are small and the endothelium is plump and metabolically active, suggesting an immature phenotype. Mulliken et al have shown that IH-derived endothelial cells form capillary-like tubes in vitro[46]. Boye et al showed that IH-derived endothelial cells are clonal and therefore suggested that they arise from a common precursor[47]. First, IH-derived stem cells differentiate into endothelial cells due to (local) hypoxia (vasculogenesis). Because of juxtacrine signalling between IH-derived endothelial cells and IH-derived stem cells via JAGGED1 signalling through the NOTCH pathway, IH-derived stem cells differentiate into pericytes[48]. There are many pericytes in the proliferating phase and they appear to undergo a maturation process concurrently with the endothelial cells. Recently it was found that pericytes in IH are pro-angiogenic[49]. This triggers angiogenesis.

Other factors

E-selectin, normally found in inflammatory skin, can also be found in proliferating IHs and its expression decreases in involuting IHs[50]. In another study, Smadja et al found evidence that α6-Integrin is increased in proliferating IHs and expressed by IH-derived stem cells[51]. This expression is decreased in involuting IHs. Integrins are receptors important for cellular adhesion to extracellular matrix and to other cells. Furthermore, α6-Integrin is also involved in angiogenesis and is required to form vascular networks in vitro. Finally, hormonal influences may be involved. Oestrogen receptors are also expressed by IH-derived endothelial cells and stimulation with oestrogen increases proliferation, migration and survival of endothelial cells[9,25]. Genetic influences may contribute as several patients with IHs show considerable loss of heterozygosity for markers in a region of chromosome 5q[22,52]. The evidence however is not conclusive and could not be confirmed in bigger studies.

Treatment based on pathogenesis: Rapamycin[53,54]

Treatment, if necessary, is usually with propranolol nowadays. Before 2008 corticosteroids were the traditional first-line therapy. Pointing at side effects and non-responders to therapy (especially in the case of corticosteroids), Greenberger et al make a plea for additional therapies that will shorten treatment duration or maybe even prevent problematic IHs from forming[53]. In their murine model they tested Rapamycin, which is an inhibitor of the mTOR pathway. They concluded that Rapamycin suppresses vasculogenesis in vivo, that self-renewal and multi-lineage differentiation are disrupted by Ra-

pamycin, that Rapamycin leads to mesenchymal maturation and impaired vasculogenic potential, and that Rapamycin stimulates regression of pre-existing vessels formed from IH-derived stem cells. Another option is the monoclonal antibody bevacizumab, which, however, has never been tested in IH[9].

OVERALL CONCLUSION

The pathogenesis of infantile haemangioma remains elusive. There are currently three competing hypotheses which are, however, not mutually exclusive: 1) the theory of tissue hypoxia, 2) the theory of embolization of placental endothelial cells, and 3) the theory of increased angiogenic and vasculogenic activity. Local hypoxia is important: laboratory findings indicate stabilisation and an increased transcription activity of hypoxia inducible factor 1α (HIF1α), leading to up-regulation of its downstream target genes (such as VEGF, vascular endothelial growth factor), which normally occurs in cases of hypoxia.

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CHAPTER 2

Educational paper: Therapy of infantile haemangioma – history and current state (part II)

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ABSTRACT

Infantile haemangioma (IH) is the most frequent tumour of infancy. Although it is benign and self-limiting, severe complications can arise due to localisation and fast tumour growth. Also, IHs leave scars after regression in more than half of the cases. Management and therapy of IH have changed greatly after 2008. This update provides an overview of the older therapy options from before 2008, which mainly consisted of the administration of corticosteroids, and discusses the modern management with new therapy options such as β-blockers (both systemically and topically). *Conclusion:* β-blockers are promising and are currently preferred above corticosteroids, but β-blockers still don't give a definitive treatment.

- This update provides an overview of the older therapy options for infantile haemangioma from before 2008, which mainly consisted of the administration of corticosteroids.
- This update also discusses the modern management with new therapy options such as β-blockers (both systemically and topically).

LIST OF ABBREVIATIONS

bFGF	Basic Fibroblast Growth Factor
HAS	Haemangioma Activity Score
HDCS	Haemangioma Dynamic Complication Scale
HSS	Haemangioma Severity Scale
IH	Infantile Haemangioma
mTOR	Mammalian Target Of Rapamycin
VEGF-A	Vascular Endothelial Growth Factor A

INTRODUCTION

The incidence of infantile haemangioma (IH) varies greatly in the literature but in any case it is the most frequent occurring tumour of infancy with incidences of 5–10% up to 20% in prematures[1-3]. They occur predominantly in the Caucasian population[4]. IHs follow a typical course: they arise within the first few days to weeks after birth and grow exponentially for up to 6 to 9 months. Thereafter, regression follows, with approximately 10% per year; at the age of five years 50% of IHs have disappeared, at the age of 7 years 70%, and most IHs have gone at the age of 10 years[5]. However, a scar can remain[5]. Moreover, a recent publication shows that scarring is present in more than half of the regressed IHs[6]. Although IHs are benign and self-limiting, severe complications can arise due to localisation and accelerated tumour growth[2,7,8]. In these cases, therapy is necessary. The management of IH can vary from conservative ('watch and wait') to radical (surgery/excision) and management and therapy have changed greatly over the years, especially after 2008[9]. This update presents an overview of the available options available over the years and discusses in more detail the treatments that are more and more used nowadays.

History

From 1930 to 1950 (and even beyond), X-irradiation therapy was an effective treatment for IH besides compression therapy[10]. However, apart from the fact that patients were exposed to radiation, X-irradiation therapy was associated with late (tissue) sequelae such as atrophy, contractures, pigmentation, and telangiectasia[11]. From the 1950s onwards a 'watch and wait' approach was advocated given the natural course of IH. Nevertheless, a small proportion of IHs were problematic and needed more than just watch and wait. In the mid-1960s systemic corticosteroids were found to be an effective treatment[12]. Optimal treatment was never defined due to uncertainty about the biology of haemangiomas (tumour or vascular malformation?) In 1982, Mulliken and Glowacki proposed a biologic classification of vascular birthmarks into haemangiomas (infantile haemangioma) and vascular malformations[13]. In the 1980s and 1990s multidisciplinary working groups were established which gained more knowledge and experience[14].

In 1989 laser therapy was further developed and the pulsed dye laser became another potential treatment for IH[15]. It can be effective in very superficial lesions and may help accelerate healing in some ulcerated IHs. However, it has no impact on deeper dermal components. In a randomized clinical trial, pulsed dye laser did not show any benefit in early uncomplicated flat IHs when compared with a wait and see policy[16]. In later stages it can be used for treating residual telangiectasia. However, treatment is painful. Physicians then went in search for better therapies and in 1991 Ezekowitz et al reported the use of interferon alpha as a novel therapy[17]. Interferon alpha is an antiangiogenic

agent that decreases the proliferation of endothelial cells by down-regulation of bFGF (basic fibroblast growth factor). The dosage varies from 1 to 3 million units/m^2/day administered by subcutaneous injection. Treatment is a long affair; it may vary from 6 to 12 months. A complete response rate of 40% to 50% has been reported. We now know that severe neurotoxicity is an important side effect of this treatment[17].

Since 2008

The serendipitous observation of Léauté-Labrèze et al in 2008 that IHs had regressed with orally administered propranolol was an important hallmark[9]. Propranolol then soon became the first choice of treatment, even though it has to be administered for some time and often is not fully effective. Still, more and more patients are now treated with both orally and topically administered β-blockers, with good results. Clinicians would do well, however, to considerer whether treatment is really necessary. Treatment has changed over the last years, but IHs are still self-limiting!

What can be expected for the future? Many research efforts are directed at the pathogenesis of IH and hypoxia seems to be crucial[18-20]. Sirolimus, an mTOR inhibitor, probably has a good effect by blocking mammalian target of rapamycin (mTOR) signalling in the hypoxia pathway[21,22]. We have discussed the pathogenesis and sirolimus in more detail in part I[23].

Alarming versus non-alarming IHs

IHs are very heterogeneous and therefore require individual assessment. Some are very small and superficial and relatively slowly growing, but others may be very big, with a part under the skin and growing very rapidly. We propose to divide IHs into two groups: non-alarming IHs and alarming IHs. Non-alarming IHs do not cause immediate danger and a watch and wait management is adequate. Alarming IHs are IHs that give immediate problems or are expected to give problems at the short term (rapid growth near the eye, rapid growth of internal haemangioma e.g. near the trachea or in the liver). In these cases, oral therapy is indicated without delay. We propose that some non-alarming IHs (especially those in the face) can be treated topically, e.g. for cosmetic reasons or given the fact that IHs leave scars in >50% of cases. It is also possible to treat deeper IHs with a visible superficial part topically in order to diminish the visible discoloration.

Systemic therapy

Corticosteroids

For a long time corticosteroids were the first option for IH treatment, even though really successful results were obtained in only a small proportion (50% or less) of IHs[24,25].

Therapy has always been empiric treatment, and only recently the working mechanism of corticosteroids in the treatment of IH was explained by Greenberger et al[26]. They showed that dexamethasone suppresses the vasculogenic potential of haemangioma-derived stem cells in a murine model. Furthermore, they showed that dexamethasone suppresses Vascular Endothelial Growth Factor A (VEGF-A) expression by haemangioma-derived stem cells in vitro and that silencing of VEGF-A expression inhibits the vasculogenic potential of these cells in vivo[26,27].

Corticosteroids can be administered either orally or intravenously. Adverse effects are reversible and include increased appetite, Cushing syndrome, behavioural changes (restlessness), increased crying, adrenal insufficiency and hypertension)[28]. A recent review showed that corticosteroids (prednisone) were usually administered at a dosage of 2–3 (and even up to 4) mg/kg/day[29]. In 2013, Nieuwenhuis et al showed that (intermittent) short course systemic glucocorticosteroid therapy is a more effective and safer treatment for IH, with a substantially lower cumulative dose of glucocorticosteroids compared to prolonged low-dose therapy[25].

Some IHs, for example periocular IHs, respond better to intralesionally administered corticosteroids, with success rates of up to 64%[30-32]. The obvious advantage of this method is that it is very safe; fewer side effects occur because often just one injection is needed. In some cases a second injection is indicated, but then the total dose is still lower than that with oral corticosteroids. The possible side effects are rare: injection pressure during intralesional injection usually exceeds systemic arterial pressure and poses a risk of corticosteroid particle embolization into the ocular circulation due to retrograde arterial flow. Serious ocular complications include ophthalmic artery occlusion, retinal embolization and central retinal artery occlusion. Others are eyelid hypo-pigmentation, linear subcutaneous fat atrophy, sclerodermiform linear atrophy, eyelid necrosis, and periocular calcification. Very rare systemic side effects include Cushingoid features, growth deceleration, and adrenal suppression[30,32]. A disadvantage is the requirement of general anaesthesia. Usually, a mixture of 3 ml Celestone 4 mg/ml and 2 ml Kenacort-A 40 mg/ml is used[8]. A maximum of 5 ml is then injected at one or several places in the IH.

Propranolol

Propranolol is a non-selective β-blocker and its effect on IHs was serendipitously discovered in 2008 in France[9]. An infant with an IH was given corticosteroids and because of cardial side effects propranolol was administered. After propranolol, quick regression was observed. The authors thereupon began treating IHs with propranolol and a follow-up study showed good results[33]. Since 2008, propranolol rapidly became the first choice of treatment for its good and fast results[34-37].

The most common serious side effects include bradycardia and hypotension but also dyspnoe, cold acra, provocation of decompensatio cordis or hypoglycemia, nightmares and decreased cardiac output[38]. Therefore, important contraindications in children are sinus bradycardia, AV-block, hypotension, asthma and decompensatio cordis, among other things. Furthermore, even caries is described as a side effect of propranolol treatment, resulting from the sweetened solution in which propranolol suspension is being produced by some pharmacists[39]. Real side effects are rare. There are some publications reporting hypoglycaemia and hypotension[40-42] in patients receiving propranolol but in fact, these are rarely directly related to propranolol. Beta-blockers act on β_1-adrenoceptors in the heart, thereby preventing the positive chronotropic and inotropic effects mediated by these receptors. Nonselective beta-blockers (e.g. propranolol) also antagonize the vasorelaxant effect that occurs after stimulation of vascular β_2-adrenoceptors. An increase in peripheral vascular resistance will occur, caused both by the direct vascular effects of propranolol and by activation of the baroreceptor reflex. Blood pressure is therefore unlikely to fall acutely; this usually occurs no earlier than after several weeks of treatment when these compensatory mechanisms have disappeared. Obviously, the effects of beta-blockade will be particularly apparent in patients in whom the sympathetic nervous system has been activated; little or no effect on blood pressure is expected in healthy, normotensive people[43]. Hypoglycaemia is another side effect. However, this occurs mostly in the neonatal period. Older infants and children are considered to be at low risk, and IH therapy is generally started after the neonatal period. A number of case reports published after 2008 describe hypoglycaemia as a side effect in children with IH treated with propranolol[36,40]. However, on close reading it appears that most patients who were hypoglycaemic had an underlying condition; often they were ill or feverish (rectal temperature <36 or >38.5 °C) and were in a fasting state[44]. This observation has been summarized by Holland et al[42]. The mechanism by which hypoglycaemia develops, aside from less oral intake, is not completely understood. Also, normal glucose homeostasis is thought to be impaired through inhibition of adrenergic mediated glycogenolysis, gluconeogenesis and lipolysis. Children (and infants) seem to be at a higher risk for this adverse effect because their glucose use is higher while fasting (attributed partly to their greater brain mass relative to their body weight). In addition, glycogen stores are lower in infants and children compared with adults, leading to a reduced fasting ability[45]. Thus, when treating healthy infants with beta-blockers, hypoglycaemia normally does not occur.

Very little is known about the mechanism of action of propranolol in IH. In 2010, Storch and Hoeger presented an overview of how propranolol interferes with endothelial cells, vascular tone, angiogenesis and apoptosis[46]. They distinguished three stages, with immediate (early), intermediate and long-term effect. These effects of propranolol on IH

can be attributed to three different pharmacological targets. Early effects (brightening of the haemangioma surface within 1–3 days after start of therapy) are attributable to vasoconstriction due to decreased release of nitric oxide. Intermediate effects are due to the blocking of proangiogenic signals (vascular endothelial growth factor, basic fibroblast growth factor, matrix metalloproteinase 2 and 9) and result in growth arrest. Long-term effects of propranolol are characterized by induction of apoptosis in proliferating endothelial cells, which results in tumour regression[46]. Kum and Khan differentiated in their research between IH-derived endothelial cells and IH-derived stem cells[47]. They were the first to find that there was no apoptosis in IH-derived stem cells. Apoptosis was however seen in IH-derived endothelial cells.

Although used in IH since 2008 and many years before in paediatric cardiology, a uniform recommendation on dosage, starting up and duration of propranolol is not yet available. Most physicians tended to start very carefully, for example at a dosage of 1 mg per kg per day and increasing this to 2 mg/kg/day during hospitalisation[48]. Blood pressure and glucose are closely monitored and an ECG is taken. Rebounds were commonly seen and therapy lasted for more than one (and even 2) years[48]. Nowadays most infants start with propranolol in a day-care unit. We commence therapy with 1 mg/kg/day in three times, after thorough physical examination, ECG, glucose, and blood pressure and pulse measurements. After propranolol, the first day, we also check glucose, and blood pressure and pulse measurements. The second day we elevate the dosage to 2 mg/kg/day in three times. Currently an international study is evaluating the optimal dosage and duration of propranolol therapy in the treatment of IH[49]. Results are not yet published but preliminary results have been presented by Léauté-Labrèze at several congresses, indicating that propranolol therapy with 3 mg/kg/day is superior with no extra side effects. In most patients, therapy can then be stopped after six months. We think that this will be an important paper for defining a uniform strategy for propranolol treatment.

Bronchial hyper reactivity is a relative contra-indication for propranolol treatment due to the effect of propranolol on the β_2 receptors in the airways. Some authors have considered for example atenolol, a selective β-blocker[50,51]. However, there is not enough evidence of the results. Given the good effect and few side effects of propranolol, other (selective) β-blockers are generally not advised in the treatment of IH.

Vincristine

Treatment of IH with vincristine was first described in 1993 by Boehm et al[52]. Vincristine is a vinca alkaloid from the plant Vinca rosea, widely used in cancer chemotherapy due to its lack of antimitotic myelosuppression at conventional dosage. The major antitumor effect of this agent appears to be related to its high-affinity binding to the basic protein

subunit of microtubules, tubulin, which results in disruption of the mitotic spindle apparatus and arrest of cells in metaphase, thus interfering mitosis and provoking cellular apoptosis. Additionally, vincristine is capable of modulating angiogenesis. Thus, in the experimental setting, it has shown high efficacy as an antiangiogenic agent due to its direct attack to the host vasculature causing endothelial retraction, swelling and disruption. Its principal limiting side effect has been neurotoxicity, which is usually manifested by a peripheral mixed sensory-motor neuropathy with symmetrical neurological signs and symptoms[53,54]. In general, adverse reactions include alopecia, rash, and local reactions such as phlebitis and necrosis[53]. Vincristine has to be administered intravenously at a dosage of 0.03–0.05 mg/kg <10kg and 1.0–1.5 mg/m^2 >10kg, once a week for at least six weeks[53].

Topical therapy

Before 2008 topical treatment of IH was rare, mainly from fear of side effects of the available options. The most used options were laser therapy and imiquimod. Imiquimod 5% cream is an inducer of cytokines and has been used since 2002, but can invoke an eczema-like reaction on and around the IH[55]. Sidbury et al showed in 2003 that imiquimod decreased tumour cell proliferation, increased tumour apoptosis, and increased expression of tissue inhibitor of matrix metalloproteinase-1 with decreased activity of matrix metalloproteinase-9[56]. The main disadvantages of laser therapy (and therefore not an option in our centre) is that the necessity of multiple sessions, all with general anaesthesia, and that it can only give good results in very superficial IHs; laser (pulsed dye) only penetrates the skin for 1 mm[16].

After 2008, topical β-blockers were produced and superficial IHs were more easily treated by these drugs. Bonifazi et al were the first to use propranolol 1% cream and they described favourable results[57]. Nowadays even case reports of propranolol 2% are published[58]. Timolol is a β-blocker that already existed in a gel (0.1%) and an ophthalmic solution (0.5%). It is being used in ophthalmology for glaucoma. Guo et al and Pope et al were the first to use timolol for superficial IH[59,60]. A recent, large randomised controlled trial on timolol[61] showed that the ophthalmic solution worked better due to a higher concentration, although this may seem illogical as it is an ophthalmic solution. It is advised to use these topical β-blockers 3–4 times daily, consistent with the half-time of β-blockers[62].

Chantasart et al have shown that β-blockers, especially propranolol, permeate through the skin[63]. On the other hand, when comparing simple posology schemes, timolol seems to be up to 10 times more potent than propranolol. However, a comparative study has not been performed until now.

Table 1 – Some common* *topical* therapy options in the treatment of infantile haemangiomas (IH) with their advantages and disadvantages

Therapy	Comments	Advantages	Disadvantages
β-blockers (timolol ophthalmic solution 0.5%, propranolol cream 1%)	For small (superficial) lesions	Local treatment, side effects are not expected	For superficial lesions only, not possible in deep IHs
Corticosteroids (Intralesional injection with a solution of 3 ml Celestone 4 mg/ml and 2 ml Kenacort-A 40 mg/ml)	Good results in periocular IHs. Inject a maximum of 5 ml of this solution	No systemic side effects	Narcosis and hospitalisation (day treatment)
Imiquimod (cream 50 mg/g)		Works good, also in slightly deeper IHs	Gives an eczema reaction around and on the IH
Becaplermine (gel 0.01%)	Is a recombinant platelet-derived growth factor		Experimental
Laser therapy (pulsed dye)	Favourable results are described		Only possible in very superficial IHs. Narcosis and at least 3 sessions are required

*Options such as surgery, radiation and sirolimus are not mentioned because of their risk of side effects and higher costs

Table 2 – Some common* *systemic* therapy options in the treatment of infantile haemangiomas (IH) with their advantages and disadvantages

Therapy	Comments	Advantages	Disadvantages
Propranolol per os	At start therapy: global paediatric physical examination, ECG, and during therapy blood pressure and glucose. Day treatment	It seems to work good It seems safe	Is still being investigated. Hypoglycaemia? Be careful in PHACES** syndrome Rebound of IH growth when stopped too early Long lasting therapy (>1 year?) Day-care unit
Corticosteroids per os		Safe if used properly (in pulses)	Systemic side effects (↓ cortisol, Cushing) Start therapy during hospital stay (3 days)
Vincristine intravenous	In case of therapy resistance		Intravenous, 6 dosages (or more) Several side effects; most frequent: obstipation

*Options such as interferon and sirolimus are not mentioned because of their risk of side effects and higher costs

**PHACES is a syndrome with one of more of: Posterior fossa (Dandy Walker) malformations, Haemangioma, Arterial cerebral anomalies, coarctatio aortae and other Cardial abnormalities, Eye abnormalities and abnormalities of the Sternum. Be careful because there might be anomalies of vessels in the brain, and propranolol acts on blood vessels. Recent publications show that in most cases there are no difficulties; further examination might be necessary before starting propranolol therapy.

Side effects are generally not seen because only (small) superficial IHs are treated. Dr. Lisa Weibel (personal communication; not published) showed at the 11th ESPD congress in 2012, Istanbul, that both propranolol and timolol are indeed systemically absorbed, but in very small harmless amounts (for example compared to the use in ophthalmology. Timolol has to be used with caution in elderly people with pre-existing heart problems[64].

In tables 1 and 2 the current systemic and topical therapies of IH are summarized. Figures 1–3 show some examples of IH evolution before and after therapy.

Evaluation of IH

Evaluation of IH seems crucial, especially nowadays with more and more infants being treated. As research in this field increases, there is also an increasing demand for an objective scoring system for severity and effect of treatment of IHs. The first described scoring system ever is from Enjolras and Mulliken in their book on vascular malformations. They are well-known from their research on vascular malformations. Their scoring system is intended to score the severity and seriousness of all vascular malformations. The obvious disadvantage of this system is that it is too extensive and complicated as

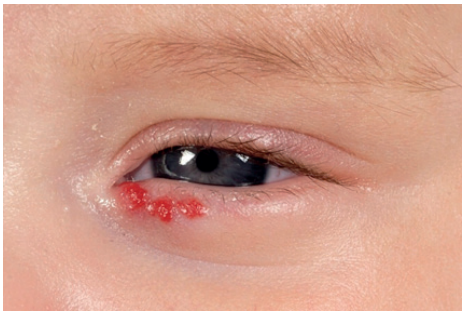

Figure 1a – Timolol ophthalmic solution 0.5% before treatment

Figure 1b – Timolol ophthalmic solution 0.5% 4 months after treatment: excellent result

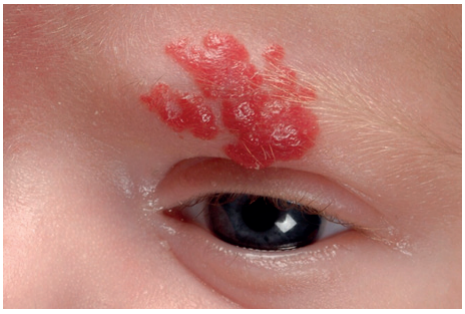

Figure 2a – Imiquimod cream 50 mg/g before treatment

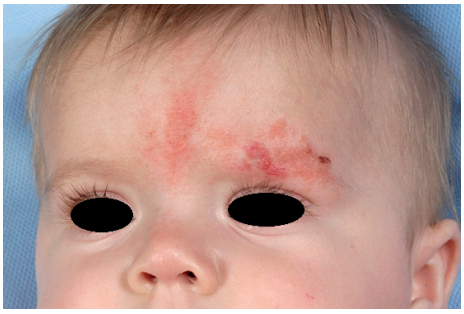

Figure 2b – Imiquimod cream 50 mg/g after several weeks; note the eczema-like reaction around the lesion which has improved

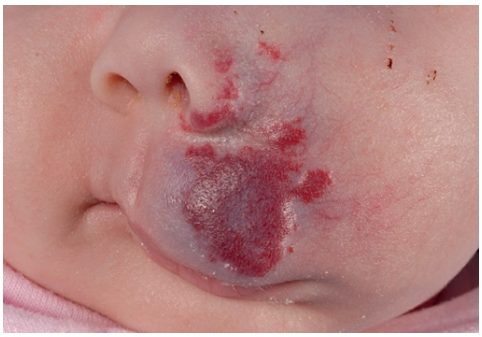

Figure 3a – Oral propranolol before treatment

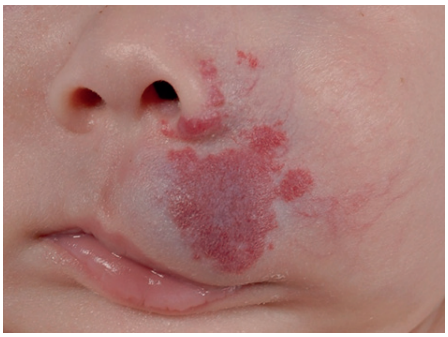

Figure 3b – Oral propranolol after 5 days: note the quick reduction of the swelling

Figure 3c – Oral propranolol after 5 months: good improvement

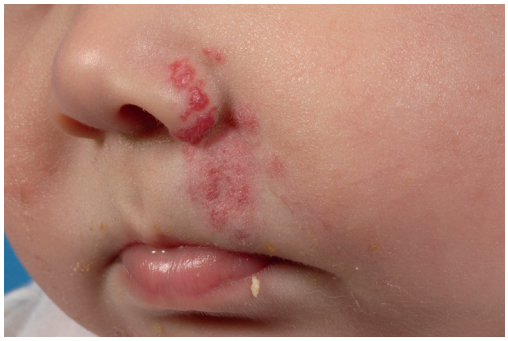

Figure 3d – Oral propranolol after 9 months: good improvement

it is intended for *all* vascular malformations. In 2011, Janmohamed et al published the Haemangioma Activity Score (HAS)[65]. This system assesses the activity of an IH based mainly on the colour of the IH. As known, IHs change from bright red in the proliferative phase to red, and to purple/blue in the regression phase. Before the skin colour returns, a greyish discoloration can be seen. To make the HAS an all-round system, also swelling and ulceration are included. The HAS is effective in prospective use (on a patient) as well as in retrospective use (on photographs). It is very objective with only one subjective measurement: estimation whether or not a swelling has shrunken with more or less than half. This item is included though to make it usable in deep IH. In table 3 items of the HAS are given with an example of the calculation. Recently, Haggstrom et al[66] published two other scoring systems. The first one, the Haemangioma Severity Scale (HSS) measures the overall severity of an IH, using both objective items (like size, location, and complications) and subjective items (pain). A main disadvantage of this system is its weakness when using it retrospectively on photographs. Their second system is the Haemangioma Dynamic Complication Scale (HDCS). This scale assigns severity grades to IH complications. Currently we are performing a study to compare the HAS system with the HSS system.

Table 3 – Haemangioma Activity Score (HAS) scoring form – Items to be scored, filled in (*italic*) as an example

Date		*t=0*	*t=1*	*t=2*
Deep swelling:	Tense IH (6)	*6*		
	'Neutral' IH at t=0 or less than 50% reduction at follow up (4)			
	>= 50% reduction at follow up (2)		*2*	
	No more swelling at follow up (0)			*0*
Bright red / shining red IH (5) OR Bright red edge* (4)		*4*		
Matt red / reddish-purple IH / matt red edge (3)		*3*	*3*	*3*
Blue IH or blue shining through in deep IH (2)		*2*	*2*	
Grey IH (1)			*1*	*1*
Skin coloured after activity** (0)				*0*
Total score		*15*	*8*	*4*
Number of items scored		*4*	*4*	*4*
Preliminary HAS = total score / number of items scored		*3.8*	*2*	*1*
Ulcer =< 1 cm^2 (+ 0.5)			*+0.5*	
Ulcer 1–25 cm^2 (+ 1)		*+1*		
Ulcer >= 25 cm^2 (+ 2)				
HAS = preliminary HAS + ulcer score		*4.8*	*2.5*	*1*

**Bright red edge* should only be scored when the IH is not totally bright red

***Skin coloured after activity*: do not score in deep IH (deep swelling) unless the IH has changed into it after activity

(number) The numbers between brackets are the points which have to be scored if this item is present in the IH

IH = infantile haemangioma

OVERALL CONCLUSION

Therapy in IH has changed considerably over the last years. Nevertheless, regarding the management of IH, it still seems better to use the watch and wait strategy in non-alarming IHs. Why treat something that will go away by itself? Nonetheless, when dealing with alarming IHs, the earlier the treatment, the better. In that case, orally administered propranolol is the first choice and just recently a large trial showed that 3 mg/kg/day is more efficient than 1 mg/kg/day. In most patients, propranolol could be tapered off after 6 months with that dosage (to be published). Nowadays also smaller IHs (for example superficial IHs around the eyes or in the face) can be treated with topical β-blockers, which are safe and show good results. Propranolol cream 1% is available, just as timolol ophthalmic solution 0.5% and these have to be administered 3 to 4 times daily for the best results! However, a comparative study between propranolol and timolol has not yet been performed. A randomised placebo controlled, double blind study is recommended with different concentrations of topical propranolol and timolol.

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The authors declare that they have no conflict of interest.

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CHAPTER 3

Aims of the thesis

AIMS OF THE THESIS

This thesis addresses the pathogenesis, evaluation, and therapy of infantile haemangiomas. The pathogenesis is not well understood, there is a lack of scoring systems for infantile haemangiomas, and the management is mostly experience-based and not evidence based. To fill the gaps in knowledge, the aims of this thesis are:

1. To summarize and give an update of the different theories proposed in the pathogenesis of infantile haemangioma and to summarize and give an update of the different therapies considered in patients with infantile haemangiomas (Introduction)
2. To evaluate the role of hypoxia in the pathogenesis of infantile haemangioma (Part I)
3. To propose a scoring system for disease activity of infantile haemangioma (Part II)
4. To evaluate different therapy options for patients with infantile haemangiomas (Part III)

PART I

Pathogenesis of infantile haemangioma

CHAPTER 4

Support for the hypoxia theory in the pathogenesis of infantile haemangioma

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ABSTRACT

The pathogenesis of infantile haemangioma (IH) is unknown. Several mechanisms have been proposed, including hypoxia, which triggers up regulation and stabilisation of hypoxia-inducible factor 1α (HIF1α). HIF1α stimulates downstream transcription of target genes that enhance angiogenesis.

In this study we aimed to identify possible involvement of hypoxia in the pathogenesis of IH, as hypoxia signalling constitutes a potential therapeutic target.

IH tissue samples collected from 1991–2011 (preserved in paraffin) were immunohistochemically analysed for the known HIF1α targets; BNIP3 (BCL2/adenovirus E1B kD-interacting protein (BNIP) family member 3), CA-IX (Carbon Anhydrase IX), GLUT-1 (Glucose Transporter 1), HIF1α, pAKT (Phosphorylated protein kinase B), pS6 (Phosphorylated S6 protein), and VEGF (Vascular Endothelial Growth Factor). Four observers independently assessed findings.

Two of the 10 IH samples appeared to be in the growth phase. GLUT-1, BNIP3, pAKT, and VEGF were positive in all samples. pS6 was positive in nine cases and negative in one. CA-IX was weakly positive and HIF1α was negative in all cases.

Several factors implied in hypoxia-induced angiogenesis may be involved in IH development. The small sample size and retrospective approach preclude definitive conclusions. Prospective studies are needed to conclusively determine which factors involved in the (hypoxia) cascade are required for an IH to grow and can thus possibly be targeted by drugs for IH treatment.

What's already known about this topic?

- Infantile haemangioma (IH) is the most common tumour of infancy and can cause severe complications.
- The pathogenesis of IH is unclear; understanding of the mechanisms governing IH-growth and development could lead to better targeted drugs.

What does this study add?

- Evidence for up regulation of hypoxia response genes, which supports the notion that hypoxia-induced signalling is an important factor driving IH growth.
- These genes might be regulated either directly by HIF signalling, or by hypoxia-induced regulation of mTORC1-signalling.

LIST OF ABBREVIATIONS

$BNIP_3$	BCL_2/adenovirus E_1B kD-interacting protein (BNIP) family member 3
CA-IX	Carbon Anhydrase IX
ERK	Extracellular signal-Related Kinase
FFPE	Formalin Fixed and Paraffin Embedded
GLUT-1	Glucose Transporter 1
$HIF_{1\alpha}$	Hypoxia Inducible Factor 1α
$HIF_{2\alpha}$	Hypoxia Inducible Factor 2α
HPF	High-power fields
IH	Infantile Haemangioma
MAPK	Mitogen-Activated Protein Kinase
MMP-9	Matrix Metallopeptidase 9
mTOR	Mammalian Target Of Rapamycin
$mTORC_1$	mTOR Complex 1
pAKT	Phosphorylated v-akt murine thymoma viral oncogene homolog 1
pS6	Phosphorylated S6 protein
TNF-α	Tumour Necrosis Factor α
VEGF	Vascular Endothelial Growth Factor
VEGF-A	Vascular Endothelial Growth Factor A

INTRODUCTION

The pathogenesis of IH remains elusive[1]. In a proliferative IH, rapidly growing endothelial cells form blood vessels. Increased apoptosis of endothelial cells in the involution phase leads to regression of blood vessels. Eventually, the thick multilaminated basement membrane surrounding the endothelial layer is replaced by adipocytes in fibrous tissue[2]. Furthermore, the significant increase in the number of mast cells during the involution phase may alter the balance of angiogenic factors, thus promoting regression[3]. Many mechanisms have been considered to explain the development of IHs[1,4-7]. Genetic influences may contribute as several patients with IHs show significant loss of heterozygosity for markers in a region of chromosome 5q[8]. Embolization of placenta has also been postulated as a possible mechanism, as many of the characteristic molecular markers of endothelial cells in IHs are also expressed by normal placental endothelial cells. Furthermore, hormonal influences may be involved: stimulation with oestrogen increases proliferation, migration and survival of endothelial cells. Stem cell theories are also proposed because multipotential stem cells can be derived from IHs. These cells can produce neovascularisation. Finally, hypoxia may be involved in the pathogenesis of IH[4,5,7,9]. In 50% of cases an anaemic macula (precursor lesion) occurs at a place where an IH will eventually develop, supporting the idea that local ischemia is important. Hypoxia triggers stabilisation at the protein level of the transcription factor HIF1α[10]. HIF1α in turn stimulates transcription of downstream target genes such as BNIP3, CA-IX, GLUT-1, pAKT, pS6, and VEGF. These target genes can be regulated either directly by HIF1α or by hypoxia-induced regulation of mTORC1 signalling[11]. mTORC1 is a key player in the mTOR-pathway, a protein complex with a central role in regulating cellular metabolism, driven by growth factors, nutrients as well as hypoxia (Figure 1). Deregulation of the mTOR-pathway may lead to disorganized growth[12]. As macrophages secrete proangiogenic molecules such as TNF-α and interleukin-1, they are also thought to be involved in the evolution of IH[2,9,13]. Of all theories discussed, the hypoxia-theory seems the most attractive given the anaemic macula often seen and the endothelial cell origin of IH (cells typically growing under hypoxic conditions). The empirically based current therapy aims to induce/accelerate the natural involution process[2]. Systemic propranolol induces quick therapeutic responses and has made corticosteroids and all other treatment options obsolete[14,15]. However, it has potential side effects and must be used for 1–2 years (own experience). Topical use also has limitations. Propranolol and corticosteroids both act on factors induced by HIF1α as a result of hypoxia[15,16], further supporting a crucial role of hypoxia in IH. To confirm this role, we analysed the expression of the most important genes in the hypoxia response using immunohistochemical staining. A better understanding of IH pathogenesis may help to develop targeted treatments that lack the disadvantages of β-blockers.

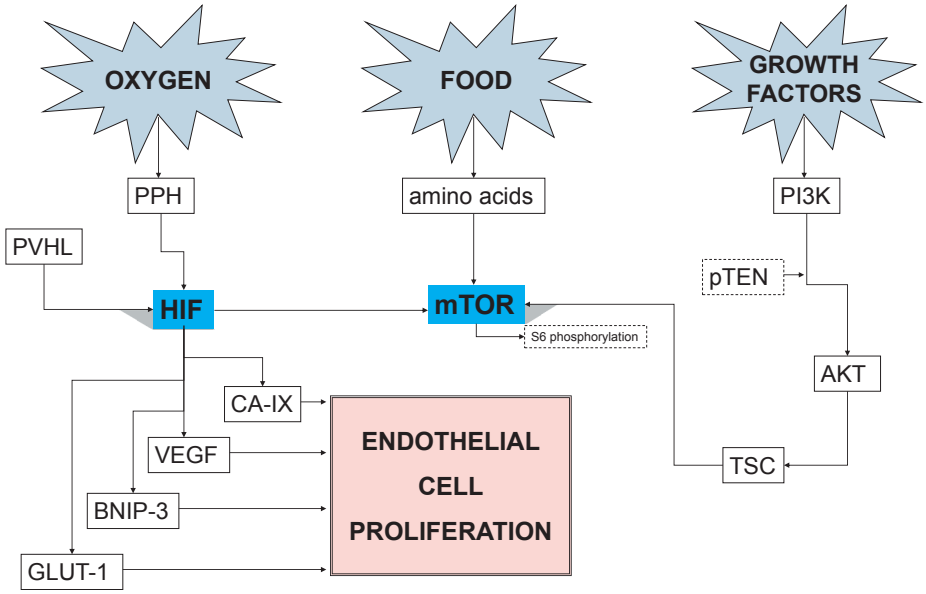

Figure 1 – Very simplified scheme of the interaction between pathways and factors discussed in this article, resulting from hypoxia into endothelial cell proliferation (proliferative infantile haemangioma)

METHODS

Tissue samples

Three-mm punch biopsies were obtained for histopathological examination as part of regular care. Most biopsies were performed to differentiate between IHs and vascular malformations. Samples were formalin fixed, paraffin embedded (FFPE) according to standard procedures and stored at the department of Pathology at room temperature. Tissue samples from 10 IH patients diagnosed from 1991 onward were selected, using the registry of the Pathology laboratory of the Erasmus MC. Tissue samples were cut into five-µm sections and collected on coated slides to optimize fixation. Collection, storage and use of all tissues and patient data were in agreement with the "Code for Proper Secondary Use of Human Tissue in the Netherlands". This study is part of the 'Strawberry Marks' project, which focuses on the pathogenesis and therapy of IH, and was approved by the Medical Ethics Review Board of the Erasmus MC in Rotterdam, the Netherlands.

Patient selection:

Inclusion criteria were characteristic microscopic appearance and GLUT-1 positivity. Sufficient material had to be available for analysis. Patients were excluded in case of GLUT-1 negativity and in case of diagnosis of (another) vascular malformation.

Antibodies:

GLUT-1 staining was performed to confirm the diagnosis. We used antibodies against HIF1α itself and one of its most important target genes, VEGF. In addition BNIP3 and CA-IX were analysed, other known important target genes of the HIF1α pathway. Finally, to assess mTOR pathway activity, phosphorylated AKT and S6 were stained. For all antibodies, tissue known to be positive was included as positive control (see table 1). Negative controls (omission of the primary antibody) were included in all experiments.

Immunohistochemical staining:

BNIP3, VEGF, and pS6

Five-µm FFPE sections were deparaffinized in xylene and rehydrated through graded ethanol concentrations. Endogenous peroxidase activity was blocked by incubation in 0.3% (BNIP3 and VEGF) or 3% (pS6) (w/v) hydrogen peroxide (H_2O_2) in methanol for 30 minutes, after which antigens were retrieved by microwave treatment using 10 mmol/L citrate buffer (pH 6) for 10 minutes (90W). Then, non-specific protein binding was blocked using 3% bovine-serum-albumin (BSA). Primary antibodies were diluted in Dako Antibody diluent (see table 1) and incubated for 1h at room temperature (BNIP3) or overnight at 4° C (pS6). Secondary detection was with the use of the Envision detection system (Dako, Heverlee, Belgium) for 30 minutes and bound antibody was visualized by using 3,3-diaminobenzidine (DAB) for 10 minutes. Tissue was counterstained with Gill II haematoxylin, dehydrated and coverslipped. Dako Wash buffer was used throughout for washing.

pAKT

For pAKT, the slides were boiled in citrate buffer (pH 6) for 20 minutes (microwave 90W), after which endogenous peroxidase was inactivated in 3% (w/v) hydrogen peroxide

Table 1 – Antibody characteristics

Antibody	Source	Order number	Positive control tissue	Dilution
BNIP3	Sigma-Aldrich	B7931	Clear cell renal carcinoma	1:400
pS6	Cell Signalling	#4858	Lung carcinoma	1:200
pAKT	Cell Signalling	#3787	Lung carcinoma	1:25
GLUT-1	Thermo Scientific	MS-10367	Combination of: appendix/colon, tonsil, pancreas and liver; the immature epithelium of the tonsil is positive; liver and pancreas are negative	1:800
VEGF	Thermo Scientific	RB9031	Capillary haemangioma	1:100
CA-IX	Novus Biologicals	100-417	Clear cell renal carcinoma	1:1000
HIF1α	BD Biosciences	#610959	Mammary carcinoma	1:50

(H_2O_2) diluted in methanol for 10 min. Primary antibody was diluted in 1% BSA in tris-buffered saline tween-20 (TBST) and incubated over night by 4° C. For all other steps the protocol stated above was used.

GLUT-1

After deparaffination with xylene and rehydration, slides were pre-treated with Tris/EDTA (ph9) in a microwave oven for 15 minutes, after which they were incubated in 3% (w/v) hydrogen peroxide (H_2O_2) for 15 min. Antibody was diluted in Normal Antibody Diluent (Klinipath, Duiven, the Netherlands) and incubated for 30 minutes at room temperature. For secondary detection, the Dako Envision Kit (K5007) was used. Remaining steps were performed as mentioned above.

CA-IX and HIF1α

The CA-IX and HIF1α stainings were performed on 5 µm FFPE sections on a Dako autostainer system with use of a pre-treatment module using EnVision FLEX Target Retrieval Solution, High pH (Dako, Heverlee, Belgium). The antibodies were applied for 20 minutes at room temperature. For HIF1α, the slides were additionally incubated with Envision Flex Mouse Linker to amplify the signal. The Dako Envision Flex kit (K8002) was used for secondary detection. Subsequently, sections were counterstained with Gill II haematoxylin, dehydrated and coverslipped.

Interpretation of the staining

Four observers independently assessed all slides: a pathologist (JH), a dermatologist (AO), and two residents (SJ and TB). Both the percentages of positive cells and the intensity of the stains were assessed. The intensity was scored as – negative, + weakly positive, ++ positive or +++ strong positive, with respect to the positive control. Different scores between observers were discussed and resolved by consensus. For mast cell count, the mean of 4 different counts (per HPF 40x, 0.239 mm^2 of tissue) was taken.

RESULTS

We identified a total of 12 samples diagnosed as 'haemangioma' in the period 1991–2010. Re-evaluation led to exclusion of two samples (misdiagnosed/GLUT-1 negative), leaving 10 samples for analysis. Three had been obtained recently (2009–2010) and 3 were older than 10 years. Two samples appeared to have been biopsied in the proliferative phase: the IHs were (shiny) red and the biopsies took place at the age of approximately two months.

Table 2 – Results of immunohistochemical staining for HIF1α, BNIP3, CA-IX, VEGF, pAKT, and pS6 (intensity of staining and percentages of positive cells), and mast cell count of samples of infantile haemangioma (IH)

Biopsy#	HIF1α	BNIP3	CA-IX	VEGF	pAKT	pS6	Mast cell count	Phase of IH	Biopsy date
1	–	90% ++	90% ++	100% +++	100% ++	50% ++	33	Regression	1998
2	–	100% +++	90% +++	100% +++	100% +++	90% +++	9	Proliferative	2009
3	–	90% +++	90% +	100% ++	100% ++	30% +++	37	Regression	2010
4	–	90% ++	90% +	100% ++	70% +	75% +	22	Regression	2006
5	–	100% ++	–	100% ++	90% +	–	53	Regression	1992
6	–	90% +++	70% +	95% +++	90% ++	90% ++	26	Regression	2010
7	–	100% +++	50% +	100% +++	100% ++	90% ++	55	Proliferative	2005
8	–	100% +++	50% +	100% +++	100% +++	90% ++	91	Regression	2003
9	–	90% +++	50% ++	100% ++	60% +	90% +	17	Regression	2005
10	–	90% ++	70% ++	100% +++	100% ++	60% ++	37	Regression	1991

Samples were scored as follows: – negative + weak positive ++ positive +++ strong positive
For mast cell count, the mean of 4 different counts (per HPF 40x, 0.239 mm^2 of tissue) was taken.

Negative and positive controls were all negative and positive, respectively. Table 2 presents an overview of the results of the staining for BNIP3, CA-IX, HIF1α, pAKT, pS6, and VEGF. Nuclear HIF1α itself could not be detected in all cases, but VEGF, the most important target gene of the HIF1α pathway, was strongly expressed in the majority of the samples. BNIP3 and pAKT were positive in all cases. CA-IX expression was (weakly) positive in 9 cases and negative in one case. CA-IX expression was also found on the surrounding cells within the lesion. pS6 was negative in one case and positive in the others (Figure 2). For all markers we noted that expression was not found on normal blood

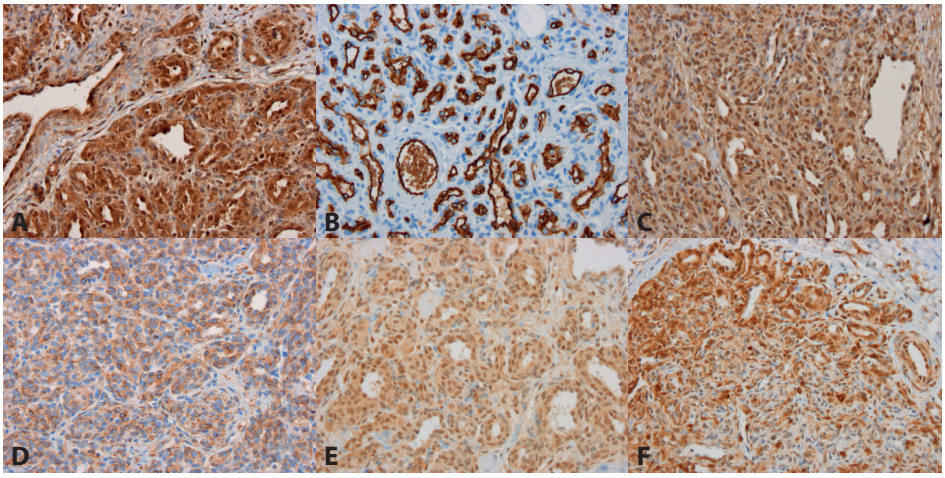

Figure 2 – Positive staining (brown) in infantile haemangioma samples (original magnification 20x). A) VEGF B) GLUT-1 C) BNIP3 D) CA-IX E) pAKT F) pS6

vessels outside the lesions. Expression patterns did not differ between the 2 proliferative IHs and the 8 IHs in regression. Mast cell count in total varied from 9 to 91 (active IH 9 and 55, IH in regression ranging from 17 to 91) per HPF 40x, 0.239 mm^2 of tissue (table 2).

DISCUSSION

Our data show expression of GLUT-1, CA-IX (weak), VEGF (strong), and BNIP3 (normal to strong), which are all up regulated by HIF1α and associated with angiogenesis under hypoxic conditions[5]. BNIP3 is actually a pro-apoptotic factor but has also been reported to be angiogenic[17]. CA-IX is a marker of hypoxia in epithelial cells but we also found expression of CA-IX in the surrounding cells within the lesion, suggesting that hypoxia extends a larger area. Also, pAKT and pS6 as part of the mTOR-pathway were positive, presumably due to the influence of hypoxia[18,19]. We did not find expression of these markers in normal blood vessels outside the lesions.

Research on the pathogenesis of IH remains important since propranolol, while speeding up regression, does not provide a definitive solution. IH can even reappear when therapy is stopped too early. Our findings are consistent with earlier studies concerning IH pathogenesis. Kleinman et al analysed 10 samples for fewer proteins, but provided additional analyses on blood samples. They also found elevated levels of VEGF and of other proteins involved in angiogenesis[9]. Chen et al reported similar results from a study (English abstract) in 28 samples[20]. However, we did not find positive staining of HIF1α itself, possibly because most analysed IHs were in regression. Our positive/negative controls were all positive/negative respectively, therefore we conclude that our findings are legitimate. Thus the question arises whether or not previous studies used proper (positive/negative) controls. Furthermore, in case of activation of HIF1α, rather than increasing, expression shifts from cytoplasm to nucleus and it is uncertain how other authors have dealt with this. It may also be possible that transcriptional activity was increased, resulting in a hypoxia response without a change in HIF1α levels[21]. Finally, we also note that negative staining is consistent with the previously published variable expression; expression of target genes can occur via HIF2α only[22,23].

As mentioned earlier, a better understanding of IH may lead to better (targeted) treatments. Storch et al hypothesized that propranolol blocks beta-adrenergic agonist induced angiogenesis, which normally proceeds by synthesis of proangiogenic factors (VEGF and MMP-9) and activation of proangiogenic (ERK/MAPK) cascades[15]. Greenberger et al found evidence that corticosteroids can inhibit VEGF-A in infantile haemangioma-derived stem cells[16]. In our study we found positive staining for pAKT and

pS6, suggesting that agents capable of inhibiting the mTOR pathway, such as Rapamycin, might be effective treatments for IH and could be considered[18,24].

This is the first systematic study on the expression of known hypoxia responders, and the results support the hypothesis that hypoxia signalling might be involved in IH pathogenesis. Two limitations of this study are the retrospective approach and the small sample size. The small sample size makes it hard to judge possible differences in IH between the active phase and the regression phase. Older IHs may contain more mast cells. One of the active IHs in this study indeed contained no more than 9 mast cells while the other one contained 55 mast cells (mean per HPF 40x, 0.239 mm^2 of tissue). To the best of our knowledge, larger studies are not available to date so our sample size can be regarded as average in comparison with other studies.

In conclusion, our data suggest that hypoxia might indeed be involved in the development of IH. There are several clues indicating that HIF1α, triggered by hypoxia, plays a role and that factors up regulated by HIF1α can be influenced. In our research, we confirmed elevated activity of these regulating factors in IH.

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PART II

Evaluation of infantile haemangioma

CHAPTER 5

Scoring the proliferative activity of infantile haemangioma: the Haemangioma Activity Score (HAS)

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ABSTRACT

Infantile haemangioma (IH) is the most frequently occurring benign tumour of infancy. A good, reliable and objective scoring system for haemangioma activity is not yet available.

We developed a simple system called the Haemangioma Activity Score (HAS) for scoring the (disease) proliferative activity of IHs. The current study was undertaken in order to validate this system.

We validated the HAS system in a comparative study of photographs taken during consultations from 2000 until 2008 (n=78). Agreement between three observers was assessed at two different time points with a minimum interval of six months (t=0 and at t=1) using the interclass correlation coefficients (ICC).

Agreement between observers was good. The average ICC of HAS at t=0 and t=1 was 0.72 and 0.76, respectively. The average ICC of HAS regarding the changes from baseline (HAS at t=0 minus HAS at t=1) was 0.69.

We concluded that the HAS was a good system for scoring the (disease) proliferative activity of haemangiomas and believe it to be useful in future investigations. Today, there is an increasing number of studies comparing different therapies for treating haemangiomas. The HAS (before and after treatment) may provide a valuable scoring system for evaluating such therapies.

What is already known about this topic?
- What an infantile haemangioma (IH) is and how it behaves
- IH may cause severe complications
- Complicated IH have to be treated

What does this study add?
- A good, reliable and objective scoring system for IH activity is not yet available
- This study provides a tool (scoring system) for scoring the disease activity of IH
- The effect of therapy can be measured objectively with this scoring system

INTRODUCTION

Infantile haemangioma (IH) is the most frequently occurring benign tumour of infancy[1]. Its incidence is 10% in the general population, with a higher incidence (20–30%) in prematures. It is 1.5–5 times more common in girls than in boys and is located on the face in 60% of the cases[1,2]. The diagnosis is usually only based on clinical observation and history[3]. The term haemangioma has often been used incorrectly for different types of vascular anomalies. This terminology is confusing and thus the term IH is preferred[4]. It becomes manifest in the first weeks of life and grows rapidly. After this proliferative phase there is a period of inactivity and subsequent involution[5,6]. IH may cause severe complications, generally because of extensive growth or location, leading to life-threatening or disabling complications such as visual impairment, compression of the airways, heart failure and ulceration[6-8].

The pathogenesis of IH still remains unknown[9]. Current therapy in IH is empirically based, but has radically changed recently because propranolol halts the growth of haemangiomas and even shrinks them[6,10-12].

The effect of treatment may be evaluated by 'scoring' haemangiomas in various ways e.g. by change in colour or change in size[6,13,14]. However, a good, reliable and valid scoring system for haemangiomas is lacking at present.

We introduce a system called HAS, Haemangioma Activity Score, for scoring the (disease) proliferative activity of haemangiomas in this study. This score can be compared with the score of an earlier visit and provide a more clinically objective evaluation. The current study was undertaken to validate this system.

METHODS

For evaluating the effect of treatment on haemangiomas, most dermatologists compare the actual haemangioma with a photograph taken at an earlier visit and then often check for any change in the size or the colour. Some dermatologists ask the parents what they think. This may be used as a 'global assessment', either by the doctor or by the parents. However, a better, more precise and reliable scoring system is needed for evaluating the effect of treatment or for comparing different treatments.

Scoring system

Two of the authors (SJ and APO) developed a system for scoring the (disease) proliferative activity of haemangiomas. The aim was to develope a simple system, which was easy to use and therefore did not require extra time; we wanted to use it during consultations without difficult calculations or procedures such as measuring the size with a ruler or ultrasound. The intention was to examine a haemangioma at various time intervals and evaluate the change in the score in the same patient. It was not intended to compare haemangiomas in different patients and therefore cannot be used to state that a haemangioma of patient A is 'worse' than that in patient B because of a higher score. However, you can compare changes in scores for evaluating effect of treatment.

We looked at haemangiomas and scored them in various ways, eventually leading to our definite system. The colour of a haemangioma changes together with its course. An active, rapidly growing haemangioma in a few weeks old infant is often very red. This redness becomes less leading to purple-red in the stabilization phase and eventually turns into grey and skin-coloured in the regression phase. Therefore, we chose the colour as the main item in our scoring system. We tested the definite system in a pilot training study of 20 haemangiomas with 2 different observers and developers of the system (SJ and APO). The interclass correlation coefficient of this pilot study was 0.97 (95% confidence interval: 0.91 – 0.99). The term HAS (Haemangioma Activity Score) was suggested by SJ for this system.

The HAS is defined as follows. The HAS scoring form at the end of this thesis may be used in clinical practice for scoring haemangiomas and filing scores. This HAS form may also be used as an example for clarifying the following.

First we assess the swelling. Swelling means that the haemangioma is larger than that visible on the outside; such haemangiomas regress slowly. If this is true (i.e. deep subcutaneous swelling), score one of the following:

- 6 points if the swelling is tight / tense
- 4 points when the swelling is 'neutral' (i.e. not tight) at t=0, or has less than 50% reduction at the follow-up
- 2 points when the swelling has a reduction of 50% or more at the follow-up
- 0 points when there is no visible swelling at the follow-up

Of course, this step can be omitted if there is no swelling.

We then assess the colour of the haemangioma. As explained before, a bright red haemangioma ('Ferrari-red') is very active and will not regress faster as compared with a

grey haemangioma in the regression phase. Therefore, a bright red haemangioma has the most points. You can score multiple items if they apply to the haemangioma. Score:

- 5 points if the haemangioma (totally) is bright / shining red
- 4 points if the haemangioma (only the edge) has a bright / shining red edge
- 3 points if the haemangioma is red, red-purple (totally or partially), or if the haemangioma has a matt red edge (only the edge)
- 2 points if the haemangioma is blue (totally or partially) or is blue shining-through in deep haemangiomas (totally or partially)
- 1 point if the haemangioma is grey (totally or partially)
- 0 points if the haemangioma is skin coloured after activity (totally or partially)

Note that if there is a bright red haemangioma, then we do not score the edge. Thus, a shining (bright) red edge should only be scored when the haemangioma is not bright red.

This adds up to a total, which we divide by the number of scored items. E.g. a haemangioma that is red-purple, grey and skin coloured (3 items only and no swelling, therefore that item is omitted) has a preliminary score of $\frac{3+1+0=4}{3} = 1.3$.

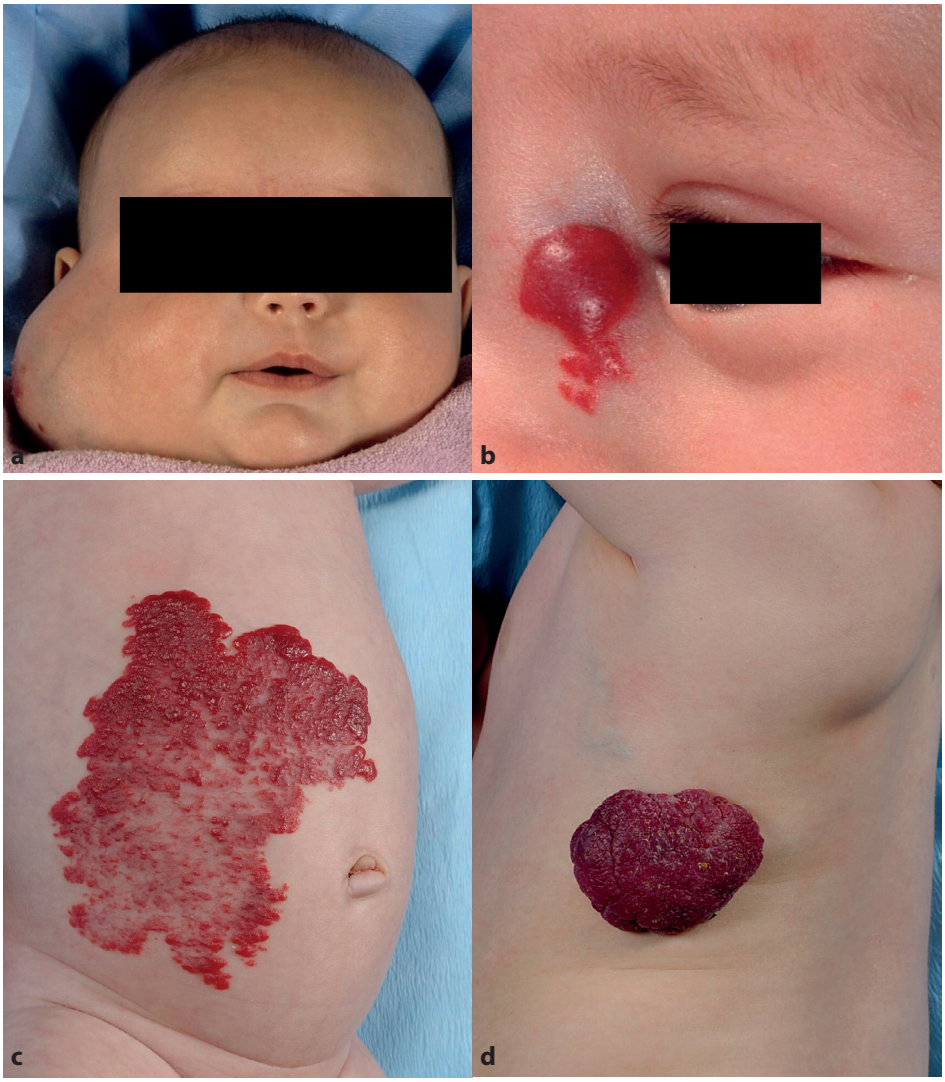
Finally we asses ulceration. If there is an ulcer, additional points are given because ulcerated haemangiomas heal slower:

- +0.5 points if there is an ulcer smaller than or equal to 1 cm^2
- +1 points if there is an ulcer of 1 cm^2 up to 25 cm^2
- +2 points if there is an ulcer of 25 cm^2 or more

These points are added up to the preliminary score. Therefore, the HAS scale goes from 0 up to and including 8.

In the example above, if there is an ulcer of 2 cm^2, then the ulcer score is 1. This adds up to a HAS (Haemangioma Activity Score) of 1.3 + 1 = 2.3.

Some examples of each item of the HAS are shown in Figure 1. The corresponding HAS scores are shown in Table 1. Note that the tight swelling (in order to calculate the HAS we assume that this is the picture of t=0) can be seen in Figure 1a, but from this angle, the aspect (redness) is difficult to score. Figures 1c and 1f can be compared to each other because they are from the same patient. From a global assessment you can see that there is clinical improvement. Note the decrease in the HAS. Furthermore, in Figure 2 an example of how a haemangioma changes after therapy, at very short follow-up (5 days) and longer follow-up (up to 13 months) is shown. HAS has also been calculated as an example and is shown in Table 1.



Study design and patient selection

We validated the HAS system by designing a comparative study of photographs taken during consultations from 2000 until 2008. Since 1993 we have a standardized approach in patients with vascular malformations (e.g. IH) in a special multidisciplinary work group (Workgroup on vascular abnormalities Rotterdam, WEVAR). This multidisciplinary team includes APO (paediatric dermatologist), PCJL (general paediatrician) and GCM (paediatric surgeon). We searched all outgoing letters to the general practitioner of patients who had visited the out patient clinic for the key word 'haemangioma' which resulted in 1815 cases. We filtered out all adults and double patient ID numbers, leaving

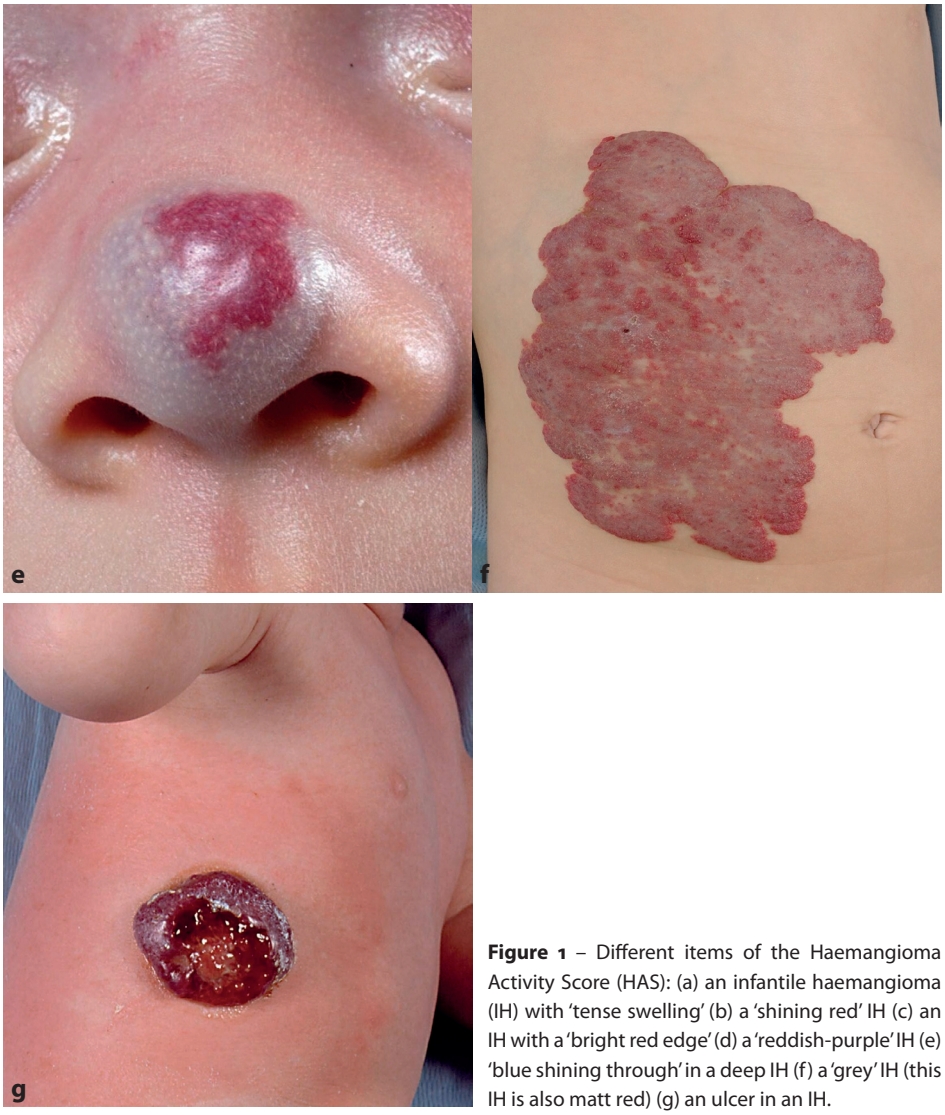


Figure 1 – Different items of the Haemangioma Activity Score (HAS): (a) an infantile haemangioma (IH) with 'tense swelling' (b) a 'shining red' IH (c) an IH with a 'bright red edge' (d) a 'reddish-purple' IH (e) 'blue shining through' in a deep IH (f) a 'grey' IH (this IH is also matt red) (g) an ulcer in an IH.

us with approximately 600 patients. We sorted these patients on the date of the first letter and began to check the patient's personal health record. Eventually, we selected those patients who were indeed diagnosed with 1) haemangiomas, 2) had photographs of the haemangiomas in the electronic file, 3) these photographs must be clear and representative, 4) photographs at t=0 must be haemangiomas in the active phase and 5) had a follow-up (with photographs) of at least six months (t=1). We scored a total of n=78 haemangiomas at t=0 and t=1, 1 to 3 photographs per haemangioma per time-point.

Table 1 – Examples of Haemangioma Activity Scores (HAS) for the photographs 1–2

Photograph number	Fig 1a	Fig 1b	Fig 1c	Fig 1d	Fig 1e	Fig 1f	Fig 1g	Fig 2a *t=0*	Fig 2b *t=1*	Fig 2c *t=2*	Fig 2d *t=3*
Deep swelling:											
Tight haemangioma (6)	6***							6			
'Neutral' haemangioma or less than 50% reduction at follow up (4)					4				4		
>= 50% reduction at follow up (2)										2	
No more swelling at follow up (0)											0
Bright red / shining red (5) OR Bright red edge* (4)		5	4					4			
Red / red-purple / matt red edge (3)	3****		3	3	3	3	3	3	3	3	3
Blue or blue shining through in deep haemangiomas (2)					2			2	2		
Grey (1)						1				1	1
Skin coloured after activity** (0)						0					0
Total	9	5	7	3	9	4	3	15	9	6	4
Number of items scored	2	1	2	1	3	3	1	4	3	3	4
Preliminary HAS = total / number of items scored	4.5	5	2.5	3	3	1.3	3	3.8	3	2	1
Ulcer =< 1 cm^2 (+ 0.5) Ulcer 1–25 cm^2 (+ 1) Ulcer >= 25 cm^2 (+ 2)	0	0	0	0	0	0	+1	0	0	0	0
HAS = preliminary HAS + ulcer score	4.5	5	2.5	3	3	1.3	4	3.8	3	2	1

*"Bright red edge" should only be scored when the haemangioma is not totally "bright red".
**"Skin coloured after activity": do not score in deep haemangioma-component (deep swelling) unless the haemangioma has changed into it after activity.
***suppose that this is t=0
****_difficult to see from this angle_

Outcome measures

To assess agreement, the HAS of these 78 haemangiomas were scored independently at two time points (t=0 and t=1) by three different physicians: an expert paediatric dermatologist, who basically developed the HAS system (APO, observer 2), another trained paediatric dermatologist (FBWS, observer 3), and a non-dermatologist also involved in the development of the HAS (SJ, observer 1). We calculated the interclass correlation coefficients of the HAS scores at t=0 and at t=1, and also for the changes of HAS (HAS at t=0 minus HAS at t=1).

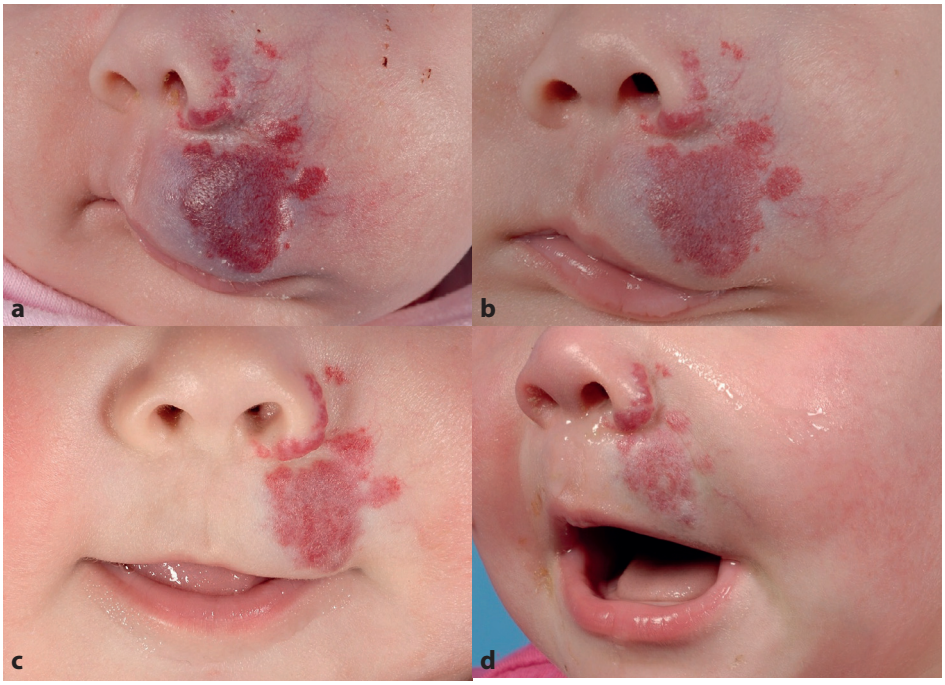


Figure 2 – (a) An infantile haemangioma (IH) at t=0 in an almost 2 months old girl, just before propranolol therapy was introduced (b) t=1, just 5 days after the start of propranolol therapy. Note the reduction of the swelling (c) t=2, 6 months after the start of propranolol there is further regression of the IH (d) t=3, 13 months after the start of propranolol therapy there is even more regression, which can also be seen in the changing HAS score.

Statistical methods

In order to evaluate the agreement between the three observers pair-wise, interclass correlation coefficients (ICC) including 95% confidence intervals were calculated. ICC values of 0.61–0.80 are generally considered to denote good agreement and ICC values >0.80 are generally considered to denote very good agreement. Mean HAS scores at the two time points were compared between observers using the paired samples t-test to assess systemic differences between observers.

RESULTS

We scored a total of 177 photographs (n=78 haemangiomas) at t=0 and t=1, 1 to 3 photographs per haemangioma per time-point. Baseline characteristics are shown in Table 2. Median age at t=0 was 4 months (10^{th} percentile 1 month, 90^{th} percentile 9 months) and median duration between t=0 and t=1 was 11 months (10^{th} percentile 7 months, 90^{th} percentile 24 months).

Table 2 – Baseline characteristics of n=78 haemangiomas, scored by three observers at time points t=0 and t=1

	Median	10th percentile	90th percentile
Age at t=0 (months)	4	1	9
Duration between t=0 and t=1 (months)	11	7	24

Table 3 – Descriptive data of the Haemangioma Activity Score (HAS) of n=78 haemangiomas, scored by three observers at time points t=0 and t=1 (data shown between parentheses represent the range)

	t = 0		t = 1	
	Mean HAS	SD	Mean HAS	SD
Observer 1	3.51 (1.3 – 5.5)	0.87	1.59 (0.0 – 3.0)	0.70
Observer 2	3.39 (1.5 – 6.0)	0.93	1.56 (0.0 – 3.0)	0.64
Observer 3	3.48 (1.8 – 5.5)	0.79	1.60 (0.0 – 3.0)	0.70

The results of HAS of the three observers at time points t=0 and t=1 are shown in Table 3. Mean HAS, range (between brackets) and standard deviation (SD) at t=0 for observer 1 was 3.51 (range 1.3 – 5.5), SD 0.87, for observer 2: 3.39 (range 1.5 – 6.0) SD 0.93, and for observer 3: 3.48 (range 1.8 – 5.5), SD 0.79. At t=1 the result for observer 1 was 1.59 (range 0.0 – 3.0), SD 0.70, observer 2: 1.56 (0.0 – 3.0), SD 0.64, and for observer 3: 1.60 (range 0.0 – 3.0), SD 0.70.

It can be seen in Table 4 that there are no significant systemic differences in the HAS of the three observers (pair-wise) at the two time points, calculated by using the paired t-test; all p-values are > 0.05.

The interclass correlation coefficients of HAS at t=0 and t=1, and of the changes in HAS between the two time points (HAS at t=0 minus HAS at t=1) are given in Table 5. The pair-wise interclass correlation coefficients at t=0 ranged from 0.67 to 0.79 (average 0.72) and can be regarded as good. The same applies to the agreement at t=1 with ICC ranging

Table 4 – Pair-wise differences of HAS between the three observers of n=78 haemangiomas at time points t=0 and t=1

	t=0				t=1			
	Mean difference	SD	95% confidence interval	p value	Mean difference	SD	95% confidence interval	p value
Pair 1*	0.12	0.58	–0.01 to 0.25	0.07	0.03	0.42	–0.06 to 0.13	0.52
Pair 2**	0.03	0.66	–0.12 to 0.18	0.69	–0.01	0.48	–0.12 to 0.10	0.87
Pair 3***	–0.09	0.70	–0.25 to 0.07	0.26	–0.04	0.52	–0.16 to 0.08	0.51

*Pair 1 = HAS observer 1 minus HAS observer 2
**Pair 2 = HAS observer 1 minus HAS observer 3
***Pair 3 = HAS observer 2 minus HAS observer 3

Table 5 – Interclass correlation coefficients (ICC) and 95% confidence intervals (95% CI) of HAS, and of the changes of HAS, of n=78 haemangiomas, independently scored by three observers at time points t=0 and t=1

	HAS at t=0		HAS at t=1		Changes of HAS[#]	
	ICC	95% CI	ICC	95% CI	ICC	95% CI
Observers 1 vs. 2	0.79	0.69 – 0.86	0.81	0.71 – 0.87	0.75	0.63 – 0.83
Observers 1 vs. 3	0.69	0.55 – 0.79	0.76	0.65 – 0.84	0.68	0.55 – 0.79
Observers 2 vs. 3	0.67	0.53 – 0.78	0.70	0.56 – 0.80	0.65	0.50 – 0.76

[#]HAS at t=0 minus HAS at t=1

from 0.70 to 0.81 (average 0.76) and to the changes of HAS between the two time points with ICC ranging from 0.65 to 0.75 (average 0.69). It can be seen in Table 5 that there is good agreement between the three observers.

DISCUSSION AND CONCLUSIONS

This study based on photographs showed that the Haemangioma Activity Score (HAS) system is useful for scoring the (disease) proliferative activity of haemangiomas in the same patient at different intervals. The pair-wise interclass correlation coefficients at t=0 ranged from 0.67 to 0.79 (average 0.72) and can be regarded as good. The same applies to the agreement at t=1 with ICC ranging from 0.70 to 0.81 (average 0.76) and to the changes of HAS between the two time points with ICC ranging from 0.65 to 0.75 (average 0.69). Studies on the value of HAS submitted for publication or in preparation support the power of the system (Retrospective studies: Nieuwenhuis K. et. al., Haemangioma of infancy: protocollar treatment with short pulse systemic corticosteroid therapy as an alternative for propranolol, submitted; Janmohamed S.R. et. al., Evaluation of intralesional corticosteroids in the treatment of periocular haemangioma of infancy: an alternative if propranolol fails, submitted. Prospective studies: Oranje A.P. et. al., Timolol eyedrops in the treatment of IH, in preparation; Janmohamed S.R. et. al., The results and complications of propranolol in infants with IH, in preparation). We think that this system is also useful for scoring minor changes in the appearance of the IH. This will be included in the last mentioned publication which will summarize the short-term and the long-term results in patients treated with systemic propranolol.

We attempted to keep the HAS as simple as possible, leaving out the most subjective parameters (e.g. we scored the colour and not the observed regression). One advantage of this system is that it can also be used in patients with deep haemangiomas. Another great advantage is that this system is quick and easy to use. It does not require extra time during consultations of patients. Furthermore, the HAS system can be used both

prospectively with patients and retrospectively on photographs. However, good quality photographs are imperative for optimal scoring.

We would like to emphasize that this system scores the (disease) proliferative activity of a haemangioma and does not take size into account (we do not measure the size of the haemangioma). Furthermore, it is intended for use to assess the haemangioma activity at different intervals in one patient and does not aim to compare the activity in different patients. However, you can compare the changes in the scores for evaluating the effect of treatment.

There are some limitations to our study. In deep haemangiomas, points must be given for the swelling. Is it tight, or has it reduced at the follow-up by more or less than 50%? This may be difficult to estimate from the photographs, keeping in mind that a patient also grows and becomes older. In analogy, the size of an ulcer may also be difficult to estimate in a photograph. Photographs do not allow palpation of the skin to assess whether the haemangioma is tight or not, but we think that you can see it from the skin or the skin area surrounding the swelling. We wanted to keep the number of estimations at a minimum in our system, but to make this 'all-round' system work in almost all haemangiomas, we had to take these parameters into account. Moreover, we also noticed that it took some time and practice before the scoring system could be mastered. All the authors were trained to evaluate using a training series of slides of haemangiomas selected by APO, which were then scored together. Another (obvious) disadvantage of this system is that is cannot be used for scoring internal and mucosal haemangiomas e.g. haemangiomas in the eye (cornea) or the liver.

As stated before, a useful scoring system has not been available until now. In a recent publication on this topic, Tsang et al tried to measure growth and size of IHs by measuring haemangiomas and by using a formula, calculating (an estimate of) the volume[14]. We think that this method may be inaccurate because by calculating the volume, you assume that a haemangioma does not have any irregularities and that in case of a partially deep haemangioma, the part under the skin is as large as the 'visible' part. For these reasons it would be better to score the proliferative activity of the haemangioma with the HAS instead of measuring and calculating an estimate of the volume of the haemangioma. Our system measures changes in the individual haemangiomas. It can still be used in clinical trials when one compares the individual percentual reductions in the scores of the haemangiomas, but not the absolute scores. We have used the HAS scoring system in two retrospective protocollar studies that were based on photographs, and it correlated well with the global assessment (Nieuwenhuis K. et. al., Haemangioma of infancy: protocollar treatment with short pulse systemic corticosteroid therapy as an

alternative for propranolol, submitted; Janmohamed S. et. al., Evaluation of intralesional corticosteroids in the treatment of periocular haemangioma of infancy: an alternative if propranolol fails, submitted).

Most ulceration in IH are superficial. Depth of ulceration is an item we did not include in this study based on photographs (retrospective). However, we propose taking depth into account in prospective studies. We propose to use the following scale: superficial, moderate deep and deep ulceration by adding respectively +0.5, +1 and +2 points.

We did not correlate our findings with the opinion of the parents and patients because of the retrospective design of the study. We are planning another prospective study, linking HAS results with 'patient-orientated HAS' in order to investigate the involuting of their haemangiomas.

We conclude that the HAS is a promising scoring system for the (disease) proliferative activity of haemangiomas in individual patients and, in our opinion, would be useful in future investigations for monitoring the clinical activity of haemangiomas after various (new) therapies[15,16]. We intend to perform a comparative study with a group of independent (international) investigators to examine the level of the interobserver agreement. For the evaluation of the severity we are planning an investigation using the Visual Analogue Scale (VAS) and ultrasound (at our institute only). The VAS is generally used to measure mood, pain, pruritus and sleep loss in a variable range of 1–10 representing the severity of the item. The VAS may be used by the doctor as well as by the parents[17] since children younger than 4 years are unable to use the VAS.

ACKNOWLEDGEMENTS

Dr. B. Tank is thanked for correcting the English.

Appendix (at the end of this thesis)

HAS scoring form

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Addendum

Scoring the proliferative activity of haemangioma of infancy: to HAS or not to HAS?

Janmohamed SR, de Waard-van der Spek FB, Madern GC, de Laat PC,
Hop WC, Oranje AP

Clin Exp Dermatol. 2013 Jan;38(1):90–1.

CORRESPONDENCE

Dear editor,

Earlier, Semkova and Kazandjieva[1] (S&K) commented on the Haemangioma Activity Score[2] (HAS) we have developed. Although every system will have its shortcomings, we think that the HAS remains a valuable option for scoring the activity of infantile haemangioma (IH).

We would like to address two clinically relevant issues raised by S&K. First, the fact that the HAS does not assess the residual lesions (telangiectatic vessels) and IH size. We understand these concerns but we believe that both IH size, as well as scoring the telangiectatic vessels, which are in fact secondary efflorescences, is not a necessity for a small, simple and quick system like the HAS. Therefore these items have not been included. The HAS, unlike other systems such as the recently published system by Haggstom et al[3], is not very time consuming and does not take much time during busy consultations. IHs grow very fast and have most change in the first year of life. After proliferation, regression starts; this can last for up to ten years. In this long period, individual IHs do not change much per year, compared to the change in the first year. Therefore, there is no need for scoring the IHs then. In the first year of life however, a scoring system is necessary to (objectively) observe (re)growth or regression and, maybe more important, to evaluate effect of treatment. In this year, the colour and swelling (if present) do change a lot. Therefore, the score will be different in IHs scored at multiple time points. This means that you do not have to take size into account. We did see the effectiveness of the HAS in early stages of treatment. Later on, it does not seem relevant that e.g. a patient at the age of 5 years should have an IH in regression with HAS score 0.5 and at the age of 6 years the HAS is zero but with telangiectatic lesions. Our score is not a severity score which tells you to treat or not, but an activity score that tells you if therapy works. Therefore this system can also be used in research. The choice of treatment is in our opinion dependent on e.g. location (among others fast growing IHs around the eye) or appearance (e.g. ulceration or telangiectatic lesions in regressed IHs with cosmetic objections). Therefore there is not a demand for severity scores.

Second, S&K proposed that consistency of the lesion should be taken into account. We deliberately left this out and in fact considered swelling of the IH as an alternative. In that context there is some overlap but we use a different terminology. Besides, consistency cannot be evaluated on photographs, and the fact that HAS scoring can be performed on photographs is an extremely important aspect of the HAS.

We are currently planning a large validation study to validate the HAS. We also encourage others to validate the HAS and, if desirable, to compare the HAS with other assessments, e.g. the change in consistency of lesions.

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CHAPTER 6

Multiple cutaneous infantile haemangioma and the risk of internal haemangioma

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Madern GC, Oranje AP

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Vredenborg AD, Janmohamed SR (both first author), de Laat PC, Madern GC, Oranje AP.
Br J Dermatol. 2013 Jul;169(1):188–91.

ABSTRACT

Infantile haemangioma (IH) is a frequently occurring tumour in infancy of which the pathogenesis is not completely understood. Although IHs are self-limiting, they can cause problems during their active growth and therapy may then be indicated. Generally, screening for internal haemangiomas is recommended when five or more cutaneous IHs are present. This recommendation, however, is lacking solid scientific evidence. In this chapter we discuss some controversies regarding the prevalence of IH, the nomenclature of 'haemangiomas', the nomenclature of multiple haemangiomas / haemangiomatosis, the therapy of IH, and the above-mentioned screening recommendation.

Since 1993, children with IHs have been evaluated in outpatient consultations by the working group on vascular anomalies Rotterdam (WEVAR), using a protocolized approach. Aiming at determining the relation between number of IHs and the occurrence of internal haemangiomas, we identified all patients presenting with five or more cutaneous IHs in the period 1993–2011. These patients had all been referred for an ultrasound study for internal haemangiomas. We distinguished between children with 10 or more IHs (*haemangiomatosis* group, group 1) and children with 5–9 IHs (*multiple IH* group, group 2).

Forty-three patients were included, 27 in group 1 and 16 in group 2. Nine infants in the *haemangiomatosis* group 1 showed internal haemangiomas, versus none in group 2. Further examination for internal haemangiomas in children with fewer than 10 cutaneous IHs is controversial and does not seem to be necessary. However, we do recommend ultrasound examination for children with 10 or more cutaneous IHs.

LIST OF ABBREVIATIONS:

$BNIP_3$	BCL_2/adenovirus E_1B kD-interacting protein (BNIP) family member 3
CA-IX	Carbon Anhydrase IX
GLUT-1	Glucose Transporter 1
$HIF_{1\alpha}$	Hypoxia Inducible Factor 1α
IH	Infantile Haemangioma
mTOR	Mammalian Target Of Rapamycin
$mTORC_1$	Mammalian Target Of Rapamycin Complex 1
pAKT	Phosphorylated v-akt murine thymoma viral oncogene homolog 1
pS6	Phosphorylated S6 protein
TNF-α	Tumour Necrosis Factor α
VEGF	Vascular Endothelial Growth Factor
VEGF-A	Vascular Endothelial Growth Factor A

INTRODUCTION

Infantile haemangioma (IH) is the most frequent benign tumour of infancy and is 1.5–5 times more common in girls than in boys[1]. Its incidence is 10% in the general population, with a higher incidence (20–30%) in prematurely born infants. They are found on the face in 60% of the cases[1,2]. The diagnosis is usually only based on clinical observation and history[3]. IHs are rarely present at birth[1]; they typically develop several days to weeks after birth and can grow very fast in a few months (known as the proliferation phase). Eventually, after a short steady phase, they regress by approximately 10% per year (known as the involution phase)[4,5].

Controversies

The prevalence of IH is controversial because a wide range has been reported in the literature, varying from 5% up to 20% (the latter in premature infants). The nomenclature is controversial because some professionals refer to several vascular anomalies as 'haemangioma'. This was common practice in the past. Nowadays we prefer the term 'infantile haemangioma' for better understanding and discrimination between IH and other vascular anomalies.

The pathogenesis of IH is still unknown[6]. IH is characterized by the presence of the erythrocyte-type glucose transporter GLUT-1[7], which is not found in other vascular malformations. In a proliferative IH, rapidly growing endothelial cells form blood vessels. Increased apoptosis in the involution phase causes endothelial cells to die, leading to regression of blood vessels. In the end, the thick multilaminated basement membrane surrounding the endothelial layer is replaced by adipocytes in fibrous tissue[8]. Furthermore, a significant increase in the number of mast cells during the involution phase may alter the balance of angiogenic factors, thus promoting regression[9]. Many different causative mechanisms have been proposed[8-12]. Genetic influences may contribute as several patients with IHs show significant loss of heterozygosity for markers in a region of chromosome 5q[13]. Placental embolization is thought to play a causative role, as many of the characteristic molecular markers of endothelial cells in IHs are also expressed by normal placental endothelial cells. Furthermore, hormonal influences may be involved: stimulation with oestrogen increases proliferation, migration and survival of endothelial cells. Also stem cell theories have been proposed because multipotential stem cells can be derived from IHs that can produce neovascularisation. Lastly, hypoxia may be involved in the pathogenesis of IH[10,11,14,15]. In 50% of cases an anaemic macula (precursor lesion) occurs at a place where an IH will eventually develop, supporting the idea that local ischemia is important. Hypoxia triggers stabilisation at the protein level of the transcription factor HIF1α. HIF1α in turn stimulates transcription of downstream target

genes such as BNIP3, CA-IX, GLUT-1, pAKT, pS6, and VEGF[16]. These target genes might be regulated either directly by HIF signalling or by hypoxia-induced down regulation of mTORC1 signalling[17]. mTORC1 is a key player in the mTOR pathway, a protein complex with a central role in regulating cellular metabolism, driven by growth factors, nutrients, and hypoxia. Deregulation of the mTOR pathway may lead to disorganized growth[18]. As macrophages secrete proangiogenic molecules such as TNF-α and interleukin-1, they are also thought to be involved in the evolution of IH[8,15,19].

Because IHs will eventually regress, the 'watch and wait' principle is advocated. However, in approximately half of all cases they will leave scars, often necessitating surgery or plastic surgery. Although of a benign nature, IHs can cause problems during the proliferation phase, generally related to extensive growth or location. Problems may be twofold. First, a gigantic IH in the face is likely to carry a heavy psychological burden. Parents have to explain it over and over again and older children can be made fun of. Second, an IH can cause life-threatening or disabling complications such as visual impairment, compression of the airways, heart failure and ulceration[5,20,21]. A multidisciplinary approach is recommended for such alarming IHs[22]. The current empirically based therapy aims to induce or accelerate the natural involution process[8]. Therapy has radically changed recently, however, with the introduction of propranolol, which halts the growth of haemangiomas and even shrinks them. Systemic therapy with oral glucorticosteroids (GCS) or intralesional therapy with GCS used to be the first-line therapy for alarming IHs[12,23-31], but it is now generally considered a second-line treatment for IHs. Propranolol (a non-selective beta-blocker), often used as therapy for high blood pressure in adults, has made corticosteroids and all the other treatments obsolete[28]. However, evidence on dosing, duration of therapy and possible adverse events is inconclusive[28,32-35]. Until recently, small IHs were treated with topical steroids, imiquimod cream or by PDL laser for cosmetic reasons[36-41]. Similar to propranolol, timolol is a non-selective beta-blocker used in ophthalmic solution and eye gel as a drug against increased ocular pressure. The available eye gel contains timolol 0.1% gel and may be used only after the age of 12 years. At present, we treat superficial IHs with timolol ophthalmic solution 0.5% or propranolol cream 1%.

Controversy

Treatment in IH is controversial because it is not evidence based. There are no generally accepted guidelines defining which patients need which treatment and how.

The use of the term haemangiomatosis is controversial, as is the number of cutaneous IHs needed for this diagnosis. In most cases the number of IHs is limited to one; but in 30% of cases there are more than one[42,43]. The presence of five or more IH is rare (3% of all cases)[44]. The term haemangiomatosis has been used without a consensual definition

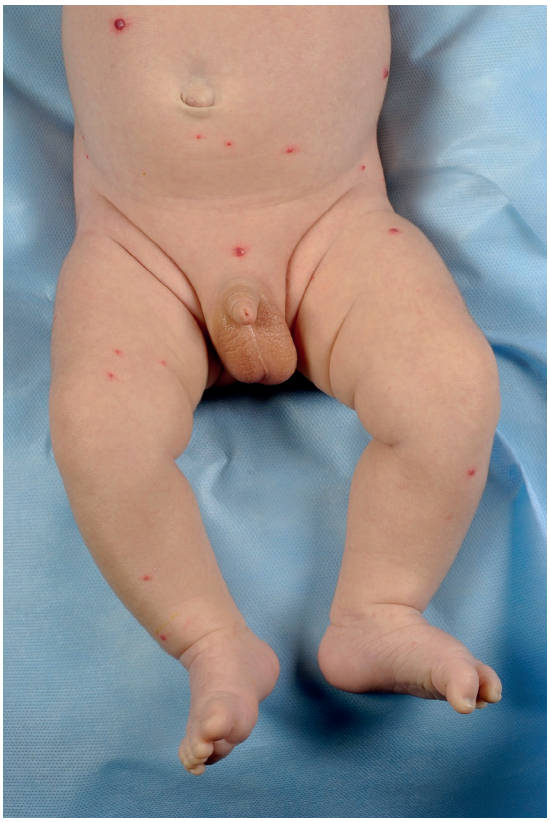

Figure 1 – Infant with multiple cutaneous infantile haemangiomas; in this shot >15 (i.e. haemangiomatosis, miliary infantile haemangioma)

(see figure 1). It is agreed that lesions must be small and multifocal, but the minimal number of lesions is debated. Often the term haemangiomatosis is used to refer to more than five or even ten to hundreds of small multifocal cutaneous IHs[45]. In a recent consensus meeting on IH (Entretien du Carla, France, 13–14 March 2014), the experts agreed to distinguish between focal IH, multiple IH, miliary IH (instead of the older term "haemangiomatosis"), and segmental IH.

Controversy

The use of the term haemangiomatosis is controversial, as is the number of cutaneous IHs needed for this diagnosis. In a recent consensus-of-experts meeting on IH (Entretien du Carla, France, 13–14 March 2014) it was agreed to distinguish between focal, multiple, miliary, and segmental IH.

The most common extra cutaneous site involved is the liver (see figure 2). Other sites include: brain, intestine, lungs, pancreas, and eyes[45]. The mucosa is not considered an internal organ. The severity of hepatic haemangioma varies from asymptomatic to life

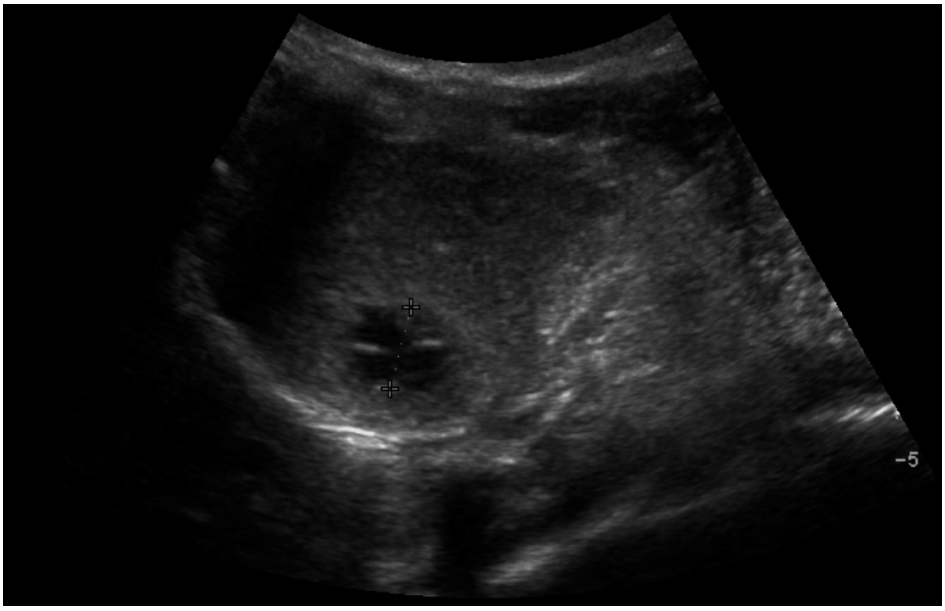

Figure 2 – Ultrasound of a patient with multiple cutaneous miliary infantile haemangiomas (haemangiomatosis). In the liver, multiple lesions (internal haemangiomas) can be seen

threatening, mostly due to congestive heart failure. Identification of infants with cutaneous IHs who are at risk for internal IHs seems crucial[46]. At present, infants are screened for internal IHs when they have multiple cutaneous IHs[47]. In the literature and in most textbooks, this strategy is recommended if there are five or more cutaneous IHs. Nevertheless, this cut-off point was evaluated in only a few studies[43,48,49]. Thus, solid scientific evidence for this policy seems to be lacking.

Controversies

It is controversial when to screen for internal haemangiomas. It is generally recommended to screen in patients with five or more cutaneous IHs but this statement is lacking solid scientific evidence. Finally, it is controversial whether or not to screen only the liver (abdominal ultrasound), or both abdomen and brains. Scientific evidence is lacking.

The purpose of this study was to determine whether further examination for internal IHs should indeed be recommended and in which patients. We distinguished between two categories: multiple IHs (five up to and including nine IHs), and haemangiomatosis (>= 10 IHs). The main controversy to be addressed is 'is screening for internal haemangiomas necessary in infants with five or more IHs?' Other controversies, discussed above, concern the prevalence of IH, the nomenclature of 'haemangiomas', the nomenclature of multiple haemangiomas / haemangiomatosis, and the therapy.

METHODS

In 1993 a multidisciplinary outpatient clinic named WEVAR (workgroup on vascular anomalies Rotterdam) was started at the Erasmus Medical Centre – Sophia Children's Hospital for children with alarming or disfiguring vascular anomalies. The core medical team consists of a paediatric dermatologist, paediatric surgeon, and a paediatrician. Other medical specialists are consulted when necessary. Treatment is standardized. All patients with five or more IHs from the WEVAR and the Paediatric Dermatology outpatient clinics seen from 1993 until 2011 were included in this study. They had all been referred for further examination for internal haemangiomas by ultrasound of the abdomen (and brain). This included Doppler measurements. This is a retrospective study based on a protocollar approach, and embedded in 'Strawberry Marks' (*'Aardbeesie'*), the WEVAR project which researches the pathogenesis and therapy of IH. The study has been approved by the Medical Ethics Review Board of the Erasmus Medical Centre in Rotterdam, the Netherlands.

Two different patient groups were created:
Group 1 (haemangiomatosis) consists of children diagnosed with 10 or more cutaneous IHs.
Group 2 (multiple IH) consists of children diagnosed with five up to and including nine cutaneous IHs.

Outcome measures and statistical methods

Demographic and clinical characteristics collected from the electronic and paper files (e.g. letters and photos) were evaluated with the use of descriptive statistics analyses. Outcome measures were abnormalities on ultrasound with or without symptoms. The Cramér's V analysis served to test significant differences in categorical demographic and clinical characteristics; the Wilcoxon rank sum (two-sample) analysis to test significant differences in numerical demographic and clinical characteristics between the two groups. SPSS Statistics 17.0.2 was used and differences were considered significant when p-values were <0.05.

RESULTS

Forty-three infants were included, 16 boys and 27 girls. Twenty-seven (62.8%) were diagnosed with haemangiomatosis (group 1, 10 or more IHs), and 16 (37.2%) with multiple IH (group 2, 5–9 IHs). Ten of all (23.3%) had been born prematurely. Demographic characteristics are given in table 1.

Table 1 – Demographic and clinical characteristics of patients with haemangiomatosis (10 or more infantile haemangiomas (IH), group 1), and multiple IHs (five up to and including nine IHs, group 2)

Descriptive values		Haemangiomatosis (N =27)		Multiple IH (N =16)	
		Number	(%)	Number	(%)
Gender	Male	10	(37.0)	6	(37.5)
	Female	17	(63.0)	10	(62.5)
Premature (<37 wks)	Yes	6	(22.2)	4	(25.0)
	No	21	(77.8)	12	(75.0)
Gestational age (wks)	Mean±SD	37.5 ± 3.7		37.5 ± 4.5	
Number of cutaneous IH					
10th percentile		12.0		5.0	
Median		16.0*		6.5*	
90th percentile		42.8		10.0	
Phase of IH	Active	(N missing = 1)			
Stable		17	(63.0)	10	(62.5)
Regression		5	(18.5)	4	(25.0)
		4	(14.8)	2	(12.5)
Aspect of IH		(N missing = 1)			
Oval		7	(25.9)	3	(18.8)
Round		15	(55.6)	4	(25.0)
Drop-shaped		1	(3.7)	2	(12.5)
Polycyclic		3	(11.1)	7	(43.8)
Size of IH (cm diameter)					
10th percentile		0.32		0.50	
Median		1.50		2.25	
90th percentile		4.40		8.0	
Treatment of cutaneous IH					
Yes		6	(22.2)	4	(25.0)
No		21	(77.8)	12	(75.0)
Kind of IH treatment					
Intralesional corticosteroids		2	(33.3)	2	(50.0)
Topical corticosteroids		1	(16.7)	0	
Systemic corticosteroids		2	(33.3)	0	
Imiquimod		0		1	(25.0)
Laser		1	(16.7)	0	
Pressure		0		1	(25.0)

*p<0.001, Wks; weeks. SD; standard deviation. Mth; month

The two groups did not significantly differ in sex, prematurity or gestational age (p-value = 0.976, 0.835, and 0.668, respectively). The median number of IH in group 1 was 16 and in group 2: 6.5 (p-value <0.001). The majority of IH were in the active phase. Phase did not significantly differ between the two groups (p-value = 0.842). The IHs in group 1 were mainly round (55.6%); those in the group 2 mainly polycyclic (43.8%) (p-value = 0.07). The median size of IHs in group 1 was 1.50 cm; that in group 2 was 2.25 cm (p-value = 0.069). Six patients in group 1 (22.2%) were treated for the cutaneous IH, mostly with intralesional or systemic corticosteroids; four patients in group 2 (25.0%) were treated, mostly with intralesional corticosteroids (p-value = 0.835).

Table 2 – Details of further examination of infants with haemangiomatosis (10 or more infantile haemangiomas (IH), group 1) compared to infants with multiple IHs (five up to and including nine IHs, group 2)

Descriptive values	Haemangiomatosis (N =27) Number	(%)	Multiple IH (N =16) Number	(%)
Age at screening (mth)				
10th percentile	0.86		2.70	
median	3.0*		6.5*	
90th percentile	8.80		19.1	
Kind of screening (ultrasound)				
Only abdomen	13	(48.1)	12	(75.0)
Both abdomen and brain	14	(51.9)	4	(25.0)
Internal haemangioma				
Yes	9*	(33.3)	0*	(0)
No	18	(66.7)	16	(100)
Localisation of Internal haemangioma				
Liver	8		n/a	
Spleen	1		n/a	
Number of Internal haemangioma				
Solitary	4		n/a	
Multiple	5**		n/a	
Symptoms of Internal haemangioma				
Yes	0	(0)	0	
No	9	(100)	16	
Treatment for Internal haemangioma				
Yes	1	(11.1)	n/a	
No	8	(88.9)	n/a	

Wks; weeks. SD; standard deviation. Mth; month. n/a; not applicable

*p<0.01

**One patient had 3 internal haemangiomas in the liver. The other four patients had more than one internal haemangioma, but this number was unknown.

Further examination in group 1 took place at a younger age than in group 2: respectively 3.0 and 6.5 months (p-value = 0.003). Fourteen patients in group 1 (51.9%) had an ultrasound of both the abdomen and brain versus four in group 2 (25.0%, not significant, p-value = 0.163). Nine patients in group 1 (33.3%) were diagnosed with internal IH versus none in group 2 (p-value = 0.009). Five of those nine patients in group 1 had more than one internal IH. Most internal IH were located in the liver (eight out of nine, one was located in the spleen). One patient was treated for the internal IH. See table 2.

DISCUSSION

This study shows that only patients diagnosed with haemangiomatosis (10 or more IHs) are likely to be at risk for developing internal haemangiomas, for one third of them had internal haemangiomas versus none of the infants with 5–9 IHs. We opted for 10 IHs as

a cut-off point because the cut-off point of five IHs for further screening is controversial and because we have never seen internal haemangiomas in patients with <10 IHs. There was no consensus, too, about the definition of haemangiomatosis until the recent expert meeting described in the *introduction*. Therefore we described haemangiomatosis as the presence of 10 or more IHs.

Prior studies have shown that infants with five or more cutaneous IHs are generally at risk for internal haemangiomas[46,47,50-54]. Horii et al found that 16% of patients with five or more IHs developed internal haemangiomas. Although they did not distinguish between two groups, from their data it can be recalculated that nine of the 87 patients with 5–9 IHs had hepatic haemangiomas (10%) and that 15 of the 64 patients with 10 or more IHs had hepatic haemangiomas (23%). The respective percentages in our study were 0% and 33%. Therefore Horii et al recommend screening when five or more IHs are present, whereas from our study it can be concluded that screening is recommended when 10 or more IHs are present. This seems the more because Horii et al found that none of their patients with 5–9 IHs needed treatment for internal haemangioma. Therefore this approach could also be more cost-effective. Conclusive evidence should come from more and larger, preferably prospective studies. Also, a meta-analysis can be performed within the existing literature. Statistically (but probably of less clinical significance), the risk (odds ratio) of internal haemangioma in the study of Horii et al is 2.7 times larger for patients with 10 or more IHs, compared to patients with 5–9 IHs (p-value 0.03, chi-square test).

In our study we included more patients in the group with 10 or more IHs than did Horii et al, probably because our study stems from a tertiary medical centre which more often treats worse cases than other hospitals. Furthermore, Horii et al found a trend for greater risk of hepatic haemangioma in patients with greater numbers of IH, which is in line with our findings[46]. Nonetheless, due to small sample size, we could neither confirm nor disprove this. A recent study from Maruani et al also showed a trend towards greater hepatic involvement when there are more cutaneous IHs[55]. Unfortunately this is also a retrospective study in only 19 patients). In that study, three patients with >10 hepatic haemangiomas were treated (because of increased arterial blood flow). Their study distinguished between benign neonatal haemangiomatosis and diffuse neonatal haemangiomatosis. The authors hypothesize that the mechanism may be different from that in IH: in their cases, lesions were almost always present at birth, and the increase was not in size but in number. Therefore there was no ulceration. However, many complications occurred during pregnancy. Unfortunately, this study did also not distinguish between 'multiple cutaneous IHs' (5–9) and 'haemangiomatosis' (10 or more IHs). Our study is to our knowledge the first to research both groups separately. In doing so, we showed

that patients with multiple IH are not at risk for developing internal haemangiomas, in contrast to patients with haemangiomatosis.

In the present study, all but one internal haemangiomas were located in the liver. One was found in the spleen. Only a few infants with internal haemangioma required treatment for liver disease. Previous studies also found low percentages of infants requiring treatment[46] and concluded that IH of the liver can be asymptomatic and not need treatment, even when multifocal hepatic lesions are present[46]. Other groups have identified an association between number of cutaneous IH and number of hepatic haemangioma identified on screening ultrasound[48]. A more recent study did not find such association[43].

Ultrasound is a non-invasive and relatively inexpensive technique to identify infants with internal haemangiomas. However this screening method is not conclusive. Haemangiomas of the liver are often difficult to distinguish from other vascular malformations. Also, the technology for diagnosing haemangiomas on ultrasound has been improved over the years. It is evident that multifocal vascular lesions in the past may have been diagnosed as internal haemangiomas whereas a different diagnosis (such as multifocal lymphangioendothelioma, haemangioendothelioma or pyogenic granuloma-like lesion) might have been more likely. This could explain why older studies describe higher incidences of internal haemangioma. Radiologists, preferably paediatric radiologists, have to be trained very well to recognize internal haemangiomas on ultrasound and to distinguish these from other types of vascular malformations.

Several limitations of this study should be addressed. First, this is a retrospective study (but based on a protocolled approach) with relatively few patients. Second, the subjects were recruited from only a specialized paediatric dermatology and multidisciplinary specialty practice in a university setting. This might have induced selection bias. Third, ages at screening differed significantly between the two groups, possibly because infants with haemangiomatosis are referred to a specialist at a younger age than infants with multiple IH. Furthermore, there are also limitations in the examinations performed in the infants with internal haemangioma. The exact number of hepatic lesions is often unknown. Retrospectively, we could only find out whether the lesions were solitary or multiple. Also, no heart echography had been performed. Most doctors would like to know whether there are internal haemangiomas or not, and specifically hepatic haemangioma as these carry a large risk of congestive heart failure. A classification system for hepatic haemangioma was proposed by Christison-Lagay[56]. We did not use this classification system because our study was started before this system was published. Apart from the limitations, this study has a particular strength; it is the first to compare two separate groups, the haemangiomatosis group (10 or more IHs), and the multiple IH

group (5–9 IHs), as we believe that an infant with five or more IHs is not comparable to one with 300 small drop-like IHs.

Imiquimod was used as a local therapy option for cutaneous IHs in patients in this study. One infant with multiple IHs was treated with this therapy. This therapy gives good results but can lead to a local (eczema-like) skin reaction. Topical corticosteroids can be used to treat this reaction[57]. Therapy of IH has changed considerably over the last years. The 'watch and wait' principle is followed less often nowadays, especially after the discovery in 2008[28] that β-blockers, both orally and topically, can also play a role in the treatment of superficial IHs. This study reports on patients selected from 1993–2011 and therefore most patients were treated by corticosteroids rather than topical or systemic propranolol. Systemic options used to be corticosteroids, with low success rates and important complications, and vincristine IV, which also can give rise to side effects. Currently propranolol is advocated in a dosage of 3 mg/kg daily for 6 months (Léauté-Labrèze et al, NEJM, submitted). Propranolol occasionally causes side effects like nightmares and cold acra, but also more serious side effects such as hypoglycaemia and hypotension have been reported. It is good to realize, however, that hypotension normally does not occur in healthy infants because β-blockers only work when the blood pressure is elevated[58]. Regarding hypoglycaemia, this is never a direct effect of propranolol, but rather the combined effect of propranolol and fasting, for example because of vomiting or fever. This can also occur with combinations of particular medicines, such as propranolol and corticosteroids. As for topical treatment, the best current options are timolol 0.5% ophthalmic solution, and propranolol 1% cream, applied 3–4 times daily[59,60]. We would like to stress that the effect of topical treatment with these β-blockers can only be seen after 2–4 months (with documented photographs) and that it is advised to apply the medicine 3–4 times daily.

CONCLUSION

Generally, screening for internal haemangiomas is recommended when five or more multiple cutaneous IHs are present, but this is controversial. Based on the results of this study, we recommend referring patients with 10 or more IHs for further examination for internal haemangiomas by ultrasound of abdomen (and brain). However, the results of our study suggest that further examination for internal haemangioma in patients with 5–9 IHs is not necessary.

Controversies surrounding infantile haemangioma (IH)

- The prevalence of IH is controversial because a wide range has been reported in the literature, varying from 5% up to 20% (the latter in premature infants).
- The nomenclature is controversial because some professionals refer to several vascular anomalies as 'haemangioma'. This was common practice in the past. Nowadays we prefer the term 'infantile haemangioma' for better understanding and discrimination between IH and other vascular anomalies.
- Use of the term haemangiomatosis is also controversial, as is the number of cutaneous IHs needed for this diagnosis. In a recent consensus-of-experts meeting on IH (Entretien du Carla, France, 13–14 March 2014) it was agreed to distinguish between focal, multiple, miliary, and segmental IH.
- Treatment in IH is controversial because it is not evidence based. There are no generally accepted treatment protocols for defining which patients need which treatment and how.
- It is controversial when to screen for internal haemangiomas. It is generally recommended to screen in patients with five or more cutaneous IHs but this recommendation is lacking solid scientific evidence. In our study we did not see internal haemangiomas in patients with up to nine cutaneous IHs.
- Finally, it is controversial whether only the liver (abdominal ultrasound) should be screened, or both abdomen and brain. Scientific evidence is lacking.

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PART III

Therapy of infantile haemangioma

CHAPTER 7

Evaluation of intralesional corticosteroids in the treatment of periocular infantile haemangioma: still an alternative besides propranolol

Janmohamed SR, Madern GC, Nieuwenhuis K, de Laat PC, Oranje AP

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ABSTRACT

Infantile haemangioma (IH) is the most frequently occurring benign tumour of infancy. Alarming IH require treatment. Current therapy is empirically based; corticosteroids are often administered but in recent publications propranolol was reported to be more effective. Periocular IHs are highly sensitive to corticosteroids. Our goal was to evaluate the effectiveness of intralesional corticosteroids in the treatment of periocular IH.

We selected all patients with periocular IH who had only been treated with intralesional corticosteroids at our hospital from 1993 until 2009. Treatment was standardized according to a prospective protocol.

A total of n=34 patients were included. There were no complications at all after therapy. A second intralesional injection was necessary in 5 patients. At follow-up after 6 and 12 months after injection, 94% and 91% of the patients, respectively had regression of the IH. Astigmatism, HAS (Haemangioma Activity Score) and global assessments all had improved after therapy.

This study shows that intralesional therapy with corticosteroids is very safe in the treatment of periocular IH. It remains a good and safe alternative besides propranolol or when propranolol therapy is not possible (e.g. asthma, PHACE syndrome, and certain cardiac diseases).

INTRODUCTION

Infantile haemangioma (IH)[1] is the most common, benign, self-limiting tumour of infancy[2,3] and therefore needs no treatment[4]. It is commonly located on the face[5]. However, IH may cause severe complications[4], generally because of extensive growth or location, leading to (besides psychological problems) life-threatening or disabling complications[6] (e.g. compression of the airways, heart failure, ulceration and visual problems). Amblyopia or reduced vision in the affected eye, secondary to astigmatism or visual deprivation affects 43% to 60% of the children with periocular IH[1]. Of course, such IH require treatment. However, the current therapy in IH is empirically based. Systemic or intralesional corticosteroid treatment is often administered[7]. Periocular IHs are particularly sensitive to corticosteroids. In recent publications, propranolol[8,9] was reported to be either more or at least equally effective in treating proliferative IH. Other alternatives among others are[10]: laser therapy, intravenous vincristine, cyslophosphamide and recombinant interferon alfa[11] (high risk for adverse events), local/intralesional imiquimod, becaplermin (recombinant platelet-derived growth factor) and bleomycin. Apart from waiting for involution or surgical resection, treatment aims at inducing or accelerating the natural involution process[12]. Although there is extensive literature, objective measures of astigmatism and anisometropia before and after treatment are limited. In this article we evaluate the effectiveness of intralesional corticosteroids in the treatment of periocular IH.

METHODS

Study design and patient selection

In 1993 Oranje, de Laat and Madern started a special outpatient clinic called WEVAR (Workgroup on Vascular Abnormalities Rotterdam) at our hospital, for children with threatening, very large, or fast growing vascular abnormalities. This outward clinic is multi-disciplinary and includes as a basic team APO (Paediatric dermatologist), PCJL (Paediatrician) and GCM (Paediatric surgeon). Other specialists are consulted if necessary. Treatment (when necessary) is standardized. One of the therapy options are intralesional corticosteroids, (a mixture of 2 ml Kenacort-A 40 mg/ml and 3 ml Celestone 4 mg/ml), and these are administered at a dose of 1 to 5 ml at one or multiple sites, depending on the size of the lesion. Corticosteroids are injected with a 23G 1" needle. During the procedure, the paediatric surgeon (visually) checks the site of injection. The position of the needle is checked both visually and by palpation. Injection in a great vessel is prevented by checking for blood return in the needle (aspirate). After the procedure, pressure on the injection site(s) with a gauze and by using the fingers was performed by the paediatric surgeon until haemostasis had been reached. Standard controls after the

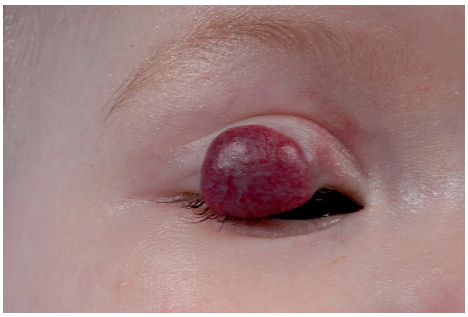

Figure 1A – A periocular infantile haemangioma (IH) in an almost 6 months old girl at t=0. Note the swelling which closes the eye

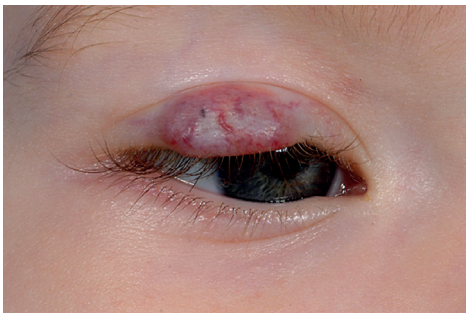

Figure 1B – 6 months after treatment with intralesional corticosteroids: the IH is in regression and the eye can be opened again

Figure 1C – 12 months after treatment with intralesional corticosteroids: the IH is almost gone

procedure consists of checking the site of injection, the eye, general condition, and, by indication, hormonal controls or eye-examinations by an ophthalmologist. Figures 1A, 1B and 1C show the course of a periocular IH, treated with intralesional corticosteroids.

Patients with alarming (threatening) periocular IH who needed treatment, e.g. because they were (at risk of) developing amblyopia, were treated according to a WEVAR protocol including ultrasonography of the periocular region and eye examinations prior to therapy. These eye examinations (cycloplegic refractions of both eyes) were also protocolized and performed by a paediatric ophthalmologist. Patients received one drop of a solution containing phenylephrine 2.5% and tropicamide 0.5% in each eye. After 15 to 20 minutes this was repeated. The examination started 15 to 20 minutes after the second drops were administered to the patients. Spherical and cylindrical abnormalities were assessed by using skiascopy. All patients were awake during the eye examinations.

Inclusion and exclusion criteria:
All of the patients with alarming periocular IH (i.e. the eye was at risk because of such threatening IH) who were treated with intralesional corticosteroids from 1993 until

2009 were included in this study. Patients who were treated with both systemic and intralesional corticosteroids were excluded, as were patients with IH at another location than periocular. Patients with intra-orbital IH are not treated with intralesional therapy. Data was obtained by investigating the patients' medical dossiers. Photographs that had been taken were assessed.

Scoring IH

The effect of treatment was evaluated by the Haemangioma Activity Score (HAS)[13]. This scoring system focuses on the (disease) proliferative activity of a IH at a certain time point. Scores at multiple time points of one IH in one patient can be compared with each other.

Outcome measures

HAS before and after treatment was calculated at t=0, t=6 and t=12 months from the photographs. We searched all patients' medical dossiers for complications. The global assessments at the check-ups on the size of the IH were collected as were the ophthalmic measurements made by the ophthalmologist.

Statistical methods

SPSS version 15.0.0 was used for the database and statistical measures. Differences in astigmatism, in matters of refractive cylindrical value, and HAS between the three time points were assessed using Friedman's test.

RESULTS

In the period 1993 to 2009 a total of n=67 patients with IH were treated with intralesional corticosteroids. After excluding patients with concomitant IH other than periocular IH (n=28) and patients who had both systemic and intralesional therapy (n=5), a total of n=34 patients were included. These patients were treated with only intralesional corticosteroids because of an alarming periocular IH. Their baseline characteristics are shown in Table 1. 88% of patients were female and 82% full term (gestational age > 37 weeks). 9% of patients were twins. The median age at which the IH started to develop was 0.4 months (10^{th} percentile = 0.0, 90^{th} percentile = 1.75). The number of IH in each patient ranged from 1 to 5 but most patients had 1 IH (median number = 1, 10^{th} percentile = 1, 90^{th} percentile = 4) and most IH were small (71% < 3 cm, 3% > 10 cm, greatest diameter taken on ultrasonography). Finally, the median age of first intralesional therapy was 3.8 months (10^{th} percentile = 2, 90^{th} percentile = 9).

Table 1 – Demographics and baseline characteristics of n=34 patients with periocular infantile haemangioma (IH) treated only with intralesional therapy

Characteristic	Rx/ Intralesional corticosteroids (n=34)
Sex	Female: 88% Male: 12%
Gestational age	>37 weeks: 82% Premature: 18%*
Gemini	No: 91% Yes: 9%
Median age of development of IH	0.4 months (10th percentile = 0,0; 90th percentile = 1.75)
Median number of IH per patient	1 (10th percentile = 1; 90th percentile = 4)
Size of IH (assessed by ultrasonography)	<3 cm: 71% 3–10 cm: 26% >10 cm: 3%
Median age at start intralesional therapy	3.8 months (10th percentile = 2; 90th percentile = 9)

*5 patients with a gestational age between 33 and 35 weeks, one patient with a gestational age of 25 3/7 weeks

Of these n=34 patients, 5 patients were given a second intralesional corticosteroid injection. Table 2 shows the outcomes of the therapy at 6 months and 12 months after the first injection. HAS declined from 3 (SD 1.3) at baseline to 2.1 (SD 0.9) after 6 months and 1.6 (SD 0.7) after 12 months after the first injection (p = 0.000). Global scores by the doctor strengthen the (reduction in) HAS: after 6 months 94% was scored as 'smaller', after 12 months this was 91% (6% was the same and 3% worse). Astigmatism shows a similar

Table 2 – Outcomes of intralesional corticosteroid therapy in n=34 patients with alarming periocular infantile haemangioma (IH) at t=0 (baseline, just before intralesional therapy), t=6 and t=12 (6 and 12 months after injection)

	T=0	T=6 months	T=12 months
Mean HAS	3.0 (SD 1.3)	2.1 (SD 0.9)	1.6 (SD 0.7)*
Refraction: mean astigmatism (Diopters)	2.57 (SD 1.49)	1.84 (SD 1.01)	1.44 (SD 1.26)**
Occlusion therapy (for amblyopia)			
YES	46%	40%	28%
NO	54%	60%	72%
Therapy effect (evaluation by the doctor)			
SMALLER		94%	91%
SAME		6%	6%
WORSE		0%	3%
Complications			
YES		0%	0%
NO		100%	100%
Plastic surgery required until 2009?			
YES			9%
NO			81%

*$p < 0.001$

**$p = 0.011$

trend as the HAS: mean cylindrical diopters dropped from 2.57 (SD 1.49) at baseline to 1.84 (SD 1.01) after 6 months and 1.44 (SD 1.26) after 12 months (p = 0.011). At baseline, 46% of the patients had occlusion therapy, after 6 months 40% and after 12 months only 28% of all patients still had occlusion therapy. Until now, 9% of patients had plastic surgery. Most important: we did not see any (lasting) complications. In one patient we did see periocular calcification, but this appeared to be reversible.

DISCUSSION

This study showed that intralesional therapy with corticosteroids is a very safe alternative in the treatment of (periocular) IH as no complications were observed by us. HAS, eye examinations, and clinical appearance all improved after intralesional therapy. Since IH is a self-limiting tumour, the expectation is that HAS and clinical appearance would eventually improve, but we were able to demonstrate that when an eye is at risk of visual impairment, intralesional therapy is highly effective: all eye examinations (especially cylindrical abnormalities) improved after treatment.

In 9% of our cases, plastic surgery was indicated in a later stage. However, this did not mean that intralesional therapy was not effective. The IH did regress faster. We think that without the intralesional therapy or with another therapy, a scar would have eventually developed that required plastic surgery. It is possible that this percentage may have been higher because some patients could have gone to another hospital or may require plastic surgery in the future.

Our results of the treatment with intralesional corticosteroids for periocular IH are excellent with a success rate of 85% after one injection and 100% after two injections, without any adverse events. These percentages are somewhat higher than those reported in the literature. Fifty percent of IH respond after therapy with corticosteroids, periocular IH are more sensitive, with rates up to 64%[10,14,15]. These differences are probably attributed to other measures of effect and success. Often, the amount of corticosteroids that were administered in those cases is also difficult to establish. We treated all patients in a standardized manner.

The possible side effects are as follows. Injection pressure during intralesional injection usually exceeds systemic arterial pressure and poses a risk of corticosteroid particle embolization into the ocular circulation due to retrograde arterial flow. Serious ocular complications include ophthalmic artery occlusion, retinal embolization and central retinal artery occlusion. Others are eyelid hypo-pigmentation, linear subcutaneous fat

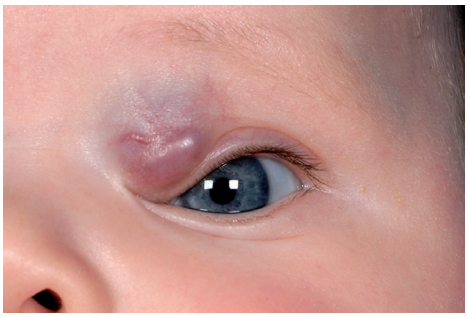

Figure 2A – A periocular infantile haemangioma (IH) in a 4 months old boy at t=0

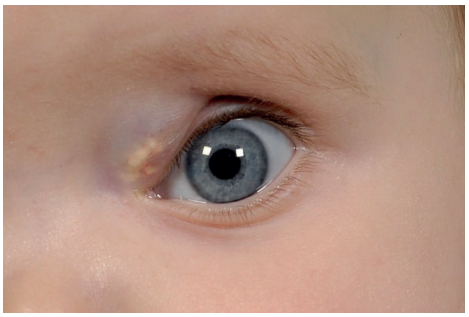

Figure 2B – 6 months after intralesional corticosteroids: the IH is in regression. Note the periocular calcification

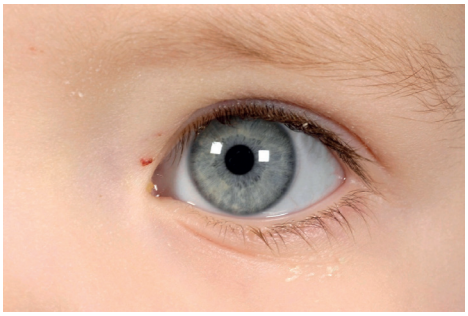

Figure 2C – 2 years after intralesional corticosteroids: the periocular calcification appeared to be reversible

atrophy, sclerodermiform linear atrophy, eyelid necrosis, and periocular calcification. Rare systemic side effects include cushingoid features, growth deceleration, and adrenal suppression[10,15]. We did not encounter any of these side effects. Periocular calcification was seen in one patient but appeared to be reversible (see figures 2A, 2B and 2C). The most important thing is to aspirate for blood after injection and inject with care. For example, if the IH is located in the eyeball, intralesional therapy is dangerous because the IH expands while corticosteroids are administered leading to compromised perfusion and visual loss. An extension of the IH into the orbital cavity can also be associated with complications, and therefore we do not treat such patients with intralesional therapy.

Furthermore, female to male ratio is slightly high in our study, but agrees with the reported findings that IH is more common in girls[12]. We also observed that most patients had a small (<3 cm) IH.

In spite of these good results, intralesional therapy of IH with corticosteroids is undergoing a transformation. Today there is an increasing interest on propranolol in the treatment of IH with promising results and until now few reported side effects. We think that it has almost become the first choice of treatment. The results of this study showed that intralesional injection with corticosteroids is still a safe alternative for treating

Table 3 – Advantages and disadvantages of intralesional corticosteroid treatment and propranolol treatment of periocular infantile haemangioma (IH)

	Intralesional corticosteroids	Propranolol
Advantages	Often just 1 injection, instead of long-term administration of medicine	Seems to work at least as good as intralesional corticosteroids without the side effects of intralesional therapy
	Local, once	
	Good response on periocular IH	
	A lot of experience with this therapy: not many side effects	
	Can be given when propranolol fails, or in PHACE syndrome	
Disadvantages	Anaesthesia is recommended to minimize side effects	Systemic
	Other IH than periocular have a success rate of only 50%	Side effects have not yet been properly investigated at this age (watch out: do not give in patients with PHACE. Several published case reports with hypoglycaemia). Blue coloured hands and feet
	One day hospitalization, limited ultrasound evaluation	One day hospitalization, expensive multiple ultrasound pre-treatment evaluation
	Sometimes the therapy has to be repeated later on (with another narcosis)	Doses and duration of therapy is still unknown. Additional investigations are necessary
	When administered by non-experienced physicians: retinal occlusion/optic nerve compression is a rare complication	Contra-indicated in vascular malformations of internal organs and PHACE syndrome[16]

(periocular) IH when propranolol is not effective or when propranolol is (relatively) contra-indicated (e.g. asthma, certain cardiac diseases, multiple medication causing extra burden, and PHACE syndrome[16]). The recent study reported by Greenberger et al elucidated the effect of corticosteroids on angiogenesis and vasculogenesis[17]. Certain advantages and disadvantages of both propranolol and intralesional therapy are summarized in Table 3. We conclude that intralesional therapy with corticosteroids still has a valuable place in the treatment of IH.

DISCLOSURE STATEMENT

The authors declare that they have no conflict of interest.

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CHAPTER 8

Infantile haemangioma: treatment with short course systemic corticosteroids as an alternative for propranolol

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ABSTRACT

Infantile haemangiomas (IHs) are increasingly being treated with propranolol or other beta-blockers, but before this therapeutic option was available, oral glucocorticosteroids (GCSs) were the criterion standard treatment and are still the alternative modality in problematic cases. Nevertheless, there is no standard treatment protocol for the dose and duration of GCSs. Long-term treatment with GCSs is associated with unwanted side effects such as growth suppression, behavioural changes, and reflux.

Twenty-one children with troublesome IHs were treated according to an algorithm with 3 mg/kg/day of oral prednisolone divided three times per day with varying duration and number of GCS courses. Two blinded investigators independently interpreted therapy results using the Haemangioma Activity Score (HAS). Side effects were determined according to reports in patient charts and parental questionnaires.

The median duration of a short course of GCSs was 2 weeks (range 1–6 weeks). The number of courses was 2 (range 1–5). The median cumulative dose was 91 mg/kg. Growth stabilized in all patients, with a good response (>50% reduction in HAS) in 62% and a favourable response (30–50% reduction is HAS) in 23%. Twelve of the 21 children (57%) had minor side effects. Persistent side effects did not occur.

Intermittent short course, systemic, high-dose GCS therapy is an effective and safe treatment modality for IH, with a substantially lower cumulative dose of GCSs compared to prolonged therapy and no major side effects. This treatment is an alternative in cases in which propranolol fails or is contraindicated.

INTRODUCTION

Infantile haemangioma (IH) is the most frequent benign soft tissue tumour occurring in infants[1]. In 2008 propranolol was reported as an effective treatment of IH, and since then many studies have supported its use[2-8]. Glucocorticosteroid (GCS) treatment was previously considered to be the first-line therapy for IH causing medical complications or with the potential of significant disfigurement[1,9-11] but it is now generally considered as a second-line treatment for IH. The mechanism of action of corticosteroids was addressed in a recent study showing that dexamethasone inhibited the vasculogenic potential of stem cells derived from human IH. Dexamethasone also suppressed the expression of vascular endothelial growth factor A (VEGFA) in haemangioma-derived stem cells implanted in immune-deficient mice. Silencing of VEGFA expression in these cells inhibited vasculogenesis[12].

The recommended starting dose for systemic GCS is 2 to 3 mg/kg of prednisone or prednisolone, usually given orally as a single morning dose for several (6–9) months[1,9-11,13-17]. Dose, duration of treatment, recommended monitoring during and after treatment, and methods of tapering vary widely[9].

Generally 30% of patients have a favourable and persistent response to orally administered GCS treatment. Tumour growth is stabilized in approximately 40%, whereas 30% are non-responders who fail to respond to higher doses or additional intravenous high-dose pulse therapy with GCS[1].

METHODS

In the setting of our multidisciplinary Workgroup on Vascular Abnormalities Rotterdam (WEVAR), children are treated according to prefixed algorithms. Treatment algorithms exist for different variations of IH and different therapy options. This study is a retrospective case series of the children treated according to the algorithm with 3 mg/kg/day of GCS with varying duration and number of GCS courses.

Using this protocol, when systemic therapy with GCS is indicated, it consists of orally administered prednisolone 3 mg/kg/day in three daily doses for two to three weeks. The dose is then tapered over a period of 10 days. The child is hospitalized for two to three days at the first administration under the supervision of a paediatrician who monitors for side effects. WEVAR evaluates treatment results at two-week intervals. A watch-and-wait strategy is adopted when the treatment is successful. A repeated short course of

Table 1 – Haemangioma Activity Score (HAS)

Date	t=0 *example*
Deep swelling: Tight haemangioma (6) 'Neutral' haemangioma or less than 50% reduction at follow up (4) >= 50% reduction at follow up (2) No more swelling at follow up (0)	6
Bright red / shining red (5) OR Bright red edge* (4)	
Red / red-purple / matt red edge (3)	3
Blue or blue shining through in deep haemangiomas (2)	
Grey (1)	1
Skin coloured after activity** (0)	
Total	10
Number of items scored	3
Preliminary HAS = total / number of items scored	3.3
Ulcer =< 1 cm^2 (+0.5) Ulcer 1–25 cm^2 (+1) Ulcer >= 25 cm^2 (+2)	0.5
HAS = preliminary HAS + ulcer score	3.8

*"Bright red edge" should only be scored when the haemangioma is not totally "bright red".
**"Skin coloured after activity": do not score in deep haemangioma (deep swelling) unless the haemangioma has changed into it after activity.

GCS is started in the case of significant rebound growth of IH. This new course is usually given at the same dose and for the same duration as before.

The final result of the systemic GCS treatment in each patient is determined according to the Haemangioma Activity Score (HAS), a validated score developed to quantify treatment results in IH (table 1)[18]. The results were evaluated by comparing each child's HAS at baseline (before treatment), two weeks after the first course, and three months after the last course. Investigators scored each photograph individually. Investigators were blinded; all photographs were coded and placed in random order.

A good response is defined as a minimum of 50% reduction in HAS three months after the final course of GCS therapy. A favourable response is defined as a 30% to 50% reduction in HAS. A patient is defined as a non-responder if the HAS has decreased less than 30% three months after the last course of GCS therapy.

Patients

From May 1994 to March 2009, 349 patients (246 female, 103 male) with troublesome IH were referred to the WEVAR outpatient clinic. Therapy was indicated in 163 (47%), and 33 of these received systemic therapy with oral prednisolone. The following inclusion

criteria were used: first WEVAR consult between May 1994 and March 2009, diagnosis of IH, treatment of first choice orally administered GCS, and follow-up longer than 12 months. The diagnosis of IH was based on clinical presentation: age, appearance, and course. In most cases, colour Doppler evaluation or magnetic resonance imaging was used to support the diagnosis and to indicate the extent of the tumour. The inclusion criteria were met in 21 (16 female, 5 male) of the 33 children treated with systemic GCSs. Nine patients were excluded because they had been treated before or simultaneously with intralesional GCS therapy. Another patient was excluded because the diagnostic biopsy was GLUT-1 negative. One patient was excluded because of a solitary liver lesion and one because systemic GCS treatment was given at another hospital. Table 2 shows an overview of the clinical characteristics of all included patients.

Patients were evaluated in terms of sex, age, location of the lesion, effect of treatment, and side effects such as hypertension, cushingoid features, behavioural changes, and diminished growth. Hypertension was defined as a systolic blood pressure more than 2 standard deviations (SD) above the norm for the age. Blood pressure was measured regularly during the first three days of the first course and at follow-up visits thereafter. We used photographs taken during the entire follow-up period and used the pre-treatment photograph as the reference to determine whether a child had developed cushingoid features. Behavioural changes were determined according to a retrospective questionnaire to the parents. We used the measurements taken at the visits to the WEVAR outpatient clinic to fill in a standard growth chart for each child. A diminished rate of growth was

Table 2 – Clinical characteristics of 21 patients with infantile haemangioma

Characteristic	N
Sex	
Female	16
Male	5
Number of lesions	
> 1	11
1	10
Type	
Superficial only	10
Deep only	2
Mixed	9
Location of the lesion	
Head-neck	19
Trunk	2
Complications	
Ulceration	18
Visual impairment	1
PHACE syndrome	2

defined as a decrease from the pre-treatment growth line. This study is part of the WEVAR project "Aardbeesie," and was approved by the Erasmus MC Medical Ethical Committee.

RESULTS

Twenty-one patients with IH treated with systemic GCSs were eligible for this study. An overview of the treatment parameters is shown in Table 3. All patients had cutaneous IH. Intralesional GCSs were also administered in three patients but more than 3 months after the last systemic GCS therapy.

Number of intermittent treatments and cumulative dose

There was no significant re-proliferation after the first treatment period in eight (38%) of the 21 children. In six children, there was rebound growth after the first treatment period but no further proliferation after the second treatment period. A third treatment episode was necessary in four children. Only three children with a prolonged proliferation phase needed more than three courses of GCSs.

Patients 15, 19, and 21 (table 3) had higher HAS scores after the last course of GCSs, but re-proliferation was not severe enough to warrant an extra course of GCSs. In patients 19 and 21, rebound growth occurred after an initial very good response. It was decided to give a lower dose of 2 mg/kg/day in the next courses, anticipating a favourable response and fewer side effects. The cumulative dose in patient nine was much higher than expected because of a first course with a dose of 5 mg/kg/day of GCS. This was the first patient we treated according to this algorithm. The higher dose was given because of the size and growth of the haemangioma; 5 mg/kg/day was a high but acceptable dosage at that time. The patient responded well to the second course at 3 mg/kg/day.

The median number of courses was 2 (range 1–5). The median duration of a short course of GCS was 2 weeks (range 1–6 weeks). The median age at the start of therapy was 10 weeks (range 5–19 weeks). Median cumulative dosage was 91 mg/kg (range 42–224 mg/kg). Children treated with GCS were followed over a period that varied from 12 to 224 months (median 61 months).

Therapeutic responses

The therapeutic responses represented as longitudinal HAS scores are shown in table 4. The proliferation of IHs was stabilized in all of the children. A good result (>= 50% reduction in the HAS) was noted in 13 (62%) of the 21 children. The results in patient 17 are shown in figure 1.

Table 3 – Treatment parameters and side effects in the 21 cases included in study

Case	Sex	Age at the start of treatment (weeks)	Localization of lesion(s)	Ulceration	Short courses (n)	Total cumulative dose (mg/kg)	Follow-up (months)	Side effects
1	Female	10	Chin, lip, neck	+	1	63	143	None
2	Female	7	Face	+	1	84	20	None
3	Female	14	Lip, nose	+	1	42	10	None
4	Female	9	Cheek, nose	+	1	84	112	None
5	Female	10	Face	-	1	63	109	↑ appetite
6	Male	6	Cheek, ear	-	1	63	53	None
7	Female	19	Cheek, ear, nose	+	1	42	81	None
8	Male	16	Back, cheek	-	1	63	61	↑ appetite, Cushing
9	Female	5	Face	+	2	224	152	None
10	Female	7	Chin, lip, cheek	+	2	126	83	None
11	Female	14	Cheek	+	2	91	88	↑ Blood pressure 1st day
12	Male	11	Cheek	+	2	105	27	↑ appetite, behaviour
13	Female	12	Face	+	2	84	26	Cushing, behaviour
14	Female	10	Nose	-	2	126	21	Cushing, adrenal insufficiency
15	Female	14	Cheek, lip, nose	+	3	84	70	↑ Blood pressure 1st day
16	Female	6	Face, chest	+	3	147	62	Cushing
17	Female	6	Lip, nose	+	3	91	48	Cushing
18	Female	12	Periocular	-	3	126	12	None
19	Male	12	Cheek	-	5	168	21	↑ appetite, Cushing, behaviour
20	Male	7	Face	+	5	210	36	Cushing, acne
21	Female	12	Cheek	+	6	119	70	↑ appetite, behaviour, Cushing

A favourable result (30%–50% reduction in HAS) was observed in 5 (24%) of the 21 children. Only 3 (14%) of the 21 children were considered to be non-responders (a less than 30% reduction in the HAS). Stabilization of growth was reached in these three children, but there was no further reduction of the HAS, so no extra course of GCS was given.

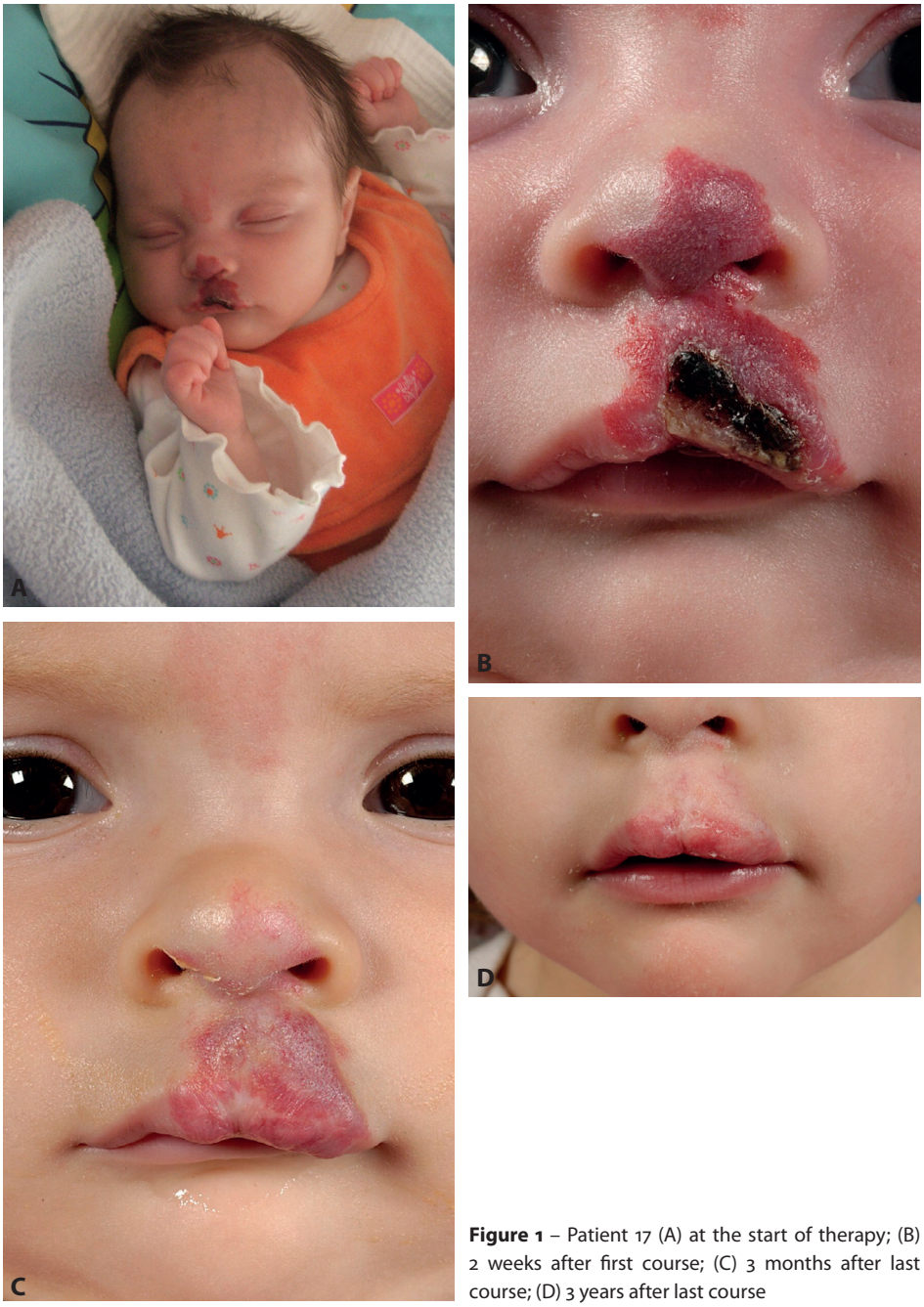

Figure 1 – Patient 17 (A) at the start of therapy; (B) 2 weeks after first course; (C) 3 months after last course; (D) 3 years after last course

Table 4 – Therapy results

Case	Short courses (n)	Age at start of treatment (weeks)	HAS before first course	HAS 2 weeks after first course	Reduction in HAS* (%)	HAS 3 months after glucocortico-steroid therapy	Reduction in HAS* (%)
1	1	10	2.7	2	26	1.3	52
2	1	7	3	1.5	50	1	67
3	1	14	3.3	1.5	55	1.2	64
4	1	9	5	2	60	2	60
5	1	10	3.7	3	19	2.5	32
6	1	6	3.8	2.5	34	2	47
7	1	19	5.5	3	45	2.5	55
8	1	16	3.7	3	19	2.5	32
9	2	5	4	3	25	1.3	68
10	2	7	4.3	3	30	2	53
11	2	14	3.8	3	21	3	21
12	2	11	5.7	4.5	21	2.3	60
13	2	12	2.5	2	20	2	20
14	2	10	4	3	25	2	50
15	3	14	4	2	50	3	25
16	3	6	6.5	2.5	62	2.4	63
17	3	6	5.5	3	45	1.3	76
18	3	12	3	3	0	2	33
19	5	12	4.2	2	52	2.4	43
20	5	7	5.5	2.8	49	2	64
21	6	12	4	1.5	63	2	50

*Reduction in Haemangioma Activity Score (HAS) from before first course to current HAS

Side effects

One or more side effects were noted in 12 (57%) of the 21 children (table 3). Five (24%) of the 21 children had increased appetite during GCS therapy. Eight (38%) of the 21 children developed cushingoid features. Behavioural changes (restlessness) were reported in four children, accompanied by increased crying in three. One child developed acne. This child received five courses of high-dose prednisolone, resulting in a higher cumulative dose than in the other patients. One patient developed a temporary adrenal insufficiency after GCS therapy. This was treated with substitution therapy. Two children developed systolic blood pressure more than 2 SD above the norm for the age on the first day of therapy. The blood pressure had normalized at the next measurement without medical intervention and GCS treatment was continued. More GCS courses resulted in more side effects. Only two of the eight children who received one course developed side effects. Children who received three or more courses all developed side effects. None of the side effects were persistent.

DISCUSSION

In cases of problematic IH in which beta-blocker therapy fails or is contraindicated, GCS therapy is a valuable alternative. In our population, propranolol has a failure rate of less than 10% (based on preliminary data to be confirmed in a prospective study). Contraindications for propranolol are heart disease, pulmonary hypertension, bradycardia, serious internal vascular malformations, bronchiolitis, and bronchospasm. Propranolol may also facilitate hypoglycaemia under distress. There is a greater risk of side effects in premature children.

Intermittent, short course, high-dose GCS systemic therapy was effective in the treatment of troublesome IH. Proliferation of IH was stabilized in all of the patients. There was no correlation between the number of courses and the final therapy result. Good and favourable responses were seen in tumour size and activity in 62% and 24% of our patients, respectively. This is much better than the results in the literature, which reported that only approximately 30% of patients treated with systemic GCSs had a good and persistent response, approximately 40% had stabilization of tumour growth, and 30% were considered to be non-responders[1]. In most studies, GCSs were usually given over a period of 6 to 9 months[14-16]. This long treatment period resulted in a high cumulative dose and a greater risk of adverse effects.

Serious adverse effects accompany prolonged use of systemic GCSs. Behavioural changes (crying, insomnia, irritability), gastric irritation and reflux, cushingoid face with hairiness, and growth suppression have been reported. Growth curves usually normalized after 2 years of age[10]. Hypertension was underestimated or not assessed at all in the literature. Some studies report that hypertension developed more quickly in patients given a higher initial dose of GCS[10,16]. Rare complications of GCSs are hypertrophic cardiomyopathy[19], cataract, infection[20], osteoporosis with protracted GCS treatment, and prolonged adrenal suppression[1]. Infants with growth suppression may be at greater risk of adrenal suppression[21].

In cases of propranolol failure and relative contraindications, we treat children with troublesome haemangiomas with GCSs in an intermittent short course of high-dose oral prednisolone. This results in a substantially lower cumulative dose of GCSs than with the prolonged treatment recommended in the literature[14-16]. WEVAR, a multidisciplinary vascular anomalies team of a paediatric dermatologist (AO), a paediatrician (PL), and a paediatric surgeon (GM) performs diagnosis, therapy, and follow-up.

This study reports the effectiveness and the adverse effects of intermittent, short course, high-dose, systemic therapy. The median cumulative dose was 91 mg/kg, which is substantially lower than the calculated cumulative dose of oral GCS therapy of 3 mg/kg/day during 6 to 9 months of continuous use (approximately 500–750 mg/kg).

In this study we hypothesized that intermittent short-courses of high-dose systemic GCSs would be a good alternative to prolonged GCS treatment for troublesome IH, with fewer side effects expected because of the lower cumulative dose. Furthermore, IH activity may be better determined when GCSs are given in courses. It is not possible to determine whether there is still proliferation when GCSs are administered over a prolonged period, which can lead to unnecessary prolongation of GCS treatment.

Pope et al[17] compared continuous oral GCSs with high-dose monthly intravenous GCSs. Continuous oral GCSs were more effective than monthly intravenous pulses, but with greater risk of adverse effects. In our study with short courses of oral GCSs, we found a response rate comparable to that of continuous GCSs reported in the literature: 80% to 85% regression or cessation of growth[9,17]. We did not compare short courses and prolonged GCS therapy in this study; further comparative studies between our approach and prolonged GCSs would be useful to strengthen our hypothesis.

George et al[16] reported minor side effects (cushingoid face, personality change, hypertension) in more than 70% of their patients treated with GCSs for a prolonged period. No persistent side effects were found in our study with short courses of GCSs. Minor side effects were recorded in 57% of the patients: cushingoid features, behavioural changes, increased appetite, and acne. These side effects disappeared soon after treatment was stopped.

Intermittent short courses of GCSs have several advantages over prolonged GCS therapy. The treatment is limited to the proliferative phase, the total cumulative dose of GCSs is much lower, and side effects of GCSs are less frequent and severe.

Nonetheless, the systemic treatment of IH is undergoing a transformation. Propranolol provides a new therapy option with promising results and few reported side effects. Propranolol has now become the first choice in the treatment of IH. Systemic GCS therapy is an effective alternative choice in case of treatment failure or contraindications to propranolol. Most IHs respond well to propranolol therapy, although propranolol must be given for a longer period than GCSs, in some cases even for several years, and regrowth is still possible after the age of 2 years[22]. The recent study reported by Greenberger et al elucidated the effectiveness of GCSs on angiogenesis and vasculogenesis[12,22]. There-

fore GCS therapy still holds a valuable place as an alternative for beta-blockers in the treatment of IH. In cases in which propranolol or other beta-blockers have failed or are contraindicated, we recommend intermittent short course GCS therapy as the preferred choice instead of long continuous therapy. It is effective and has fewer side effects because of the lower cumulative dose than with prolonged GCS therapy.

The limitations of our study are its retrospective nature and the fact that it is a single-centre study with a limited number of cases. To prove our hypothesis that short course GCS therapy is as effective as prolonged GCS therapy, or to compare short course GCS therapy with propranolol, further comparative studies are necessary, preferably in multiple centres.

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CHAPTER 9

Treatment of small superficial infantile haemangioma with timolol 0.5% ophthalmic solution. A series of 20 cases

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ABSTRACT

Infantile haemangioma (IH) in the face may be disfiguring and alarming for parents. Usually they are not treated when they are small. Treatment of IH with propranolol is a breakthrough. Timolol (topical treatment) and propranolol are closely related.

We considered topical treatment with timolol 0.5% ophthalmic solution 3–4 times daily in patients with small IH. Twenty patients with small mostly superficial IH were included.

A series of 20 patients with IH treated with timolol 0.5% ophthalmic solution are described. The treatment was effective in all superficial IH after 1–4 months. A quick direct inhibitory effect on the growth of the IH followed by slower regression was observed. The children had to be treated during the whole proliferative phase. Deep IH on the nose (two cases) and lower eyelid (one case) showed no response.

Topical timolol 0.5% ophthalmic solution is effective in superficial IH. Safety and effectiveness of drugs like topical timolol and topical propranolol require further investigation but seems very safe when used in small IH. We recommend that small superficial IHs should be treated in an early proliferative phase.

This study shows that topical application of timolol ophthalmic solution 0.5% is an effective treatment for small superficial infantile haemangiomas.
Frequent application more than twice daily, at least three to four times daily, gives a better result.
Because of low resorption the treatment is safe.
This study adds that glucose and blood pressure measuring is not necessary during application of timolol ophthalmic solution 0.5% on small superficial infantile haemangiomas.

INTRODUCTION

Infantile haemangioma (IH) is the most common tumour of infancy. Its incidence is estimated at 10%[1]. To date, it is recommended to wait and observe the spontaneous course of innocent IHs. A multi-discipline diagnostics and treatment is recommended for alarming IHs[2].

Systemic therapy with oral corticosteroids or intralesional therapy with corticosteroids is chosen for life-threating or disabling IH. Propranolol is making corticosteroids and all the other treatments obsolete[3]. It is highly important that propranolol is effective in ulcerating IHs. However, published data on the dose, the duration of therapy and the eventual adverse events are insufficient[3-8]. Alarming IHs are treated using multi-disciplinary approach.

Small IHs were treated until recently with topical steroids, imiquimod cream or by PDL laser because of cosmetic reasons[9-14]. Similar to propranolol, timolol is a non-selective beta-blocker, which is used in ophthalmic solution and eye gel as a drug against increased ocular pressure. The available eye gel contains timolol 0.1% gel and may be used only after the age of 12 years (www.kinderformularium.nl). We use preferably timolol 0.5% ophthalmic solution up to four times daily, but application and frequency of application are off-label. Taking into account the effect of systemically administered propranolol, one may also expect that topical timolol may be effective. Guo and Ni were the first who reported the positive effects of the use of topical timolol in treating capillary infantile IH in a 4-month-old infant[9]. At the World congress of Paediatric Dermatology in Bangkok in 2009, Pope et al reported later a pilot study showing also that topical timolol had a successful effect in the treatment of superficial IH. In the meantime, additional studies have been reported on this aspect[14-17]. Recently, Bonifazi et al reported promising results with topical propranolol in a 1% cream in 23 cases of superficial IH. Good reduction of the IH was achieved in 9 out of the 23 cases[15]. Ni and others published more data on the use of topical timolol for periocular IH[12]. Khunger and Pahwa published positive results in a large flat IH in PHACE syndrome[16].

At present, we treat superficial IH with timolol 0.5% ophthalmic solution. Earlier we also treated children with timolol 0.1% gel 3–4 times daily, but was noted to be less effective in a small try-out study (article in Dutch)[18]. In this article, we report a case series of 20 patients, who were prospectively treated with topical timolol 0.5% ophthalmic solution (3–4 times daily) using a standard protocol.

METHODS

Patients with small IHs who attended the outpatient clinic of the WEVA (working Group on vascular abnormalities – Oranje AP, De Laat PCJ and Madern GC) and the outpatient clinic of the Paediatric Dermatology Division of the Department of Paediatrics during 2010 were treated with timolol 0.5% ophthalmic solution. Off-label use of timolol 0.5% ophthalmic solution was started after oral consent. All the parents were advised to use the drops 4 times based on an early try-out. From earlier pilots we observed that twice daily application shows intermediate reddening of the IH. Treatment was continued during 2–4 months, and advised to use during 6–12 months. Therapeutic effects were evaluated by a Global Score (Excellent, Good, Sufficient, No response), patients/parents Global Score and Haemangioma Activity Score (HAS). The details of HAS have been reported elsewhere[19]. In all patients we tried to check the blood pressure, however because of crying of the child this was often not reliable. Parameters such as glucose were also checked in several patients but is actually theoretically not necessary to measure. This study is a part of the project Strawberry marks ('Aardbeesie'), a study project focussing on diagnosis and therapy of IH, and has been approved by the Medical Ethical Committee of the Erasmus MC Rotterdam.

Patients

During the last year we have treated a series of 20 patients with disfiguring IHs on the face and sometimes elsewhere. Patients were included after oral and written consent for photographic documentation. The age of the patients varied from 1 to 11 months. The characteristics of the children are shown in table 1. Case 4 is described in details. All patients used less than one bottle of 5 ml timolol ophthalmic solution 0.5% per month.

Case 4

The patient was born after a full term pregnancy. The patient visited the out-patient unit of the paediatric dermatology at an age of 2 months and 3 weeks in connection with a plaque on the face under the left eye. The plaque had developed after the birth, whereby the patient has had a very teary eye. The plaque was absent at birth. The patient was further in good health except for intermittent ear infections.

Red, slightly swollen, button-like raised papulous lesions with intense redness were observed at dermatological examination (figure 1). The diagnosis of an IH was established. Topical treatment with timolol 0.5% ophthalmic solution 3 times daily was started. The growth of the lesion stopped after 1 week and the parents were enthusiastic. The patient was re-evaluated after 3 months; the abnormality had disappeared except for a few minimally red points (figure 2). The Haemangioma Activity Score (HAS) was used for the

Table 1 – Characteristics of children with infantile haemangioma treated topically with timolol 0.5% ophthalmic solution 3–4 times[#] daily with a follow-up of at least 3 months

Case	Location	Age	Global score caregiver/ physician	Diameter (mm)	HAS at follow-up (time in months)			
					0	2–4	>5–7	>10
1	el~	2 mths	e/e	5x2	4.5	2	1–0	
2	el	10 mths	g/g	9x6	2.7	1.7	1.5	
3	el	4 mths	g/g	8x4	2.5	1.3	cured	
4	el	3 mths	e/e	3x2	5		1.5	
5	el	1–2 mths	g/e*	20x5	4.5	2	1.5	
6	hd~	9 mths	e/e	10x5	3	1.5	1.3	Alm cured
7	nose	2 mths	s/g *	20x20	4.5	1.5	1.2	&
8	hand	1 mth	n/n	50x60	2	1.3	Stopped	&
9	nose	1 mth	n/n	20x20	1	3	failure, systemic therapy started	
10	el	1 mth	g/g	14x3	5	1.5	1.3	
11	hd	2 mths	g/g	10x5	5	1.5	1.3	
12	hd	6 mths	e/e	30x20	3.6	3	1.5	nearly cured
13	arm	2 mths	g/g	100x50	3	2	1.3	
14	el	3mths	g/g	12x8	2.7	1.3		
15	hand	11mths	g/g	50x70	2	1.5	& (mother preferred 0.1% gel)	
16	el	2mths	g/g	13x5	5	2		
17	el	4mths	e/e	15x4	3.5	1.5		
18	el	2mths	e/e	3x3	3.7	2.3		
19	hd	4mths	e/e	3x6	5	Cured		
20	el	2mths	n/n	10x15	3.3	3.8 (after 3 weeks)	Failure	

[#]Our advice was application 4 times daily but in practice caregivers sometimes forgot to apply one time, therefore we have described 3–4 times.
~el= eyelid; hd=head
*score caregiver/parents different from physicians score
& used first gel with lower concentrations
In cases 1, 3, 5, 6, 10, 12, 13, 14, 16, 17, 18, 19 and 20 (13 out of 20) we could measure the blood pressures and glucose; those measurements were completely normal.
n=no response; s=sufficient response; g=good response; e=excellent response

evaluation of the therapy (table 2)[19]. The HAS decreased from 3.5 (swelling 2, intense redness 5, together 7/2=3.5) to 1.3 (swelling 0, dull red 4, skin coloured after abnormalityo; 4/3=1.3)[10]. The parents were convinced and very happy. The therapy was still continued till the end of the proliferation phase to prevent any re-growth (6–12 months duration).

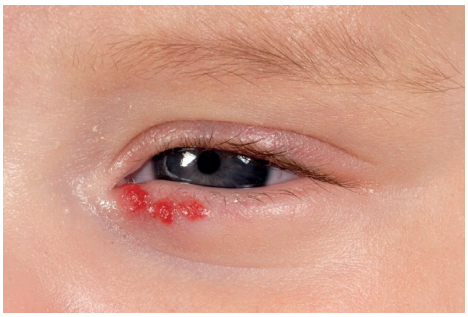

Figure 1 – Infantile haemangioma. Clinical aspect before treatment (patient 4)

Figure 2 – Infantile haemangioma. Effect of timolol 0.5% ophthalmic solution 3–4 times daily on the infantile haemangioma after 3 months (patient 4)

RESULTS

The characteristics of the 20 patients and the results (global score, patient's global score and Haemangioma Activity Score [HAS]) are presented in table 1. All patients were at least evaluated by the main author (APO). Patients were aged 2 to 10 months. IHs were superficial in 8 cases and mixed in 4. Six were superficial and were located on the eyelids, 3 on the nose (all mixed), and 3 on the head (1 superficial and 1 mixed). All the 6 super-

Table 2 – Haemangioma Activity Score (HAS)[#]

Date			
Deep swelling:	Tense IH (6) 'Neutral' IH at t=0 or less than 50% reduction at follow up (4) >= 50% reduction at follow up (2) No more swelling at follow up (0)		
Bright red / shining red IH (5) OR Bright red edge (4)			
Matt red / reddish-purple IH / matt red edge (3)			
Blue IH or blue shining through in deep IH (2)			
Grey IH (1)			
Skin coloured after activity (0)			
Total score			
Number of items scored			
Preliminary HAS = total score / number of items scored			
Ulcer =< 1 cm^2 (+ 0.5) Ulcer 1–25 cm^2 (+ 1) Ulcer >= 25 cm^2 (+ 2)			
HAS = preliminary HAS + ulcer score			

[#]Janmohamed SR, de Waard-van der Spek FB, Madern GC, de Laat PC, Hop WC, Oranje AP. Scoring the proliferative activity of haemangioma of infancy: the Haemangioma Activity Score (HAS). Clin Exp Dermatol 2011 Oct;36(7):715–23.

ficial IHs on the eyelid responded with excellent response in 2 cases and good response in 4 cases. Only 2 of the 3 IHs on the nose responded not well. Insufficient or no response at all was seen in 2 cases and systemic propranolol therapy was started in 1 of them. Superficial IH on the head responded very well, while those with a deep component did not. The global score of the parents differed only in 2 patients from the physician global score (good versus excellent by the physician and sufficient versus good). The final results of the global score were in concordance with the HAS.

DISCUSSION

In the treatment of IH, propranolol as a non-selective beta-blocker provides a rapidly reducing effect on the IH. Although no major adverse events from systemic beta-blocker treatment have been reported, the incidence of potential adverse events such as bronchospasm, hypoglycaemia, heart block, bradycardia and congestive heart failure is partly unknown because of the novelty of the treatment[12]. However, hypoglycaemia only occurs when the patient is fasting[20]. When using topical timolol ophthalmologic solution in such a small amount, no serious systemic side-effects have to be expected. Timolol does not penetrate deeply and can therefore only be used in superficial IH. Besides, the absorption rate is extremely low. All our patients used less than 5 ml timolol solution per month and that is an extremely low amount of this solution. Small IH in the facial area are not strictly medically spoken an indication for treatment, but based on negative psychological impact of visible abnormalities, there is a certain necessity for treatment. Intralesional steroids, Pulse Dye Laser (PDL) treatment, topical steroids, imiquimod 5% cream, and recently topical propranolol hydrochloride or timolol maleate are all possible therapies[9,11]. The PDL has a superficial effect. Psychological drawbacks may be huge in some of these children. Sorrell et al (2010) recommended the use of oral sucrose for pain relief in young infants with IHs treated with intralesional steroids[21]. This has also been used in PDL treatment, because more than one treatment session is required. Timolol maleate 0.5% ophthalmic solution is an agent that is used relatively often in ophthalmology. After the first publication of Ni et al[12], Pope et al reported positive results in a small group of patients at the World congress of Paediatric Dermatology in Bangkok in 2009. Recently, they also published these favourable results[14]. At present, we have also treated a series of patients with a positive inhibitory effect. The treatment can be evaluated properly only after 2–4 months. It is important to apply 3–4 times maybe even more to have a constant effect on the IH. Several parents noted even then for example more redness early in the morning after the night. That can be diminished by more frequently application. This was especially remarkable in patients in which in our pilot study with timolol gel 0.1% were treated twice daily. Propranolol hydrochloride

and timolol maleate are hydrophilic and their absorption through the skin is low. For a systemic effect a transdermal delivery system has been developed for propranolol hydrochloride[22]. Topically applied ophthalmic drugs are a potential cause of allergic contact dermatitis of the periorbital region and the face[23]. Several cases of contact allergy to timolol and related drugs have been described. Contact allergy for propranolol has been reported sporadically[24]. At first sight, this issue is not a contra-indication for their use.

In conclusion, treatment with timolol 0.5% ophthalmic solution at least 3 times daily is worth trying in small IHs. The frequency of administration has not been investigated scientifically, but is based on the observation by the parents and is preferably chosen. Therapy is particularly recommended especially in the early proliferative phase if the IH is superficial.

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CHAPTER 10

Scoring the therapeutic effects of systemic propranolol for infantile haemangioma: a prospective study comparing the Haemangioma Activity Score (HAS) with the Haemangioma Severity Scale (HSS)

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ABSTRACT

Background: propranolol is thought to be a safe and efficacious treatment for infantile haemangioma (IH). Validated and reliable instruments to measure disease severity are needed to substantiate this. To date, only two purpose-made systems have been described: the Haemangioma Activity Score (HAS) and the Haemangioma Severity Scale (HSS).

Objective: comparing the HAS with the HSS in terms of ease of use, accuracy, and outcome in infants treated with systemic propranolol.

Methods: prospective study with 54 infants with IH from October 2009 – December 2012. Systemic propranolol treatment was based on a cautious protocol (propranolol 0.5 mg/kg/day on day one and increased to 2 mg/kg/day at day three). The HAS and the HSS were applied independently by two observers.

Results: intraclass correlation coefficients (ICC) of the HAS and HSS between the observers was comparable but we noted that HSS scores often remain the same upon improvement of the IH and therefore does not really reflect severity. HAS scores decreased over time, with a dramatic drop in the first week reflecting the immediate therapeutic responses.

Conclusion: this study demonstrates that the HAS is to be preferred over the HSS.

Capsule summary

- To date, two good systems are available to measure infantile haemangioma (IH) severity: the HAS (Haemangioma Activity Score) and the HSS (Haemangioma Severity Scale)
- The HAS has some advantages over the HSS
- We recommend to use the HAS for scoring both treated and untreated IH

LIST OF ABBREVIATIONS

HAS	Haemangioma Activity Score
HDCS	Haemangioma Dynamic Complication Scale
HSS	Haemangioma Severity Scale
ICC	Intraclass Correlation Coefficient
IH	Infantile Haemangioma
PHACE syndrome	Syndrome with one or more of the following: Posterior fossa malformations, Haemangioma, Arterial cerebral anomalies, Coarctatio aortae and other cardiac defects, Eye abnormalities
SD	Standard deviation

INTRODUCTION

The beneficial effect on infantile haemangiomas (IHs) of propranolol, a non-selective β-blocker, was serendipitously discovered in France in 2008[1]. Our French colleagues thereupon began treating IHs with propranolol and a follow-up study showed good results[2]. Propranolol soon became the first choice of treatment for its good and fast results[3-5]. It has several side effects, however, including bradycardia and hypotension, but also dyspnea, cold acra, provocation of decompensatio cordis or hypoglycaemia, nightmares, and decreased cardiac output[6]. Although used in IH since 2008 and in paediatric cardiology even longer, a uniform recommendation on dosage, starting up and duration of therapy is not yet available.

There is also an increasing demand for an objective scoring system for evaluation of severity and effect of treatment of IHs. To date, only two systems have been described for this goal in IHs. One is the Haemangioma Activity Score (HAS) developed by our own group in 2011, which is based mainly on the colour of the IH[7]. As known, IHs change from bright red in the proliferative phase to red, and to purple/blue in the involution phase. Before the normal skin colour returns, a greyish discoloration can be seen. Moreover, swelling and ulceration are assessed. The HAS is effective when used prospectively (on a patient) and when used retrospectively (on photographs). It is very objective with only one subjective measurement: estimation whether in deep IH a swelling has shrunk by more or less than half. In 2012, Haggstrom and colleagues[8] developed two other scoring systems. One, the Haemangioma Severity Scale (HSS), measures the overall severity of an IH, using both objective items (size, location, and complications) and subjective items (pain). Also, the risks for associated structural anomalies and disfigurement are included. It follows that this system is less valuable when using it retrospectively on photographs. The second, the Haemangioma Dynamic Complication Scale (HDCS), assigns severity grades to IH complications.

Validated and reliable instruments are needed to measure disease severity given the increasing number of studies of IH. We compared ease of use, accuracy, and outcomes of the HAS and the HSS in our patients treated with propranolol and evaluated the treatment outcomes.

METHODS

This is a prospective study in our first IH-patients treated with systemic propranolol, included from October 2009-December 2012. All patients were evaluated until April 2013. This study is part of the "Aardbeesie" (strawberry) project, which focusses on the patho-

genesis and therapy of IH, and was approved by the Medical Ethics Review Board of the Erasmus Medical Centre in Rotterdam, the Netherlands, and is conducted according to the Declaration of Helsinki principles. All parents gave informed consent for off label use of systemic propranolol in the treatment of IH.

All patients requiring systemic therapy with propranolol for their IHs were included. Reasons for systemic therapy in our first patients were mainly functional impairment or ulceration (not cosmetic). We typically treat patients with actively growing IHs. Exclusion criteria were the following: earlier systemic or intralesional therapy, only internal haemangioma, or start of propranolol therapy elsewhere. Also, patients in which propranolol was contra-indicated (sinus bradycardia, AV-block, hypotension, asthma and decompensatio cordis) or patients with PHACE syndrome (which is not a strict contra-indication anymore, see *Discussion*) were excluded[3] (PHACE syndrome is a syndrome with one or more of the following: Posterior fossa malformations, Haemangioma, Arterial cerebral anomalies, Coarctatio aortae and other cardiac defects, Eye abnormalities[9]).

All patients were treated following a predefined protocol. Because propranolol therapy for this indication had just been started and there was only little experience, we used a very cautious protocol. Therapy was started after thorough (physical) examination by a paediatrician, and the work-up included blood pressure measurements, glucose controls, and ECG (and if necessary consultations from the paediatric cardiologist). All patients started therapy during hospitalization. At day 1, propranolol 0.5 mg/kg/day was given in 3 times with regular blood pressure controls. At day 2, the dose was increased to 1 mg/kg/day and at day 3 it was increased to 2 mg/kg/day. At day 4, patients were discharged if daily ECG, blood pressure measurements, and glucose controls were satisfactory. Patients were regularly seen at our outpatient clinic: 2 weeks, 6–8 weeks, 12 weeks, 0.5 year, 1 year, 1.5 year, 2 years, and 2.5–3 years after baseline. Propranolol was tapered off after approximately 1 year.

Photographs were taken at all visits and these were independently assessed (HAS and HSS) by two observers (SJ and AO). Scoring of the HAS[7] and the HSS[8] is described elsewhere. In addition, global scores of both physician and parents were recorded (1=very good result, 2=good result, 3=stable, 4=deterioration).

Baseline characteristics are reported by descriptive statistics. To show the effectiveness of propranolol we used scatter plots of mean HAS and mean HSS over time and fitted these with local regression (Loess) lines. A sophisticated longitudinal statistical model is not necessary for this relatively simple question. No patients were lost to follow-up. Furthermore we used intraclass correlation coefficients (ICC) per visit interval to determine inter-

observer agreement for the HAS and the HSS. Finally, to assess differences in different types of IHs (superficial, deep or mixed), we made separate scatter plots. Ultimately, to assess the safety and effectiveness of propranolol, we used descriptive statistics to show the side effects and the numbers of times that restarting propranolol was necessary.

RESULTS

A total of 68 patients were eligible for inclusion. Fourteen of these patients were excluded for the following reasons: six received systemic or intralesional corticosteroid therapy prior to propranolol therapy, five patients had only internal haemangioma, and propranolol therapy had been started elsewhere in two patients, and in one patient therapy was eventually not started. Thus, data of 54 patients were included in the analysis.

Of these 54 patients, 80% were female and 19% were born prematurely (<37 weeks). Mean birth weight was 3032 grams (SD 830 grams). The median number of visits per

Table 1 – Baseline characteristics of 54 patients who received systemic therapy with propranolol for infantile haemangioma (IH)

Characteristic	Number
Gender	Female: 80% Male: 20%
Gestational age	19% born prematurely (<37 weeks) 81% term born
Mean birth weight	3032 grams (SD 830 grams)
Median # of visits per patient (including baseline)	6 (range 2 – 18, 25th percentile = 5; 75th percentile = 9)
Median length of follow up	50 weeks (range 4 – 152, 25th percentile = 28; 75th percentile = 92)
Mean age at start therapy	17 weeks (SD 7 weeks)
Type of IH	Deep: 31% Superficial: 4% Mixed: 65%
Location of IH	Periorbital: 24% Face (excluding periorbital): 37% Genital area: 13% Rest of the body: 26%
Reason for therapy	Eye involvement: 20% Ulceration: 41% Fast growth/other: 39%
Mean size of IH	3.9 cm (SD 2.2 cm)
Precursor lesion	YES: 23% NO: 77%

SD = standard deviation

patient was 6, including baseline (range 2–18, 25th percentile=5; 75th percentile=9). Median duration of follow up was 50 weeks (range 4–152, 25th percentile=28; 75th percentile=92). Mean age at start therapy was 17 weeks (SD 7 weeks). Thirty-one per cent of IH were deep, 4% were superficial and 65% were mixed. IH was located: periorbital (24%), in the face (excluding periorbital, 37%), in the genital area (13%), and on the rest of the body (26%). Reasons for therapy were: eye-involvement (20%), ulceration (41%), fast growth/other (39%). Mean size of IH at baseline was 3.9cm (SD 2.2cm). Twenty-three per cent of patients had a precursor lesion prior to IH development (table 1).

Figures 1 and 2 show the evolutions of the mean HAS scores of two observers and mean HSS scores of two observers respectively, with regards to time from baseline. The HAS scores clearly show a decreasing trend with a dramatic drop after initiation of treatment. This pattern is less pronounced for the HSS scores. Figures 3 and 4 show the global scores from the physician and the parents, respectively. Both figures show a good result after initiation of treatment, which gradually changes to "stable" over time. Furthermore, figures 5 and 6 show the evolutions of the mean HAS scores of deep IH and mixed IH, respectively. It is clear that deep IH tend to respond better than do mixed IH. The graph for the subgroup of patients with only superficial IH is not shown as the sample size is too small.

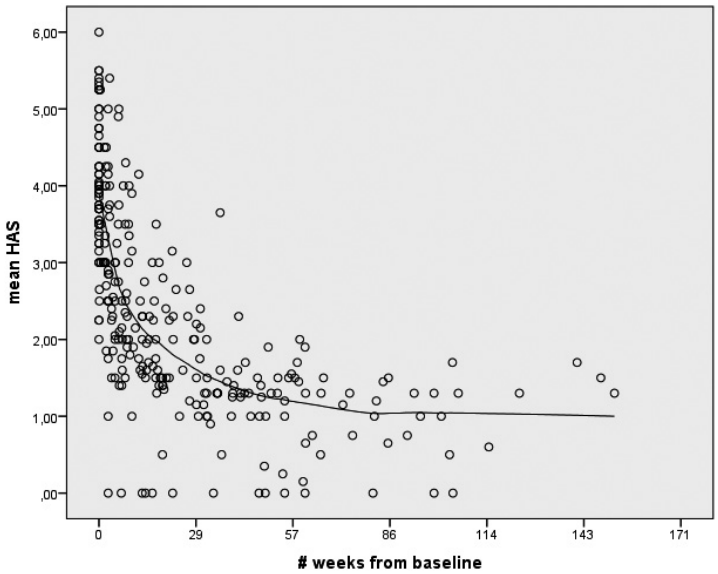

Figure 1 – The evolution of the mean Haemangioma Activity Score (HAS) of two observers with regards to time from baseline in patients with infantile haemangioma treated with systemic propranolol. There is a clear improvement of the HAS, especially after initiation of treatment, showing the quick responses of propranolol therapy.

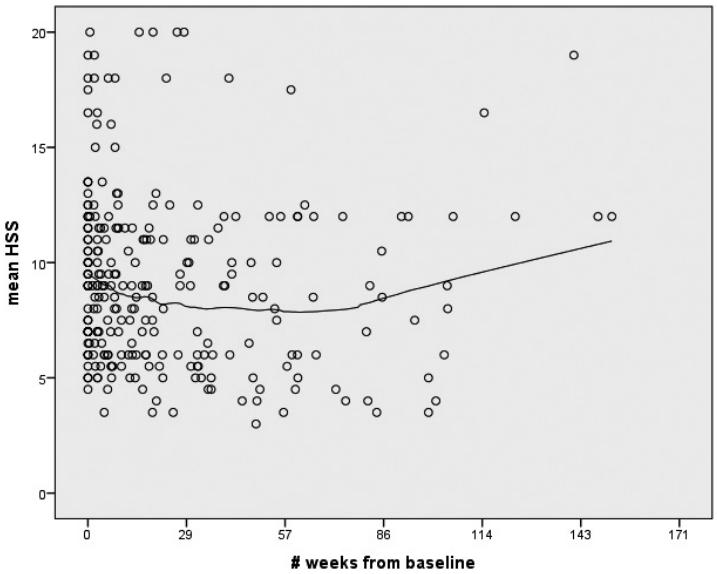

Figure 2 – The evolution of the mean Haemangioma Severity Scale (HSS) of two observers with regards to time from baseline in patients with infantile haemangioma treated with systemic propranolol. In contrast with figure 1 the score changes less clearly.

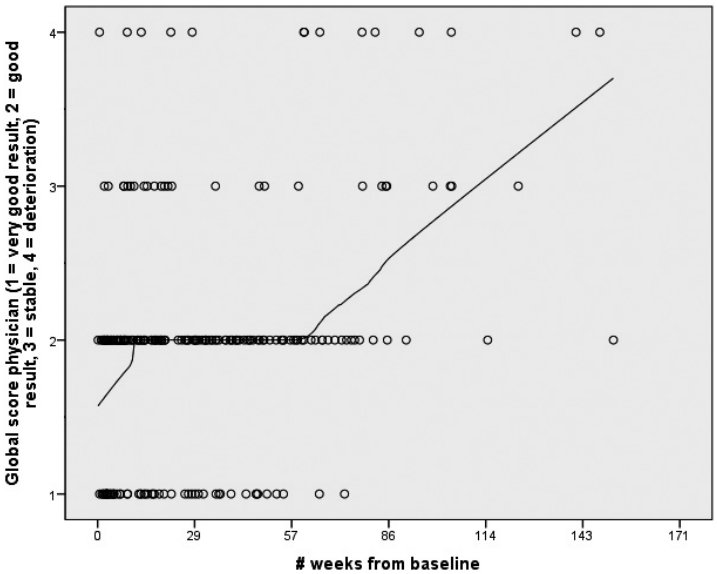

Figure 3 – The evolution of the Global scores (physician) with regards to time from baseline in patients with infantile haemangioma treated with systemic propranolol. In the beginning there is a good result (score 2), which gradually changes to a "stable" situation (score 3).

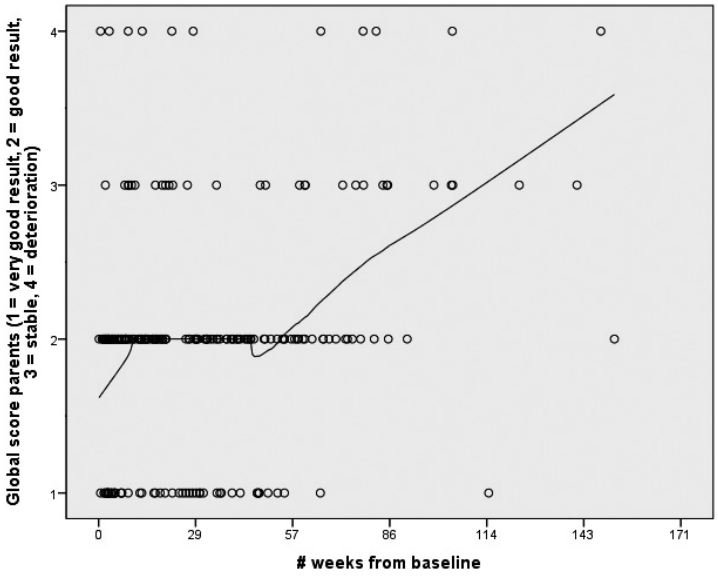

Figure 4 – The evolution of the Global scores (parents) with regards to time from baseline in patients with infantile haemangioma treated with systemic propranolol. In the beginning there is a good result (score 2), which gradually changes to a "stable" situation (score 3).

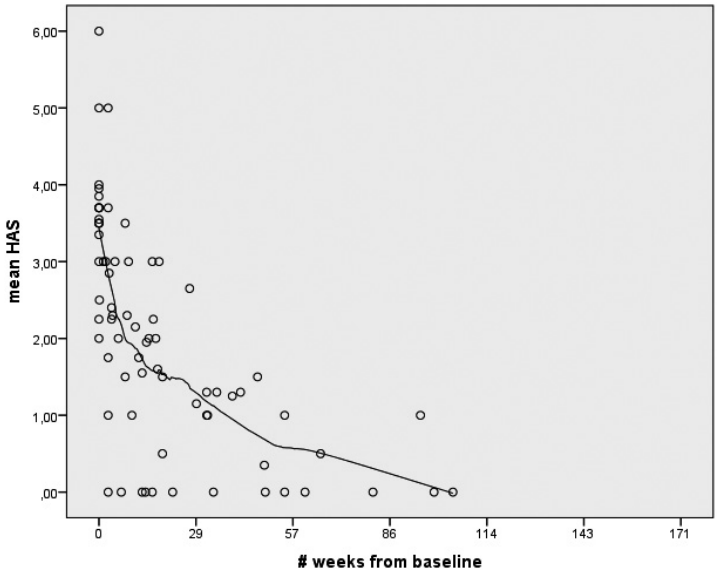

Figure 5 – The evolution of the mean Haemangioma Activity Score (HAS) of two observers with regards to time from baseline in patients with *deep* infantile haemangioma treated with systemic propranolol. Clearly there is a good result (HAS score 0).

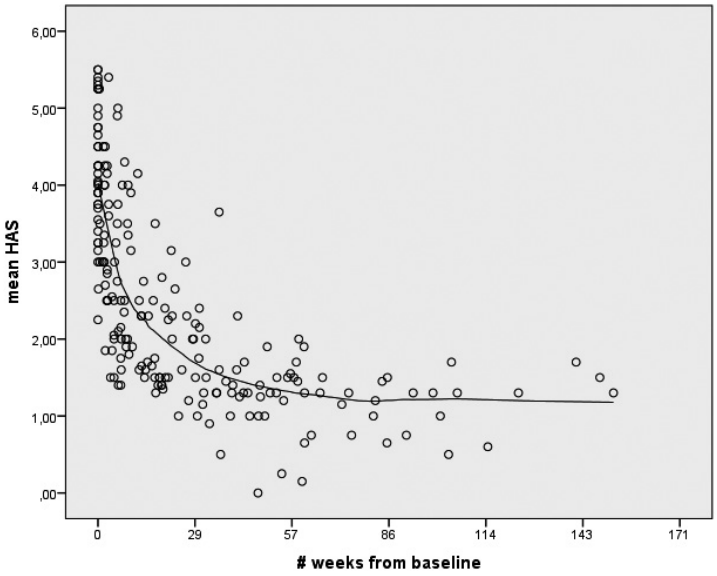

Figure 6 – The evolution of the mean Haemangioma Activity Score (HAS) of two observers with regards to time from baseline in patients with *mixed* (deep and superficial) infantile haemangioma treated with systemic propranolol. There is a good result but in contrast to figure 5 there is no complete resolution.

Mean ICCs of the HAS and the HSS per time interval were comparable (around 0.5) and are therefore not given in more detail.

Side effects were seen in 10 cases: 5x nightmares or sleeping disturbances, 4x cold extremities, and 1 patient showed hypoglycaemia (see *Discussion*, this was due to diminished intake). Other problems with glucose monitoring and problems with blood pressure measurements or ECG were not noticed (table 2).

Propranolol was stopped in 17 patients (after 1 year therapy). Propranolol was restarted after relapse of the IH in three of these patients: in one of these patients once, in another twice (with success), but the third case did not yield the expected result; propranolol failed after initial good response: there was growth of a subglottic haemangioma resulting in

Table 2 – Observed number of side effects of systemic propranolol therapy in 54 children with infantile haemangiomas

Side effect	Number of times observed
Nightmares / sleeping disturbances	5
Cold extremities	4
Hypoglycemia*	1

*Actually due to decreased intake and not as a direct result of propranolol therapy

stridor and breathing difficulties. This patient, who was prematurely born, was admitted to the IC-unit and prednisone and vincristine were complementary given with good result.

DISCUSSION

This study describes the treatment of IH with systemic propranolol. Patients demonstrated positive responses and side effects seemed mild. Treatment failed in only 1 patient.

Furthermore, two different disease severity scales for IH were compared; namely the HAS and the HSS. Although ICCs of the HAS and HSS were comparable, we found the HAS easier to use. Also the scatterplots in figures 1 and 2 show that the HAS reflects improvement of IH (proven by the global scores) whereas the HSS does not. These observations suggest that the HAS is superior to the HSS in monitoring IH-treatment.

A possible limitation of this study is the fact that the authors have more experience in using the HAS. Nevertheless, we applied the HSS conscientiously as intended. On the other hand, this is a relatively long (median 50 weeks) and large (54 patients, median 6 visits per patient) study.

Over the years several scoring systems have been described. Often global assessments are used, but these are very subjective. Some researchers have used an adapted visual analogue scale, but this is originally intended for scoring pain[10]. The first described scoring system is from Enjolras and Mulliken in their book on vascular malformations. This system was designed to score the severity and seriousness of *all* vascular malformations, and therefore is too extensive and complicated. In 2006, Tsang and colleagues provided a formula to estimate the volume of IHs[11]. However, often the part of the IH on the skin is not the same as the part under the skin. Therefore measuring is not always an option and ultrasonography in every patient with IH is expensive. A recent pilot study described the technique of three-dimensional stereo photogrammetry for volumetric measurements[12]. In 2011 the HAS was developed[7] with only one subjective item to make it an all-round system and in 2012 the HSS[8] was developed, consisting of both objective and subjective items. These authors also proposed another system, the HDCS, which assigns severity grades to IH complications. Since this is a totally different indication, we did not use this scale.

There are two main advantages of the HAS. First, it can be used both prospectively (on patients) and retrospectively (on photographs). The HSS cannot be used retrospectively

because information needed for scoring is missing. Furthermore, we noted that while HAS scores often decreased (as one would expect as a result of propranolol therapy) the HSS scores often remained the same (one of the items is location, which does not change) and therefore does not really indicate the severity. The HSS is little discriminative and the score often remains the same, whereas the HAS scores reflect the quick response to propranolol therapy shortly after initiation.

The good results of systemic propranolol therapy for IHs are in line with the literature[2,3,13-15]. We stratified for IH type but unfortunately we had only two superficial IHs. However, when comparing deep IHs with mixed IHs, deep IH tended to respond better than mixed IH. An explanation could be that in mixed IHs the superficial part can cause prolonged visibility.

Side effects were seen in 10 cases and were generally mild: 5x nightmares or sleeping disturbances, and 4x cold extremities, which are known side effects. One patient developed hypoglycaemia, which is often thought to be a result of propranolol therapy. In fact, hypoglycaemia in patients with propranolol only occurs in infants with diminished caloric intake due to intercurrent disease (diminished gluconeogenesis)[16]. This held also true in our case.

In 7 of the 17 cases in which propranolol was stopped after 1 year therapy the lesion became a little bit more red/active. However, most lesions were already in the involution phase and in some cases topical therapy (timolol ophthalmic solution 0.5% or propranolol cream 1%) was given. This was also noticed by parents who stopped for 2 weeks because they had run out of propranolol. Nonetheless, in 3 cases systemic propranolol was restarted.

In our first patients we used a very cautious treatment protocol because of lack of experience. Nowadays we follow the less cautious guidelines of Léauté-Labrèze and colleagues[13]. These have not been published yet but preliminary results have been presented at several congresses, indicating that propranolol therapy with up to 3 mg/kg/day is superior with no extra side effects (no hospitalization needed). In most patients, therapy can then be stopped after six months. In the beginning it was also advised not to use propranolol in PHACE syndrome[3]. However, later on it was used in patients with mild cerebral abnormalities[17,18].

Little is known about the mechanism of action of propranolol in IH. Storch and Hoeger presented an overview of how propranolol interferes with endothelial cells, vascular tone, angiogenesis and apoptosis. They distinguished three stages, with immediate

(early), intermediate, and long-term effects. These effects of propranolol on IH can be attributed to three different pharmacological targets. Early effects are attributable to vasoconstriction due to decreased release of nitric oxide. Intermediate effects are due to the blocking of pro-angiogenic signals (among others vascular endothelial growth factor) and result in growth arrest. Long-term effects of propranolol are characterized by induction of apoptosis in proliferating endothelial cells, which results in tumour regression[19]. In fact, we indeed showed a quick effect in the beginning of therapy as illustrated by the HAS scores. Long-term effects of early and prolonged β-blocker therapy in infants have to be investigated.

This study has demonstrated that the HAS has significant advantages over the HSS, for example when scoring photographs (retrospectively). Perhaps other options will be available in the future such as computer software that calculates the size and 'values' of the various colours in the IH (just like a greyscale) from a digital photograph with pre-set defaults, offering the opportunity to assessing improvement or deterioration from serial photographs. For the future, we recommend big international studies evaluating different scoring systems for IH that would enable to reach consensus on a single system.

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Conflict of interest

SJ and AO have developed the Haemangioma Activity Score (HAS).

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PART IV

Discussion and summary

CHAPTER 11

General discussion

LIST OF ABBREVIATIONS

$BNIP_3$	BCL_2/adenovirus E_1B kD-interacting protein (BNIP) family member 3
CA-IX	Carbon Anhydrase IX
GLUT-1	Glucose Transporter 1
HAS	Haemangioma activity scale
HemSCs	Haemangioma derived stem cells
HemECs	Haemangioma derived endothelial cells
$HIF_{1\alpha}$	Hypoxia inducible factor 1α
$HIF_{2\alpha}$	Hypoxia inducible factor 2α
HSS	Haemangioma severity scale
IDO	Indoleamine 2,3 dioxygenase
IGF	Insuline-like growth factor
IH	Infantile haemangioma
IHs	Infantile haemangiomas
mTOR	Mammalian target of rapamycin
$mTORC_1$	Mammalian target of rapamycin complex 1
pAKT	Phosphorylated protein kinase B
pS6	Phosphorylated S6 protein
VEGF	Vascular endothelial growth factor

GENERAL DISCUSSION

Vascular anomalies are subdivided into vascular malformations and vascular tumours[1]. The latter group includes the infantile haemangiomas. Infantile haemangioma (IH) is considered to be a tumour of the skin; benign due to its typical course with eventual regression. IH occurs in up to 10% of children in the western population and therefore is the most frequent childhood tumour. Typical for IH is its development shortly after birth in most cases and its exponential growth, followed by regression starting after 9 to 12 months and which can last for up to 7–10 years[2].

The first important aspect to consider is the use of correct terminology. For a long time, and even today, many clinicians have referred to different vascular anomalies as being 'haemangioma'. Therefore, when speaking of these typical strawberry-like tumours – which mostly develop after birth, grow exponentially and thereafter go into spontaneous regression – one should use the term 'infantile haemangioma'[3].

Part I: Pathogenesis

Despite all research efforts in this field, the pathogenesis is still not fully understood. It is very well possible that multiple mechanisms play a role in the pathogenesis, however, hypoxia seems crucial[4]. After all, clinically, in approximately half of the cases an IH is preceded by an anaemic macula, or a so-called precursor lesion, which is a sign of local ischemia. Laboratory findings in earlier research indicate stabilization and an increased transcription activity of HIF1α, leading to up-regulation of its downstream target genes (such as VEGF), which normally occurs in cases of hypoxia[5]. Three main hypotheses have been proposed in IH pathogenesis, namely 1) the theory of tissue hypoxia, 2) the theory of embolization of placental endothelial cells, and 3) the theory of increased angiogenic and vasculogenic activity. These hypotheses are, however, not mutually exclusive[6]. An hypoxic environment triggers stabilization at the protein level of the transcription factor HIF1α. HIF1α in turn stimulates transcription of downstream target genes such as BNIP3, CA-IX, GLUT-1, pAKT, pS6, and VEGF. These target genes might be regulated either directly by HIF1α signalling or by hypoxia-induced regulation of mTORC1 signalling. mTORC1 is a key player in the mTOR-pathway, a protein complex with a central role in regulating cellular metabolism, driven by growth factors, nutrients as well as hypoxia. Deregulation of the mTOR-pathway may lead to disorganized growth[5,7-10]. Placental embolization is thought to play a causative role. Chorionic villus sampling has been associated with an increased incidence of IHs. GLUT-1 is strongly expressed in IHs but not in other vascular malformations; GLUT-1 is also expressed in the placenta. Furthermore, IHs and the placenta also express other molecular markers such as merosin, laminin, Lewis Y antigen, FcγR2, IDO, and IGF-2. Therefore it has been hypothesized that IH precursor cells originate

from the placenta, although subsequent molecular genetic investigations revealed no evidence for maternal-foetal microchimerism[6,11-13]. Both vasculogenesis and angiogenesis have been proposed as mechanisms contributing to the neovascularization in IH. Vasculogenesis is the de novo formation of blood vessels from stem cells. It was long believed that this occurs in foetal life only. Angiogenesis on the other hand is the growth of new blood vessels from pre-existing vessels, which includes migration of endothelial cells[4]. Evidence was found that mesenchymal cells, isolated from proliferative IHs, are capable of differentiating into endothelial cells, pericytes (perivascular cells), and adipogenic lineages. When implanted into immune-deficient mice, these haemangioma stem cells (HemSCs) formed GLUT-1 positive vessels (vasculogenesis) and eventually an IH. This did not work with HemECs. Because of juxtacrine signalling between HemECs and HemSCs via JAGGED1 signalling through the NOTCH pathway, HemSCs differentiate into pericytes. There are many pericytes in the proliferating phase and they appear to undergo a maturation process concurrently with the endothelial cells. Recently it was found that pericytes in IH are pro-angiogenic[4,14,15].

In our research we investigated the role of hypoxia in the pathogenesis of IH. Indeed we found elevated levels of GLUT-1 (not surprisingly), VEGF, and BNIP3, which are part of the HIF pathway, but also elevated levels of pAKT and pS6, which are part of the mTOR pathway. The latter finding implies that Rapamycin (sirolimus) could be a therapeutic option[16,17]. This was completely in line with previous research. However, we did not find up regulation of HIF1α itself, for several possible reasons. First of all, most analysed IHs were in regression. Furthermore, in case of activation of HIF1α, rather than increasing, expression shifts from cytoplasm to nucleus and it is uncertain how other authors have dealt with this. It may also be possible that transcriptional activity was increased, resulting in a hypoxia response without a change in HIF1α levels. Finally, we also note that negative staining is consistent with the previously published variable expression; expression of target genes can occur via HIF2α only. We are currently involved in prospective research dealing with the same question but also focusing on HIF2α. These results are of course interesting, but unfortunately this research lies beyond the publication of this thesis.

Part II: Evaluation

The number of studies comparing different therapies in the treatment of IH is increasing. Other than a global score and an adapted form of the visual analogue scale, which obviously are subjective or less useful, a validated, reliable, and objective scoring system for haemangioma activity was lacking. We proposed a scoring system based on the colour(s) of the IH: the haemangioma activity score (HAS)[18]. Our intention was to keep it as short as possible, as objective as possible, and useful both retrospectively (on photographs) and prospectively (on the patient). We believe that we have succeeded and in all clinical

studies in this thesis we have used the HAS. Unfortunately for us, but good for science, another system was proposed by the group of Ilona Frieden, called the haemangioma severity scale (HSS)[19].

We feel that this scale has some important disadvantages: it takes longer to complete, has subjective items and cannot be used retrospectively. In one of our studies which we will discuss in part III, i.e. treatment with systemical propranolol, we have compared the HAS with the HSS and concluded that the HSS is not as sophisticated as the HAS, especially after start of treatment and upon improvement. Big international studies are needed in order to validate the HAS system further. The HAS scoring form can be found in the *appendix*.

Part of the evaluation of IH is the management of patients with multiple haemangiomas. During a recent haemangioma expert meeting in France (European Expert group on Infantile Haemangioma, Entretien du CARLA), IHs were divided into focal, multiple, miliary, and segmental. We showed that in patients with multiple IHs (in our group up to and including 10 IHs), further screening for internal haemangiomas is not necessary. In miliary IHs ('haemangiomatosis'), it is recommended to screen for internal haemangiomas. However, our sample size was limited, therefore more research is necessary, with more patients, to definitively preclude the group of multiple IHs from screening for internal haemangiomas. Another question is whether this is only true for liver/abdominal haemangiomas or for all internal haemangiomas, for example haemangiomas in the brain.

Part III: Therapy

Although IH is benign and self-limiting, severe complications can arise due to localization and fast tumour growth[20-22]. In these cases therapy is necessary. Therapy of IH has changed considerably over the last years. Both the old-fashioned and the more recent therapy options are summarized and commented on in the *introduction*. IH often needs no treatment but the 'watch and wait' principle is followed less often nowadays, especially after the discovery in 2008[23] that β-blockers can also play a role in the treatment of IHs, both orally and topically in superficial IHs. On the other hand, IHs associated with complications or IHs without complications but with great risk of developing complications but also ulcerated IHs or IHs giving serious cosmetic concerns, obviously require immediate systemic treatment. For non-alarming IHs the "watch-and-wait" strategy is still advocated. Nevertheless, small (superficial) IH in the face may be treated topically with topical β-blockers.

Systemic options used to be corticosteroids, with low success rates and important complications, and vincristine IV, which also can give rise to side effects. Currently proprano-

lol is advocated in a dosage of 3 mg/kg daily for 6 months (Léauté-Labrèze et al, NEJM, submitted). Propranolol occasionally causes side effects like nightmares and cold acra, but also more serious side effects like hypoglycaemia and hypotension are reported. It is good to realize, however, that hypotension normally does not occur in healthy infants because β-blockers only work when the blood pressure is elevated[24]. Regarding hypoglycaemia, this is never a direct effect of propranolol, but rather the combined effect of propranolol and little nutritional intake, for example because of vomiting or fever. This can also occur with combinations of particular medicines, such as propranolol and corticosteroids. When analysing all cases described, you will always find another underlying situation[25]. As for topical treatment, the best current options are timolol 0.5% ophthalmic solution, and propranolol 1% cream, applied 3–4 times daily[26,27]. We would like to stress that the effect of topical treatment with these β-blockers can only be seen after 2–4 months (with documented photographs) and that it is advised to apply the medicine 3–4 times daily. Systemic side effects are not to be expected given the small surface and the limited resorption through the skin, as discussed at several congresses by Dr. Lisa Weibel (data not published, abstract for the 11th Congress of the European Society for Paediatric Dermatology (ESPD), Istanbul, Turkey, 2012). Of course, this is the reason why this treatment only works in superficial IHs. As known, propranolol is contra-indicated in some children, for example children with bronchial hyper reactivity. It has been postulated that atenolol, a selective β-blocker, might work as well as propranolol. This has to be investigated in more detail because it is known that atenolol does not

Table 1 – Some common* *topical* therapy options in the treatment of infantile haemangiomas (IH) with their advantages and disadvantages

Therapy	Comments	Advantages	Disadvantages
β-blockers (timolol ophthalmic solution 0.5%, propranolol cream 1%)	For small (superficial) lesions	Local treatment, side effects are not expected	For superficial lesions only, not possible in deep IHs
Corticosteroids (Intralesional injection with a solution of 3 ml Celestone 4 mg/ml and 2 ml Kenacort-A 40 mg/ml)	Good results in periocular IHs. Inject a maximum of 5 ml of this solution	No systemic side effects	Narcosis and hospitalization (day treatment)
Imiquimod (cream 50 mg/g)		Works good, also in slightly deeper IHs	Gives an eczema reaction around and on the IH
Becaplermine (gel 0.01%)	Is a recombinant platelet-derived growth factor		Experimental
Laser therapy (pulsed dye)	Favourable results are described		Only possible in very superficial IHs. At least 3 sessions are required

*Options such as surgery, radiation and sirolimus are not mentioned because of their risk of side effects and higher costs

Table 2 – Some common* *systemic* therapy options in the treatment of infantile haemangiomas (IH) with their advantages and disadvantages

Therapy	Comments	Advantages	Disadvantages
Propranolol per os	At start therapy: global paediatric physical examination, ECG, and during therapy blood pressure and glucose. Day treatment	It seems to work good It seems safe	Is still being investigated. Hypoglycaemia? Be careful in PHACES** syndrome Rebound of IH growth when stopped too early Long lasting therapy (>1 year?) Day treatment
Corticosteroids per os		Safe if used properly (in pulses)	Systemic side effects (↓ cortisol, Cushing) Start therapy during hospital stay (3 days)
Vincristine intravenous	In case of therapy resistance		Intravenous, 6 dosages (or more) Several side effects; most frequent: obstipation

*Options such as interferon and sirolimus are not mentioned because of their risk of side effects and higher costs

**PHACES is a syndrome with one of more of: Posterior fossa (Dandy Walker) malformations, Haemangioma, Arterial cerebral anomalies, coarctatio aortae and other Cardial abnormalities, Eye abnormalities and abnormalities of the Sternum.

Table 3 – Management of patients with infantile haemangiomas

Non-alarming	Treatment by general practitioner or interested paediatrician (watch and wait policy)
Cosmetic problems and/or possibly alarming	Refer to (paediatric) dermatologist For example: superficial IH in the face or precursor lesion
Alarming	Refer to specialized (paediatric) dermatologist or multidisciplinary team

pass the blood-brain barrier and therefore will not give a central nervous system effect to reduce sympathetic activity. On the other hand, given the fact that β-blockers have an effect on IH growth, it would be interesting to know whether α-agonists, which also give peripheral vasoconstriction, also work in topical IH therapy. Research in this field is thriving and trials comparing different concentrations of propranolol and timolol could be helpful. Tables 1 and 2 give a summary of topical (table 1) and systemic (table 2) options in the treatment of IHs. Table 3 shows how to manage patients with different IHs.

Concluding remarks

- Three main hypotheses have been proposed in IH pathogenesis, namely 1) the theory of tissue hypoxia, 2) the theory of embolization of placental endothelial cells, and 3) the theory of increased angiogenic and vasculogenic activity. These hypotheses are, however, not mutually exclusive.
- Hypoxia seems crucial in the pathogenesis of IH.

- The HAS is a valuable system for scoring the activity of IHs.
- Different topical and systemic therapy options for patients with IHs are summarized in tables 1 and 2.
- Propranolol is the first choice for patients who need systemic treatment.
- For topical use, timolol 0.5%, and propranolol 1% or comparable products are the most elegant solutions.

Furthermore this thesis has shown that we are not finished with our research. There are still many questions to be answered, and with research from all over the world we will understand the pathogenesis even better. Hopefully one day, with the understanding of the pathogenesis, we can develop a more definitive therapy for patients with IHs.

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CHAPTER 12

Summary

INFANTILE HAEMANGIOMA: PATHOGENESIS, EVALUATION, AND THERAPY

Vascular anomalies are subdivided into vascular malformations and vascular tumours. Infantile haemangioma (IH) is considered to be a vascular tumour, occurring in up to 10% of children. Typical for IH is its development shortly after birth in most cases and its exponential growth, followed by regression which starts after 9 to 12 months and can last for up to 7–10 years.

The *introduction* includes an update of the current knowledge with regard to the pathogenesis and the therapy of IH. In **chapter 1**, which addresses the pathogenesis of IH, we discuss the possibility that several mechanisms act together. Clinically, in about half of the cases, an IH is preceded by an anaemic macula (local ischemia) or a so-called precursor lesion. Laboratory findings indicate stabilization and an increased transcription activity of hypoxia inducible factor 1α (HIF1α), leading to up-regulation of its downstream target genes (such as VEGF, vascular endothelial growth factor), which normally occurs in cases of hypoxia. Three main hypotheses have been proposed, namely 1) the theory of tissue hypoxia, 2) the theory of embolization of placental endothelial cells, and 3) the theory of increased angiogenic and vasculogenic activity. Tissue hypoxia seems to be crucial. Although IH is benign and self-limiting, severe complications can arise due to localization and fast tumour growth. Also, IHs leave scars after regression in more than half of the cases. Management and therapy of IH have changed greatly after 2008. **Chapter 2** provides an overview of the older therapy options from before 2008, which mainly consisted of the administration of corticosteroids, and discusses the new therapy options such as β-blockers (both systemically and topically). Corticosteroids were not always effective and could give many side effects. β-blockers are promising and are currently preferred above corticosteroids, but β-blockers still do not provide definitive treatment. **Chapter 3** provides the aims of this thesis.

Part I covers the pathogenesis of IH and in **chapter 4** we report on a retrospective study investigating the tissue hypoxia theory described in chapter 1. The pathogenesis of IH is unclear. Several mechanisms have been proposed, including hypoxia, which triggers up regulation and stabilization of hypoxia-inducible factor 1α (HIF1α). HIF1α stimulates downstream transcription of target genes that enhance angiogenesis. We aimed to identify possible involvement of hypoxia in the pathogenesis of IH, as hypoxia signalling constitutes a potential therapeutic target. IH tissue samples collected from 1991–2011 (preserved in paraffin) were immunohistochemically analysed for HIF1α and the following targets: BNIP3 (BCL2/adenovirus E1B kD-interacting protein (BNIP) family member 3), CA-IX (Carbon Anhydrase IX), GLUT-1 (Glucose Transporter 1), pAKT (Phosphorylated protein kinase B), pS6 (Phosphorylated S6 protein), and VEGF (Vascular

Endothelial Growth Factor). Four observers independently assessed findings. Two of the 10 IH samples appeared to be in the growth phase. GLUT-1, BNIP3, pAKT, and VEGF were positive in all samples. pS6 was positive in nine cases and negative in one. CA-IX was weakly positive and HIF1α was negative in all cases. Our data suggest that hypoxia might indeed be involved in the development of IH and that factors up regulated by HIF1α can be influenced.

Part II covers the evaluation of IH. The number of studies comparing different therapies in the treatment of IH is increasing. Other than a global score and an adapted form of the visual analogue scale, a validated, reliable, and objective scoring system for haemangioma activity was lacking. In **chapter 5** we report on the development of an easy system called the Haemangioma Activity Score (HAS) for scoring the disease (proliferative) activity of IH. We validated the HAS system in a comparative study of photographs taken during consultations from 2000 until 2008 (n=78). Agreement between three observers was assessed at two different time points with a minimum interval of six months (t=0 and at t=1) using the interclass correlation coefficients (ICC). Agreement between observers was good. The average ICC of HAS at t=0 and t=1 was 0.72 and 0.76, respectively. The average ICC of HAS regarding the changes from baseline (HAS at t=0 minus HAS at t=1) was 0.69. We concluded that the HAS is a good system for scoring the (disease) proliferative activity of haemangiomas and that it can be useful in future investigations, for example comparing different therapies for treating haemangiomas. The necessity of screening for internal haemangioma in patients with multiple IH is addressed in **chapter 6**. Screening for internal haemangioma is recommended when five or more multiple cutaneous IH are present). This recommendation, however, is lacking solid scientific evidence. Since 1993, children with IH have been evaluated in outpatient consultations by the working group on vascular anomalies Rotterdam (WEVAR), according to a protocolled approach. Off all these patients (1993–2011) we selected all patients with five or more IH. These patients had all been referred for further examination with ultrasound for internal haemangioma. We distinguished between two groups. Group 1 (*haemangiomatosis*) consisted of 27 children with >= 10 IHs and group 2 (*multiple IH*) consisted of 16 children with 5 up to and including 9 IHs. Nine infants in group 1 showed internal haemangioma, whereas none in group 2 showed internal haemangioma. Further examination for internal haemangioma in children with fewer than 10 IHs is controversial and does not seem to be necessary. However, we do recommend further examination (using ultrasound of the abdomen) for children with 10 or more IHs.

In *part III* we evaluate some therapy options. **Chapter 7** starts with the evaluation of intralesional corticosteroids. Periocular IHs are highly sensitive to corticosteroids. Our goal was to evaluate the effectiveness of intralesional corticosteroids in the treatment

of periocular IH at our hospital from 1993 until 2009. We selected all 34 patients with periocular IHs who had been treated with intralesional corticosteroids only. There were no complications at all after therapy. A second intralesional injection was necessary in 5 patients. At follow-up after 6 and 12 months after injection, 94% and 91% of the patients, respectively had regression of the IH. Astigmatism, HAS, and global assessments all had improved after therapy. This study shows that intralesional therapy with corticosteroids is a good and safe alternative in the treatment of periocular IHs besides propranolol or when propranolol therapy is not possible (e.g. asthma, PHACE syndrome, and certain cardiac diseases). **Chapter 8** evaluates therapy with systemic corticosteroids. IHs are increasingly being treated with propranolol or other β-blockers, but before this therapeutic option was available, oral glucocorticosteroids (GCSs) were the criterion standard treatment and are still the alternative modality in problematic cases. Nevertheless, there is no standard treatment protocol for the dose and duration of GCSs. Long-term treatment with GCSs is associated with unwanted side effects such as growth suppression, behavioural changes, and reflux. Twenty-one children with troublesome IHs were treated according to an algorithm with 3 mg/kg/day of oral prednisolone divided three times per day with varying duration and number of GCS courses. Two blinded investigators independently interpreted therapy results using the HAS. Side effects were determined according to reports in patient charts and parental questionnaires. The median duration of a short course of GCSs was 2 weeks (range 1–6 weeks). The number of courses was 2 (range 1–5). The median cumulative dose was 91 mg/kg. Growth stabilized in all patients, with a good response (>50% reduction in HAS) in 62% and a favourable response (30–50% reduction in HAS) in 23%. Twelve of the 21 children (57%) had minor side effects. Persistent side effects did not occur. Intermittent short course, systemic, high-dose GCS therapy is an effective and safe treatment modality for IH, with a substantially lower cumulative dose of GCSs compared to prolonged therapy and no major side effects. This treatment is an alternative in cases in which propranolol fails or is contraindicated. **Chapter 9** evaluates the topical use of timolol 0.5% ophthalmic solution 3–4 times daily in 20 patients with small superficial IHs. The treatment was effective in all superficial IHs after 1–4 months. A quick direct inhibitory effect on growth of the IH was observed, followed by slower regression. The children had to be treated during the whole proliferative phase. Deeper IH on the nose (two cases) and lower eyelid (one case) showed no response. Therefore we concluded that topical timolol 0.5% ophthalmic solution is effective in superficial IH. Safety and effectiveness of timolol require further investigation but this therapy seems very safe when used in small IHs and effective when started in the early proliferative phase. Finally, **chapter 10** reports on the systemic therapy with propranolol, with a special focus on the comparison of the HAS with another scoring system recently published, namely the HSS (haemangioma severity scale). Propranolol is thought to be a safe and efficacious treatment for IH. But to make proper statements,

validated and reliable instruments are needed to measure disease severity. However, to date only two systems have been described for this goal: the HAS and the HSS. We performed a prospective study with 54 infants with IH treated with systemic propranolol from October 2009–December 2012. Treatment was based on a cautious protocol (day one propranolol 0.5 mg/kg/day and increased to 2 mg/kg/day at day three). All IHs were independently scored by two observers. Intraclass correlation coefficients (ICC) of the HAS and HSS between the observers were comparable. We noted that the HSS scores often do not change when IHs did improve and concluded therefore that the HSS does not really indicate the severity. We noted that the HAS decreased over time, with a dramatic drop in the first week, following the quick response to propranolol. This study demonstrates that the HAS performs better than the HSS. Regarding the therapy itself, side effects were seen in 10 cases (5x nightmares/sleeping disturbances, 4x cold extremities, and 1 time hypoglycaemia but actually due to diminished intake). Other problems with glucose and problems with blood pressure measurements or ECG were not noticed. In our series, propranolol was stopped 17 times (after 1 year therapy). In 3 cases there was rebound growth of the IH and propranolol was restarted; in two cases with success and in one case unsuccessful. Therefore we concluded that systemic propranolol treatment is indeed safe (only mild side effects were seen in our patients) and effective (only 1 failure).

In conclusion, hypoxia is important in the pathogenesis of IH but the exact mechanism is not clear yet. As for the therapy, when treatment is necessary, propranolol has become the first choice of treatment but for topical use the best option and concentration is not yet clear. Therefore more research is still necessary!

CHAPTER 13

Summary (Dutch)

HET INFANTIEL HEMANGIOOM: PATHOGENESE, EVALUATIE EN THERAPIE

Vasculaire afwijkingen worden onderverdeeld in de vasculaire malformaties en de vasculaire tumoren. Het infantiel hemangioom (IH) wordt beschouwd als een vasculaire tumor. IH's ontstaan bij 10% van de pasgeborenen. Typisch voor IH's is het ontstaan kort na de geboorte in de meeste gevallen en de exponentiele groei gevolgd door langzame regressie die begint na 9 tot 12 maanden en 7 tot 10 jaar kan duren.

De *introductie* beschrijft de huidige stand van zaken met betrekking tot de pathogenese en de therapie van IH's. **Hoofdstuk 1** gaat in op de mogelijkheid dat meerdere mechanismen een rol spelen bij de pathogenese. Klinisch is er in ongeveer de helft van de gevallen op de plek waar later een IH ontwikkelt een anemische macula (lokale ischemie) of zo genoemde precursor-laesie te zien. Eerder laboratoriumonderzoek liet zien dat er sprake is van stabilisatie en verhoogde transcriptie-activiteit van hypoxia inducible factor 1α (HIF1α), resulterend in een up-regulatie van doelgenen zoals vascular endothelial growth factor (VEGF), wat normaal onder hypoxische condities plaatsvindt. Momenteel worden er drie belangrijke hypothesen genoemd, namelijk 1) de theorie van weefselhypoxie, 2) de theorie van embolisatie van placentaire cellen en 3) de theorie van verhoogde angiogene en vasculogene activiteit. Weefsel hypoxie lijkt cruciaal. Hoewel IH's goedaardig en self-limiting zijn, kunnen er ernstige complicaties optreden ten gevolge van de locatie en snelle groei. Ook laten IH's in meer dan de helft van de gevallen littekens achter na regressie. Het beleid en de behandeling van IH's is sterk veranderd na 2008. In **hoofdstuk 2** worden de oudere therapie-mogelijkheden van voor 2008 samengevat, waarbij voornamelijk corticosteroïden werden gebruikt. Ook geeft dit hoofdstuk de huidige stand van zaken met de nieuwere mogelijkheden: systemische en topische β-blokkers. Corticosteroïden waren niet altijd effectief en konden veel bijwerkingen geven. β-blokkers zijn veelbelovend en hebben momenteel de voorkeur boven corticosteroïden, maar β-blokkers geven ook nog niet een definitieve genezing. In **hoofdstuk 3** zijn de doelen van dit proefschrift beschreven.

Deel I behandelt de pathogenese van IH's en in **hoofdstuk 4** beschrijven wij een retrospectief onderzoek naar de eerder genoemde theorie van weefselhypoxie (hoofdstuk 1). De pathogenese van IH's is nog niet helemaal opgehelderd. Er zijn meerdere mechanismen beschreven, waaronder hypoxie, dat een up-regulatie en stabilisatie geeft van hypoxia inducible factor 1α (HIF1α). HIF1α stimuleert daarop weer downstream transcriptie van doelgenen die uiteindelijk zorgen voor angiogenese. Het was ons doel om de rol van hypoxie in de pathogenese van IH's te onderzoeken, omdat de hypoxie-pathway mogelijk een therapeutische target kan zijn. Weefsel van patiënten met een IH van de periode 1991–2011 (bewaard in paraffine) werd opgezocht en middels immuunhisto-

chemie geanalyseerd voor HIF1α en enkele bekende targets van HIF1α, te weten: BNIP3, CA-IX, GLUT-1, pAKT, pS6 en VEGF. Vier beoordelaars hebben onafhankelijk de resultaten gescoord. Twee van de tien weefselstukjes bleken in de groeifase afgenomen te zijn. GLUT-1, BNIP3, pAKT en VEGF waren positief in alle weefselstukjes. pS6 was positief in negen weefselstukjes en negatief in een. CA-IX was zwak positief en HIF1α was negatief in alle gevallen. Onze resultaten wijzen erop dat hypoxie inderdaad een rol kan spelen in de ontwikkeling van IH's en dat factoren die door HIF1α up-gereguleerd worden kunnen worden beïnvloed.

Deel II behandelt de evaluatie van IH's. Gedurende de laatste jaren is er een toename van studies die verschillende therapiemogelijkheden vergelijken. Er was, naast het afnemen van een globale score en een aangepaste vorm van de visual analogue scale, nog geen gevalideerd, betrouwbaar en objectief score systeem voor IH activiteit beschikbaar. **Hoofdstuk 5** beschrijft de ontwikkeling van een eenvoudig systeem, de Haemangioma Activity Score (HAS), voor het scoren van proliferatieve activiteit van IH's. Dit systeem werd gevalideerd in een vergelijkende studie met foto's van IH's genomen tijdens consultaties van 2000 tot en met 2008 (n=78). Agreement tussen drie beoordelaars werd gemeten op twee verschillende tijdspunten met een minimum interval van zes maanden (op t=0 en op t=1) middels de interclass correlation coefficients (ICC). Agreement tussen de beoordelaars was goed. De gemiddelde ICC van de HAS op t=0 en op t=1 was 0.72 en 0.76, respectievelijk. De gemiddelde ICC van de HAS met betrekking tot het verschil vanaf baseline (HAS op t=0 minus HAS op t=1) was 0.69. We concluderen dat de HAS een goed systeem is om de proliferatieve activiteit van IH's te scoren en dat het kan worden gebruikt in toekomstige onderzoeken bijvoorbeeld om de verschillende behandelingen te evalueren. Een ander punt is de noodzaak van aanvullend onderzoek naar interne IH's in de evaluatie van patiënten met (multipele) IH's. Dit wordt besproken in **hoofdstuk 6**. Screening voor interne IH's wordt geadviseerd wanneer er multipele cutane IH's zijn, over het algemeen vijf of meer. Echter, sterke wetenschappelijke onderbouwing ontbreekt voor deze aanbeveling. Kinderen met IH's zijn sinds 1993 poliklinisch geëvalueerd door de werkgroep vasculaire afwijkingen Rotterdam (WEVAR) volgens vaste protocollen. Alle patiënten van dit spreekuur met vijf of meer IH's werden geselecteerd (periode 1993–2011). Allen waren doorgestuurd voor aanvullend onderzoek middels echografie voor interne IH's. Wij maakten onderscheid tussen twee groepen. Groep 1 (*hemangiomatose*) bestond uit 27 kinderen met >= 10 IH's en groep 2 (*multipele IH's*) bestond uit 16 kinderen met 5 tot en met 9 IH's. Negen kinderen in groep 1 hadden interne IH's in tegenstelling tot 0 in groep 2. Aanvullend onderzoek voor interne IH's in kinderen met minder dan tien IH's is controversieel en lijkt niet nodig. Wij raden echter wel aanvullend onderzoek aan (middels echografie van de buik) voor kinderen met 10 of meer IH's.

In *deel III* evalueren wij enkele mogelijkheden bij de behandeling van patiënten met IH's. **Hoofdstuk 7** begint met de evaluatie van intra-laesionale corticosteroïden. Perioculaire IH's reageren goed op corticosteroïden. Onze doelstelling was het evalueren van de effectiviteit van intra-laesionale corticosteroïden als behandeloptie voor perioculaire IH's. Wij hebben alle patiënten geselecteerd met perioculaire IH's die in ons ziekenhuis behandeld werden van 1993 tot en met 2009 met alleen intra-laesionale corticosteroïden. Deze therapie was gestandaardiseerd volgens een prospectief protocol. Er warden in totaal n=34 patiënten geïncludeerd. Er werden geen complicaties na therapie gezien. Een tweede intra-laesionale injectie was nodig in 5 patiënten. Bij de follow-up na 6 en 12 maanden na de injectie werd bij respectievelijk 94% en 91% van de patiënten regressie van het IH gezien. Astigmatisme, HAS en globale scores waren allemaal verbeterd na therapie. Dit onderzoek toonde aan dat intra-laesionale therapie met corticosteroïden een zeer veilige optie is voor de behandeling van perioculaire IH's, naast propranolol, of wanneer therapie met propranolol niet mogelijk is (bijvoorbeeld bij astma, PHACE syndroom en sommige hartaandoeningen). In **hoofdstuk 8** wordt de therapie met systemisch toegediende corticosteroïden geëvalueerd. IH's worden steeds vaker behandeld met propranolol of andere β-blokkers, maar voordat deze geïntroduceerd werden, waren orale glucocorticosteroiden (GCSs) de standaardbehandeling en het is nog steeds een alternatief in probleemgevallen. Er is echter geen standaard behandelprotocol voor wat betreft de dosering en duur van GCSs. Langetermijnbehandeling met GCSs kan bijwerkingen geven zoals groeiachterstand, gedragsveranderingen en reflux. Eenentwintig kinderen met alarmerende IH's werden behandeld volgens een algoritme met 3 mg/kg/dag oraal prednisolon, verdeeld over 3 doses met variërende duur en aantal GCS pulsen. Twee geblindeerde onderzoekers hebben onafhankelijk van elkaar de resultaten voor en na therapie beoordeeld door gebruik te maken van de HAS. Bijwerkingen werden geëvalueerd via ingevulde vragenlijsten door de ouders en de medische dossiers. De mediane duur van een korte puls GCSs was 2 weken (range 1–6 weken). De mediaan van het aantal pulsen was 2 (range 1–5). De mediaan van de cumulatieve dosis GCSs was 91 mg/kg. De groei van het IH stabiliseerde in alle patiënten: Een uitstekende respons (>50% reductie in HAS) werd gezien in 62% van de gevallen en een goede respons (30–50% reductie in HAS) in 23% van de gevallen. Twaalf van de eenentwintig kinderen (57%) hadden milde bijwerkingen. Persisterende bijwerkingen werden niet gezien. Intermitterende korte-puls, systemische, hoge-dosis GCS-therapie is dus een effectieve en veilige optie voor de behandeling van IH's, met een aanzienlijke lagere cumulatieve dosis van GCSs vergeleken met de ononderbroken (lange) therapie. Er zijn ook geen grote bijwerkingen. Deze behandeling is een alternatief in gevallen waarin propranolol niet werkt of niet mogelijk is. In **Hoofdstuk 9** evalueren wij het topisch gebruik van timolol 0.5% oogdruppels, 3–4 keer per dag, bij 20 patiënten met kleine, oppervlakkige IH's. De therapie was effectief in alle oppervlakkige IH's na 1 tot

4 maanden. Een snel direct remmend effect op de groei van het IH werd gevolgd door een langzamere regressie. Kinderen moeten gedurende de hele proliferatieve fase behandeld worden, Dieper gelegen IH's op de neus (2 patiënten) en op het onderste ooglid (1 patiënt) vertoonden geen effect. Daarom concluderen wij dat topische timolol 0.5% oogdruppels effectief is in oppervlakkige IH's. Veiligheid en effectiviteit van timolol moeten verder onderzocht worden maar deze therapie lijkt zeer veilig bij gebruik in kleine IH's en is effectief als het vroeg gestart wordt in de proliferatieve fase. Ten slotte werd in **hoofdstuk 10** systemische therapie met propranolol onderzocht, waarbij ook de HAS vergeleken werd met een ander, recentelijk gepubliceerd scoresysteem, namelijk de HSS (haemangioma severity scale). Propranolol wordt geacht een veilige en goede therapie te zijn voor de behandeling van IH's. Maar om goede conclusies te trekken heb je een gevalideerd en betrouwbaar systeem nodig om de activiteit van IH's te meten. Helaas zijn er tot nu slechts twee systemen beschreven voor dit doel: de HAS en de HSS. Wij hebben een prospectieve studie uitgevoerd met 54 kinderen met IH's, behandeld met systemisch propranolol in de periode oktober 2009–december 2012. De behandeling was toen nog gebaseerd op een voorzichtig protocol (propranolol 0.5 mg/kg/dag op dag 1, ophogen naar 2 mg/kg/dag op dag 3). Alle IH's werden onafhankelijk gescoord door twee beoordelaars. Intraclass correlation coefficients (ICC) van de HAS en HSS tussen de beoordelaars waren vergelijkbaar. Wij merkten dat de HSS vaak dezelfde waarde houdt terwijl het IH verbetert en daarom dus niet echt de activiteit aangeeft. We concludeerden dat de HAS afnam over de tijd, met een enorme afname in de eerste weken, conform de snelle reactie op propranolol. Deze studie laat zien dat de HAS een beter scoresysteem is dan de HSS. Wat betreft de therapie, bijwerkingen werden gezien in 10 gevallen (5x nachtmerries/slaapproblemen, 4x koude extremiteiten en 1x hypoglycemie maar eigenlijk door een verminderde intake). Andere problemen met betrekking tot glycemie, tensie en ECG werden niet gezien. In onze serie was propranolol inmiddels gestopt in 17 patiënten (na 1 jaar therapie). In 3 patiënten was er hergroei van het IH en werd propranolol herstart; in twee gevallen met succes maar in 1 geval zonder succes. We concludeerden dat systemische behandeling met propranolol inderdaad veilig is (er werden slechts milde bijwerkingen gezien) en effectief (slechts 1 failure).

De slotconclusie luidt dat hypoxie belangrijk is in de pathogenese van IH's, maar dat het precieze mechanisme nog onduidelijk is. Voor wat betreft de therapie, als actieve therapie nodig is, is propranolol de eerste keus geworden maar voor topisch gebruik is de beste keus en concentratie nog onduidelijk. Daarom blijft er nog steeds onderzoek nodig!

PART V

About the author

CHAPTER 14

Curriculum Vitae

Personalia

Naam:	Janmohamed
Voornamen:	Sherief, Rahit
Geslacht:	Man
Nationaliteit:	Nederlandse
Geboortedatum:	6 juni 1981
Geboorteplaats:	Eindhoven (Nederland)
Burgerlijke staat:	Gehuwd
Woonplaats:	Brussel (België)
E-mail adres:	sherief.janmohamed@uzbrussel.be

Opleidingen

2014 – heden	Master na Master in de Specialistische Geneeskunde Vrije Universiteit Brussel
2013	Cursus Injectable Specialist Kliniek Laurium BV / Entercare BV te Linschoten **Certificaat: 30 januari 2013**
2012 – 2013	Master of Science in Health Sciences Netherlands Institute for Health Sciences (NIHES) / Erasmus MC **Master of Health Sciences (MHS) diploma: 30 augustus 2013**
2010 – 2014	Promotietraject Afdelingen Kindergeneeskunde en Kinderchirurgie Erasmus MC – Sophia Kinderziekenhuis te Rotterdam These: Infantile Haemangioma: Pathogenesis, Evaluation, and Therapy **Doctoraat (PhD): 4 december 2014**
2004	In het kader van de NIHES opleiding MSc in Clinical Epidemiology: Management in Public Health course (cijfer: A) Clinical Epidemiology course (cijfer: A) Harvard University, Boston, MA, USA
2002 – 2004	Master of Science in Clinical Epidemiology Netherlands Institute for Health Sciences (NIHES) / Erasmus MC **Master of Science (MSc) diploma: 27 Augustus 2004**

2000 – 2006	Geneeskunde Erasmus Universiteit Rotterdam **Arts diploma (MD): 18 Augustus 2006** **Doctoraal diploma (MSc): 13 Mei 2004** **Propedeuse diploma: 31 Augustus 2001**
1999 – 2000	Bedrijfswiskunde & Informatica Vrije Universiteit Amsterdam **Propedeuse diploma: 16 Augustus 2000**
1993 – 1999	Voorbereidend Wetenschappelijk Onderwijs (VWO) dr. Knippenbergcollege te Helmond (jaar 1–4) Penta College CSG Blaise Pascal te Spijkenisse (jaar 5–6) **VWO diploma: 30 Juni 1999**

Werkervaring

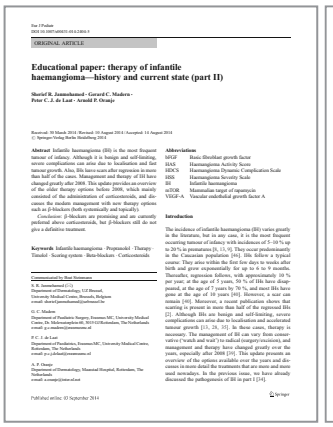
2014 t/m heden	Universitair Ziekenhuis Brussel te Brussel (België) Dermatoloog i.o.
2013 t/m heden	Oprichter en eigenaar van CosmoDermic Kliniek voor injectables (Botox en Fillers)
2012 t/m heden	Intermedica kliniek voor Dermatologie te Boxmeer Arts trichologie
2010 t/m 2014	Erasmus MC te Rotterdam Arts-onderzoeker / PhD student (Kinderdermatologie)
2009 t/m 2013	Havenziekenhuis te Rotterdam Arts op de afdeling KinderHaven (Kinderdermatologie)
2008 t/m 2011	Erasmus MC te Rotterdam Arts op de afdeling Kinderchirurgie
2007	Sint Franciscus Gasthuis te Rotterdam Arts op de afdeling Kindergeneeskunde
2006 t/m 2007	Maasstad Ziekenhuis te Rotterdam Arts op de afdeling Interne Geneeskunde

CHAPTER 15

List of publications

ARTICLES INCLUDED IN THIS THESIS

Introduction – Chapter 1



Introduction – Chapter 2

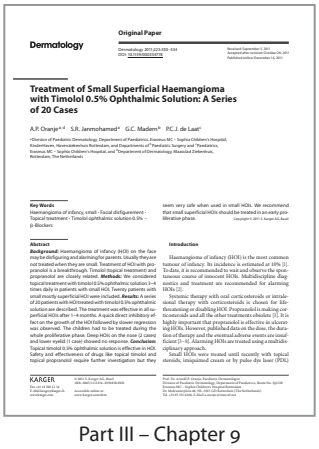
Part I – Chapter 4

Part II – Chapter 5

Part II – Chapter 6

Part III – Chapter 7

Part III – Chapter 8



Part III – Chapter 9

Part III – Chapter 10

LIST OF PUBLICATIONS

Full articles

1. Oranje AP, Janmohamed SR, Madern GC, de Laat PC. *The treatment of small superficial hemangiomas with timolol eye drops 0.5% or 0.1% gel.* Nederlands Tijdschrift voor Dermatologie en Venereologie. 2010 Dec;20(11):711–713.

2. Stalder JF, Barbarot S, Wollenberg A, Holm EA, De Raeve L, Seidenari S, Oranje A, Deleuran M, Cambazard F, Svensson A, Simon D, Benfeldt E, Reunala T, Mazereeuv J, Boralevi F, Kunz B, Misery L, Mortz CG, Darsow U, Gelmetti C, Diepgen T, Ring J, Moehrenschlager M, Gieler U, Taïeb A; PO-SCORAD Investigators Group (including Janmohamed SR). *Patient-Oriented SCORAD (PO-SCORAD): a new self-assessment scale in atopic dermatitis validated in Europe.* Allergy. 2011 Aug;66(8):1114–21.

3. Janmohamed SR, Madern GC, de Laat PC, Oranje AP. *Haemangioma of infancy: two case reports with an overdose of propranolol.* Case Rep Dermatol. 2011 Jan;3(1):18–21.

4. Janmohamed SR, de Waard-van der Spek FB, Madern GC, de Laat PC, Hop WC, Oranje AP. *Scoring the proliferative activity of haemangioma of infancy: the Haemangioma Activity Score (HAS).* Clin Exp Dermatol. 2011 Oct;36(7):715–723.

5. Oranje AP, Janmohamed SR (both first author), Madern GC, de Laat PC. *Treatment of small superficial haemangioma with timolol 0.5% ophthalmic solution: a series of 20 cases.* Dermatology. 2011;223(4):330–4.

6. Janmohamed SR, Madern GC, Nieuwenhuis K, de Laat PC, Oranje AP. *Evaluation of intra-lesional corticosteroids in the treatment of peri-ocular haemangioma of infancy: still an alternative besides propranolol.* Pediatr Surg Int. 2012 Apr;28(4)393–8.

7. Nieuwenhuis K, de Laat PC, Janmohamed SR, Madern GC, Oranje AP. *Infantile Hemangioma: treatment with short course systemic corticosteroid therapy as an alternative for propranolol.* Pediatr Dermatol. 2013 Jan-Feb;30(1):64–70.

8. Janmohamed SR, Madern GC, de Laat PC, Oranje AP. *Hemangiomen en vasculaire malformaties: hoe en door wie te behandelen?* Tijdschrift voor Kindergeneeskunde. 2013 Feb;81(1):14–22.

9. Janmohamed SR, Oranje AP, Devillers AC, Rizopoulos D, van Praag MC, Van Gysel D, Goeteyn M, de Waard-van der Spek FB. *The proactive wet-wrap method with diluted corticosteroids versus emollients in children with atopic dermatitis: a prospective, randomised, double-blind, placebo-controlled trial.* J Am Acad Dermatol. 2014 Jun;70(6):1076–82.

10. Janmohamed SR, Madern GC, de Laat PC, Oranje AP. *Educational paper: Pathogenesis of infantile haemangioma, an update 2014 (part I).* Eur J Pediatr. 2014. In press.

11. Janmohamed SR, Madern GC, de Laat PC, Oranje AP. *Educational paper: Therapy of infantile haemangioma – history and current state (part II).* Eur J Pediatr. 2014. In press.

12. van Oosterhout M, Janmohamed SR, Spierings M, Hiddinga J, de Waard-van der Spek FB, Oranje AP. *Correlation between Objective SCORAD and Three Item Severity (TIS) score by physician, and Objective PO-SCORAD by parent/patient in children with atopic dermatitis.* Dermatology. 2014. In press.

13. Janmohamed SR, Brinkhuizen T, den Hollander JC, Madern GC, de Laat PC, van Steensel MA, Oranje AP. *Support for the hypoxia theory in the pathogenesis of infantile haemangioma.* Clin Exp Dermatol. 2014. In press.

14. Janmohamed SR, van Oosterhout M, de Laat PC, van Rosmalen JM, Madern GC, Oranje AP. Scoring the therapeutic effects of s*ystemic propranolol for infantile hemangioma: a prospective study comparing the Hemangioma Activity Score (HAS) with the Hemangioma Severity Scale (HSS).* 2014. Submitted.

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15. Janmohamed SR, de Laat PC, Madern GC, Oranje AP. *Do we have to check glucose in patients with haemangioma of infancy treated with beta-blockers?* J Eur Acad Dermatol Venereol. 2011 Dec;25(12):1490.

16. Janmohamed SR, de Laat PC, Madern GC, Dorresteijn EM, Jan Danser AH, Oranje AP. *Treating hemangioma of infancy with beta-blockers: is there really a risk of hypotension?* J Am Acad Dermatol. 2012 Aug;67(2):315–6.

17. Janmohamed SR, de Waard-van der Spek FB, Madern GC, de Laat PC, Hop WC, Oranje AP. *Scoring the proliferative activity of haemangioma of infancy: to HAS or not to HAS?* Clin Exp Dermatol. 2013 Jan;38(1):90–1.

18. Vredenborg AD, Janmohamed SR (both first author), de Laat PC, Madern GC, Oranje AP. *Multiple cutaneous infantile haemangioma and the risk of internal haemangioma.* Br J Dermatol. 2013 Jul;169(1):188–91.

19. Janmohamed SR, Madern GC, de Laat PC, Oranje AP. *Intractable vascular autonomic dysregulation (Harlequin phenomenon) in two brothers: another indication for propranolol?* Dermatol Ther. 2014 Jul;27(4):230–2.

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20. Vredenborg AD, Janmohamed SR (both first author), de Laat PC, Madern GC, Oranje AP. *Multiple cutaneous infantile haemangioma and the risk of internal haemangioma.* In: Oranje AP and Al-Mutairi N (editors), Controversies in Paediatric Dermatology. 2014. In preparation.

21. Janmohamed SR, Chandran NS, Oranje AP. *Propranolol versus atenolol in the treatment of infantile haemangioma.* In: Oranje AP and Al-Mutairi N (editors), Controversies in Paediatric Dermatology. 2014. In preparation.

CHAPTER 16

PhD portfolio

Portfolio Wetenschappelijk *(10 ECTS totaal)*	**ECTS**
Congressen	
European Society for Paediatric Dermatology (ESPD) – Istanbul 16–19 mei 2012	
European Academy for Dermatology and Venereology (EADV) – Praag 27–30 september 2012	
European Paediatric Surgeons' Association (EUPSA) – Leipzig 5–8 juni 2013	
Children's Skin and Allergy (3rd ESPD Summer School) 27–29 juni 2013 *Organizing committee en secretaris van satellietsymposium hemangiomen*	
European Society for Paediatric Dermatology (ESPD) – Kiel 12–14 juni 2014	
European Academy for Dermatology and Venereology (EADV) – Amsterdam 8–12 oktober 2014	
Poster presentaties	
Diagnostiek en Therapie in de Kinderdermatologie – 18e artsencursus 24 juni 2011	
European Academy for Dermatology and Venereology (EADV) – Praag 27–30 september 2012	
European Paediatric Surgeons' Association (EUPSA) – Leipzig 5–8 juni 2013	
Voordrachten	
Wetenschappelijke vergadering AVM en hemangiomen, Nederlandse Vereniging voor Kinderchirurgie 23 maart 2011	*1*
5-Jarig Jubileumcongres van de HEVAS 3 maart 2012	*1*
European Society for Paediatric Dermatology (ESPD) – Istanbul 16–19 mei 2012	*1*
Diagnostiek en Therapie in de Kinderdermatologie – 19e artsencursus 1 juni 2012	*1*
European Academy for Dermatology and Venereology (EADV) – Praag 27–30 september 2012	*1*
European Paediatric Surgeons' Association (EUPSA) – Leipzig 5–8 juni 2013	*1*
3rd ESPD Summer School (3x presentatie) 27–29 juni 2013	*1*
Symposium Pomeranian Medical University – Szczecin 26 november 2013	*1*
Dermatologie symposium Poznan, Polen Maart 2013	*1*
European Academy for Dermatology and Venereology (EADV) – Amsterdam 8–12 oktober 2014	*1*

Portfolio Onderwijs *(10 ECTS per onderdeel)*	**ECTS**
Gevolgd onderwijs	
Diagnostiek en Therapie in de Kinderdermatologie – 15e artsencursus 30 mei 2008	
Diagnostiek en Therapie in de Kinderdermatologie – 16e artsencursus 25 september 2009	
Methodologie van Patiëntgebonden Onderzoek en Voorbereiding van Subsidieaanvragen 16 februari 2010	
Diagnostiek en Therapie in de Kinderdermatologie – 17e artsencursus 25 juni 2010	
Monitoring and patient safety in investigator initiated clinical trials 11 maart 2011	
Diagnostiek en Therapie in de Kinderdermatologie – 18e artsencursus 24 juni 2011	
BROK cursus (geslaagd met 88%) 14–18 november 2011	
Diagnostiek en Therapie in de Kinderdermatologie – 19e artsencursus 1 juni 2012	
Master of Science in Health Sciences (specialisation Public Health) – NIHES 2012–2013	*10*
Nascholing "Allergie KinderHaven voor huisartsen" 2 oktober 2012	
Wetenschappelijke vergadering NVDV / 100 jaar Dermatologie Groningen 20 september 2013	
Gegeven onderwijs	
Eczeemschool 2009–2013	*2*
College aan co-assistenten 2010–2013	*5*
Onderwijsavond KinderHaven 19 augustus 2010	*0.2*
Grand round voor staf Kindergeneeskunde 1 april 2011	*0.2*
Onderwijs aan verpleegkundigen (specialisatie Kinderverpleegkunde) 7x in 2011–2013	*0.5*
Hoorcollege Kinderdermatologie aan studenten Geneeskunde 23 mei 2012	*0.1*
Begeleiden van oudste co-assistenten (onderzoeksstage) 2x in 2012–2013	*2*

CHAPTER 17

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Sherief Janmohamed
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APPENDIX

The haemangioma activity score (HAS) scoring form

HAEMANGIOMA ACTIVITY SCORE (HAS)

Patient name:
Patient date of birth:
Patient ID #:
Location of infantile haemangioma (IH):

'Bright red edge' should only be scored when the IH is not totally 'bright red'
'Skin coloured after activity': do not score in deep IH (deep swelling) unless the IH has changed into it after activity

Date				
Deep swelling: Tense IH (6) 'Neutral' IH at t=0 or less than 50% reduction at follow up (4) >= 50% reduction at follow up (2) No more swelling at follow up (0)				
Bright red / shining red IH (5) OR Bright red edge (4)				
Matt red / reddish-purple IH / matt red edge (3)				
Blue IH or blue shining through in deep IH (2)				
Grey IH (1)				
Skin coloured after activity (0)				
Total score				
Number of items scored				
Preliminary HAS = total score / number of items scored				
Ulcer =< 1 cm^2 (+ 0.5) Ulcer 1-25 cm^2 (+ 1) Ulcer >= 25 cm^2 (+ 2)				
HAS = preliminary HAS + ulcer score				

Date				
Deep swelling: Tense IH (6) 'Neutral' IH at t=0 or less than 50% reduction at follow up (4) >= 50% reduction at follow up (2) No more swelling at follow up (0)				
Bright red / shining red IH (5) OR Bright red edge (4)				
Matt red / reddish-purple IH / matt red edge (3)				
Blue IH or blue shining through in deep IH (2)				
Grey IH (1)				
Skin coloured after activity (0)				
Total score				
Number of items scored				
Preliminary HAS = total score / number of items scored				
Ulcer =< 1 cm^2 (+ 0.5) Ulcer 1-25 cm^2 (+ 1) Ulcer >= 25 cm^2 (+ 2)				
HAS = preliminary HAS + ulcer score				