LESS IS MORE IN KELOID DISEASE

A CLINICAL STUDY

Eveline Byland

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A Clinical Study

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Een klinische studie

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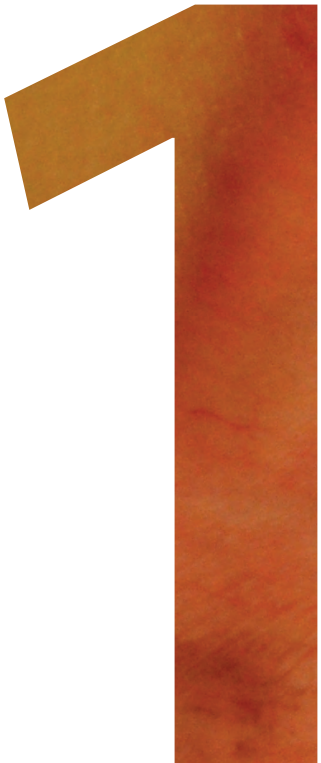
General Introduction



Wound healing and scarring

Each year, about 10% of the Dutch population undergoes surgery [1], resulting in a scar after wounds have healed. In addition, during life virtually everybody will at least once experience a minor trauma such as a fall, cut or burn resulting in one or more scars as well. Thus, everyone has scars. However, having scars is not a problem per se; it is a sign of the healing power of our body. But if wound healing is disturbed problematic scars may develop.

Skin is the largest organ of the human body, which has three main layers: the epidermis, the dermis and the subcutis (Figure 1). The epidermis is the most outer layer which has a barrier function to protect our body's interior from influences from outside and which prevents water and nutrient loss from inside our body, and helps to maintain a stable body temperature [2]. The most inner layer of the epidermis consist of basal cells, also known as stratum basale, that produces new cells and gets nutrients from the dermis. These cells are pushed outwards, flatten, lose their nucleus and form a sealing top layer (stratum spinosum, granulosum, lucidum (very thin) and corneum, respectively) [3]. Underneath the basal layer are the papillary and reticular dermis; rich of collagen, blood vessels and containing most skin appendages (exocrine glands, hair follicles, sensory corpuscles). If skin damage extends into the deeper reticular dermis this will cause scarring [3]. The dermis covers the subcutis that consist of subcutaneous fat and fascia layers that give the skin mobility relative to the muscles, tendons and bones in our body and isolates our body to prevent heat loss [2].

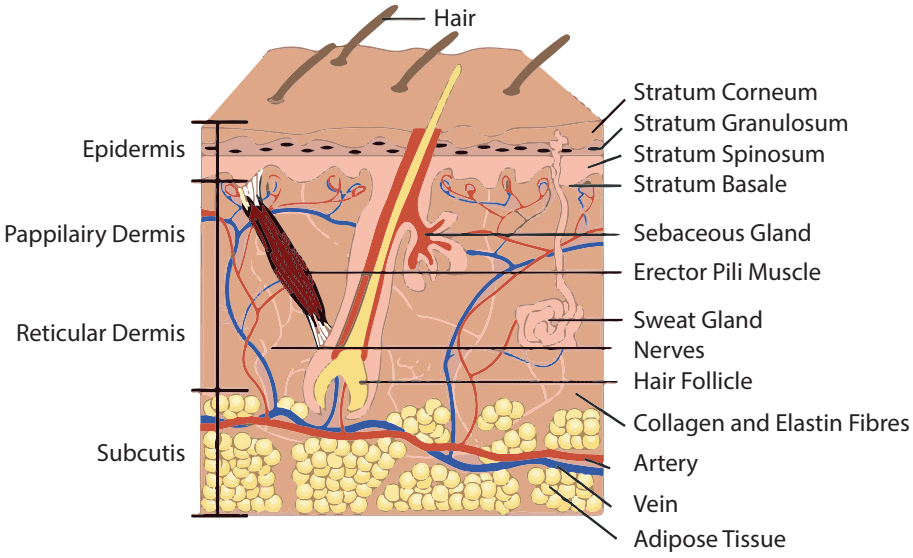


Figure 1. Sectional view of the skin

Wound healing is a complex and well-orchestrated process that follows several overlapping phases (Figure 2). Briefly, after the skin has been damaged healing starts with

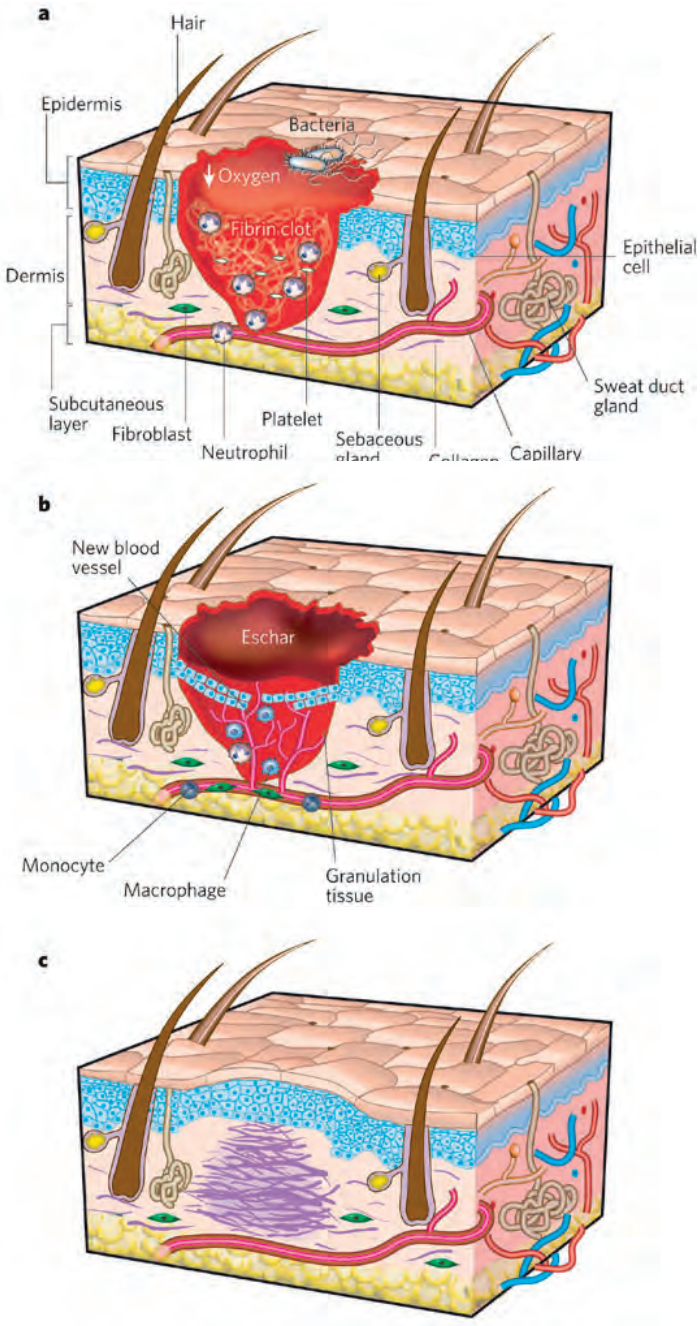


Figure 2. Sectional view of skin during wound healing

Figure 2. Sectional view of skin during wound healing (continued)
Classic stages of wound repair: inflammation (a), proliferation (b) and remodelling (c). a, Inflammation. Depicted is a skin wound at about 24–48 h after injury. A fibrin clot has formed. Bacteria, neutrophils and platelets are abundant in the wound. Normal skin appendages (such as hair follicles and sweat duct glands) are still present in the skin outside the wound. b, Proliferation. Depicted is a skin wound at about 5–10 days after injury. An eschar (scab) has formed on the surface of the wound. Most cells from the previous stage of repair have migrated from the wound, and new blood vessels now populate the area. The migration of keratinocytes can be observed under the eschar. c, Remodelling. This stage lasts for a year or longer. Depicted is a skin wound about 1–12 months after repair. Disorganized collagen has been laid down by fibroblasts that have migrated into the wound. The healed region does not contain normal skin appendages. From: Gurtner et al. Wound repair and regeneration. Nature. 2008;453:314-321 with permission of Nature publishing group

hemostasis, which is followed directly by an inflammatory phase that lasts up to 4 to 6 days. The resulting fibrin cloth, which is rich of released cytokines and growth factors serves as a scaffold for neutrophils, monocytes and fibroblasts. Lymphocytes clear the debris and bacteria, macrophages enter the wound and promote angiogenesis, fibroplasia and keratinocyte formation. After clearing the wound, the proliferative phase (day 4-14) starts during which new tissue is formed and the epidermis is restored. Keratinocytes reconstitute the epidermis. Fibroblasts, stimulated by platelet derived growth factor (PDGF) and epidermal growth factor (EGF), produce collagen type III, glycosaminoglycans and fibronectin that form a matrix. Vascular endothelial growth factor (VEGF) stimulates blood vessels to grow which supply this matrix. In the last phase, this provisional matrix is remodeled and matures (day 8 to 1 year) to an organized and strong scar. During the first 4 to 5 weeks, collagen builds up and thin collagen parallel to the epidermis is replaced by thicker type I collagen which is organized along stress lines. Eventually, scar strength is around 80% of uninjured skin strength [3, 4].

Pathologic scarring

As soon as the previously described delicate healing processes get disrupted, this may result in problems from chronic wounds to excessive scar formation like hypertrophic and keloid scars. Normal scars first appear as a thin red line, but after maturation they turn into a slightly broadened white line. In pathologic scar formation too much tissue is formed (Figure 3).

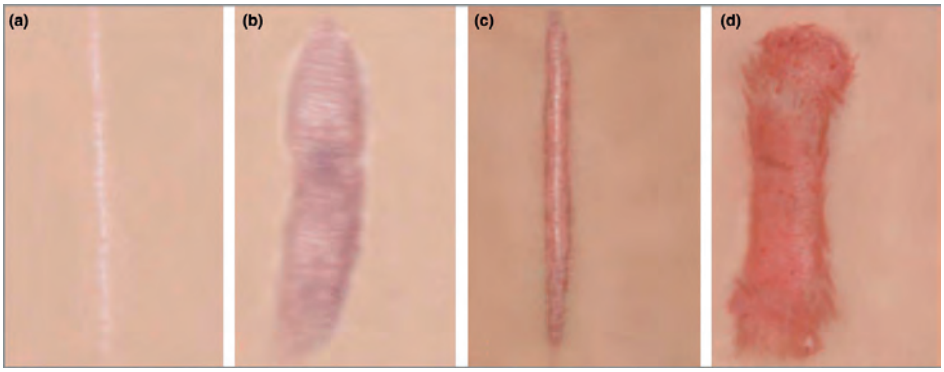


Figure 3. Normal scar (a), atrophic scar (b), hypertrophic scar (c), and keloid scar (d)
From: Ud-Din and Bayat. Strategic management of keloid disease in ethnic skin: a structured approach supported by the emerging literature. British Journal of Dermatology. 2013;169 (Suppl. 3):71–81 with permission of John Wiley and Sons.

Pathologic scarring covers two clinical conditions: hypertrophic and keloid scars. Hypertrophic scars (HTS) are raised, thick, firm and red scars that may itch or give pain. They develop early in the maturation phase, are quite common, and tend to regress spontaneously after a prolonged maturation phase. Keloid scars are rare (prevalence 0.1-8.3%, incidence 1-160/1000 patient years)[5-8], and may develop months to even years after injury, growing outside of original wound borders. They do not regress spontaneously and are refractory to many treatments. Beside a very prominent appearance they usually cause itch and pain. A higher incidence is found in darker skin types and specific sites like the anterior chest, shoulders, and ears are more often affected [9-11].

Although keloids have distinct clinical features, in clinical practice it can be hard to distinguish keloids from HTS and sometimes both conditions even seem to appear in one scar. Opinions differ on whether HTS and keloids are truly different conditions or whether they are two gradations of the same pathologic process. Aside from clear differences in clinical behavior, histological differences have also been shown. Collagen bundle thickness is vastly increased in keloids while in normal scars and HTS bundle thickness is decreased compared to normal skin [12]. Myofibroblasts are present in HTS but not in keloids; reversely, mucin is present in keloids but not in HTS [13].

Arguments that both conditions are similar pathological entities with different quantitative deviations have been made [14, 15]. There are many similarities between both conditions: 1) collagen production is increased, resulting in excess extracellular matrix, 2) collagen orientation in keloids, HTS, and normal scars is more parallel than in normal skin, and 3) mast cells are increased [12, 13]. Histological differences can be explained by site specific structures and mechanical forces of surrounding tissues. Common theory is that ongoing inflammation, which is significantly longer than the standard inflam-

matory phase during normal wound healing, causes excessive scar formation. Various immuno- and neuro-inflammatory processes and cytokine leakage by vascular damage have been proposed as etiologic factors for this prolonged inflammation [14, 15]. Although the exact etiology of pathologic scar formation is not entirely clear, several factors that affect keloid scarring have been identified (Table 1).

Table 1. Intrinsic and extrinsic factors influencing keloid scarring

Extrinsic factors	Intrinsic factors	Local factors
Repeated trauma	Skin type	Body area
Wound infection	Genetic factors	Wound tension
	Age	
	Hypertension	
	Hormonal state	
	Syndromal	

While opinions on differences and similarities on HTS and keloids differ, I am convinced that in clinical research a difference must be made. Different definitions have been used, however, the most important factor of keloids is growth outside of the original wound borders. Continuous growth, symptoms like pain and itch, and no spontaneous regression are also used in keloid definitions. In this research project scars were judged on growth history (starting late after trauma, continuous growth), shape (invasive in healthy skin), size (>0.5 cm beyond the original lesion), and symptoms, if most keloid features were present the lesion was classified as a keloid. For experienced observers classification on clinical features is usually very clear, and there are major implications on treatment choices based on the different clinical behavior of keloids and HTS [9].

Burden of keloids

Historically wound healing research has focused on objective measurements, like clinical appearance and histological features of different scar types or keloid size reduction after various treatments. While these areas are important, the patients' perspective and perception is equally important and nowadays is a standard part of most study outcomes. Skin conditions can have just as much impact on quality of life as some life-threatening conditions [16]. Ten years ago the impact of scars on quality of life was gaining attention and it was shown that scars may burden patients [17]. Scars affect physical comfort and functioning, self-acceptability, social functioning and emotional well-being. Patients with pathologic scars also report distress induced by the uncertain nature and treatment options of their condition. Obviously, keloids potentially cause a

bigger burden than normal scars do by their appearance and symptoms. But specifically to what extent keloids affect quality of life was unclear.

Treatment of keloids

Evidence-based keloid treatment is hampered by scarcely available evidence. While there are many papers on current and future keloid treatments, the ideal treatment has not been found yet and there is no consensus on the best treatment currently available. Over the past few decades no major changes in treatment modalities have taken place [9, 18]. There are several reasons for this:

- Preclinical research is difficult because keloids only exist in humans and no research animal model is available. Therefore, optimization of new treatments in a standardized living model is not available. Because keloids are affected by many different factors, treatment effects cannot be accurately simulated with a fibroblast culture or even a fibroblast/keratinocyte double culture. The giant step from *in vitro* results to clinical studies in patients seems to impair treatment development.
- Keloids on itself are very heterogenic, with a wide variety of clinical appearance from single small lesions to multiple and very large lesions covering entire body parts (Figure 4). This makes it hard to compare different groups of keloid patients because group sizes are rarely large enough to reach an even distribution.
- The heterogeneity between studies is exaggerated by not clearly differentiating between HTS and keloids. HTS are known to spontaneously regress. With adequate follow-up length it is impossible to measure treatment effect apart from natural regression if all types of scars are combined.
- There is a plethora of small case series showing good treatment results of one or a combination of several different treatments, but there is a lack of comparative studies informing clinicians which treatment strategy is most effective for which keloid and/or patient.

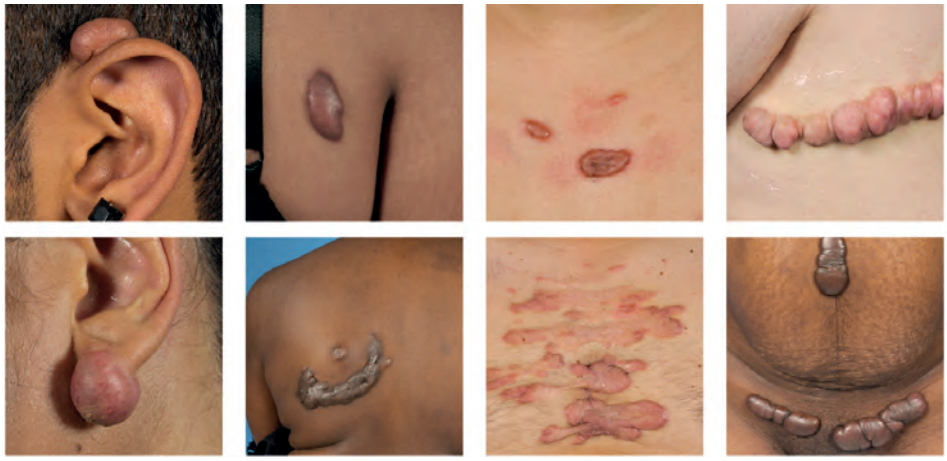


Figure 4. Examples of keloid scars
Different fenotypes of keloids. Upper row left to right: keloid of helix, keloid on upper back, presternal keloids, infra-axillairy keloid. Lower row left to right: earlobe keloid, upper back keloid, presternal keloids, abdominal keloids.

Treatment of keloids is challenging; treatment response is poor or keloids recur afterwards. Specifically surgery is infamous for recurrences because the new wound ends up in the adjacent and equally keloid prone skin area, starting the same pathologic scar derailment right away. Current therapies are based on empirical knowledge, but their treatment mechanism is not always completely understood. Table 2 shows the most commonly used treatment strategies, the expected results and the treatment specific drawbacks [9, 18]. Publications on many other treatment modalities are available, including bleomycin, imiquimod, 5-fluorouracil, onion extract, photodynamic therapy, electrical stimulation, calcium antagonists and interferon therapy [9, 11, 18, 19].
Most clinicians have experience with a few modalities depending on their specialty (general practitioner, skin therapeutic, dermatologist, plastic surgeon) and offer a limited treatment selection to patients seeking solution for their keloid problems. A stepped care approach is often used, starting with a mildly invasive treatment, escalating to more invasive treatments if necessary.

Table 2. Overview of keloid treatment options most used

Treatment	Outcomes	Draw backs
Corticosteroid injections	50-100% response 9-50% recurrence	Injection pain, skin atrophy, hypopigmentation
Laser (PDL 585)	57-85% response	Painful
Contact cryotherapy	51-76% response	Painful, hypo- and hyperpigmentation, skin atrophy
Surgery	45-100% recurrence	Recurrences often bigger than original scar
Surgery followed by pressure therapy	0-10% recurrence	6-24 months treatment duration, high treatment adherence needed, not applicable on all body areas, depends on good pressure garment manufacturing
Surgery followed by corticosteroid injections	0-100% recurrence	Injection pain, skin atrophy, hypopigmentation, recurrences often bigger than original scar
Radiation	10-94% response	Stochastic carcinogenic effects, growth interference in children
Surgery followed by radiation	1-35% recurrence	Stochastic carcinogenic effects, growth interference in children, wound healing problems

Historical background of this thesis

At the Erasmus MC keloid treatment has been performed for many years by the departments of dermatology, plastic surgery and radiation oncology in collaboration with plastic surgery. As a third line referral center we see many problematic keloids that are often treated with excision and brachytherapy, which is a very invasive, time consuming, and costly treatment with some specific complications. Potentially carcinogenic stochastic effects are an important issue when treating benign lesions in young patients, therefore we searched for keloid treatments that reduce the use of ionizing radiation. We tried photodynamic therapy as a promising alternative, but it was very painful and less effective for improving scar appearance [19]. Consequently, we have not incorporated this as a standard alternative for post-excisional brachytherapy.

Some patients do not react well to intralesional corticosteroids, leading to early surgical treatment. Because surgery with adjuvant corticosteroids in these cases is also not likely to prevent recurrence, treatment with brachytherapy is often indicated sooner than usual. If more effective non-surgical treatments would be available, this early jump to invasive, last resort treatment could be prevented.

When a new device for intralesional cryotherapy was promoted the department of plastic surgery became enthusiastic based on available evidence [20-23] and was attracted to the possibility of effective keloid treatment preventing toxic radiation and hospital admittance of our patients. However, before implementing intralesional cryotherapy as a standard treatment at our center we wanted to see further proof of its efficacy in a randomized clinical trial.

As for many other conditions in the field of plastic surgery, we deemed patient reported outcomes and quality of life improvement of very high importance. Quality of life was not well incorporated in literature on keloid treatment at that time, but now is of increasing importance in a time of increasing healthcare costs and need to prioritize decisions on treatment compensation.

Aims and outline of this thesis

The overall aim of the present research project was to improve health care for keloid patients. We started by determining the burden of keloid disease and analyzing what factors add to the disease burden. After identifying which factors cause the greatest quality of life impairment, treatments can be aimed to improve these factors affecting quality of life most. Many keloid patients report pain as their main symptom, however, often no treatment is prescribed to relief pain. To explore symptomatic therapy options we need to know more of the etiology and kind of pain in keloid patients.
Our aim to reduce the use of brachytherapy in keloid patients stimulated us to investigate whether other treatments could be effectively used as well and whether radiation doses could be reduced. In this way we wanted to provide scientific evidence guiding clinicians in their choice of treatment, mainly based on patient reported outcome measures.

Part I Burden of keloids

Chapter 2
It has been proven that scars in general may impair quality of life [17]. Because keloid disease is a severely pathologic scar we wanted to establish how much impact such a scar has on quality of life and which patient characteristics or aspects of the scars play an important role in this burden. We investigated this in a multi-center cross-sectional self-administered questionnaire study.

Chapter 3
Pain is an important symptom of many keloid patients, while many matured scars are asymptomatic and only some patients suffer from painful scars. To acquire more knowledge on the extent and possible reasons for this problem, we performed a systematic review of all available literature on prevalence, etiology and pathophysiology of pain in dermal scars.

Chapter 4

Because knowledge on pain in keloid patients is very limited, we wanted to investigate the characteristics of pain experienced by keloid patients and see whether this correlated to skin innervation patterns of keloids. This might give insight in possibilities for symptom relief therapy.

In a pilot study, eight patients completed questionnaires on neuropathic pain and on cold intolerance. Afterwards the keloid and a matching control site of normal skin were clinically investigated on eleven different sensation modalities that corresponded to a certain type of nerve fibers. Shortly after these tests, surgical keloid removal was planned and tissue was obtained. After processing we stained, quantified and assessed the upper dermal and epidermal nerve fibers in these keloids. We hypothesized that clinical and histological findings would correlate.

Part II Treatment of keloids

Chapter 5

In a stepped care approach patients start with non-invasive treatment strategies. Fortunately, surgery is not necessary for all patients with keloids. The most used modalities in the Netherlands are silicone gels or sheets and injections with corticosteroids. Corticosteroids are widely used and although outcomes differ widely in literature, in general good results can be obtained. However, some keloids do not respond to corticosteroids, often requiring surgery with brachytherapy because no effect of postoperative corticosteroids is expected after surgery if there was no effect of corticosteroid injections only. In Asia and other areas around the world injections with other agents as the chemotherapeutic agent 5-fluorouracil (5-FU) are also available, but in the Netherlands this off-label use of 5-FU is uncommon because of a lack of irrefutable proof of efficacy. We systematically reviewed previous research on the use of 5-FU in keloid treatment to see whether 5-FU injections should be made available to keloid patients in the Netherlands.

Chapters 6 and 7

Very good results have been reported with intralesional cryotherapy [20-23]. But this therapy was not formally compared to other treatments that are often used for keloids. There were clear advantages of intralesional cryotherapy over our current practice in terms of treatment time and costs. A randomized clinical trial comparing intralesional cryotherapy to excision with adjuvant corticosteroids, or for more resistant keloids, to excision with adjuvant brachytherapy was designed. The trial protocol is presented in **chapter 6**. After unexpectedly disappointing results of intralesional cryotherapy compared to the surgical treatments and due to a problematic inclusion rate the trial was

prematurely stopped, but we were still able to analyze our data as planned with mixed models described in **chapter 7**.

Chapter 8

Apart from the search for alternative treatment options to reduce ionizing radiation, it is also feasible to reduce the treatment dose instead of the number of treatments. Radiotherapy is a worldwide used treatment modality, but many different dose schemes and sources are in use. In the Netherlands several hospitals use Iridium-192 high dose rate brachytherapy, but all centers use different radiation dosing schemes. A retrospective analysis of the results in three Dutch University Medical Centers was done to establish the preferred dose scheme for brachytherapy.

In the general discussion (**chapter 9**) the results of the present thesis are put into a broader perspective and compared to other recent work on the topic of keloid treatment. In addition, I share my opinion on current practice in keloid treatment, the biggest challenges, the way I think we can improve patient care and how we can reach evidence based medicine in keloid treatment. This thesis contains a summary in English (**chapter 10**) as well as in Dutch (**chapter 11**).

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PART I

Burden of Keloids

Emotional Quality of Life is Severely Affected by Keloid Disease: Pain and Itch Are the Main Determinants of Burden

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Mureau MAM

* contributed equally

Composed of
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And

Burden of Keloid Disease: A Cross-sectional Health-related Quality of Life Assessment
Acta Dermato Venereologica. 2017;97(2):225-229.

Abstract

Keloid scars may be painful, itch severely and be cosmetically disturbing. The burden of keloid disease, however, has not yet been determined. This study evaluated the association of keloid disease with health-related quality of life (HRQL) and identified indicators of burden using a cross-sectional survey study, with one disease-specific HRQL measure (Skindex-29) and 2 generic HRQL measures (SF-36 and EQ-5D-5L). A total of 106 keloid patients with no other skin diseases participated in the study. Having keloid disease was associated with a considerable impairment of emotional wellbeing, with most impairment on the emotional and mental HRQL. Pain and itch were the strongest indicators of HRQL impairment in keloid patients. Having painful or itchy keloids was related to low mental and emotional HRQL, implying that patients with keloids require access to effective treatment aimed at alleviating physical symptoms.

Background

Keloids are abnormal scars, which act like benign tumours growing beyond the margins of the original wound [1]. Additional physical symptoms, such as itch and pain, occur in up to 80% of patients [1, 2]. Keloid disease can lead to aesthetic, physical and psychological complaints in affected individuals [3, 4]. Treatment has varying results and is associated with a high degree of resistance to treatment and recurrence [5].

The advent of health-related quality of life (HRQL) measures has greatly improved our insight into the burden of skin diseases. Skin diseases (e.g. psoriasis) may negatively affect HRQL to a degree comparable to or exceeding that of life-threatening illnesses, such as myocardial infarction and heart failure [6]. Thus, the impact of skin diseases should not be underestimated, and HRQL research is warranted for all skin conditions.

The limited research available on HRQL of patients with scars shows negative effects on physical, psychological and social well-being [3, 4, 7-9]. These studies, however, have substantial limitations, most importantly, a failure to differentiate between hypertrophic scars (HTS) and keloids, while these conditions are distinctly different from one another. HTS stay within the original wound margins, are self-limiting, and respond considerably better to treatment with lower recurrence rates [10]. Preservation of HRQL is more likely with favourable symptoms, prognosis and duration of HTS compared with keloids. Research on these two conditions combined probably underestimates the burden of the more severe condition, keloid disease. In addition, none of these studies evaluated the effect of keloids on HRQL using both disease-specific and generic health measures. Generic health measures allow comparison of the burden of keloids with that of other major diseases. Thus, the degree of burden of keloids can be illustrated and the need for effective treatment can be formally prioritized. The combined use of different HRQL instruments provides such a broad and sensitive assessment of the burden of skin diseases [11].

In the current study we aimed: (I) to determine the HRQL of patients with keloid disease; and (II) to identify indicators of high disease burden.

Methods

The present study was a multi-centre cross-sectional online survey.

Participants

All adults diagnosed with keloid disease by an experienced physician from the participating Departments of Plastic and Reconstructive Surgery and Dermatology at two university hospitals were eligible. Diagnosis of keloid disease was made on clinical

presentation, most important continuous growth, beyond wound borders, without spontaneous regression after one year. Patients were excluded if there was any doubt about the diagnosis, or if the diagnosis differed between physicians. Patients, who were not proficient in Dutch, no longer had a keloid, or had additional skin diseases, were excluded. All respondents completed an online informed consent form and a series of self-administered questionnaires between February and May 2014. Non-responders were contacted by telephone after three weeks to invite them to participate.

Questionnaires

Patient and Observer Scar Assessment Scale (POSAS). Scar severity was subjectively assessed using the patient scale of this validated scar assessment tool (PSAS), consisting of 6 items on pain, itch, colour, stiffness, thickness, and irregularity, as well as an overall opinion on the scar. All items as well as the overall opinion were rated on a 10-point scale [12]. Higher scores represent a more severe scar. A threshold of >3 on the pain and itch items was used to indicate substantial symptoms [13, 14].

Skindex-29. This dermatology specific quality of life questionnaire consists of 30 questions with a five-point Likert-scale. HRQL was scored on an emotional, functional, symptomatic, and summary scale, ranging from 0 to 100 with higher scores indicating worse HRQL [15]. For improved interpretability of the results, patients were grouped into having minimal, mild, moderate, or severe impairment of HRQL, according to anchor-based cut-off scores reported by Prinsen et al. [16].

SF-36. This widely used generic HRQL questionnaire contains 36 questions that provide scores on 8 different dimensions of functional health and well-being. Scores are given on a 100-point scale, with higher scores indicating better quality of life. Norm-based physical component summary scores (PCS) and mental component summary scores (MCS) (mean 50, SD 10) were calculated using pooled-age-matched norm scores from a Dutch urban (Amsterdam) reference population [17].

EuroQol 5D (EQ-5D-5L). This utility measure describes mobility, self-care, usual activities, pain/discomfort, and anxiety/depression on a 5-point scale. All possible answer combinations correspond to a single number, where 1 corresponds to perfect health and 0 to death. It complements other HRQL measures by representing HRQL in a single number, allowing easy comparison of health-states, and it is extensively used in healthcare decision-making and health economics [18].

Independent measures

Socio-demographic and clinical characteristics, including sex, age, skin colour as described by Fitzpatrick, location and visibility of the keloid, number of keloids (quantity), disease duration, origin of the keloid, previous treatments, and comorbidities, were collected.

Statistical analysis

Characteristics of the study population were analysed using descriptive statistics. Outcomes of keloid disease were compared with the Student's t-test to values of other diseases as reported in literature. Two-sided p-values ≤ 0.05 were regarded statistically significant. Cohen's d effect sizes were calculated; values > 0.20 were considered small effects, > 0.50 medium effects, and > 0.80 large effects [19].

Correlations were calculated between the independent variables and the 4 Skindex-29 scales, the SF-36 PCS and MCS scales, and EQ-5D-5L index scale. Pearson's correlation coefficients (r_p) were calculated for normally distributed data, and Spearman's correlation coefficients (r_s) for not normally distributed data.

Seven multiple linear regression models were made to assess the predictive value of the independent variables (sex, age, visibility of the keloid, number of keloids as well as all the PSAS variables: pain, itch, colour, stiffness, thickness, and irregularity) on HRQL outcomes. Non-normal dependent variables were root-transformed in order to obtain a normal distribution. Data were entered, followed by a backward procedure, in which non-significant effects (p > 0.10) were removed from the models. Regression coefficients were standardized (betas) to allow for better comparison of different factors in the model, independent of the units of measurement of the variables. A beta of 0.1 indicates a small effect, 0.3 a medium effect, and 0.5 a large effect. R^2 represents the amount of variability in the outcome that is accounted for by indicators used in the model.

All analyses were executed using IBM® SPSS Statistics version 22 for Mac OSX. Two-sided p-values ≤ 0.05 were regarded as statistically significant.

Ethical considerations

The Ethics Board Committees of both participating academic hospitals concluded that this study was exempt from approval because of absence of any risk to participants.

Results

Patient characteristics

Of the 280 eligible patients who were invited by post to participate in the study, 70 could not be reached after 3 attempts, 17 no longer had a keloid, 38 indicated they did not wish to participate, 8 had another skin disease besides keloids, and 41 did not complete the online survey, leaving 106 patients who successfully completed the questionnaires (36 from a dermatology department, and 70 from a plastic surgery department). The response rate was 57%. Socio-demographic and clinical characteristics of the responders are shown in Table 1. Of the non-responders, 49% were male and the

mean age was 39.1 years (SD 13.0, range 18–72 years), which was comparable to the responders (Table 1).

Table 1. Characteristics of the keloid patients analyzed in the paper

Characteristic	N=106	Mean (SD)	Range
Age (years)		38.6 (11.9)	18-63
Disease duration (years)		13.8 (10.3)	1-40
		N	**Percentage**
Sex	Male	51	48.1
	Female	55	51.9
Skin color (Fitzpatrick)	1-2 Light	23	21.7
	3-4 Colored	44	41.5
	5-6 Dark	38	35.8
Number of keloids	1	39	36.8
	2-4	31	29.2
	5 or more	36	34.0
Secondary Symptoms	Pain & itch ≤3	27	25.5
	Pain >3	3	2.8
	Itch >3	22	20.8
	Pain & itch >3	54	50.9
Location of keloids *	Head	9	8.5
	Ear	16	15.1
	Neck	7	6.6
	Shoulders	40	37.7
	Chest	63	59.4
	Back	11	10.4
	Abdomen	17	16.0
	Arm	9	8.5
	Leg	7	6.6
Keloids visible (wearing normal clothing)	No	40	37.7
	Yes	66	62.3
Origin of keloid	Surgical procedure	36	34.0
	Piercing	7	6.6
	Vaccination	3	2.8
	Acne	29	27.4
	Traumatic injury	6	5.7
	Unknown	25	23.6
Previous keloid treatment *	None	4	3.8

Table 1. Characteristics of the keloid patients analyzed in the paper (*continued*)

Characteristic	N=106	Mean (SD)	Range
	Silicone sheets	56	52.8
	Pressure therapy	4	3.8
	Intralesional corticosteroid	82	77.4
	Excision	25	23.6
	Excision with additive	27	25.5
	Radiation therapy	12	11.3
	Cryotherapy	27	25.5
	Laser	32	30.2
		Mean (SD)	**Range**
PSAS	Pain	4.21 (2.82)	1-10
	Itch	5.84 (2.97)	1-10
	Color	6.68 (2.52)	1-10
	Stiffness	7.00 (2.43)	1-10
	Thickness	7.73 (2.05)	1-10
	Irregularity	7.54 (2.21)	1-10
	Overall opinion	8.13 (2.05)	2-10

N: number of patients. SD: standard deviation of the mean. PSAS: Patient part of the Patient and Observer Scar Assessment Scale. * Multiple answers were possible, summed percentages exceed 100.

Dermatology specific HRQL of keloid patients

Almost half of the patients (and 60% of the females) had severe emotional symptoms and about a quarter reported severe problems on the symptomatic and functional scale of skin symptoms and functional problems as assessed with the Skindex-29 questionnaire (Table 2). Skindex-29 scores of keloid patients from the present study were compared with those of eight other skin diseases and with subjects without a skin disease (Table 3) [20]. All subscales were affected in keloid patients comparable to other skin diseases.

Table 2. Overview of Skindex-29 outcomes of the keloid patients analyzed

		Impairment Level (%)			
Skindex-29	**Mean (SD)**	**Minimal**	**Mild**	**Moderate**	**Severe**
Emotional scale	40.9 (26.6)	26.4	17.9	7.5	48.1
Symptomatic scale	36.9 (22.0)	51.9	4.7	16.0	27.4
Functional scale	22.9 (23.9)	62.3	10.4	2.8	24.5
Sum scale	32.5 (22.1)	41.5	17.9	15.1	25.5

SD: standard deviation of the mean. Skindex-29 (dermatology specific quality of life instrument) scores on an 1-100 scale, higher scores indicate worse health related quality of life (HRQL). Keloid patients are grouped into minimal, mild, moderate and severe impairment on the Skindex-29 scale using cut-off scores reported by Prinsen et al. (2010), the distribution over impairment levels for each skindex-29 scale is shown.

Table 3. Skindex-29 scores of patients with keloid disease compared to other skin diseases adapted from Klein et al. (2011).

	Sample size	Emotional scale Mean (SD)	Rank	Symptomatic scale Mean (SD)	Rank	Functional scale Mean (SD)	Rank
Keloid disease	106	41 (26)	5	37 (22)	5	23 (24)	5
Without skin disease	107	9 (13)	1	14 (12)	1	4 (8)	1
NMSC/AK	136	20 (19)	2	29 (20)	2	9 (14)	2
Rosacea	29	33 (20)	3	33 (20)	4	16 (18)	3
Psoriasis	44	39 (27)	4	42 (21)	7	23 (27)	5
Acne vulgaris	63	41 (25)	5	30 (19)	3	16 (16)	3
Eczema	102	41 (27)	5	48 (23)	9	26 (26)	7
Dermatomyositis	22	45 (27)	8	42 (25)	7	28 (29)	8
CLE	157	48 (28)	9	40 (23)	6	28 (25)	8
Vulvodynia	280	50 (20)	10	50 (17)	10	44 (22)	10

SD: standard deviation of the mean. NMSC/AK: non-melanoma skin cancer and actinic keratosis. CLE: cutaneous lupus erythematosus. Skindex-29 subscale scores for each dermatologic disease (adapted from Klein et al. 2011) compared with those in keloid disease. Higher scores and rank indicates better health related quality of life.

Generic health-related quality of life of keloid patients

The outcomes for all the individual dimensions of the SF-36, as well as the PCSs and MCSs of the keloid patients were compared with an age-matched Dutch reference population, including healthy and unhealthy subjects with a living area and sex distribution comparable to our study population (Table 4) [17]. Compared with the reference population, keloid patients scored considerably lower on the SF-36 dimensions bodily pain, vitality, and social functioning as well as on the MCS, meaning that keloid patients reported a worse mental HRQL. The effect size for the MCS was –0.28, indicating a small effect [19]. This is in contrast to the PCS, which was similar to that of the reference population (Table 4).

Table 4. Overview of SF-36 scores of the keloid patients compared to an age-matched reference population.

	Keloid disease population N=106 Mean (SD)	Reference population N=3800 Mean (SD)	Effect size Cohen's d	Student's t test p-value
Physical Function	90.4 (16.3)	88.6 (19.0)	0.12	0.17
Role Physical	86.8 (29.1)	81.5 (33.4)	0.16	0.07
Bodily Pain	72.8 (25.2)	81.7 (23.3)	-0.38	<0.01
General Health	73.4 (19.0)	72.6 (19.9)	0.04	0.67
Vitality	63.3 (21.2)	68.7 (18.9)	-0.28	0.01
Social Function	80.1 (24.1)	85.9 (20.2)	-0.29	0.02
Role Emotion	81.4 (36.0)	83.2 (32.5)	-0.05	0.63
Mental Health	72.0 (20.7)	75.7 (17.5)	-0.21	0.07
PCS	50.4 (8.7)	50.0 (10.0)	0.05	0.60
MCS	47.2 (12.0)	50.0 (10.0)	-0.28	0.02

SF-36: the 36-item Short Form Health Survey. N: number of patients. SD: standard deviation of the mean. PCS: physical component summary score. MCS: mental component summary score. Pooled-SD, age-matched, urban (Amsterdam) reference population adapted from Aaronson et al. (1998) with comparable proportions males (46%). PCSs and MCSs are norm transformed to the reference population with a mean=50 and SD=10. The study population is compared to the reference population; the effect size is given with Cohen's d (0.20 small effect, 0.50 moderate effect, 0.80 large effect).

To put the effect of keloid disease on HRQL further into perspective, the PCSs and MCSs of the keloid patients were compared to those of other common chronic medical conditions as well as a group of healthy adults (Table 5) [6, 21]. Physical HRQL of keloid patients was best of all conditions (mean 50.45, SD 8.7), with only healthy adults scoring better. Mental HRQL (mean 47.2; SD 12.0), on the other hand, was affected to a degree comparable with other chronic medical conditions like psoriasis, dermatitis, arthritis, and cancer.

Table 5. SF-36 summary scores of patients with keloid disease compared to other medical conditions adapted from Rapp et al. [6].

	Sample size	PCS Mean (SD)	Rank	MCS Mean (SD)	Rank
Keloid disease	106	50.5 (8.7)	2	47.2 (12.0)	8
Healthy adults	468	55.3 (5.1)	1	53.4 (6.3)	1
Dermatitis	214	46.9 (11.5)	3	46.2 (12.1)	9
Cancer	105	45.1 (11.6)	4	48.8 (11.1)	6
Depression	504	45.0 (12.1)	5	34.8 (12.2)	12
Hypertension	2089	44.3 (10.8)	6	52.2 (9.3)	2
Arthritis	826	43.2 (11.6)	7	48.8 (11.1)	7
Myocardial infarction	107	42.6 (10.0)	8	51.7 (8.2)	4
Chronic lung disease	182	42.3 (14.1)	9	44.5 (12.3)	11
Type 2 diabetes	541	41.5 (11.3)	10	51.9 (9.6)	3
Psoriasis	317	41.2 (14.2)	11	45.7 (11.4)	10
Congestive heart failure	216	34.5 (12.1)	12	50.4 (11.1)	5

SF-36: the 36-item Short Form Health Survey. SD: standard deviation of the mean. PCS: Physical Component Summary of SF-36. MCS: Mental Component Summary of SF-36. Higher scores and rank indicates better health related quality of life. PCS and MCS scores are norm transformed to the reference population, with a mean=50 and SD=10. Summary scores for each chronic medical condition (respondents were asked whether their doctor had ever told them that they had the condition or they now have the condition, adapted from Rapp et al. (1999)) compared with those in keloid disease.

Table 6. Bivariate correlations of health related quality of life outcomes and patient characteristics of keloid disease.

	Skindex-29 (N=106)				SF-36 (N=106)		EQ-5D-5L (N=100)
	Emotional Scale	**Functional Scale**	**Symptomatic Scale**	**Sum Scale**	**PCS**	**MCS**	**Index value**
Correlation	r (p)	r (p)	r (p)	r (p)	r (p)	r (p)	r (p)
Sex	[P]**0.23 (.017)**	[P]0.13 (.188)	[P]**0.24 (.013)**	[P]**0.23 (.017)**	[P]0.01 (.961)	[P]-0.10 (.309)	**-0.18 (.073)**
Age	[P]-0.02 (.809)	[P]0.11 (.248)	[P]0.15 (.131)	[P]0.06 (.524)	[P]**-0.23 (.016)**	[P]0.10 (.331)	-0.02 (.819)
Skin color [D]	[P]-0.03 (.729)	[P]0.05 (.599)	[P]-0.01 (.957)	[P]-0.01 (.896)	[P]-0.13 (.177)	[P]-0.04 (.666)	0.01 (.956)
Visibility	[P]**0.16 (.100)**	[P]0.14 (.156)	[P]-0.02 (.877)	[P]0.12 (.240)	[P]-0.07 (.497)	[P]**-0.18 (.074)**	-0.03 (.736)
Number [D]	[P]**0.24 (.014)**	[P]**0.28 (.019)**	[P]0.14 (.143)	[P]**0.23 (.016)**	[P]-0.05 (.606)	[P]-0.05 (.624)	-0.14 (.181)
Disease duration	[P]0.09 (.356)	[P]0.12 (.226)	[P]0.15 (.139)	[P]0.13 (.198)	[P]-0.01 (.902)	[P]0.03 (.800)	-0.04 (.670)
Pain	**0.55 (<.001)**	**0.47 (<.001)**	**0.69 (<.001)**	**0.60 (<.001)**	**-0.24 (.012)**	**-0.26 (.006)**	**-0.54 (<.001)**
Itch	**0.52 (<.001)**	**0.44 (<.001)**	**0.75 (<.001)**	**0.60 (<.001)**	**-0.29 (.003)**	**-0.28 (.003)**	**-0.54 (<.001)**
Scar Color	**0.27 (.004)**	**0.18 (.063)**	**0.29 (.002)**	**0.28 (.004)**	-.00 (.985)	-0.09 (.388)	-0.08 (.437)
Stiffness	**0.33 (.001)**	**0.24 (.012)**	**0.44 (<.001)**	**0.35 (<.001)**	**-0.19 (.054)**	-0.15 (.133)	**-0.29 (.004)**
Thickness	**0.40 (<.001)**	**0.32 (.001)**	**0.50 (<.001)**	**0.44 (<.001)**	**-0.21 (.031)**	**-0.18 (.061)**	**-0.32 (.001)**
Irregularity	**0.43 (<.001)**	**0.39 (<.001)**	**0.44 (<.001)**	**0.46 (<.001)**	-0.12 (.235)	**-0.17 (.085)**	**-0.24 (.014)**

All bivariate correlations are shown, correlations with p<.10 are printed bold. The presented correlation coefficients are Spearman correlation coefficients unless indicated by [P] in which case it represents Pearson correlation coefficients. Skin color [D]: a dichotomized variable on skin color with light skin (Fitzpatrick type 1-2) and colored skin (Fitzpatrick type 3-6). Number [D]: a dichotomized variable on number of keloids with 1 keloid and 2 or more keloids. N: number of patients in analyzed group. SF-36: the 36-item Short Form Health Survey. PCS: Physical Component Summary of SF-36. MCS: Mental Component Summary of SF-36.

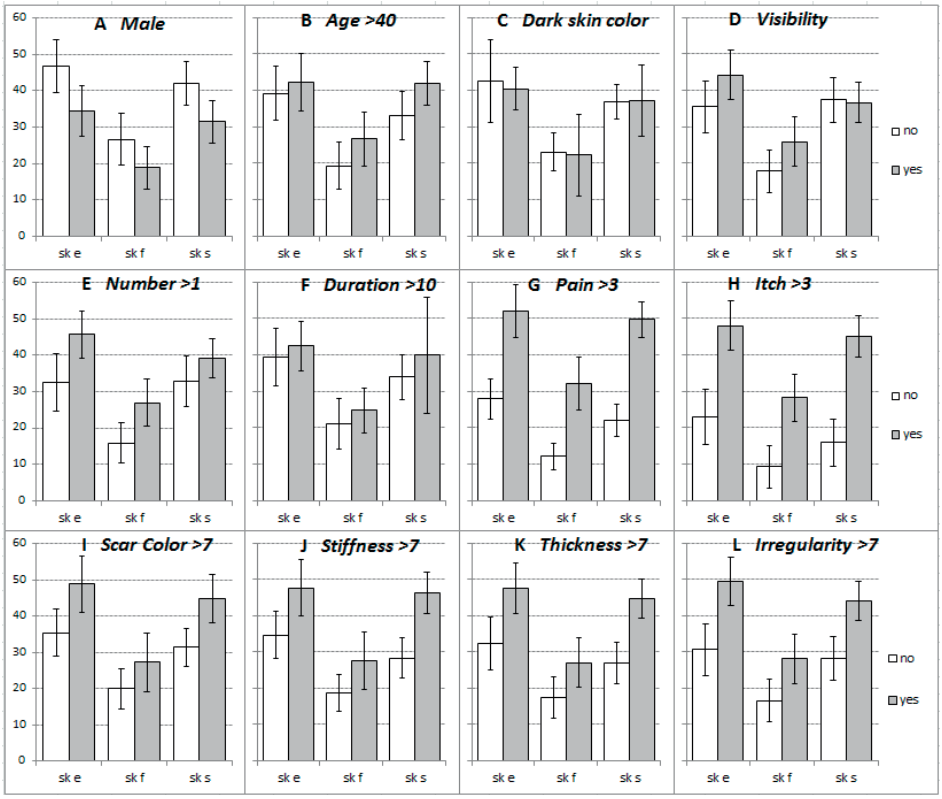

Figure 1. Skindex-29 scores for groups divided by patient and keloid characteristics
Skindex-29 scores are represented as means with 95% confidence intervals. B age in years. D visibility of keloids while wearing normal clothing. E number of keloids. F duration of keloid disease in years. G-L are values derived from the Patient Scar Assessment Scale. Sk e: emotional scale of Skindex-29. Sk f: functional scale of Skindex-29. Sk s: symptomatic scale of Skindex-29.

Associated factors and predictors of health-related quality of life of keloid patients

The relationship between HRQL and the individual independent variables sex, age, skin colour, visibility of keloids, quantity of keloids, disease duration, hospital department, and all PSAS items were analysed.

Pain and itch were correlated to all Skindex-29 scales (ranging from r_s 0.44 to 0.75, $p < 0.001$), to both SF-36 component summary scores (ranging from r_s –0.24 to –0.29, $p < 0.012$) and the EQ-5D-5L index (–0.54, $p < 0.001$), meaning pain and itch were associated with nearly all HRQL measures. In addition, scar stiffness, thickness, and irregularity showed high correlations with HRQL outcomes. Duration of disease, skin colour, and department type (dermatology vs plastic surgery) showed no significant association

with HRQL. Female sex correlated with worse outcomes on emotional, symptomatic, and sum scores of the Skindex-29 (r_p 0.23–0.24, $p < 0.017$).

Regression analyses revealed that pain was a negative HRQL indicator in 6 (moderate effect on all Skindex-29 scales, small effect on PCS, and moderate effect on EQ-5D-5L index), and itch in 4 (large effect on symptomatic Skindex-29 and moderate effect on Skindex-29 sum, MCS and EQ-5D-5L) models, respectively, making these the most consistent and strongest indicators of HRQL. Besides pain and itch, other indicators were age (moderate effect on PCS), keloid visibility (small effect on MCS), number of keloids (small effect on emotional, functional and sum Skindex-29), scar stiffness (moderate effect on PCS and EQ-5D-5L index) and irregularity (small effect on emotional, functional and sum Skindex-29). Keloid colour was present as indicator in 2 models, but a more aberrant scar colour improved the HRQL, while there was no proof of multi-collinearity. The Skindex-29 models could explain between 29% and 60% of the variability in outcome (R^2) and for the PCS, MCS, and EQ-5D-5L index it was 17%, 11%, and 34%, respectively (Table 7).

Table 7. Summary of the multivariate regression analyses for the health related quality of life outcomes as dependent variable and patient characteristics as dependent variables, in the total group and for males and females.

	Skindex-29 (N=106)				SF-36 (N=106)		EQ-5D-5L (N=100)
	Emotional Scale	**Functional Scale**	**Symptomatic Scale**	**Sum Scale**	**PCS**	**MCS**	**Index value**
Regression R^2	0.36	0.29	0.60	0.47	0.17	0.11	0.34
	β (p)	*β* (p)	*β* (p)	*β* (p)	*β* (p)	*β* (p)	*β* (p)
Sex							
Age					-0.25 (0.008)		
Visibility						-0.19 (0.048)	
Number	0.19 (0.21)	0.18 (0.030)		0.16 (0.028)			
Pain	0.45 (<0.001)	0.35 (<0.001)	0.29 (0.002)	0.32 (0.004)	-0.18 (0.077)		-0.28 (0.027)
Itch			0.53 (<0.001)	0.26 (0.030)		-0.28 (0.004)	-0.26 (0.059)
Scar Color					0.31 (0.014)		0.40 (0.001)
Stiffness					-0.33 (0.013)		-0.30 (0.017)
Thickness							
Irregularity	0.19 (0.029)	0.23 (0.013)		0.17 (0.054)			

N: number of patients in analyzed group. *β*: regression coefficient. R^2: proportion of variance explained by the model. SF-36: the 36-item Short Form Health Survey. PCS: physical component summary score of SF-36. MCS: mental component summary score of SF-36. Number: number of keloids.

Discussion

In this study HRQL, specifically emotional wellbeing, of patients with keloid disease was considerably lower than in a reference population. On the emotional scale of the Skindex-29, 48% of all keloid patients and even up to 60% of women reported a severe HRQL impairment. On the other hand, impact on the symptomatic and functional Skindex-29 scales was less, with 27% and 25% of patients' HRQL severely affected, respectively, and over 50% of patients' HRQL only minimally impaired. Also, the generic HRQL instrument SF-36 showed impairment of mental HRQL in keloid patients and a physical HRQL comparable to that of the reference population. Reinholz et al. [9] found similar results on HRQL of keloid patients using the Dermatology Life Quality Index (DLQI); specifically symptoms and feelings were affected.

Factors associated with worse HRQL of keloid patients were pain and itch symptoms that are more prominent in keloids than in other scar types. Remarkably, cosmetic issues correlated less, or even inconsistently with HRQL. These findings support priority setting, as surgery for cosmetic issues can be interpreted as "luxury healthcare", instead of a medical need, which relates to its current lower priority in health policy decision-making [22]. The current study showed that HRQL can be considerably impaired in patients with keloid disease, causing reasonable doubt on current priority setting.

Skin colour, age, and disease duration did not interact with HRQL. Generally, pain and itch are frequently reported symptoms of keloid disease [1]. Of the patients with pain and itch scores >3, 70% had severe emotional HRQL impairment (Skindex-29) compared with 16% in the group of patients that had low pain and itch scores. Moreover, we showed that pain and itch were consistently and strongly associated with HRQL impairment.

The negative effect of keloid disease on the Skindex-29 was much more pronounced in women than in men (Figure 1). This sex difference was similarly found on the scar assessment scale (PSAS). In contrast, the generic instrument scores were not affected by sex. Sex did not improve the multivariate regression models. Further analysis showed that women rated their pain and itch higher, suggesting that these pain and itch symptoms may have accounted for the sex difference on HRQL.

A previous study on HRQL in patients with scars found less pain (26%) and itch (44%) complaints and minimal correlations of pain and itch with emotional HRQL [4]. These discrepancies can be explained by the large differences between keloids and other scar types, which are less severe than keloids.

Furtado et al. [8] specifically studied keloid patients and similarly found that pain and itch correlated with worse HRQL. They showed non-visible keloids resulted in worse physical HRQL, while we could not find an effect of keloid visibility on physical HRQL. However, we did find impairment of mental HRQL in patients with a visible keloid. Vis-

ible keloids could affect HRQL because they are socially more disturbing. Furtado et al. [8] explained that in their population non-visible keloids were long-existing recalcitrant pre-sternal keloids, resulting in worse HRQL in this group.

High-profile reviews on pathological scarring focused mainly on the morphological and disfiguring aspects of keloid disease, and considered the accompanying symptoms of secondary concern [23-25]. However, the results of our study challenge this view by clearly showing that itch and pain symptoms are the main indicators of HRQL impairment. Consequently, we believe that these symptoms should be of primary concern in the evaluation and treatment of keloid disease, as well as in scientific research on this topic.

A limitation of the current study is that all patients were recruited from academic hospitals, possibly resulting in a selection bias towards patients with a relatively high burden of disease. This could limit the generalizability of the current results to the entire keloid patient population. On the other hand, the patients from the present study represent those who seek treatment from a medical specialist. The sample was evenly distributed on sex and contained a variety of skin types, disease durations, age groups, and other clinical characteristics and had similar composition to other population-samples described in the literature [9]. The incomplete response rate could also have introduced selection bias.

Another issue could be the cross-sectional design with online questionnaires for scar quality and HRQL assessment that completely relied on self-reports. Patients may think that exaggerating their burden may result in better treatment, a higher chance of insurance fees or sick leave. These disease induced benefits, or secondary gains, may have influenced their answers. However, a large part of our study group was not currently under treatment or clinically re-evaluated, and was probably less affected by secondary gains.

Conclusion

Keloid disease is strongly associated with mental and emotional HRQL impairment, which suggests a high need for effective treatment and thus a priority in healthcare policymaking. HRQL is most severely affected in patients with an itching or painful keloid, suggesting that, in these patients, cosmetic appearance is less of a concern than physical symptoms.

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A Systematic Review on Prevalence, Etiology and Pathophysiology of Intrinsic Pain in Dermal Scar Tissue

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Abstract

Background Scars can cause pain, even without symptoms of underlying nerve damage. A lack of knowledge on intrinsic scar pain hampers effective treatment of these complaints.

Objective Aggregate current knowledge on prevalence, etiology and pathophysiology of intrinsic pain in dermal scars.

Study Design Systematic review

Setting University Medical Center

Methods We searched the Embase, Medline, Cochrane central, CINAHL, Web-of-Science and Pubmed databases with search terms: scar, skin, pain, and etiology/pathology, adding all synonyms of these terms. Relevant papers were selected and analyzed by three reviewers.

Results Intrinsic pain in scars has low prevalence. However, pathologic scars and burns regularly cause pain of high intensity. Etiology is multifactorial, the extent of trauma was an important predicting factor. Nerve fiber density did not explain the intrinsic pain when pan-neuronal markers were used, while a correlation with an increased number of a subtype of C-fibers seems plausible. Nerve growth factor (that stimulate these C-fibers) plays an important role in wound healing. Thereby, it also sensitizes neurons and promotes inflammation, releasing even more neurotrophic factors. Central sensitization causes a long-lasting effect even after wounds are healed. Furthermore, the opioid-system, that influences inflammation and healing and possible systemic sensory alterations after injury are discussed.

Limitations Liberal selection criteria challenged the systematic selection of papers.

Conclusions Burn and pathologic scars often lead to high intensity pain symptoms. This pain has many characteristics of neuropathic pain that could be caused by an imbalance of C-fibers subtypes. The scar tissue itself may alter the nerve fiber distribution; the imbalance results in ongoing neuro-inflammation and pain symptoms.

Background

After injury our skin heals by forming scar tissue. Dermal scars can decrease quality of life by esthetic, psychological and physical complaints. The latter consists of movement restriction, itch and pain, of which pain gives rise to the most severe burden [1, 2]. Burn patients and patients with pathologic scars often visit dermatologists or plastic surgeons, who often experience difficulties treating the pain complaints. This leaves these patients without proper treatment. A better understanding of the mechanisms involved in painful scars and their treatment options could improve pain relief for these patients.

Wounds heal by a complex process involving several phases. During the inflammatory phase, which starts directly after injury, a blood clot is formed and pro-inflammatory cytokines produced by cells like macrophages and mast cells attract neutrophils to the wound. Around the fourth day after trauma debris and bacteria are removed by the macrophages and neutrophils, furthermore angiogenesis starts, and fibroblasts get activated. Hereafter, the proliferative phase lasts for around two to six weeks. Several growth factors stimulate the fibroblasts and keratinocytes. Angiogenesis makes growth of granulation tissue possible and epithelialization takes place. After the wound is completely closed, the scar matures for approximately one year. New tissue with collagen fibers arranged at random transforms to a well-organized network under mechanical stress adding strength to the scar. This occurs by a continuous process of degradation and generation of collagen [3].

A normotrophic scar appears as a thin white line in plane with the surrounding skin. Scars can present with different, abnormal phenotypes, like atrophic scars (stretched out and thinner than surrounding skin), contracted scars (shorter than the original wound), hypertrophic scars (raised, red and itching or painful) or keloids (growing into surrounding skin and forming, discolored and itching or painful tumors). Abnormal scar types are most likely the result of dysregulation in the wound healing process, but the exact mechanisms are not clarified yet.

Pain is a common symptom during wound healing and it generally occurs in the initial phase as a result of tissue damage. Pain complaints generally fade during the phases of wound healing and ceases in the maturation phase of healing when a scar has formed. If a matured scar is still painful, one possible cause of this pain is a neuroma, which originates from a regenerating nerve trapped in fibrotic dermal scar tissue. Neuromas have a typical clinical presentation (positive Tinel sign, numbness in the innervation area of the injured nerve) and treatment can be directed to eliminate the neuroma [4]. Unfortunately, some patients have painful scars without the typical symptoms accompanying a neuroma. The prevalence of pain symptoms in different scar types like hypertrophic scars and keloids and the mechanisms behind it are largely unknown [2].

In order to generate an effective treatment against painful scars we need to know the extent of the problem and the underlying mechanisms causing pain. Therefore, we aimed to perform a comprehensive systematic review on the prevalence and intensity of pain, and the knowledge of pathophysiology and etiology of pain in dermal scars.

Methods

Literature Search Methods

We searched the Embase, Medline, Cochrane central, CINAHL, Web-of-Science and Pubmed databases. In each database, we used the following search terms: scar, skin, pain, and etiology/pathology, adding all synonyms of these terms. The full search terms used can be found in the appendix. The search was performed from inception of the databases until December 2, 2014.

We included all English articles that mentioned the prevalence, intensity or a cause of pain in dermal scar tissue. To exclude pain caused by wounds and neuromas, we used the following definition of a painful scar: persistent pain in a healed scar over 3 months' duration, with allodynia and hyperalgesia adjacent to the scar, and with no sensory loss other than over the scar itself [5]. In order to include all relevant information we also selected reviews. We excluded papers solely based on the treatment of (painful) scars, scars in other than dermal tissue, neuromas, and papers otherwise irrelevant to the research question.

First, to select relevant papers two reviewers (EB, LU) independently assessed titles and abstracts. In cases of disagreement, a third reviewer (CK) also assessed title and abstract and made the final decision on eligibility. To make a final decision about inclusion, the full-text of the eligible articles were assessed by two reviewers (EB, LU), if there was no consensus the article was discussed by the reviewers until consensus was reached. The references of all selected articles were crosschecked and relevant papers were included (Figure 1).

Literature Analysis Methods

For all included papers, two reviewers (EB, LU) independently extracted relevant information to answer our research question. We searched data on; 1) prevalence and intensity of pain in all types of dermal scars, 2) etiology, what biological, psychological or environmental factors aggravate pain in dermal scars, and 3) pathophysiology, what is the underlying biological mechanism that causes pain in some scars and not in others. The collected information of both reviewers was verified by the other reviewer.

The papers that described pain intensity used different instruments, often the visual analog scale (VAS, range 0-10) or numeric rating scale (NRS, written or verbal, range

0-10) were used, if other scales were used (i.e. scores ranging 1-5) score were converted to a 0-10 scale to improve comparability between papers.

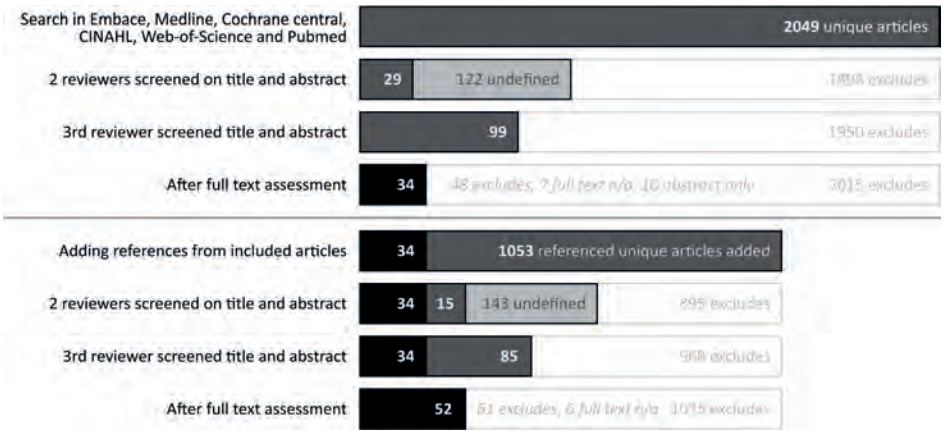

Figure 1. Diagram illustrating the systematic review of literature for selection of studies.
n/a: not available

Results

Our search resulted in 2049 records and after completion of the entire selection process 52 papers remained with relevant information about painful scars (Figure 1).

Epidemiology

We found 18 papers with reference to prevalence of scar pain and pain intensity (Table 1) [6-23]. Most studies used questionnaires not suitable for differentiating between neuromas and painful scars. Many studies investigated a specific subgroup of dermal scars with a high prevalence of pain symptoms, mainly burn scars. These studies did not report the prevalence of pain in other scar types.

Of the 14 papers that reported on pain prevalence eight used a questionnaire, of which only four included validated questionnaires. Others used merely a VAS scale, single questions, or chart notes. Of the patients with a burn scar 25-68% suffered from pain. However, after reviewing the results from these studies nerve damage underneath the scar could not be ruled out. Scar pain after surgery with low chance of nerve damage occurred in about 10% of patients, with 2% experiencing substantial pain (>3/10). Surgery with higher risk of nerve damage, like amputation and thoracotomy, more often caused painful scars (30-50%) [12]. Many of these patients (80% as described by Hoimyr et al. [10]) showed sensory disturbances distal to the scar, suggesting nerve damage. These

results indicate a prevalence of painful scars without nerve damage in less than 2% of patients.

Fourteen papers reported on pain intensity; in five papers pain intensity was recorded with a self-completed anchored visual analog scale ranging from 0-10cm. Four other papers used a numeric rating scale from 0-10 or from 1-10. The intensity of pain reported by burn patients varied from 1.3 to 5.6 on a 10-point scale. Patients with hypertrophic scars reported a lower intensity of pain (2.2) compared to patients with keloids (5.4-6.3) (Table 1). Patients experience pain from their scars, while sensibility and pain thresholds in the scars are raised when they are objectively tested [16, 22]. Some studies found that pain reduced with time [15], while other studies could not confirm this [17].

None of the studies did a validated quality of life assessment to assess the burden of a scar, but some did inquire information regarding impairment of daily life functioning. For surgical scars Hoimyr et al. [10] found 6.6% of patients that had a surgical scar had pain that impacted their daily life while Maguire et al. [15] found 18% of the patients after thoracotomy (40% of patients with pain) had pain that limited daily activities. In studies looking at burn scars several aspects are described like difficulties sleeping, or problems performing work or social activities, prevalence ranges from 10-20 % with exception of Choiniere et al. [7] and Dauber et al. [8] who show a prevalence of 45% and 55-75% respectively on these topics [7, 8, 17-19].

In summary, pain in dermal scars without nerve damage appears to have low prevalence among patients with scars [12], while specific subgroups of scars, like pathologic scars and burns, regularly cause pain. Patients with pathologic scars are also affected by pain of higher intensity.

Etiology

We found different factors that raise the risk of painful scars. Like many conditions also painful scars are considered multifactorial and genetic susceptibility has its role (although no specific genes are identified). In post-surgical scars, the surgical procedure and technique are evidently of influence [12, 14, 15]. Further, younger patients are more prone to develop painful scars after surgery [10, 12, 15]. We found contradicting results on whether the length of time after surgery influences pain [10, 15]. However, the length of time after burn injury did not reduce pain [7, 16, 17, 22]. In burns, size and the depth of the burn predicted painful burn scars in most studies [7, 16, 17, 21]. In contrast to post-surgical scars, there was no relation between post-burn pain and age [11, 17, 21, 22]. Kehlet et al. [12] state that females are more often affected with painful scars, but others found no difference with gender [7, 10, 11, 15-17, 24].

Table 1. Overview of reported prevalence and intensity of pain in dermal scars.

	Publication	N	Age (yr)	Sex male (%)	Prevalence of pain	Pain intensity Described or on a 0-10 scale	Study design	Scar age (mo)	Neuromas excluded	Remarks
Surgical scars	Maguire (15)	600	55 (14-85)	56.5	27% 3.6%	Had pain Rated pain severe	Cross-sectional Study Questionnaire	7-84	No	After VATS or thoracotomy
	Hoimyr (10)	349	60.9 ± 16	47.1	9.7% 1.7%	2.0 (median) rated pain >3.0	Cross-sectional Study Questionnaire	μ 23 [4-52]	No	Cutaneous melanoma resections
	Lynch (14)	13	33.1 (9-69)	21.4	27%	Mild	Cross-sectional Study Questionnaire	μ 31 [9-66]	No	Plantar scars only
	Kehlet (12)	-	-	-	30-50% 5-10% 20-40% 5-10% 10% 2-4%	Had pain after Had severe pain Had pain after Had severe pain Had pain after Had severe pain	Review	-	No	Amputation/ Sternotomy Breast surgery/ Thoracotomy Caesarian section/ Inguinal hernia repair
Burn scars	Dauber (8)	358	41 (3-92)	52.8	52%	-	Cross-sectional Questionnaire	μ 136 [0-732]	No	response rate 24% TBSA 59%
	Van der Wal (21)	359 327 232	22.5 ±12	61	-	2.3 2.2 1.9	Longitudinal Observational Study	3 6 12	Unclear	40% child age 0-4 22% child age 5-18
	Malenfant (17)	236	41.3 (18-97)	76	36%	3.4 +/- 2.5 6.1 +/- 2.4 (at worst)	Cross-sectional Questionnaire	μ 47 [12-102]	No	71% had paresthesia 28% had no sensory problems 27% had pain more than once a week. Pain intensity was <2.0 in 72%, 2.0 – 3.0 in 9%, and >3.0 in 19%.

Table 1. Overview of reported prevalence and intensity of pain in dermal scars. (*continued*)

	Publication	N	Age (yr)	Sex male (%)	Prevalence of pain	Pain intensity Described or on a 0-10 scale	Study design	Scar age (mo)	Neuromas excluded	Remarks
Burn scars	Malenfant (16)	121	39.8 ±11.2	80	65%	had pain	Clinical Study	μ 60 [18-121]	No	Caucasians only, quantitative sensory testing (QST), 27% had pain more than once a week
	Choiniere (7)	104	42.3 ±14.1	76	35%	4.1+/- 2.0 6.3+/-2.1 (at worst)	Cross-sectional Telephone Interview	μ 37 [12-77]	No	21% had pain daily. TBSA 19+/-16%
	Schneider (19)	72	43.6 ±1.5	67	40%	-	Retrospective Chart Review	>14	Unclear	43% hypertrophic scars. Pain starts around 4 mo post burn with intensity of 7/10 and decreases around 7 mo.
	Ward (22)	60	34 (18-65)	95	25%	-	Clinical Study	μ 27 [8-126]	Yes	Full thickness burns. 97% sensory impairment in the scar.
	Choi (6)	37	26 (1-78)	54	-	2.4+/-2.1	Cross-sectional	μ 88 [3-360]	Yes	Hypertrophic post burn scars, many on the hand (>50%)
	Parnell (18)	23	>18	61	-	1.3+/-0.8 2.3+/-1.7	Cross-sectional Study	<6 >6	Unclear	10-70% TBSA, Itching burn scars
	Isoardo (11)	22	45.8 ±12	68	59%	5.6+/-1.8	Clinical Study	-	Yes	Post burn hypertrophic scars on hands
	Widgerow (23)		-	-	68%	-	Review	-	No	

Table 1. Overview of reported prevalence and intensity of pain in dermal scars. (*continued*)

	Publication	N	Age (yr)	Sex male (%)	Prevalence of pain	Pain intensity Described or on a 0-10 scale	Study design	Scar age (mo)	Neuromas excluded	Remarks
Pathologic scars	Lee (13)	28	34 (20-53)	46	46%	6.3	Clinical Study	<24 (n=4) >24 (n=24)	Yes	Keloids, QST
	Tey (20)	13	25 (14-43)	54	31%	5.6	Clinical and Histologic Study	>6	Yes	Keloids
	Eishi (9)	6	50.2 (19-76)	50	-	5.4+/-1.4	Clinical Study	μ 82 [10-180]	Yes	Keloids on trunk

N: number of patients. Mo: months. Scar is presented as μ: mean [range]. VATS: video assisted thoracoscopic surgery. TBSA: total body surface area, used to expressed area burned. Pain intensity was converted to a 0-10 scale if another scaling was used in reference paper.

Another possible factor related to pain after injury and scar formation are traumatic memories associated with the scars. Both anxiety and stress correlate well with the perception of pain sensations [10, 25, 26]. Pre-operative anxiety is correlated with post-operative pain experience. However, although pre-operative catastrophizing scores (tendency to exaggerated pessimism on outcome) correlated with acute post-operative pain, they did not correlate with chronic post-operative pain or long lasting painful scars [12]. Conditions associated with neuropathic post-burn pain are post-traumatic stress disorder, substance abuse and depression [19].

Pathology

Most studies retrieved from the search studied the underlying mechanism of painful scars.

Numerous specialized structures are present in the skin to detect various stimuli. Merkel cells in the epidermis and Meissner corpuscles in the dermal papillae both are able to detect light touch. Pacini corpuscles, which are specialized to detect pressure, are found deep within the dermis or even in the subcutaneous tissue. Pain is transmitted through naked nerve endings located in the basal layer of the epidermis. Krause bulbs detect cold, whereas Ruffini corpuscles detect heat. Heat, cold, and proprioception also are located in the superficial dermis (Figure 2).

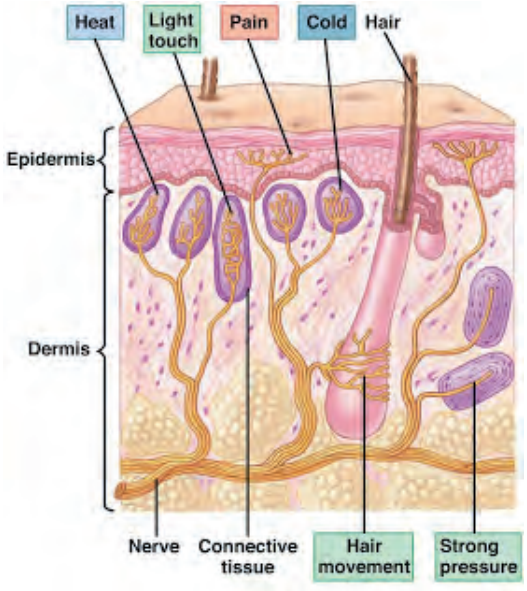

Figure 2. General view of skin and subcutaneous tissue, including several skin appendages and different sensory organs such as Ruffini bulbs (heat), Meissner corpuscles (light touch), free nerve endings (pain), Krause bulbs (cold), and Pacini corpuscles (strong pressure).

Free nerve fibers are responsible for pain transmission. They consist of both fast transmitting, myelinized Aδ-fibers and slower, unmyelinized C-fibers. Nerves fibers are present all over the skin. The density of these fibers differs per body area, varies between individuals and between childhood and adulthood. Another but a very relevant factor is the technique that is used to visualize the fibers.
This makes it hard to directly compare results between tests and studies, and only ratios and conclusions can be used to compare outcome. In neuropathic disease fiber density can be affected.[27, 28]

Nerve fiber density

Abnormal nerve fiber density has often been suggested as the cause for painful scars, but evidence is conflicting and weak (Table 2) [20, 29-40]. Many differences found in fiber density can be attributed to differences in methods of fiber staining, different skin layers, scar types, and scar age [33, 34, 36, 37, 41]. Nonetheless, several factors may increase nerve sprouting, such as neonatal age and low opioid receptor availability [38, 42]. High nerve fiber density can cause pain by inappropriate cross-stimulation as receptors are in much closer proximity, resulting in central sensitization [34]. However, most painful peripheral neuropathies show decreased fiber density, as in diabetic neuropathy or HIV [43].
Skin nociception is transmitted through myelinated, fast conducting Aδ-fibers that transmit sharp acute pain and by unmyelinated slow conducting C fibers, that represent about 70% of all epidermal fibers and conduct diffuse, burning, aching and dull type pain. In general, normotrophic matured scars seem to have less innervation than normal skin [30, 33, 37, 39-41, 44]. Pathologic and painful scars seem to have richer innervation than normal skin, as shown by an increased nerve fiber density, specifically in the peptidergic (substance P (Sub-P) or calcitonin gene-related peptide (CGRP) immunoreactive (IR)) fibers [33, 34, 36, 39-41, 45]. The C-fibers are generally divided in peptidergic and non-peptidergic fibers, the former are CGRP and Sub-P IR and respond to nerve growth factor (NGF), while the latter is P2X3 and IB4 IR and responds stronger to glial derived neurotrophic factor (GDNF), the functional difference between these types of C-fibers is not yet clarified. However, other groups found lower or no change in nerve fiber density in painful scars [30, 32, 46].
The natural course of wound healing results in increased innervation in the first weeks of scar formation, gradually returning to normal or lower levels [29, 35, 41, 45]. A disruption in the physiologic decrease of nerve fibers might cause painful scars. For example, Henderson et al. [35] showed that Sub-P remains elevated for more than 3 months after trauma. At that moment the scar contains 89% peptidergic fibers (compared to 57% in uninjured skin), which provide pain transmission. This illustrates the disturbed balance between peptidergic and non-peptidergic fibers, which is supported by several studies [30, 35, 40].

Table 2. Overview of reports on nerve fiber density in scars compared to control skin.

Marker stained	Publication	N	Sensory Symptoms	Nerve Fiber Density	Skin Layer	Scar Age (mo)	Remarks
PGP9.5 Panneuronal Marker	Reynolds (38)	3	Hyperalgesia	>	Dermis and Epidermis	1,5	Animal study, following wound innervation in time
	Kwak (36)	43	NR, pain likely	> =	Basal Epidermal Layer Dermis	>12	Hypertrophic burn scars, Asian population. No sex differences in fiber density or pain and itch complaints
	Anderson (31)	31	NR	=	Dermis and Epidermis	>18	Burn scar unilateral
	Tey (20)	13	Pain in 30%	=	Dermis and Epidermis	>6	Keloids, no correlation between itch/ pain and fiber density found
	Hamed (34)	12 pain 31 co scar	Pain No pain	=	NR NR	>24 >24	Burn scar extremity
	Henderson (35)	6 scar 6 co	NR	=	Dermis	3	Animal study
	Altun (30)	18 NSc 10 HSc	NR, pain unlikely NR, pain likely	< <<	Dermis and Epidermis	7 7	Burn scars. No difference between NSc and HSc in epidermis, but there was in dermis.
	Nedelec (37)	15	Hypoalgesia	<	Upper and Deep Dermis and Epidermis	>20	Grafted burn wounds and control skin No correlation with sensory tests
	Ward (40)	13	Hypoalgesia	<	Dermis and Epidermis	>6	Grafted burn wounds and control skin

Table 2. Overview of reports on nerve fiber density in scars compared to control skin. (*continued*)

Marker stained	Publication	N	Sensory Symptoms	Nerve Fiber Density	Skin Layer	Scar Age (mo)	Remarks
Neurofilament Panneuronal Marker	Wang (41)	15 young 15 mature 30 control	Pain Pain in 70% No pain	>co, >> mature < co, << young <young, >mature	NR	1-6 6-24	HSc, 4 of 15 in mature group were not painful, all other scars were painful. No correlation between pain and neurofilament-100 innervation.
	Zhang (39)	2 HSc 2 NSc 2 co	NR, pain likely NR NR	> co, >> NSc < co, << HSc <HSc, >NSc	NR	0-264 5-13	Scars from different origin
	Nedelec (37)	15	hypoalgesia	>	Deep Dermis	>20	Grafted burn wounds and control skin But fewer separate fibers in one bundle in grafted skin than in control skin
CGRP Peptidergic Neuron Marker	Kwak (36)	43	NR, pain likely	> >	Epidermis Reticular Dermis	>12	Hypertrophic burn scars, Asian population. CGRP in reticular dermis correlated to pain.
	Hamed (34)	12 pain 31 co scar	Pain No pain	>	NR NR	>24 >24	Burn scars on extremities. Both injured as uninjured skin in patients with pain have higher number of CGRP fibers
	Henderson (35)	6 Scar 6 co	NR	= (slightly higher)	Dermis	3	Animal study
	Crowe (33)	9 HSc 5 NSc 3 co	Pain Insensitive NR	+ in 8/9 + in 0/5 + in 3/3	Dermis	NR	Density HSc > controls

Table 2. Overview of reports on nerve fiber density in scars compared to control skin. (*continued*)

Marker stained	Publication	N	Sensory Symptoms	Nerve Fiber Density	Skin Layer	Scar Age (mo)	Remarks
Sub-P Subpopulation of Peptidergic Neurons	Kwak (36)	43	NR, pain likely	> >	Epidermis Reticular Dermis	>12	Hypertrophic burn scars, Asian population. Sub-P in dermis showed correlation with pain.
	Henderson (35)	6 Scar 6 co	NR	>	Dermis	3	Animal study. Sub-P fibers tripled, while proportion of non-peptiderg fibers decreased with 75%
	Ward (40)	13	Hypoalgesia	>	Dermis and Epidermis	>6	Grafted burn wounds and control skin
	Altun (30)	18 NSc 10 HSc	Pain unlikely Pain likely	=	Dermis and Epidermis	7 7	Burn scars
	Crowe (33)	9 HSc 5 NSc 3 co	Pain Insensitive NR	+ in 7/9 + in 0/5 + in 2/3	Dermis	NR	Density HSc > controls Penetrated in most dense collagen
HRP Sensory Axon Marker	Aldskogius (29)	18	NR	=	Dermis and Epidermis	3	Split skin graft wound. Anterograde tracer injected in L4-L5 ganglion.. After 2-3 weeks temporally hyper innervation is seen.
S100 Myelinated Neuron Marker	Biyani (32)	22	NR, both present	=	Dermis	NR	Scars after carpal tunnel release. Proximal part usually painful (4.4/mm2), distal part not painful (4.2/mm2).
DBH Sympathetic Neuron Marker	Crowe (33)	9 HSc 5 NSc 3 co	Pain Insensitive NR	+ in 6/9 + in 0/5 + in 3/3	Dermis	NR	Density HSc > controls

N: number of patients. Mo: months. > more than. = same as. < less than. PGP9.5: protein gene protein 9.5. NR: not reported. NSc: normotrophic scar. HSc: hypertrophic scar. Co: control group. CGRP: calcitonin gene related peptide. Sub-P: substance-P. HRP: horseradish peroxidase. DBH: dopamine beta hydroxylase.

Pain symptoms did not correlate with nerve fiber density consistently when pan-neuronal markers were used (PGP9.5, S100), while a correlation with an increased number of peptidergic fibers (CGRP-IR/Sub-P-IR), either absolute or relative, is thought to be likely [34, 47]. Clinical sensibility studies in keloid scars showed deficits matching with small fiber neuropathy (which is characterized by a lower epidermal nerve fiber density) [13]. However, in burn scars a fiber specific sensibility impairment was not found [16].
Finally, independent of nerve fiber density, damage or mechanical compression of Aδ-fibers and C-fibers by dense scar tissue can also be the cause of pain [11, 26].

Neurotrophic factors

Nerve growth factor (NGF) is an important neurotrophic factor that is required to maintain neurons viable. NGF is produced by Schwann cells, but also by keratinocytes at the basal-epidermal junction of the skin. NGF is present in high concentrations during wound healing as it stimulates keratinocytes to migrate until contact to other keratinocytes is re-established. NGF also stimulates melanocytes to form dendrites to transport pigment that colors the skin [5]. Both wounds that took longer to re-epithelize and persons with pigmented skin had higher risk on making pathologic scars and might have had higher levels of or prolonged NGF exposure [2].
Besides its functions in the skin, NGF triggers neuronal sensitization in several ways. First, NGF directly affects primary sensory neurons, resulting in hyperexcitability of the neurons [48]. Second, NGF stimulates sympathetic neurons to produce more neurotransmitters to the branches of these fibers that are in close proximity to the cutaneous nociceptors [5, 48, 49]. Third, it also activates mast cells, lymphocytes and leucocytes, which release inflammatory factors as Sub-P, CGRP and platelet activating factor. CGRP has direct stimulating effects on nociceptors and potentiates the effects of other factors. Platelet activating factor releases serotonin from the platelets. Serotonin injected in skin causes pain at the injection site, and in hypertrophic and red scars histamine, as well as serotonin levels are increased [50]. Increased vascularization and red appearance of scars correlated with more pain symptoms reported by patients [51]. The inflammatory response stimulates the primary sensory nerves in many ways. On the other hand, while Choi et al. [6] found more mast cells in scar tissue than in control skin, they found no correlation between mast cells and pain or between itch and pain.
The amount of NGF produced by keratinocytes is sufficient to affect neuronal growth and pain behavior. Experimental studies with laboratory animals showed that damaged nerve fibers near keratinocytes caused local NGF levels to rise. This resulted in directed and abundant sprouting and hyperexcitability of the damaged axons, resulting is clear neuropathic pain behavior [52, 53]. Persistent elevated NGF levels, and persistent inflammation, can cause permanent hyperinnervation and hyperalgesia [5, 48]. This could explain why prolonged wound healing, and continued release of inflammatory sub-

stances (interleukine-1, tumor necrosis factor-alfa) more often results in symptomatic pathologic scars [12, 48, 54]. Altering this response has been tried in several ways, starting as simple as preventing stimulation of free nerve endings, to reduce neuropeptide and inflammatory substance release. An occlusive dressing, for example, can reduce pain and scar tissue formation [23].
In summary, NGF plays an important role in wound healing and therefore scar formation, but it also sensitizes neurons and promotes inflammation, a process that releases other neurotrophic factors. When the sensitization is distinct this is a long lasting effect, and complaints can be present after the skin restored its continuity.

Pain and itch in scars

Pain and itch often co-exist in pathologic and burn scars, possibly because their mechanisms are closely related. Itch is partly transmitted by C-fibers, which also transmit pain. It is hypothesized that weak stimuli of C-fibers produces itch and stronger stimulus produces pain sensation [26, 47]. Mast cell degeneration can lead to itch as well as pain by releasing histamine, leukotrienes, Sub-P, prostanoids and growth factors that activate peptidergic C-fibers [9, 45, 47]. All these factors result in neuroinflammation that causes itch or pain. Histamine levels are high in young burn scars and return to normal levels when the scar matures [45, 50].
Another mechanism that influences itch and pain is the opioid system. Opioid mediated regulations are widely expressed in the central nervous system, but opioid receptors (MOR, KOR and DOR) are also expressed in the skin where they influence skin homeostasis and pain and itch [42, 55]. Nerve fiber density (PGP9.5) was increased and fiber morphology was changed in MOR and KOR knockout mice. It seems likely that the opioid system caused these effects, because there were no apparent signs of increased inflammation (like increased mast cell count and CD4+ count) [42]. In humans, opioid antagonists can reduce itch that does not respond to antihistamines, but may give rise to pain. Opioids reduce pain and can induce itch, which hardly responds to either opioid antagonists or antihistamines, indicating that opioid induced pruritus is not solely transmitted by histamine [47, 48, 55, 56]. In pathologic scars MOR, KOR and DOR were all increased, as was anti-nociceptive beta-endorfin (MOR ligand). This activation influences peripheral nociception and pruritus [47, 55, 56]. Long-term opioid use increases pro-inflammatory cytokines in an existing wound, while opioids administered before surgery diminish pro-inflammatory response [55]. Use of opioids impaired scar strength after secondary healing by inhibition of neo-angiogenesis. However, it increased strength of incisional wounds by enhanced scar remodeling, with up-regulation of transforming growth factor-beta and metametalloproteinase-2 [57].
In summary, opioids influence inflammatory response and wound healing; the direction is dependent on wound type and opioid timing [55, 57]. The coexistence of itch and

pain that are both affected by the opioid system, does warrant further research in this direction.

Systemic effects

Although scars are well-bounded local lesions, several reports suggested that not only local processes play a role in pain perception. Studies investigating sensory functions and nerve fiber density using both the contralateral side and other subjects as controls, found sensory functions were not only disturbed at the scar area but also at the uninjured contralateral skin [16, 31, 34, 46].

Aberrant sensory functions and increased vulnerability to pain are long-term complications after neonatal surgery and these effects are not restricted to the scar area [58, 59]. It seems that the developing peripheral nerve system in neonates is highly influenced by painful stimuli. This could affect synaptic connectivity in the central nervous system that may have long lasting effects [38, 58].

In adults, nociceptor activity in burn wounds can activate dorsal horn microglia. This activation is key to neuropathic pain development and will also affect pain perception in skin adjacent to the scar [60]. Patients with chronic pain after burn injury have a high amount of CGRP-IR fibers in both the scar and uninjured skin, while in patients without pain the scar and uninjured skin both have little CGRP-IR fibers [34]. An animal study on burn wounds, showed nerve fiber density (PGP9.5) was diminished 2 weeks after injury. This effect was seen at both the injured and non-injured site, albeit stronger at the injured site. This decrease in nerve fiber density was present until 12 weeks and maybe lasted much longer [31].

Another experimental study, using a painful mechanical scar model did not find neuronal activation in the dorsal horn. No neuronal activation (C-fos staining), which can occur following the noxious stimuli, was found in their experiment. The activation of dorsal horn neurons depends on both mechanical stimulation force and depth of anesthesia, which could have influenced these results. They did find aberrations in the myelin sheet of the spinal nerve innervating the scarred area matching Wallerian degeneration. This can be related to the mechanical hyperalgesia that was found [46].

There exists some evidence of a more than local response after burn injuries. However, the exact location of changes in the central nervous system that cause a different sensory ability in burn patients is unclear. By testing the burn scar, the uninjured contralateral dermatome, as well as another uninjured site, a spinal segmental change can be detected. However, spinal and supra-spinal integrating systems are hard to test [16].

On the other hand, there also exist arguments against a systemic effect on pain perception. Isoardo et al. [11], for example, found that in patients with burns on both sides of the body, only one side was painful, which is hard to explain if the pain response in the entire body would be affected the same way.

Discussion

We performed a comprehensive systematic review on the prevalence, etiology and pathophysiology of pain in dermal scars to give an overview of current knowledge. Although we performed a wide search in six databases, we cannot guarantee inclusion of all available papers with any relevance to the subject. We vastly improved our inclusion guarantee by checking titles and abstracts of all references from the included papers. Also, the liberal selection process, that enabled us to include papers with unexpected viewpoints, resulted in a large variety in available literature that made it challenging to analyze in a systematic way.

It is remarkable how little is known about the prevalence of painful scars. Most papers addressed burn scars, a specific type of scar known to give rise to pain symptoms. The only estimate of the prevalence of painful scars (excluding neuromas) in a general population with a surgical scar was 2%. The prevalence of painful scars found in specific populations like burn patients, or patients with pathologic scars was studied more and is much higher (30-68%). It would be valuable to study pain in scars in a design similar to that of Hoimyr et al. [10] to establish whether their results in a general population are reproducible and to determine whether pain is solely a problem for burn scars, pathologic scars and neuromas or nerve entrapment.

Although a relationship between the number of nociceptor fibers and pain sensations is plausible, various researchers question this hypothesis [20, 41, 61]. The highly variable nerve fiber densities found in scars cannot clarify this issue. However, the findings are directing towards an imbalance between non-peptidergic unmyelinated fibers (IB4-IR of N2X3-IR) and peptidergic (CGRP-IR and Sub-P-IR) fibers in painful scars. The same imbalance is also present after nerve dissection with neuropathic pain [62, 63]. Neuropathic pain might also appear when epidermal fibers are directly damaged during injury. The density of the scar tissue can hamper restoring a physiologic balance between the two types of epidermal fibers and cause permanent symptoms [29, 33]. Decreasing the scar adhesions by, for example, lipofilling can alleviate scar pain according to the study of Huang et al. [60]. Studying the nerve fiber density and balance in a clinical setting is challenging but necessary. Neuropathic pain should be objectively assessed, with extensive sensory testing and questionnaires, in order to correlate pain with nerve fiber density. Consequently, patients have to consent to supply tissue of the scar and control tissue. In addition to pan-neuronal markers, also markers differentiating between peptidergic and non-peptidergic fibers should be used.

The neuroinflammatory response is most likely important in the development of painful scars. But what is the ideal level of inflammation and its perfect timing? Even if we would know this, interfering in these processes is not without risks, because factors like

NGF and opioids play a role in many more processes than re-innervation and wound healing.

In general it is assumed that pain and underlying causes are local processes, as most authors use a control site within the same patient. We presented existing evidence on systemic effects of pain on a specific site on the body. Fitzgerald [58] concluded that pain early in life causes long term effects on sensibility and pain behavior due to the developing nervous system. If ongoing neuronal development would be the sole reason for the systemic effects of pain in early life, this systemic effect would not exist in adults, with complete development of the peripheral nerve system. In order to further investigate systemic effects of pain in experimental studies separate control animals can be used. In clinical studies control tissue form another subject would, by our opinion, introduce too much heterogeneity.

Conclusions

Our conclusion is that, normotrophic scars are rarely painful (estimated at less than 2%), while burn and pathologic scars more often lead to pain symptoms (30%-68%) with high intensity (means of 1.9-6.4 on a 10 point scale). With surgical scars the procedure, surgical technique and patient age are etiologic factors. For burn scars the size and depth of the burn and post traumatic psychological disorders are important. The pain in scars, which has many characteristics of neuropathic pain, could be caused by an imbalance of peptidergic and non-peptidergic fibers in the scar area. The increased density and hard penetrability of the scar tissue may cause the different nerve fiber distribution. The latter may also be caused by ongoing neuro-inflammation that attracts and stimulates peptidergic fibers. Future research should try to confirm these theories and attempt to alter these reactions to improve the pain in the scar area, without interfering with other body systems like the immune system.

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Appendix - Search Terms

Embase

(scar/exp OR (scar OR scars OR scarr* OR scarif* OR cicatri*):ab,ti) AND (skin/exp OR 'skin injury'/exp OR burn/exp OR (skin* OR derma* OR dermi* OR cutis OR cutan* OR epiderm* OR burn*):ab,ti) AND (pain/exp OR 'pain threshold'/de OR 'pain assessment'/exp OR (pain* OR allodyn* OR hyperalges* OR hyperesth* OR ache* OR aching*):ab,ti) AND (etiology/exp OR immunohistochemistry/exp OR physiology/exp OR pathology/exp OR pathophysiology/de OR histology/exp OR psychophysiology/exp OR (etiolog* OR aetiology* OR etiopath* OR pathogen* OR caus* OR nature* OR origin* OR physiopath* OR pathophysiol* OR pathol* OR physiol* OR immunohistochem* OR histolog* OR psychophysiol*):ab,ti)

Medline

(exp "Cicatrix"/ OR (scar OR scars OR scarr* OR scarif* OR cicatri*).ab,ti.) AND (exp "skin"/ OR exp "burns"/ OR (skin* OR derma* OR dermi* OR cutis OR cutan* OR epiderm* OR burn*).ab,ti.) AND (exp "Pain"/ OR "Pain Measurement"/ OR (pain* OR allodyn* OR hyperalges* OR hyperesth* OR ache* OR aching*).ab,ti.) AND (exp "Causality"/ OR exp "immunohistochemistry"/ OR exp "physiology"/ OR exp "pathology"/ OR exp "histology"/ OR exp "psychophysiology"/ OR etiology.xs. OR physiology.xs. OR pathology.xs. OR "anatomy and histology".xs. OR (etiolog* OR aetiology* OR etiopath* OR pathogen* OR caus* OR nature* OR origin* OR physiopath* OR pathophysiol* OR pathol* OR physiol* OR immunohistochem* OR histolog* OR psychophysiol*).ab,ti.)

Cochrane central

((scar OR scars OR scarr* OR scarif* OR cicatri*):ab,ti) AND ((skin* OR derma* OR dermi* OR cutis OR cutan* OR epiderm* OR burn*):ab,ti) AND ((pain* OR allodyn* OR hyperalges* OR hyperesth* OR ache* OR aching*):ab,ti) AND ((etiolog* OR aetiology* OR etiopath* OR pathogen* OR caus* OR nature* OR origin* OR physiopath* OR pathophysiol* OR pathol* OR physiol* OR immunohistochem* OR histolog* OR psychophysiol*):ab,ti)

CINAHL

(MH "Cicatrix"+ OR (scar OR scars OR scarr* OR scarif* OR cicatri*)) AND (MH "skin"+ OR MH "burns"+ OR TX (skin* OR derma* OR dermi* OR cutis OR cutan* OR epiderm* OR burn*)) AND (MH "Pain"+ OR "Pain Measurement"+ OR TX (pain* OR allodyn* OR hyperalges* OR hyperesth* OR ache* OR aching*)) AND (MH "immunohistochemistry"+ OR MH "physiology"+ OR MH "pathology"+ OR MH "histology"+ OR MH "psychophysiology"+ OR TX (etiolog* OR aetiology* OR etiopath* OR pathogen* OR caus* OR nature*

OR origin* OR physiopath* OR pathophysiol* OR pathol* OR physiol* OR immunohistochem* OR histolog* OR psychophysiol*))

Web-of-Science

TS=(((scar OR scars OR scarr* OR scarif* OR cicatri*)) AND ((skin* OR derma* OR dermi* OR cutis OR cutan* OR epiderm* OR burn*)) AND ((pain* OR allodyn* OR hyperalges* OR hyperesth* OR ache* OR aching*)) AND ((etiolog* OR aetiology* OR etiopath* OR pathogen* OR caus* OR nature* OR origin* OR physiopath* OR pathophysiol* OR pathol* OR physiol* OR immunohistochem* OR histolog* OR psychophysiol*)))

PubMed as supplied by publisher

(((scar[tiab] OR scars[tiab] OR scarr*[tiab] OR scarif*[tiab] OR cicatri*[tiab])) AND ((skin*[tiab] OR derma*[tiab] OR dermi*[tiab] OR cutis[tiab] OR cutan*[tiab] OR epiderm*[tiab] OR burn*[tiab])) AND ((pain*[tiab] OR allodyn*[tiab] OR hyperalges*[tiab] OR hyperesth*[tiab] OR ache*[tiab] OR aching*[tiab])) AND ((etiolog*[tiab] OR aetiology*[tiab] OR etiopath*[tiab] OR pathogen*[tiab] OR caus*[tiab] OR nature*[tiab] OR origin*[tiab] OR physiopath*[tiab] OR pathophysiol*[tiab] OR pathol*[tiab] OR physiol*[tiab] OR immunohistochem*[tiab] OR histolog*[tiab] OR psychophysiol*[tiab]))) AND publisher[sb]

Sensory Perception and Nerve Fiber Innervation in Patients with Keloid Scars

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Submitted

Abstract

Keloid scars often cause pain and itch sensations impairing quality of life. Unfortunately, keloid patients respond poorly to standard analgesics and anti-pruritic medication. It remains unclear why keloid patients experience pain and whether nociceptive or neuropathic mechanisms are playing a role. In an explorative study, we examined eight patients with keloid scars to identify the type of pain using questionnaires and quantitative sensory testing (QST) according to the protocol of the German Research Network on Neuropathic Pain before their planned keloid scar excision. After surgery skin biopsies were obtained to perform immunohistochemistry for identification of nerve fibers with protein gene product 9.5 (PGP 9.5). Pain and sensory characteristics were combined with nerve fiber density to explore a correlation possibly providing new insights in the pathophysiology. This could be a first step towards a more mechanism based improved treatment.

Our study revealed a wide variation of possible pain mechanisms playing a role in keloid tissue; about 50% of the patients experienced neuropathic pain. Using standardized questionnaires on neuropathic pain and the effect of cold temperature on pain symptoms as well as QST, pain experiences varied widely in individual patients. In keloid scars, PGP 9.5-immunoreactive nerve fibers showed a decrease in both the center and border regions as compared to control skin, which was uniformly significant in the epidermis. The border showed a higher nerve fiber density when compared to the center.

The present results suggest that somatosensory differences in keloid scars may show aspects of neuropathic pain and are extremely heterogenic. In this small explorative study, it is impossible to correlate these findings to the nervous innervation of keloid scars to provide an explanation for the pain that is experienced. For the patients experiencing neuropathic keloid pain, a local symptomatic pain treatment seems attractive, but its efficacy should first be investigated.

Introduction

Keloid scars are defined as benign tumors that extend beyond the confines of the original wound [1]. Their etiology is multifactorial, since several local or systemic factors resulting in an ongoing inflammatory process after trauma to the dermis add to keloid growth [2]. Keloid scars may cause distress because of their aesthetically unpleasant appearance [3]. However, patient burden is mainly caused by pain and itch sensations, which occur in a majority of the patients [4, 5], resulting in severe emotional symptoms in 48% of the patients (such as low self-confidence, feelings of despair, frustration). Unfortunately, these patients respond poorly to standard analgesics and anti-pruritic medication and consequently, keloids may seriously affect quality of life [6, 7].

Although many experts have described pain symptoms in patients with keloid scars, only few have studied this in detail [4, 8]. So far, the cause of pain sensations in keloid scar tissue is not well understood. It is unclear whether the pain is nociceptive or neuropathic. Nociceptive pain results from tissue damage or potentially tissue-damaging stimuli, while neuropathic pain is initiated by nervous system lesions [9]. Damaged nerves can result in loss of signals, additional signals (ectopic activity), or a combination of both. In keloid scars inflammation could result in nociceptive pain. Neuropathic pain could occur if nerves get damaged by either inflammation or dense scar tissue.

To characterize the type of pain, specific questionnaires for neuropathic pain and cold intolerance, and psychophysiological tests like quantitative sensory testing (QST) can be used. QST is a set of clinical tests that determines sensory tresholds, e.g. mechanical or temperature; different testing protocols are in use for different purposes. The QST protocol by the German Research Network on Neuropathic Pain assesses types of nerve fibers, allowing us to point out the underlying mechanisms for these phenotypes [10]. Sensory information reaches the central nervous system through primary afferents in the epidermis. Afferents are divided into Aβ, Aδ and C-fiber nociceptors; in QST these are represented by the different sensory thresholds [10]. It was previously found that spontaneous pain is mostly felt at the center of the keloid (77%) and that all patients experience pruritus, mostly at the edge of the keloids (92%)[4]. When provoked, 43% experienced allodynia (pain to a non-painful stimulus) and 14% alloknesis (itch to a non-itching stimulus). Moreover, thermosensory thresholds to warmth and cold were assessed in the keloids and showed a loss of sensory perception in keloids suggesting small nerve fiber neuropathy [4].

While QST allows demonstrating nerve fiber function, skin biopsy with quantification of nerve fibers is used to histologically identify these fibers, for which protein gene product 9.5 (PGP 9.5), a common pan-neuronal marker, can be used [11]. In the clinical setting, this is considered the gold standard for determining small fiber neuropathy [12]. Tey et al. [8] have not found any significant changes in nerve fiber density in keloid scars,

while differences in nociceptive sensations between the center and periphery of the keloid scar have been found [4, 8]. Therefore, it is not clear whether nerve fiber density patterns may correlate with experienced pain, elucidating the pain mechanism.
Up till now, there is sparse literature on the description of pain in keloid scars, its characteristics and whether these are accompanied by nerve fiber abnormalities. The aim of the study was to explore the correlation between painful sensations and nerve fiber density to get more understanding of the mechanisms playing a role in pain in keloid patients. This can be a first step towards a more effective mechanism based therapy.

Material and methods

Study setting and participants

The study was conducted at the Erasmus MC, University Medical Center Rotterdam in the Netherlands. Patients who were planned for surgical treatment of their keloid scar at our department, between November 2013 and August 2015, were invited to participate. The study was approved by the institutional review board as part of a larger trial on keloid treatment (NL40235.078.12) and registered (Dutch Trial Register NTR4151). All participants provided written informed consent. Inclusion criteria were as follows: patients over 18 years old, keloid diagnosed based on clinical characteristics (continuous growth, size, color, physical symptoms of pain and pruritus), proficient in English or Dutch.
Patients completed the DN4 [13, 14], PainDetect [15, 16] and the CISS questionnaires [17] before we performed the QST measurements. After surgery, skin biopsies from the keloids and normal skin as control were processed, whereafter stained for PGP9.5 and analyzed.

Pain evaluation questionnaires

The DN4 was developed in France to compare signs and symptoms in patients with chronic pain associated with neurological (peripheral or central) or somatic tissue injuries. It has been used often to evaluate pain caused by malignancies. The questionnaire consists of only four questions, two for the patient on pain characteristics and accompanying symptoms, and two for the physician on hypoesthesia and allodynia. Scores range from 0-10, with a score of four or more indicating probable neuropathic pain in the original version. After validation of the Dutch language version it became clear a score of five or more indicates probable neuropathic pain [13, 14].
The PainDetect was developed in Germany to identify the neuropathic component of patients with back pain. This questionnaire contains nine self-subscribed questions. Seven items for pain are graded from 0 to 5, one item on pain course in time is graded −1 to +1, and an item on pain radiation is graded 0 to 2. A total score ranging from −1

to 38 can be calculated, with higher scores indicating probable neuropathic pain (-1 to 12: unlikely, 13-18 possible, 19-38 likely neuropathic pain) [15, 16].
The cold intolerance symptom severity questionnaire (CISS), with a score ranging from 0-100 contains six questions that give detailed information on the effect of cold temperature on pain symptoms. Because it was mainly designed for cold intolerance of the hand, two questions specific for hands were removed, resulting in a maximum score of 0-86. A score of 30 or higher is considered abnormal cold intolerance [17, 18].
Scores of questionnaires are given (mean and range) with their interpretation.

Quantitative Sensory Testing

Quantitative sensory testing (QST) is a noninvasive method to determine the sensation and pain thresholds for cold and warm temperatures, and the mechanical sensation thresholds by stimulating the skin. Different protocols are used to measure different aspects of our sensory system [19]. The protocol of the German Research Network on Neuropathic Pain (DFNS) [20] was developed to assess the type of fiber. QST testing was undertaken by a specialized research nurse (EAMB) using a strict protocol concerning time of day during testing, controlled room temperature, phrasing of test instructions. We tested on the border of the keloid, which in our experience is the most active part of the keloid and also is the location of most physical symptoms [21]. Our protocol included the cold detection threshold (CDT), warmth detection threshold (WDT), thermal sensory limen (TSL), cold pain threshold (CPT), heat pain threshold (HPT), mechanical detection threshold (MDT), mechanical pain threshold (MPT), mechanical pain sensitivity (MPS), dynamic mechanical allodynia (ALL) and wind-up ratio (WUR) according to protocol [10]. For all temperature tests a thermo-sensory analyzer TSA II (Medoc Advanced Medical Systems, Durham, NC, USA) was used with a probe of 1.6x1.6 cm. In the MPS we excluded the 512N pinprick and when the keloid and control spot were on sensitive skin, like facial skin, we also excluded the 256N pinprick. We did not test the vibration detection threshold (VDT), because the keloid location often does not have a bony prominence, or this prominence was covered by keloid tissue, making it impossible to perform this test. Also, the pressure pain threshold (PPT) was not performed, as the location of some keloids prevented testing keloid tissue only, since underlying sensitive tissue would have affected the outcome when pressure was applied.
QST outcome does differ for different body areas, for the different sexes and with age. Reference values have been studied for a few body areas often affected, like the face, hands and feet. Even in similar sex and age groups the reference values have large variation [10]. Keloids can arise in both men and women, at different ages, and anywhere on the body, but hands and feet are not often affected [22]. Therefore, it is unlikely to compare keloid QST outcome to the reference values available. However, QST values can be compared to the lateral side of the body [10, 20]. We used the contralateral site of

a keloid site as a control, or when the keloid was situated midline we used the nearest unaffected dermatome that was also in the midline. Because the test and control sites were different per patient due to where the keloid was located, we provided a qualitative indication of the difference between the test and control site (from --- to +++) for each test [10]. For all subjects combined, an average qualitative score was given.

Immunohistochemistry

Skin biopsies were obtained after keloid scar excision. The high risk on keloid formation after skin biopsies prevented us to take biopsies of keloid patients' normal skin as a control to the keloid biopsies [22]. Therefore, we included one normal skin biopsy, obtained from a different patient, as a control for the staining method. All biopsies were immersion-fixed in 2% paraformaldehyde-lysine-periodate (PLP) for 24 hours at 4° C after removal [23]. Thereafter, the dissected tissues were embedded in gelatin blocks. Frozen sections (40 µm) were cut with a freezing microtome and collected serially 1:6 in glycerol containing vials.

For immunohistochemistry, sections were processed as previously described by Saffari et al [24]. In short, the following steps were applied with rinsing between the steps. Sections were first treated for antigen unmasking with sodium citrate, followed by endogenous peroxidase blocking using 3% hydrogen peroxide. Subsequently, sections were pre-incubated in a blocking solution containing bovine serum albumin (BSA) and incubated for 48 hours at 4° C with the primary antibodies against PGP 9.5 (1:10.000, Rabbit, Enzo Life Sciences, Lausen, Switzerland) and CGRP (1:30.000, Rabbit, Calbiochem, Darmstadt, Germany). Thereafter, sections were incubated with the appropriate secondary biotinylated antibody (1:200, Vector Laboratories, Burlingame, CA) for 90 minutes, followed by Avidin-Biotin Complex (Vector Laboratories, Burlingame, CA) overnight at 4° C. The 3,3'-diaminobenzidine reaction [25] was used to reveal antigenic sites whereupon the sections were randomly mounted on gelatinized slides, stained with 1% Thionin, dehydrated and cover slipped.

All slides were scanned in single layer of 8 µm into digital images using a Nanozoomer series system (Nanozoomer 2.0 series, Hamamatzu, Japan). These images were quantified using digital microscope software (NDP view) with a 20x to 40x objective. This software provides frames (0.2 mm^2) in order to count epidermal and dermal nerve fibers in center and lateral sides of the keloid scar. The localization region of these frames (center or border) was chosen based upon the images taken from the keloid scars, whereafter frames were placed at low magnification. For each person, the PGP9.5 IR-nerve fibers within the epidermis and upper dermis were counted in two sections [26]. From these counts, the results were averaged and expressed as the number of fibers per mm^2 per person. Finally, the results were compared to control skin.

Analyses

One observer performed the analyses. In order to determine statistical differences in fiber density between patients and control skin, the one-way analysis of variance (ANOVA) with a Dunnett's post hoc test was used for intergroup comparisons. Errors in variations were determined as standard error of the mean (SEM) and statistical significance was assumed at $p < 0.05$.
By employing descriptive correlation analysis, the correlation between nociceptive sensations and nerve fiber density was explored.

Results

We included eight patients that completed the questionnaires and QST tests before their planned surgery. The patients consented on participating in the study and use of the keloid tissue for research (Table 1). Control skin was collected from the chest wall of a healthy woman without keloid scars or any other skin diseases.

Neuropathic pain and cold intolerance questionnaire

The pain which was experienced by keloid patients differed extensively. Using the DN4 (50% neuropathic pain) as well as the PainDetect (25% neuropathic pain, 38% maybe), some patients were likely to suffer neuropathic pain, while others did not. Unfortunately, there was no good agreement between both questionnaires: only 50% of the patients showed agreement on DN4 and PainDetect outcomes. In one out of eight patients, cold intolerance was experienced assessed with the CISS, while three patients scored no points at all on the CISS, reflecting no reaction on cold (Table 1).

Quantitative sensory testing (QST)

The somatosensory tests in keloids showed very different results between patients. Cold detection, warmth detection and cold pain thresholds were more sensitive in some, but less sensitive in other keloid scars. The heat pain threshold was not affected in any keloid, and the cold or warm discrimination threshold was equal or less sensitive in keloids. All keloid scars had impaired mechanical detection thresholds, but mechanical pain perception differed between more and less sensitive. Allodynia, pain due to a stimulus that does not usually provoke pain, was found in three keloid patients, and increased sensitivity of keloid tissue to multiple stimuli (WUR) was not a uniform finding. After averaging the QST findings, overall similar or diminished sensitivity was found in keloid scars, compared to the control areas. However, values varied so widely that individual patients sometimes had completely different sensory perception (Table 1).

Table 1. Overview of patients' pain and somatosensory characteristics

Patient	Sex	age	location	Test dermatome	Control dermatome	DN4 score	NP DN4	PD score	NP PD	CISS	CI	CDT	WDT	TSL	paradox	CPT	HPT	MDT	MPT	MPS	ALL	WUR
1	M	46	pre-sternal	T4 midline	T3 midline	7	yes	21	yes	0	no	++	+	-	-	--	=	---	---	---	+	-
2	F	54	thorax	T4 left	T4 right	4	no	15	maybe	26	no	---	---	---	--	=	=	---	++	+++	+	+
3	M	29	pre-sternal	T2 mid line	T4 midline	4	no	13	maybe	3	no	+	=	-	+	-	=	---	---	--	=	=
4	F	53	upper arm	C5 right	C5 left	4	no	10	no	0	no	--	---	---	=	=	=	---	---	=	=	m
5	M	30	pre-sternal	T2 midline	T3 midline	7	yes	19	yes	4	no	+	++	=	=	+	=	---	-	++	=	+
6	M	44	pre-sternal	T5 midline	T6 midline	7	yes	12	no	0	no	-	=	=	++	=	=	---	--	---	=	-
7	F	26	pre-sternal	T2 midline	T3 midline	6	yes	17	maybe	48	yes	--	--	-	=	+++	=	---	+++	+++	+	m
8	F	28	Earlobe	C3 right	C3 left	2	no	3	no	14	no	---	---	---	=	-	=	---	-	m	=	+
overall	50% male	38.8 [26-54]				4.88 [2-7]	50% yes	12.6 [3-21]	44% yes	11.9 [0-48]	13% yes											

DN4: Douleur Neuropathique 4 questionnaire (scores range 0-10, score 5 or more is neuropathic pain), NP DN4: neuropathic pain based on DN4. PD: PainDetect questionnaire (scores range -1-38 score, neuropathic pain score -1-12: no, 13-18 maybe, 19-38 yes), NP PD: neuropathic pain based on PD. CISS: Cold Intolerance Severity Score (scores range 0-86, more than 30 indicates cold intolerance), CI: cold intolerance based on CISS. QST tests keloid compared to control: CDT: cold detection threshold, WDT: warmth detection threshold, TSL: thermal sensory limen, CPT: cold pain threshold, HPT: heat pain threshold, MDT: mechanical detection threshold, MPT: mechanical pain threshold, MPS: mechanical pain sensitivity, ALL: dynamic mechanical allodynia, WUR: wind-up ratio. M: male, F: female. Qualitative QST values: positive (+) keloid is more sensitive, negative (-) keloid is less sensitive. =: values <25% difference, -/+ 25-100% difference, --/++ >100% difference, ---/+++ >200% difference, m: missing.

PGP 9.5-IR nerve fiber density

PGP 9.5 stains all types of nerve fibers in the skin. In keloid scars, immunoreactive (IR) nerve fibers were identified in the various sections. It was noted that PGP 9.5-IR were easily identified in the various sections. In the keloid tissue, epidermal fibers were sparse and therefore not presented in all counted slides (Figure 1).

In the keloid scar of all patients, the epidermal nerve fibers immunoreactive to PGP 9.5 showed a significant decrease in both the center and border regions as compared to control skin ($p<0.0001$, Figure 2).

Nerve fibers immunoreactive to PGP 9.5 in the upper dermis of the keloid center were significantly lower when compared to control skin. In the border region, only QST 2 ($p = 0.0015$), QST 3 and QST 4 ($p<0.0001$) showed a significant decrease compared to control skin. In the other keloid scars, no significant differences in fiber density were found at the border (Figure 3).

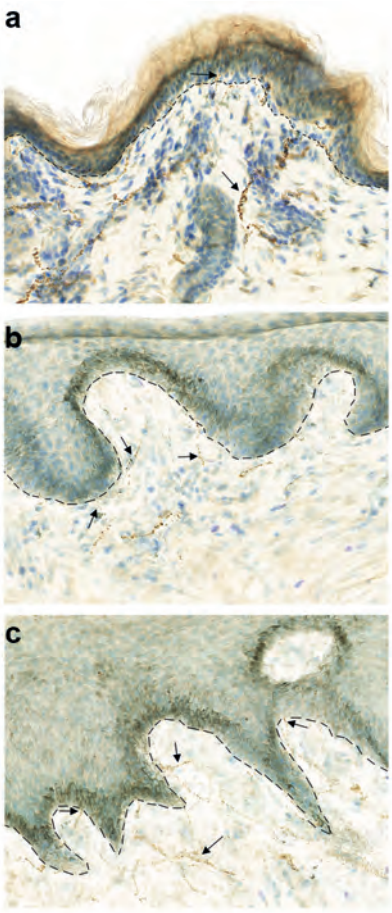

Figure 1. Micrographs of PGP 9.5-immunoreactivity in the skin.
Light micrographs showing PGP 9.5-IR nerve fibers in the epidermis and upper dermis in control skin (a), center of the keloid scar (b) and the border of the keloid scar (c). The dotted line indicates the boundary between the epidermis and the upper dermis. Arrowheads indicate several of the nerve fibers that are found in the skin. Magnification, 20x.

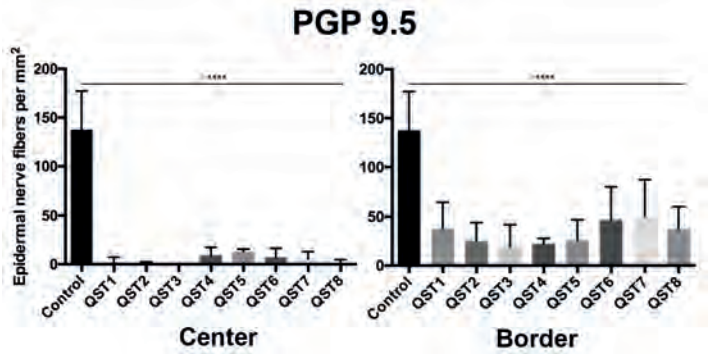

Figure 2. Density of PGP 9.5-IR epidermal nerve fibers.
Histograms showing the number of PGP 9.5-IR epidermal nerve fibers per mm^2 in the center and border regions of the keloid scar as com- pared to the control skin. Analysis was performed by using the one-way analysis of variance with a Dunnett's post hoc test. All comparisons were made with the control skin (**** P<0.0001). (One-Way ANOVA). The error bars denote the mean ± SEM.

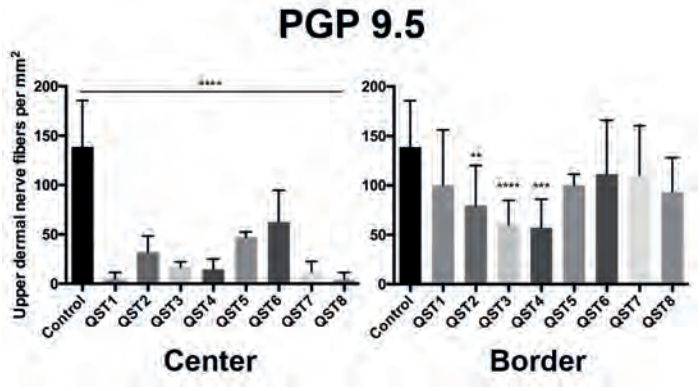

Figure 3. Density of PGP 9.5-IR upper dermal nerve fibers.
Histograms showing the number of PGP 9.5-IR upper dermal nerve fibers per mm^2 in the center and border regions of the keloid scar as compared to control skin. Analysis was performed by using the one-way analysis of variance with a Dunnett's post hoc test. All comparisons were made with the control skin (**P<0.01, ****P<0.0001). The error bars denote the mean ± SEM.

In the epidermis, a higher nerve fiber density was noted in the border regions of the keloid scar when compared to the center regions, which was significant in all keloid scars except for QST 4 and 5. Whereas in the dermis, a significantly higher nerve fiber density in the border was found in all keloid scars.
No correlation could be found between nociceptive sensations and nerve fiber density.

Discussion

To improve our understanding of painful keloids, we explored the underlying mechanism of pain in keloid patients by investigating the sensory perception and nerve fiber density in the present study. About half of the keloid patients reported neuropathic pain symptoms on the questionnaires. The DN4 classified neuropathic pain more often than the PainDetect. The PainDetect was developed specifically for back pain, the DN4 to identify (malignant) neuropathic pain over the entire body. The latter could be more fitting to assess pain in keloid patients.

The QST outcomes showed that in general in keloid scars, mechanical detection (Aβ fibers) was impaired, heat pain perception was spared (C fibers) and changes in temperature differentiation and detection varied (C and Aδ fibers). Unfortunately, the outcomes varied widely and no specific type of affected fiber or sensory modality that could predict the pain symptoms of keloid patients could be identified. Our QST findings did not confirm previous findings of impaired cold/warmth perception and heat pain perception [4].

The results on sensory perception did not even show a trend towards a specific sensory modality or fiber type that was affected. The perception of keloid patients was found to be heterogenic. In part, this may be explained by the large heterogeneity of the condition. Keloids have many phenotypes; there can be a single small lesion, or patients can be covered with many large keloids, appearing anywhere on the body [22]. In the current study, all keloids were painful and most keloids were located at the chest and of similar size (except for patient 4 and 8), controlling some, but not all, heterogeneity. When comparing the five presternal keloid scars, these patients did not show similar perception, neither in raw values nor in qualitative evaluation. Sensory perception is a complex process that is hard to fathom, because multiple signaling pathways are involved.

Lee et al [4] found that pain occurred more in the keloid center and itch more at the border. Using QST tests they compared keloids with skin next to the keloid and a contralateral control. They did not find differences between skin next to keloid and the contralateral control site. However, their probe was 9 cm^2, which means a lot of unaffected tissue was also covered by the probe and tested, while only the skin next to the keloid was targeted. Although we assessed nerve fiber density in the center and at the border of the keloid, we measured QST on one spot of the keloid (near the border), because the keloid size did not allow measuring multiple sites, even with a smaller probe size of 2.6 cm^2.

In keloid patients, pain symptoms should be evaluated more extensively. When neuropathic features are present, an anti-neuropathic pain symptomatic treatment could be an option. As keloids are a local problem, local therapy would be the first choice. One

report of botulinum toxin A (BTA) injection described a good effect on pain reduction, an effect that lasted longer than the motoric effect of BTA injections. Other treatment options could be capsaicin or lidocaine ointment.

In the present study, a higher nerve fiber density was found in the border regions of keloid scars compared to the keloid center. Contradictory, Tey et al. [8] found no significant differences in nerve fiber density between the center and border regions of the keloid scar. Our findings may explain the different sensations in the border and center regions, itch and pain respectively found by Lee et al. [4]. Unfortunately, this study could not confirm this because itch was not measured separately.

Intra-epidermal nerve fiber density (IENFD) is often decreased in conditions with neuropathic numbness (negative symptom) or neuropathic pruritus or pain (positive symptom) and indicates small fiber neuropathy when decreased below a certain value [27]. Moreover, Tey et al. [8] investigated 13 keloids of which nine were painful, they did not find a correlation between the innervation density of the keloid scar and the intensity of itch and pain. In another study, no differences in IENFD were found between operated and non-operated side in patients with persistent pain after thoracotomy. Based on these findings and other recent literature, they claim there is no correlation between pain perception and nerve fiber density [28]. Furthermore, if a correlation between pain perception and nerve fiber density would exist, it can only be shown if very large groups are studied.

An important limitation of this study was its explorative character. We only studied a very small number of patients. Based on our first findings, much larger studies should be developed, in order to describe a correlation in this heterogenic group. Beside the small groups size, another limitation of the present study was the control skin that was not taken from the keloid patients but from another patient, because high risk of keloid formation made it unethical to take a skin biopsy as control.

In conclusion, somatosensory differences in keloid scars may show aspects of neuropathic pain and are extremely heterogenic, which makes it impossible to identify a specific subgroup of nerve fibers that are affected in keloid disease, or to correlate symptoms to nerve fiber density that was uniformly decreased. Because 50% of keloid patients experienced neuropathic pain, future studies should evaluate which treatment modalities that specifically target neuropathic pain of keloids are effective and whether these treatments can decrease disease burden.

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PART II

Treatment of Keloids

Intralesional 5-Fluorouracil in Keloid Treatment: A Systematic Review

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Abstract

In the 1990s, 5-fluorouracil (5-FU) was introduced as a treatment for keloids; however, there is still no consensus on its use. In order to guide clinical practice, a systematic review of the clinical evidence on the effectiveness of 5-FU in keloid treatment was carried out. Eight databases were searched on 10 September 2014 using the terms "keloid" and "5-FU", together with all synonyms of these terms. Two reviewers selected original research reports using 5-FU alone or combined with a maximum of 2 other therapies. Eighteen papers were found that reported either on intralesional 5-FU alone, or on 5-FU combined with triamcinolone acetonide (TAC:5-FU) or excision, including 482 patients. 5-FU treatment was effective in 45–96% of patients, but only TAC:5-FU may perform better than TAC alone. Due to a poor level of evidence, further research should establish the superiority of repeated intralesional TAC:5-FU injections over TAC alone with several doses and injection schedules.

Background

Excessive scarring is a burden for both patients and specialists. Keloids are painful and itchy and, together with their aesthetic burden, have a major impact on patients' quality of life. Although they are benign lesions, they grow into healthy surrounding skin and resemble malignant growth patterns. There are wide differences in phenotype due to differences in the location, amount and size of the raised, pigmented, pruritic and painful lesions [1, 2].

There are many treatments currently in use for keloids; silicone dressings are least invasive, but strong and reliable evidence for its efficacy is lacking. Corticosteroid injections have been the mainstay of treatment, but are not effective in all cases. More invasive therapy, such as cryosurgery or conventional surgery with additional corticosteroids or radiotherapy, unfortunately has a high risk of side-effects, recurrence and deterioration [1-4]. High levels of therapy resistance, risk of recurrence, and the wide variety of treatment options all mean that treatment of keloids is challenging.

The resemblance of keloids to malignant growth patterns was used in searching for other minimally invasive, low-risk treatments; this led us to the chemotherapeutic drug 5-fluorouracil (5-FU). 5-FU blocks synthesis of the pyrimidine thymidine, which is a nucleoside necessary for DNA replication. Scarcity of thymidine monophosphatase results in thymidineless death in rapidly dividing cells [5]. 5-FU has already proved effective and safe in the treatment of glaucoma, another benign condition [6].

Even though there is no consensus on its value, 5-FU is used internationally to treat keloids. We therefore performed a systematic literature review on the effectiveness of treatment of keloids with 5-FU.

Methods

In order to collect all available evidence EMBASE, MEDLINE, Web of Science, Scopus, CINAHL, the Cochrane Library, Google Scholar and PubMed Publisher were searched on 10 September 2014, using the terms "keloid" and "5-fluorouracil" together with all synonyms of these terms (i.e. search term EMBASE #1 Keloid: 'keloid'/exp OR keloid*:ti,ab OR cheloid*:ti,ab; #2 5-fluorouracil: 'Fluorouracil'/exp OR 'fluorouracil':ti,ab OR '5 fluorouracil':ti,ab OR '5fluorouracil':ti,ab OR '5 FU':ti,ab OR '5FU':ti,ab OR Adrucil:ti,ab OR Carac:ti,ab OR Efudex:ti,ab OR Fluoroplex:ti,ab; #3: #1 AND #2).

Original research reports of randomized controlled trials (RCTs), prospective clinical trials and case series involving keloid treatment using intralesional 5-FU alone or combined with a maximum of two other therapies were included. We put no limitations on the date of publication, the age, gender, ethnicity of study participants, or the duration of

disease. Exclusion criteria were: case reports ($n \leq 2$), animal studies, studies combining more than three different treatments, and language other than English.
First, two reviewers (SS, EB) independently assessed the titles and abstracts of potentially eligible studies. In cases of no agreement, a third reviewer (FBN) decided whether the article should be selected. Two reviewers independently extracted data from full-text copies of all selected studies. To identify other relevant studies, the reference lists of all included studies were examined (Figure 1).
We extracted patient characteristics, treatment protocol, and outcomes that were reported as recurrence rates, the percentage of observer-rated reduction or improvement, the percentage of patient-rated improvement, and the presence of side-effects. The outcomes were converted into 5 levels: no response; 1–25% as poor; 26–50% as fair; 51–75% as good; and 76–100% as excellent improvement or flattening of keloids. The quality of included studies was assessed on the basis of reproducibility and study design.

Results

The literature search identified 284 references to keloid and 5-FU. After the selection process, 18 articles were included for critical appraisal. Two papers reported on the same cohort of patients; 1 of them was excluded [7, 8]. The other reasons for exclusion are given in Figure 1. Among the references of the included articles no new original research papers were found.
In 1999, Fitzpatrick was the first to report on his wide experience with 5-FU in keloids, although not in a scientific setting [9]. His publication prompted others to start collecting evidence. A total of 482 patients participated in 17 studies dating from 2001 to 2014. These studies examined several different methods of treatment with 5-FU. To evaluate the efficacy of 5-FU, we used the outcomes of 3 types of treatment: intralesional 5-FU alone, 5-FU combined with triamcinolone acetonide (TAC), and excision with 5-FU with or without TAC (Tables 1 and 2).

5-FU efficacy in keloid treatment

The use of intralesional 5-FU alone achieved a good or excellent outcome in 45–78% of patients. Only one patient was reported as a complete non-responder. Injections with 5-FU and TAC resulted in 50–96% good or excellent outcomes, and neither non-responders nor recurrence were reported. 5-FU was reported as less, as well as more, effective in direct comparison with TAC. Sadeghinia & Sadeghinia [10] who used TAC tattooing, an uncommon method of administration, showed better results with 5-FU than with TAC. Prabhu et al. [11] showed better volume reduction with TAC, also

Table 1. Overview of studies using intralesional 5-fluorouracil injections or intralesional 5-fluorouracil/triamcinolone acetonide combined injections in keloid treatment

Reference (year)	Treatment used	Patients/keloids, n	Evidence level[a]	Definition keloid	Measurement method	Conc. (mg/ml)	Max dose/injection (mg)	Injections, n	Injection interval, weeks	Follow-up[b]	Outcome	Recurrence, %
Intralesional 5-fluorouracil injections												
Gupta & Kalra (2002) (21)	5-FU	24 P, 39 K	4-	NR	NR	50	150	16	1	>38	16.6% poor, 25% fair, 25% good, 33.3% excellent flattening[c]	0
Manuskiatti & Fitzpatrick (2002) (14)	5-FU	10 P	2b-	NR	R	50	NR	10	2	32	On average good flattening[c] Patient self-assessment: 70% fair, 30% good improvement	0
	RCT control TAC:5-FU	10 P				1:45	NR	10	2	32	On average good flattening[c] Patient self-assessment: 10% poor, 40% fair, 50% good improvement	0
	RCT control TAC	10 P				20	NR	6	4	32	On average excellent flattening[c] Patient self-assessment: 30% fair, 40% good, 30% excellent improvement	0
Nanda & Reddy (2004) (23)	5-FU	28 P	2b	R	NR	50	100	12	1	24	7.1% poor, 14.3% fair, 71.4% good 7.1% excellent improvement[d]	0
Kontochristopoulos, et al. (2005) (22)	5-FU	20 P	4-	NR	NR	50	100	±7	1	52	5% no, 10% poor, 40% fair, 40% good, 5% excellent improvement[d]	47
Mutalik & Patwardhan (2008) (25)	5-FU	24 P	4-	R	NR	50[e]	75	4	4	52	33% no/poor, 67% excellent flattening[c]	25

Table 1. Overview of studies using intralesional 5-fluorouracil injections or intralesional 5-fluorouracil/triamcinolone acetonide combined injections in keloid treatment (*continued*)

Reference (year)	Treatment used	Patients/keloids, n	Evidence level[a]	Definition keloid	Measurement method	Conc. (mg/ml)	Max dose/injection (mg)	Injections, n	Injection interval, weeks	Follow-up[b]	Outcome	Recurrence, %
Sadeghinia & Sadeghinia (2012) (10)	5-FU	20 P	1b-	NR	R	50 T	NR	3	4	44	On average good flattening[c] Patient self-assessment: 15% fair, 35% good, 50% excellent improvement[d]	NR
	RCT control TAC	20 P				40	NR	3	4	44	On average good flattening[c] Patient self-assessment: 15% poor, 45% fair, 35% good, 5% excellent improvement[d]	NR
Sharma, et al. (2012) (13)	5-FU	25 K	1b	R	R	50	NR	±10	1/2/4[f]	52	12% poor, 16% fair, 40% good, 32% excellent flattening[c]	0
	RCT control TAC:5-FU	25 K				4:45	NR	±10	1/2/4[f]	52	0% poor, 4 % fair, 44% good, 52% excellent flattening[c]	0
Saha & Mukhopadhyay (2012) (12)	5-FU	20 P	1b	NR	R	50	100	±5	1	56	0% no, 15% poor, 20% fair, 55% good, 10% excellent flattening[c]	35
	RCT control TAC	24 P				40	80	± 4	1	56	0% no, 8% poor, 25% fair, 54% good, 13% excellent flattening[c]	36
Prabu (2012) (11)	5-FU	14 P	1b	NR	R	50	100	4	1	29	0% no–poor, 36% fair, 50% good, 14% excellent flattening[c]	NR
	RCT control TAC	15 P				40	80	4	1	29	0% no–poor, 13% fair, 40% good, 47% excellent flattening[c]	NR

Table 1. Overview of studies using intralesional 5-fluorouracil injections or intralesional 5-fluorouracil/triamcinolone acetonide combined injections in keloid treatment (*continued*)

Reference (year)	Treatment used	Patients/keloids, n	Evidence level[a]	Definition keloid	Measurement method	Conc. (mg/ml)	Max dose/injection (mg)	Injections, n	Injection interval, weeks	Follow-up[b]	Outcome	Recurrence, %
Intralesional 5-fluorouracil/triamcinolone acetonide combined injections												
Manuskiatti & Fitzpatrick (2002) (14)	TAC:5-FU	10 P	2b-	NR	R	1:45	NR	10	2	32	On average good flatteningc Patient self-assessment: 40% fair improvement and 50% good improvement	0
	RCT control 5-FU	10 P				50	NR	10	2	32	On average good flatteningc Patient self-assessment: 70% fair, 30% good improvement.	0
	RCT control TAC	10P				20	NR	6	4	32	On average excellent flatteningc Patient self-assessment: 30% fair, 40% good, 30% excellent improvementd	0
Darougheh, et al. (2007) (8)	TAC:5-FU	20 P	2b-	NR	R	4:45	8:90	8	1	12	On average good flatteningc Patient self-assessment: 45% fair, 55% good improvementc	NR
	RCT control TAC	20P				10	20	8	1	12	On average good flattening (70%)c Patient self-assessment: 20% poor, 60% fair, 20% good improvementd	NR

Table 1. Overview of studies using intralesional 5-fluorouracil injections or intralesional 5-fluorouracil/triamcinolone acetonide combined injections in keloid treatment (*continued*)

Reference (year)	Treatment used	Patients/keloids, n	Evidence level[a]	Definition keloid	Measurement method	Conc. (mg/ml)	Max dose/injection (mg)	Injections, n	Injection interval, weeks	Follow-up[b]	Outcome	Recurrence, %
Davison, et al. (2009) (18)	TAC:5-FU	52 K	4	NR	R	10:37.5	NR	3	4	2–313	On average excellent flattening (81%)[c]	NR
Sharma, et al. (2012) (13)	TAC:5-FU	25 K	1b	R	R	4:45	NR	±10	1/2/4[f]	52	0% poor, 4% fair, 44% good, 52% excellent flattening[c]	0
	RCT control 5-FU	25 K				50	NR	±10	1/2/4[f]	52	12% poor, 16% fair, 40% good, 32% excellent flattening[c]	0
Khan, et al. (2014) (15)	TAC:5-FU	25 P	2b	NR	R	4:45	8:90	8	1	26	32% no–poor, 68% good–excellent improvement[d]	0
	RCT control TAC	33 P				10	20	8	1	26	39% no–poor, 61% good–excellent improvement[d]	0

[a] Level of evidence rated by Centre for Evidence-Based Medicine criteria, March 2009 (www.cebm.net). [b] From 1st injection, weeks. [c] No 0%, poor 1–25%, fair 26–50%, good 51–75%, excellent 76–100% keloid flattening. [d] No 0%, poor 1–25%, fair 26–50%, good 51–75%, excellent 76–100% keloid improvement. [e] In cases of inflamed or hard keloids TAC (40 mg/ml) was added to injection ratio 1:1, and silicone dressings were used for 3 months. [f] Injections were weekly for 4 weeks, then bimonthly for 2 months, then monthly. K: keloids; NR: not reported; P: patients; R: reported; T: tattooed; 5-FU: 5-fluorouracil; TAC: triamcinolone acetonide; RCT: randomized controlled trial.

more pain reduction and less adverse events, although the last 2 were not significant. Saha & Mukhopadhyay [12] showed comparable size reduction and recurrence rates, but less pain reduction and more adverse events in the 5-FU group. The combination of 5-FU and TAC (TAC:5-FU) proved more effective than 5-FU alone [13]. Also, TAC:5-FU was more or equally effective and resulted in fewer adverse events than TAC alone [8, 14, 15]. Most authors reported no recurrence of disease, while others reported recurrence in no less than 25–47% of patients (Table 1). Excision with 5-FU achieves a good result, with recurrence rates between 4–19% [16-20]. Keloid-free outcome after excision was 43% and after excision with 5-FU 75%, when TAC:5-FU was used after excision keloids were reduced by 92% [16, 17, 20] (Table 2). A correlation between duration of keloids and treatment response, where younger keloids respond favourably, was found in 2 studies [21, 22], while others did not find this correlation [23].

5-FU treatment protocols

Fitzpatrick [9] tried different injection intervals and recommended starting with once-weekly injections, advice which many others followed [8, 11-14, 17, 21-23]. Others used 2- or 4-week intervals [10, 15, 16, 24, 25] or only once around surgery [18-20]. The outcomes do not indicate a preference for a specific injection-interval. Where serial injections were used, 6 studies used 3–6 injections and 8 used 8–16.

None of the authors reported serious side-effects. Six studies found no side-effects at all [8, 10, 14, 17, 24, 25]. Reported were purpura (20-40%), ulceration (1-65%), and transienthyperpigmentation (90%) [9, 11, 12, 15, 21-23]. In 6 surgical studies complications of necrosis, wide scars (14%) and dehiscence (1–18%) were rarely found [18, 20]. No systemic reactions were found after local injection [8-10, 12, 17, 21, 22, 24].

Without exception the manufacturer concentration of 50 mg/ml was used when 5-FU was used alone. Mild side-effects, due to local toxicity advise against using higher concentrations. Lower concentrations would require more volume for the same active dose, which increases pain on injection. In combination therapy, the TAC concentrations were very low (TAC:5-FU of 1:45 mg/ml or 4:45 mg/ml); only Davison et al. [16] tested TAC:5-FU in 10:37.5 mg/ml and noticed more side-effects than they had with TAC (23% vs. 15%, not significant).

Table 2. Overview of studies using 5-fluorouracil in combination with surgical excision in keloid treatment

Reference (year)	Treatment used	Patients/keloids in this group, n	Evidence level[a]	Definition keloid	Measurement method	Conc. injections (mg/ml)	Max dose/injection (mg)	Time of 1st injection post surgery, weeks	Injections, n	Injection interval, weeks	Follow-up from surgery, weeks	Outcome	Recurrence, %
Uppal, et al. (2001) (19)	excision+5-FU	6 P	2b	R	NR	50	NR	0	NI	–	26	50% fair, 50% good improvement[c]	NR
	RCT control excision	61 P				–	–	–	–	–	26	87% poor, 13% fair improvement[c]	NR
Davison, et al. (2009) (18)	excision+TAC:5-FU	24 K	2b	NR	NR	10:37.5	NR	0	4	2	26–313	On average excellent flattening (92%)[b]	NR
	RCT control excision+TAC	26 K				40	NR	0	4	2	26–313	On average good flattening (73%)[b]	NR
Haurani, et al. (2009) (24)	excision+5-FU	32 P	2b	R	R	50	50	2	10	4	100	10% no, 27% fair/good, 63% excellent improvement[c]	19
Hatamipour, et al. (2010) (16)	excision+5-FU+SS	25 P	2b	R	NR	50	50	1	5	1/2/4[d]	100	21% fair/good, 75% excellent improvement[c]	4
	RCT control excision + SS	25 P				–	–	–	–	–	100	35% fair/good, 43% excellent improvement[c]	22
Khare & Patil (2012) (20)	excision+5-FU	28 P	4	NR	NR	NR	150	0	1	–	>52	96% excellent result	4
Wilson (2013) (17)	excision+5-FU:Botox	80 P	4	R	NR	50:50IE	500	0	1	–	>52	16% no–poor, 84% good–excellent improvement[c]	4

Besides excision and intralesional 5-FU, a maximum of one other treatment modality has been used when indicated (triamcinolone acetonide (TAC), botulinum toxin, silicone sheets). [a] Level of evidence rated by Centre for Evidence Based Medicine criteria, March 2009 (www.cebm.net). [b] No 0%, poor 1–25%, fair 26–50%, good 51–75%, excellent 76–100% keloid flattening. [c] No 0%, poor 1–25%, fair 26–50%, good 51–75%, excellent 76–100% keloid improvement. [d] Injections were at week 1, 2, 4, 8, 12. Botox: botulinum toxin; K: keloids; NI: no injection (5-FU soaked sponge pledged intraoperative for 5 min); NR: not reported; P: patients; R: reported; SS: silicone sheets; RCT: randomized controlled trial.

Discussion

This systematic review indicates that the combination of TAC:5-FU may be more effective than TAC alone in keloid treatment (level C evidence). After keloid excision, 5-FU reduces recurrence rates to 4–19%, both on its own and in combination with TAC.

Our literature search resulted in a remarkably high number of reviews (126 of 284 papers), most of which were mainly on scar or pathological scar treatment, and mentioned 5-FU only in passing. Due to the unambiguity of our search terms the risk of missing relevant publications was minimal, as reflected by the absence of additional includes in our reference check. There were, however, several papers in the Asian literature that were not in English or that we could not retrieve.

The level of evidence was poor, there were 10 RCTs [8, 10-17, 19], some of which were unfortunately executed very poorly, 4 prospective single-arm trials [16, 18, 20, 25], 4 case series and an expert opinion [9, 21-24]. The problems included a lack of definitions, suboptimal study designs and follow-up periods. The studies we found on the novel treatment 5-FU were small, wherefore the good efficacy reported at first is probably influenced by publication bias. More recent studies on 5-FU are less positive in their results [11, 12].

Due to the large heterogeneity between studies, a meta-analysis could not be performed. This is reflected in the lack of a good definition of keloids in 11 of the 18 articles. Here less severe hypertrophic scars could be included that positively influence the results [8, 15, 26-28]. Similarly, outcome measurement technique was poorly described, and outcomes were classified in wide ranges ("good result" or "improvement 75–100%"). This forced us to do the same [12-25].

With intralesional 5-FU a good to excellent response was found in 45–79% of treated cases, and even up to 96% if TAC was added. It is unclear what caused the lowest response (45%) [22]: it cannot be explained by dose, follow-up time, or number of injections. The wide range of effectiveness we found is recognized from research on intralesional corticosteroid use alone, where a 50–100% response is reported [3]. A favourable response was seen in small and previous untreated lesions; this phenomenon is also known in other keloid treatments [2, 21, 22].

Recently the synergetic effect of TAC and 5-FU was proven in an *in vitro* study on keloid fibroblasts [29]. Although the evidence is weak, TAC:5-FU is more effective than 5-FU alone and seems to have advantages over TAC alone. The beneficial results of TAC:5-FU compared with TAC are, however, highly dependent on the dose and injection scheme of TAC and TAC:5-FU. Khan et al. [14] used low concentrations of TAC, which are less effective in keloid treatment, and weekly injections, that due to the long duration of action of TAC might cause more atrophy. For TAC:5-FU there is very little evidence on the efficacy and safety of TAC concentrations greater than 4 mg/ml, therefore we recommend the most frequently used and investigated concentration of 4:45 mg/ml TAC:5-FU. There

is insufficient evidence for a statement on the maximum allowed dose in total or per scar-surface area.
Weekly injections are mostly used; therefore most evidence is based on this injection interval. Although Fitzpatrick [9] states that longer intervals are less effective, this is not reflected by the studies we present. However, none of the studies directly compared different injection intervals. Also, the number of injections varied widely between studies (1–16) without a clear correlation with the outcome. When more injections were allowed clinical evaluation was used to determine the need for additional treatment.
Even though keloid recurrence is a major problem, some studies fail to report recurrence rates. Others have less than a year follow-up period, which is too short to draw a valid conclusion on recurrence rates [8, 10, 11, 14-16, 19, 21, 23, 30]. Five studies (follow-up 13–52 weeks) remarkably found no recurrence [13-15, 21, 23]. Higher recurrence rates of 25–47% were found after 52 weeks or longer follow-up [12, 22, 25]. The low recurrence risks found can be partly explained by the inclusion criteria or study designs, many studies selected patients with more favourably characteristics than the keloid-patient group that is usual in most clinics.

Conclusions

Based on this systematic review, we recommend 4:45 mg/ml TAC:5-FU combination therapy, injected intralesionally, until a satisfactory response is reached. It is likely that approximately 8 injections are needed. However, in order to formulate valid clinical guidelines on how to use TAC:5-FU in keloid treatment, more high-level clinical evidence is needed. This will help to establish preferred doses and injection schedules.

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Intralesional Cryotherapy versus Excision and Corticosteroids or Brachytherapy for Keloid Treatment: Study Protocol for a Randomised Controlled Trial

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Abstract

Background Keloids are a burden for patients due to physical, aesthetic and social complaints and treatment remains a challenge because of therapy resistance and high recurrence rates. The main goal of treatment is to improve the quality of life (QoL); this implies that, apart from surgical outcomes, patient-reported outcome measures (PROMs) need to be taken into account. Decision making in keloid treatment is difficult due to heterogeneity of the condition and the lack of comparative studies.

Methods/Design This is a multicentre, randomised controlled open trial that compares 1) intralesional cryotherapy versus excision and corticosteroids for primary keloids, and 2) intralesional cryotherapy versus excision and brachytherapy for therapy-resistant keloids. The primary outcome is the Patient and Observer Scar Assessment Scale (POSAS), a 12-item scale (with score 12 indicating the best and 120 indicating the worst scar imaginable). A difference of six points on the total score is considered to be of clinical importance. Secondary outcomes are recurrence rates, volume reduction, Skindex-29 scores, SF-36 scores and complication rates. Primary and secondary outcome measurements are taken at baseline, and at 2, 12, 26 and 52 weeks postoperatively. For analysis, a linear mixed model is used. A total of 176 patients will be included over a period of 2.5 years. The protocol is approved by the Medical Ethics Committee of the Erasmus University Medical Centre Rotterdam and follows good clinical practice guidelines.

Discussion The outcomes of this study will improve evidence-based decision making for the treatment of keloids, as well as patient education.

Trial registration Dutch Trial Register NTR4151.

Background

Keloids are pathologic scars that grow beyond wound borders and act as a benign tumour. The physical, aesthetic and psychological complaints that they cause are of great concern [1, 2].

After injury, the skin heals by forming a scar. Dysregulation of signalling molecules in the complex healing process can result in keloid formation, with several times more collagen synthesis than for normal skin and normotrophic scars, and a higher ratio of type 1 to type 3 collagen [3-7]. The etiology of keloids remains unknown. Although it is suggested that a relation exists with wound tension, sex hormones, sebaceous gland activity, melanocyte concentration and overlying keratinocytes, as well as with genetic predisposition, no single theory has proven of value in all aspects of keloids [8-12]. The highest incidence is seen in patients with a dark skin tone, whereas Mediterraneans, South Americans, and Asians are slightly less affected, and Caucasians are the least affected (<1%) [2, 12, 13]. It was shown that scars give acceptability problems (91%) and influence social functioning (82%) in a mixed group of scar types that were mainly on visible body sites [1]. At least as much problems can be expected for patients with keloids, as keloids are often visible on earlobes or so large that they are visible through clothing. Besides this major psychosocial burden, keloids give rise to pain and pruritus in 80% of keloid patients [1, 2].

Treatment of keloids is challenging because of therapy resistance and high recurrence rates, resulting in the search for more treatment options for keloids. Over decades, systematic reviews included zero to only three randomised controlled trials per treatment option, with a lot of heterogeneity between the studies [2, 14, 15]. In the absence of sufficient numbers and methodologically sound randomised trials, no consensus for a treatment of first choice has been reached.

In our clinical practice a 'stepped-care approach' is generally used; that is, initially, the least invasive and safest treatments are used, which are changed to more radical treatments in case of resistance or recurrence.

Because keloid is a benign condition, the main treatment goal should be to relieve the burden, which consists mainly of pruritus, pain and aesthetic complaints; all these are subjective symptoms. To objectively measure subjective burden (that is, in a reproducible way) the effects of the treatment options should be assessed using validated scar assessment scales and patient-reported outcome measures (PROMs), in addition to surgical results such as volume reduction, recurrence and complication rates.

In our centre, the 'stepped-care approach' implies that the first step is generally conservative treatment with corticosteroid injections. Other conservative methods, like laser therapy and silicone occlusive dressings, have not proven to achieve patient satisfaction in keloid treatment as is clear from recent systematic reviews [14-17]. If keloids are of

such a size that conservative treatment may not be sufficient, the next step is surgical treatment.
Because excision as monotherapy gives a recurrence rate of ≥70%, an adjuvant treatment should be used. The most frequently used adjuvants are corticosteroid injections, pressure therapy and brachytherapy (interstitial radiotherapy) [14, 18]. Another surgical keloid treatment option that gained popularity some years after several case series were published, is intralesional cryotherapy [19-21]. As no trials have compared intralesional cryotherapy with established conventional therapies, we aimed to explore the position of intralesional cryotherapy in the 'stepped-care approach'.

Objectives

Beginning in November 2012, we initiated a randomised clinical trial in which we compare frequently used keloid therapies in the Netherlands: excision and intralesional steroid injections, excision and brachytherapy, and intralesional cryotherapy. Outcomes are surgical results and PROMs. The results will assist in producing a better 'evidence-based' treatment algorithm for keloid patients.

Methods

Trial design

The design is a multicentre, randomised controlled open trial, which used minimisation to control for skin type, location and duration of disease. The trial consists of two parts: one for primary keloids and one for resistant keloids. Primary keloids are keloids that have not been surgically treated and, to some extent, have responded to corticosteroids. Resistant keloids are keloids that recurred after excision or those that did not respond to corticosteroids (progression within six weeks after corticosteroid injection). For the primary keloids we randomise between either intralesional cryotherapy or excision and additional corticosteroids. The resistant keloids are randomised between either intralesional cryotherapy or excision and brachytherapy. Follow-up assessments are in weeks 2, 12, 26, and 52 post-treatment. The follow-up period is based on scar maturation, which lasts about one year, and the chance of recurrence that usually occurs within the first year [22, 23].

Patient recruitment

Patients who present with a keloid at an outpatient clinic of the four participating centres are considered for the study. A keloid is a clinical diagnosis and is distinguished from hypertrophic scars by the clinician's judgement. The judgement between hypertrophic scars and keloid is based on: the growth history, starting early versus late after

trauma, remaining stable versus still growing; shape, following initial lesion versus not following the initial lesion; and size, <0.5 cm versus >0.5 cm beyond the original lesion. At a later stage, we will report how many patients with keloids were seen and how many were eligible for the study.

Inclusion criteria are:
1. Keloid with a surgical indication.
2. One to three keloids that can be treated in one session.
3. Minimal size of 1 × 1 cm.
4. Suitable for excision and primary closure.
5. Patient aged between 18 and 75 years.
6. Fully mentally competent.
7. Sufficient knowledge of the Dutch or English language.

Exclusion criteria are:
1. Hypertrophic scars.
2. Scars after burn wounds.
3. Keloids less than one year old.
4. Pregnancy.
5. Use of systemic chemotherapeutics or chronic use of systemic corticosteroids or immunosuppressive medication.
6. Hypersensitivity for local anaesthetics, adrenaline, or triamcinolone (primary keloids).
7. Patients not sufficiently fit for brachytherapy (resistant keloids).
8. Severe comorbidity with life expectancy under one year.

Consent procedure

At the first outpatient visit, eligible patients are informed about the study by a member of the research staff and written information is provided. After a consideration period of at least two weeks, the patient is contacted and registered in the study database when the patient wants to participate. Before treatment, a witnessed, written informed consent is obtained from all participants, following the guidelines of the local ethical committee.

Randomisation (treatment allocation)

Previous studies on keloid treatment showed that specific characteristics are predictors of recurrence. Therefore, we want to assure homogeneity between treatment groups regarding these characteristics, such as duration of the keloid existence (dichotomous; <5 years or ≥5 years) and location of the keloid (categorical; sternal region, auricular region and other). In addition, we try to match for skin type because of the strong asso-

ciation with the development of keloids, but the doubtful relation with recurrence rate (categorical; Fitzpatrick type 1 and 2, type 3 and 4, type 5 and 6). Because of the many different strata that would be formed and considering the total number of patients to be included, we choose not to use permuted blocks but will use the more sophisticated technique of minimisation. We will minimise on the three factors previously mentioned. The allocation of a new subject is determined by the allocation of the subjects already enrolled. We apply a 20% random chance factor to keep allocation predictability at a minimum. The software used is the open source program MinimPy (http://minimpy.sourceforge.net/).

When a patient agrees to participate in the study they irrevocably receive a unique identification number, which cannot be changed or removed from the database. After completion of baseline measurements, treatment allocation is conducted through a central computerized allocation using the locked database for all participating centres. Then the physician and the patient are informed of the assigned condition and the treatment is planned. In this way, allocation concealment is guaranteed.

Trial interventions

Excision with additional corticosteroid injections

Extralesional excision is performed with minimal margins, and absorbable monofilament sutures or permanent monofilament sutures are used for closure (Monocryl™, Ethilon™, Ethicon Inc, Somerville, NJ, USA). Surgery is performed by either surgical residents who have three years minimum experience, or by plastic surgeons. This standardised surgical procedure is not demanding, and we expect no learning curve. Many different surgeons (>20) reflect usual clinical practice in keloid treatment. After 2 weeks, an injection of triamcinolone acetonide 40 mg/ml is given in the newly formed scar. The injections can be repeated at 8 and 12 weeks postoperatively.

Excision with additional brachytherapy

Extralesional excision is performed with minimal margins, and absorbable monofilament sutures or permanent monofilament sutures are used for closure (Monocryl™, Ethilon™, Ethicon Inc, Somerville, NJ, USA). During the procedure, brachytherapy catheters are placed direct subcutaneously in order to cover the affected area. Next, a target dose of 600 to 900 cGy is given followed by one or two doses on the day of operation or the day after. After completion of brachytherapy, the catheter is removed [24].

Intralesional cryotherapy

The Cryoshape needle (Etgar Group International, Kfar Saba, Israel) is positioned in the centre of the keloid to guarantee total coverage of the keloid when it is visually frozen.

If necessary the Cryoshape needle is repositioned to achieve this. Our procedure differs slightly from Har-Shai *et al*. as we administer lidocaine with epinephrine around the keloid instead of intra- or translesional infiltration [21], because in our experience injecting through the keloid can be difficult and unnecessary painful for the patient. The cryotherapy can be repeated after three months if the desired effect has not been achieved.

During the study follow-up patients are not allowed to use additional keloid treatments. If treatment was not effective other treatments will be performed after at least 26 weeks follow-up. Follow-up measurements will continue as planned and patients will receive a request for an additional follow-up measurement 52 weeks after the additional treatment.

Blinding

Neither physicians nor patients are blinded for treatment. They cannot be blinded due to surgery under local anaesthesia and differences in postoperative selfcare instructions. Furthermore, during the follow-up assessments, a physician or layperson would immediately recognize the treatment type by the resulting wound or scar.

Safety concerns

Treatments applied in the current study are conventional rather than experimental. The hospital's local safety procedures are followed. Possible side-effects are treated according to current best practice. No serious adverse events are expected; however, these will be reported to the Medical Ethics Committee supervising this study and registered with EudraVigilance within two weeks after the investigator is notified of such an event. The protocol is approved by the Medical Ethics Committee of the Erasmus University Medical Centre Rotterdam and follows good clinical practice guidelines and current Dutch legislation.

Outcomes

Our primary outcome measure is the Patient and Observer Scar Assessment Scale (POSAS), a 6-item patient questionnaire and a 6-item observer questionnaire. The patient and at least two observers (clinician and investigator) will independently assess the scar. The scores of the patient will range from 6 for the best imaginable scar to 60 for the worst scar imaginable, which is a PROM, the average of the observers scores will also range from 6 to 60. The score of the patient and observers will be added to form the total score that is our primary outcome, but the scores will also be analysed separately. A difference of six points on the total score is considered to be of clinical importance. The POSAS is a sensitive instrument that includes both physician and patient opinions

of the scar, it has been previously validated, and performs well in a population of mostly dark-skinned keloid patients [25-29].

When one or two of the 12 POSAS items are missing at baseline we imputed the mean of the other scores of the same assessor. When a follow-up item was missing we imputed the last value carried forward. In cases where an item of the second observer was missing, we imputed the score of the first observer on the same item.

Secondary outcomes are keloid volume measured using a plaster mold, made before treatment, after the skin had completely healed at 12, 26 and 52 weeks. Time to recurrence is determined; the physician assesses recurrence at each follow-up visit; and, in case of recurrence, the patient is asked how many weeks after treatment the keloid recurred. As well as the diagnosis of keloid disease the diagnosis of keloid recurrence is a clinical diagnosis based on new growing scar tissue with features of keloid disease as described earlier. Photographs are taken at all visits, which will be used for additional (partial) observer scores on the POSAS and recurrence assessments. The additional observers will reduce bias in these outcomes. For assessing quality of life (QoL) we use a disease-specific and a general instrument: the Skindex-29 and the Short Form/RAND-36 (SF-36), respectively. The Skindex-29 was originally developed for psoriasis patients. It consist of 29 questions concerning symptoms, emotions and functioning and is, therefore, also suitable for other skin conditions. Questions are rated on a 5-point Likert scale. Scores range from 0 to 100, with 0 indicating no compromise on quality of life. A score ≥40 indicates a significant negative influence of the skin condition on QoL [30, 31]. Worldwide, the SF-36 is the most frequently used general QoL questionnaire. It consists of 36 questions (scored on a Likert scale) addressing eight dimensions (vitality, physical functioning, bodily pain, general health perceptions, physical role functioning, emotional role functioning, social role functioning, mental health). The dimension scores are transformed to a 0 to 100 score, with a higher score indicating a higher QoL [32, 33].

We will also analyse single item scores of our primary outcome measure POSAS, especially itch and pain from the patient questionnaire, and items on skin colour, pigmentation and vascularisation.

Data collection

Data are collected at baseline, before randomisation, and at follow-up assessments 2, 12, 26, and 52 weeks after treatment. The questionnaires are preferably filled out online, although a paper version is also available. All paper questionnaires are scanned and stored on a secure disk. The online questionnaires are saved by the online questionnaire program, and a backup is made regularly.

Photographs are taken at all visits. Volume of the keloid is measured at baseline and after the skin has completely healed at 12, 26, and 52 weeks after treatment.

Statistical analysis

Baseline demographic and clinical characteristics will be presented as proportions or means and standard deviations (SD) where appropriate. Because of the longitudinal data with multiple influencing factors, a sophisticated model is necessary. Mixed models (also called multilevel linear regression analysis) is a technique that efficiently uses longitudinal data and can work with patients' data even though measures at certain time points may be missing.

The units of analysis are the repeated measurements of the patient (first level), not the keloid, because we use several QoL measures that are not measurable for the unit keloid. The second level will be the individuals participating in the study. If necessary, a third level of subgroups with specific characteristics (skin type, duration of keloid disease and location of the keloid) can be added. If the third level is shown to improve the fit of the model, it will be incorporated in the model. We will use backward elimination and start with an unstructured covariance structure for intercept and time (slope). Simplifications of the random part of the model will be tested using the deviance statistic with restricted maximum likelihood. For the fixed part we will postulate a saturated model. We take in account time, logarithm of time and squared time, treatment condition and its interactions with time. Nonsignificant effects will be excluded using Wald tests. The fit of the final fixed model will be compared with the saturated model and will be checked using ordinary maximum likelihood. When characteristics like sex, age, skin type, duration of keloid disease and location of the keloid make a significant contribution to the model, they will be incorporated in the model [34-37]. Differences between treatment effects will be expressed in terms of effect sizes, standard errors and *P* values. Effect sizes will be calculated by dividing the estimated differences by the estimated standard deviation. An effect size of 0.20 is considered a small effect, 0.50 a medium effect and 0.80 as a large effect [38]. All analyses will be performed on an intention-to-treat basis. IBM SPSS version 21.0 and SAS version 9.3 will be used to perform the analyses.

Sample size calculation

There are no meaningful rules of thumb to estimate the sample size needed for a mixed models analysis, because, with random and fixed effects estimations, too many factors of uncertainty are involved. Therefore, a standard sample size calculation with a correction for the design effect based on the intercorrelation was used.

These calculations were performed in SPSS version 20.0 using the mixed-model ANOVA procedure as described by Aberson [39]. Type 1 error (alpha) was set at 0.05, and power (1-beta) on 0.80.

To estimate the effect size and correlation we analysed data of a natural cohort collected by one of the authors (FBN) containing general features of keloid patients and

POSAS values before and after treatment with intralesional cryotherapy. This natural cohort contains measurements at baseline, and at 12, 26, and 52 weeks after treatment. It comprised POSAS observer values from one or two observers and patient values; however, many patients lacked values for some time points (56% of follow-up complete). Only a small amount of items were missing, in total 15/3378 (0.44%) items of the POSAS data were imputed following the rules described previously.
The assumed medium-sized effect of 0.5, based on a SD of around 15 (Table 1), corresponds to 7.5 points on the POSAS scale; this is slightly more than the 6 points that is regarded as a clinically significant difference. We assumed a correlation of 0.75 between time points; this was difficult to verify in the data of the natural cohort because of many incomplete cases.

Table 1. Descriptive statistics

	N	Minimum	Maximum	Mean	SD
POSAS baseline	73	30	93	60.45	12.276
POSAS 12 wk post op	44	18	85	50.65	15.474
POSAS 26 wk post op	46	17	83	49.23	15.854
POSAS 52 wk post op	33	24	81	46.18	13.959
POSAS overall	196	17	93	53.32	15.20

POSAS = Patient and Observer Scar Assessment Scale score; Wk = weeks; post op = postoperative

The analysis, based on the observed SD and expected correlation and effect size, resulted in a group size of 33 patients, taking into account a 25% loss to follow-up and the four treatment groups; this results in a total sample of 176 subjects.
No interim analysis is planned because we do not expect any severe side-effects and, by the time a sufficient part of the participants has finished follow-up, almost all participants will have had their intervention. No rules related to stopping/withdrawal from the study have been specified.

Ethical issues

The risks of undergoing surgical treatment include complications due to undergoing anaesthesia and surgery; however, these risks are equivalent to the risks of surgical treatment without participating in the study. Only patients not responsive to conservative treatment and who opt for surgical treatment, despite knowing the risks, are enrolled in the trial.
Anticipated benefit for the medical world is improved outcome for future patients. The results will improve decision making, helping evidence-based guidelines to be developed for keloid treatment. We aim to determine the place of intralesional cryotherapy in the 'stepped- care approach' (that is, whether it should be used as second step, be

added as an extra step, or has no place at all in the treatment of keloids). If cryotherapy is shown to be effective for resistant keloids, then savings can be made by avoiding the costly brachytherapy treatment. If cryotherapy is not effective, the patient will receive appropriate treatment sooner.

Time frame

We will include patients over a period of 2.5 years and will follow every patient for one year, resulting in a total study period of 3.5 years.

Discussion

We have described a trial protocol; this is becoming standard practice when conducting clinical trials, although surgical trials are somewhat behind medical trials. The importance of publishing an extensive protocol is that it addresses questions (that may not be answered in the Methods section due to limited space) that might arise on how the trial was organised after publication of the results. It also prevents publication bias due to inconclusive or negative results. This will be the first randomised controlled trial comparing surgical keloid treatments using validated PROMs.

We have presented the problems with power analysis and sample size calculations for a more complex but sophisticated statistical model. In this corrected analysis we did not rely on assumptions only, but used previously collected data to determine the effect size and intercorrelation. The effect size we have chosen is a conservative one in order to ensure clinical relevance. When the effect size appears in fact to be smaller, we expect to be underpowered. Due to the quantity of work involved in logistics and data collection, we made this decision despite the risk of a negative result.

Trial status

At the time of submission of this protocol (August 2013), this study was recruiting patients to participate in the study.

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Intralesional Cryotherapy versus Excision with Corticosteroids or Brachytherapy for Keloid Treatment: A Randomized Controlled Trial

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Abstract

Background Keloids are a burden for patients due to physical, aesthetic and social consequences. Treatment remains a challenge due to therapy resistance and high recurrence rates. The main goals of treatment are to improve scar appearance and symptoms and patients' quality of life (QoL).

Methods A multicentre, randomized controlled open trial that compared 1) intralesional cryotherapy with excision and corticosteroid injections for primary keloids, and 2) intralesional cryotherapy with excision and brachytherapy for therapy resistant keloids. Primary outcome was scar appearance assessed with the Patient and Observer Scar Assessment Scale. Secondary outcomes were patient reported QoL (Skindex-29, SF-36, EQ-5D-5L), recurrence rates and scar volume reduction. For analysis, a linear mixed model was used. Power analysis indicated 33 patients in each group were needed.

Results The trial was prematurely terminated after inclusion of 26 patients due to unexpectedly inferior outcomes after intralesional cryotherapy. To increase statistical power both surgical treatments combined were compared with intralesional cryotherapy. Excision followed by corticosteroid injections or brachytherapy improved scar appearance and scar symptoms significantly while cryotherapy did not ($p<0.001$ and $p=0.005$, respectively). No statistically significant improvement in QoL was observed after both treatments.

Conclusions Intralesional cryotherapy is inferior to keloid excision followed by brachytherapy for resistant keloids. In primary keloids, intralesional cryotherapy resulted in mild keloid improvement and, therefore, may be used in these patients and specific cases. However, further research on the efficacy of intralesional cryotherapy for primary keloids is warranted.

Background

Keloids cause a burden on health related quality of life that justifies adequate treatment [1, 2]. Both patients and physicians are challenged due to therapy resistance and keloid recurrences. Current opinion is that treatment should follow a stepped care approach from conservative, non-invasive treatment to surgical treatment followed by adjuvant treatment in case of unsatisfactory results [3, 4]. In 2003 a new application of cryotherapy for treatment of keloids was introduced by Har Shai et al. [5], after which no recurrences were reported. Also, in several other studies equally promising and remarkable results were found for both primary and recurrent keloids [6-8], suggesting this treatment could replace surgical treatment.

Because intralesional cryotherapy previously had not been compared directly to other treatments, we designed a randomized controlled trial to compare outcomes of intralesional cryotherapy to excision and adjuvant treatment, starting inclusion in 2012 [9]. Since the start of our trial three studies have been published on intralesional cryotherapy, which showed results inferior to the first reports, however, outcomes were still reasonable with recurrence rates of 12%, 17%, and 24% [10-12].

During the course of the present trial patient inclusion was difficult and we unexpectedly encountered strikingly inferior outcomes following cryotherapy. Therefore, after careful consideration we decided to stop further enrolment of the trial. In the current report we present the results of this terminated randomized controlled clinical trial.

Methods

A randomised non-blinded clinical trial was designed to compare intralesional cryotherapy to extralesional keloid excision followed by adjuvant treatment divided in two groups:

- For primary keloids (not previously treated with surgery) we compared intralesional cryotherapy to excision followed by adjuvant triamcinolone acetonide injections.
- For resistant keloids (recurrence after previous surgical treatment or refractionary to corticosteroid injections) we compared intralesional cryotherapy to excision followed by brachytherapy.

Adult patients were eligible if they had a burdensome keloid that had not responded well to minimally invasive treatment and, therefore, had an indication for excision. Keloids had to be minimally 1 by 1 cm, and feasible for primary closure after excision. The trial started at two University Medical Centres, during the trial two other centres were added. Treatment allocation was conducted through a central computerised allocation.

The trial was approved by the local institutional review board (IRB) and all patients gave written informed consent.

Treatments

Excision with additional corticosteroid injections: Extralesional excision was performed with minimal margins. After 2 weeks, an injection of triamcinolone acetonide 40 mg/ml was given in the newly formed scar. If needed, the injections were repeated at 8 and 12 weeks postoperatively.

Excision with additional brachytherapy: Extralesional excision was performed with minimal margins. During the procedure, brachytherapy catheters were placed subcutaneously in order to cover the affected area. A target dose of 9 Gy was given followed by a second dose on the same day. After completion of brachytherapy, the catheter was removed.

Intralesional cryotherapy: The Cryoshape needle (Etgar Group International, Kfar Saba, Israel) was positioned in the centre of the keloid to guarantee total coverage of the keloid during treatment. When necessary the Cryoshape needle was repositioned. The treatment was repeated once after three months when the desired effect has not been achieved.

If treatment was not effective, choice of another treatment was allowed after at least 26 weeks follow-up.

Outcomes

Primary outcome was scar appearance, assessed with the Patient and Observer Scar Assessment Scale (POSAS) score, ranging from 12 to 120 and including a six item patient (PSAS) and a six item observer (OSAS) part with separate item scores ranging from 1 (resembling normal skin) to 10 (worst scar imaginable) [13, 14]. Based on a clinical significant difference of 6 points and a standard deviation of 15 we aimed at a size of 33 patients per treatment arm [9].

Secondary outcomes were patient reported quality of life (QoL), assessed with the disease specific instrument Skindex-29, and generic SF-36, and EuroQol 5D (EQ-5D-5L) [15, 16]. Skindex-29 is a reliable and validated self-report questionnaire of 29 items with subscales for symptoms, emotions, and functioning and a sum scale [17, 18]. Questions are rated on a 5-point Likert scale. Scores range from 0 to 100, with 0 indicating no compromise on QoL and a score ≥ 40 indicating a significant negative effect of the skin condition on QoL. SF-36 is a reliable and validated self-report questionnaire with 36 questions addressing eight dimensions of QoL and with a physical and a mental component score (PCS and MCS) [19, 20]. The scores are transformed to a 0 to 100 score, with a higher score indicating a higher QoL. EQ-5D-5L is an outcome measure that is often used in health-economics. It has five questions on mobility, self-care, usual activities,

pain/discomfort, and anxiety/depression scored on a 5-point scale. All possible answer combinations correspond to a single number, where 1 corresponds to perfect health and 0 to death [16].

Other secondary outcomes were scar volume reduction measured by plaster moulding, and pain and itch symptoms that were reported as separate items in the PSAS. We measured at baseline, 2, 12, 26 and 52 weeks after treatment.

Statistical Analyses

Baseline demographic and clinical characteristics are presented as percentages and means with standard deviations (SD) and ranges where appropriate. The surgical and intralesional cryotherapy groups for primary and resistant keloids were merged because of small group sizes. For each outcome a mixed model (multilevel linear regression analysis) was used. The repeated measures in a patient were the first level, the second level were the patients. The covariance structures for intercept and time (slope) were tested using the deviance statistic with restricted maximum likelihood. The fixed parts of the models included time, logarithm of time and squared time, treatment condition and its interactions with time [21-23]. Characteristics like sex, age, skin type [24], duration of keloid disease, keloid type (primary or resistant) and location of the keloid were tested whether they made a significant contribution to the models. Differences between treatment effects are expressed in terms of effect sizes, and p-values. Per protocol analyses were performed with IBM SPSS version 22.0.

To confirm that scar symptoms (pain and itch) had a strong effect on QoL, as previously reported by our group [1], Spearman correlations between differences in scar symptoms (pain and itch scores), aesthetic outcomes (patient reported scar colour, thickness, stiffness, and irregularity scores), and differences in QoL measures (Skindex-29, SF-36, and EQ-5D-5L) were calculated.

More detailed information on methods is available in a previously published study protocol [9].

Results

During the inclusion period 179 consecutive keloid patients with an indication for surgery were referred to the participating centres. Using the inclusion criteria, 74 were eligible for our study, but only 26 patients gave informed consent for randomisation. Many patients expressed strong treatment preferences withholding them from trial participation. Ten patients with primary keloids were included of whom five were allocated to keloid excision and corticosteroid injections and five to intralesional cryotherapy. In the resistant group 16 patients were included, of whom seven were allocated to

keloid excision and brachytherapy (all treated with 2x9Gy) and nine to intralesional cryotherapy (Table 1). Most patients had been previously treated with corticosteroid injections. If resistant keloids had been excised previously, in 50% intraoperative or postoperative corticosteroid injections also had been used.

Table 1. Patient characteristics

	Primary keloid		Resistant keloid	
	Excision w/ TAC	**Intralesional cryotherapy**	**Excision w/ brachytherapy**	**Intralesional cryotherapy**
Number of patients	5	5	7	9
Male	2 (40%)	1 (20%)	3 (43%)	5 (56%)
Age in years mean ±SD (range)	30.2±9.6 (18-40)	37.6±17.9 (19-57)	35.0±11.0 (19-48)	32.7±7.9 (22-44)
Skin type I-II*	2 (40%)	1 (20%)	0	2 (22%)
Skin type III-IV*	0	0	1 (14%)	3 (33%)
Skin type V-VI*	3 (60%)	4 (80%)	6 (86%)	4 (44%)
Location thorax	2 (40%)	2 (40%)	0	3 (33%)
Location ear	2 (40%)	1 (20%)	2 (29%)	2 (22%)
Location other	1 (20%)	2 (40%)	5 (71%)	4 (44%)
Duration of keloid in years mean ±SD (range)	8.2±9.6 (1-25)	2.6±1.9 (1-6)	6.4±3.7 (2-12)	9.6±9.8 (3-35)
Keloid volume at baseline in ml[B] Mean ±SD	1.4±0.8	5.8±6.4	5.7±7.7	8.1±10.4

w/: with postoperative. TAC: triamcinolone acetonide 40mg/ml. FU: follow-up. SD: standard deviation. *According to Fitzpatrick [24]. [A] Other locations were upper back (2x, 1 cryotherapy group) and lower extremity for primary keloids and for resistant keloids upper back (2x cryotherapy group), abdomen (3x, 1 cryotherapy group), cheek (2x), neck and lower extremity (cryotherapy group). [B] Keloid dimensions and volume varied widely depending on location; differences were not statistically significant (primary keloid p=0.054, resistant keloid p=0.265).

One patient with a primary keloid was lost to follow-up. During inclusion we unexpectedly noticed a high number of failed intralesional cryotherapy treatments after three months follow-up (Table 2); most of these patients opted for treatment from the other study arm. Therefore, the inclusion was stopped in accordance with the IRB.

Table 2. Scar volume reduction and recurrence between baseline and last on protocol measurement

	Primary keloid		Resistant keloid	
	Excision w/ TAC	**Intralesional cryotherapy**	**Excision w/ brachytherapy**	**Intralesional cryotherapy**
Number of patients	5	4	7	9
% of baseline volume at last FU mean±SD (range)	90±133 (0-300)	60±38 (33-116)	5±13 (0-33)	99±47 (29-186)
Complete scar flattening	20%	0%	86%	0%
Recurrence	80%	25%	0%	22%
Keloid larger than baseline	40%	25%	0%	44%
Treatment satisfaction	60%	25%	100%	0%
Additional treatment	40%	50%	14%	67%

w/: with postoperative. TAC: triamcinolone acetonide 40mg/ml. FU: follow-up. SD: standard deviation.

After follow-up of all included patients had been completed, we performed mixed model analyses as planned (Table 3 and Supplementary Table 1). Only treatment and time effects were included in the models, because none of the patient and keloid characteristics (sex, age, skin type, duration of keloid disease, keloid type, and location of the keloid) made a significant contribution to the models. Despite the small group sizes, we found a major and clinically relevant improvement of scar appearance after surgery (measured by the POSAS, Cohen's effect size (d) = -2.03, p<0.001), but not following intralesional cryotherapy (d=-0.49, p=0.122). These results suggest that after surgery scar appearance improved significantly more than after intralesional cryotherapy. In both treatment groups, observers (OSAS) rated the scars better than patients did (PSAS), at baseline as well as after treatment (Table 3). Although PSAS scores also improved after intralesional cryotherapy (d=-0.40, p<0.001), they showed much more improvement after surgery (d=-1.41, p<0.001).

Pain and itch symptoms significantly decreased after surgery (d=-0.73, p<0.001 and d=-0.90, p=0.005, respectively), while after intralesional cryotherapy no statistically significant improvement could be found (Table 3).

Only small and statistically non-significant QoL changes were observed after keloid treatment using the Skindex-29, SF-36 and EQ-5D-5L questionnaires and no differences were found between surgery and cryotherapy (Table 3).

Table 3. Mixed Model outcome over time for both treatment groups and the difference between the treatments

	Surgery			Cryotherapy			Difference		
Primary outcome	**Estimate**	***d***	***p-value***	**Estimate**	***d***	***p-value***	**Estimate**	***d***	***p-value***
POSAS									
Baseline	71.3			71.6					
52 weeks	37.7	**-2.03**	**<0.001**	63.5	-0.49	0.122	25.5	**1.54**	**0.001**
PSAS									
Baseline	40.6			41.6					
52 weeks	22.6	**-1.41**	**<0.001**	36.5	**-0.40**	**<0.001**	12.85	**1.01**	**0.022**
OSAS									
Baseline	30.5			30.0					
52 weeks	15.1	**-2.39**	**<0.001**	27.1	-0.45	0.174	12.5	**1.94**	**<0.001**
Secondary outcomes	**Estimate**	***d***	***p-value***	**Estimate**	***d***	***p-value***	**Estimate**	***d***	***p-value***
Itch score									
Baseline	6.3			6.2					
52 weeks	3.9	**-0.90**	**0.005**	5.4	-0.28	0.405	1.7	0.61	0.185
Pain score									
Baseline	4.5			5.3					
52 weeks	2.7	**-0.73**	**<0.001**	4.8	-0.21	0.592	1.3	0.52	0.324
Skindex-29									
emotional	33.0			43.8					
Baseline	26.2	-0.28	0.265	36.9	-0.28	0.298	0.07	0.00	0.994
52 weeks									
SF-36 MCS									
Baseline	46.3			46.0					
52 weeks	42.6	-0.32	0.188	48.1	0.18	0.495	5.83	0.50	0.164
EQ-5D-5L									
Baseline	0.84			0.72					
52 weeks	0.80	-0.19	0.558	0.79	0.31	0.407	0.10	0.50	0.315

Model was developed by backwards selection, first model included sex, age, skin color, location, keloid duration, treatment and all interactions with time, time^2 and the logarithm of time. POSAS: Patient and Observer Scar Assessment Scale (12 items, range 12-120, higher scores indicate poorer scars). PSAS: patient part of POSAS (6 items, range 6-60, higher scores indicate poorer scars). OSAS: observer part of POSAS (6 items, range 6-60, higher scores indicate poorer scars). Itch and pain scores are single items of the PSAS (range 1-10, 10 worst). Skindex-29 is a disease specific quality of life (QoL) instrument (30 items, scores 0-100, lower values represent better QoL) with 3 subscales and a sum scale. SF-36 MCS: SF-36 mental component score, the SF36 is a general QoL instrument with 36 items (scores range 0-100, with 100 representing better QoL, scores are standardized for the normal population with mean of 50 and SD of 10). EQ-5D-5L index: EuroQol 5 dimension 5 level test is a utility measure (5 item health state with population based preference scores, 0 to 1 with 1 representing best QoL). Model values are given for outcomes with highest expected differences. The other outcome measures (Skindex-29 functional, symptomatic and sum scales, and SF-36 Physical Component Score) did not show significant differences over time for each treatment or between treatments.

Supplementary Table 1. Mixed linear model parameters

	Intercept		time linear		time quadratic		time logarithmic	
Outcome	estimate	p-value	estimate	p-value	estimate	p-value	estimate	p-value
POSAS								
Surgery	71.3	<0.001	3.98	<0.001	-0.046	<0.001	-29.5	<0.001
Additional cryotherapy	0.311	0.961	-3.53	0.005	0.040	0.016	25.5	<0.001
PSAS								
Surgery	40.6	<0.001	1.68	0.017	-0.019	0.035	-13.5	<0.001
Additional cryotherapy	0.977	0.844	-0.930	0.331	0.011	0.393	8.16	0.103
OSAS								
Surgery	30.5	<0.001	2.10	<0.001	-0.024	<0.001	-15.3	<0.001
Additional cryotherapy	-0.498	0.842	-2.42	<0.001	0.027	<0.001	16.8	<0.001
Itch score								
Surgery	6.28	<0.001	0.169	0.280	-0.001	0.484	-1.86	0.025
Additional cryotherapy	-0.123	0.906	-0.011	0.958	0.000	0.969	0.644	0.567
Pain score								
Surgery	4.49	<0.001	-0.131	0.421	0.002	0.450	0.183	0.830
Additional cryotherapy	0.763	0.417	0.151	0.500	-0.001	0.638	-0.721	0.538
Skindex 29 emotional scale								
Surgery	33.0	<0.001	1.08	0.357	-0.010	0.505	-8.99	0.139
Additional cryotherapy	10.8	0.266	-0.360	0.823	0.007	0.751	0.165	0.984
SF-36 MCS								
Surgery	46.3	<0.001	-0.443	0.421	0.003	0.715	3.02	0.281
Additional cryotherapy	-0.316	0.945	0.557	0.456	-0.005	0.624	-2.58	0.504
EQ-5D-5L index								
Surgery	0.843	<0.001	0.018	0.178	-0.000	0.141	-0.073	0.284
Additional cryotherapy	-0.120	0.161	-0.028	0.149	0.000	0.150	0.143	0.141

Model was developed by backwards selection, first model included sex, age, skin color, location, keloid duration, treatment and all interactions with time, time^2 and the logarithm of time. POSAS: Patient and Observer Scar Assessment Scale (12 items, range 12-120, higher scores indicate poorer scars). PSAS: patient part of POSAS (6 items, range 6-60, higher scores indicate poorer scars). OSAS: observer part of POSAS (6 items, range 6-60, higher scores indicate poorer scars). Itch and pain scores are single items of the PSAS (range 1-10, 10 worst). Skindex-29 is a disease specific quality of life (QoL) instrument (30 items, scores 0-100, lower values represent better QoL), the skindex-29 has 3 subscales and a sum scale. SF-36 MCS: SF-36 mental component score, the SF-36 is a general QoL instrument with 36 items (scores range 0-100, with 100 representing better QoL, scores are standardized for the normal population with mean of 50 and SD of 10). EQ-5D-5L index: EuroQol 5 dimension 5 level test is a utility measure (5 item health state with population based preference scores, 0 to 1 with 1 representing best QoL). Model values are given for outcomes with highest expected differences. The other outcome measures (Skindex-29 functional, symptomatic and sum scales, and SF36 Physical Component Score) did not show significant differences over time for each treatment or between treatments.

Mean scar volume reduction was 95% after surgery with brachytherapy, but only 10% after surgery with corticosteroid injections, due to recurrences that were sometimes even three times larger than the original keloid (Table 2). This shows that keloid excision followed by corticosteroid injections has considerable risk on making the condition worse. Recurrences were more common after treatment for primary keloids, because most recurrences were found after keloid excision followed by corticosteroid injections (Table 2). Intralesional cryotherapy, on average gave a 40% scar volume reduction in primary keloids and merely 1% in resistant keloids. Thus, primary keloids seemed to respond better to cryotherapy than resistant keloids did.

Correlations between changes in scar symptoms, aesthetic outcome and changes in QoL are shown in Table 4. Changes in Skindex-29 sum and emotional subscale scores positively correlated to both changes in scar symptoms and aesthetic outcome. Changes in the Skindex-29 symptomatic subscale only correlated to changes in scar symptoms, and the Skindex-29 functional scale showed a moderate correlation to changes in scar symptoms, although not statistically significant (Table 4). Changes in the generic SF-36 scores correlated to neither changes in physical symptoms nor aesthetic outcome, while changes in EQ-5D-5L scores only showed a moderate non-significant correlation with changes in physical symptoms.

Table 4. Non parametric correlations between changes in scar symptoms, aesthetic outcome and quality of life outcomes

	Δ scar symptoms		**Δ aesthetic outcome**	
	r	**p**	**r**	**p**
Δ skindex-29 emotional scale	**0.621**	**0.001**	**0.561**	**0.004**
Δ skindex-29 functional scale	0.374	0.065	0.224	0.282
Δ skindex-29 symptomatic scale	**0.698**	**<0.001**	0.331	0.106
Δ skindex-29 sum scale	**0.633**	**0.001**	**0.539**	**0.005**
Δ PCS	-0.217	0.297	-0.231	0.266
Δ MCS	-0.285	0.168	-0.163	0.437
Δ EQ-5D-5L	-0.396	0.062	0.008	0.970

Δ: difference in outcome score between baseline and last available complete measurement. r: Spearman's rho correlations. p: p-value. PCS: SF-36 physical component scale. MSC: SF-36 mental component scale. EQ-5D-5L: EuroQOL 5 dimension utility measure.

Discussion

We performed a randomised controlled trial to compare the effectiveness of different invasive keloid treatments. Due to unexpectedly inferior results following intralesional cryotherapy, we prematurely stopped inclusion even before 20% of planned inclusion was reached. Nevertheless, after surgery with brachytherapy we still found statistically significant positive effects on scar appearance, our primary outcome. Unfortunately, we were not able to demonstrate an improvement of QoL following different keloid treatment strategies. Also, no differences in QoL between keloid treatments was found, although scar appearance and symptoms improved significantly more after surgical treatment with adjuvant therapy than following intralesional cryotherapy. Our trial fell short of statistical power due to prematurely stopping the inclusion.

The results of the present randomized controlled trial showed that intralesional cryotherapy is inferior to surgical excision with brachytherapy for treatment of resistant keloids, which is in contrast to results from previous reports on intralesional cryotherapy that showed much better results for treatment of recurrent keloids [5, 8]. There are several explanations for the difference between our unsatisfactory results of intralesional cryotherapy compared to the previous favourable results in other series. First, selection and indication bias may have occurred in previous case series, which was precluded by randomisation in our study, although it is unclear which keloids would respond better to intralesional cryotherapy. Second, outcome measures used were not the same. Previously, a non-validated 4-point one question scale was mainly used for patient satisfaction, while in the current trial we used the POSAS with good reliability and validity. Third, after intralesional cryotherapy we encountered permanent pigmentation differences that were more striking than after corticosteroid injections or brachytherapy. The first reports on intralesional cryotherapy noted no hypopigmentation but only had treated fair skinned patients [5, 7]. Later reports that included dark skinned patients also found permanent hypopigmentation after intralesional cryotherapy resulting in less patient satisfaction [10, 11]. Finally, publication bias may be a possible explanation why only publications showing favourable results of intralesional cryotherapy could be found, while reports of other groups that found poor results were not published or even written down.

Scar appearance was rated worse by patients than by observers probably because of their different perspective and expectations. Observers may have seen many poor scars professionally while patients only had their own scar as a reference. Patients may have hoped and expected that treatment would give them a normal looking scar, while physicians knew the challenges of keloid treatment and expected aberrant scar appearance even after effective treatment. Analogous to other areas of plastic surgery, patient education on treatment goals and expected outcomes is key in reducing dis-

satisfaction [25]. Also, high hopes of patients may in part explain the high number of additional treatment needed in the cryotherapy group. All patients knew about the alternative treatment when they gave informed consent, and the hope for a completely flattened scar may have fed the wish for additional treatments.

We expected scar appearance to correlate with patients' QoL, but we were not able to demonstrate QoL improvement after different keloid treatment strategies. We found a reduction in POSAS score of 47%, but possibly the scar was still so bothersome after treatment it did not show QoL improvement, or we lacked statistical power as a result of prematurely terminating the study. Obviously, QoL is related to more factors than the effects of keloid treatment only. While the effects of keloid treatment on one scar can be assessed with the POSAS, this is not possible for QoL measures. Because many patients had more than one keloid, these other keloids may still have negatively affected QoL after treatment of only one keloid. This bias may even have increased after expanding the inclusion criteria in order to improve patient inclusion.

Intralesional cryotherapy did seem to improve primary keloids; we found similar volume reduction compared to other groups (30-63%), while the keloids treated with cryotherapy tended to be larger at baseline (borderline significant p=0.054) [5, 8, 11]. Van Leeuwen et al. [11] who treated a series of primary keloids with intralesional cryotherapy also found a relevant POSAS improvement (39% reduction). Because of the inclusion of many resistant keloids in the present trial we could not confirm this finding (11% POSAS reduction). Intralesional cryotherapy may be a good option for primary keloids, specifically if patients do not want to risk a recurrence larger than the original keloid. Further proof of intralesional cryotherapy efficacy compared to other primary keloid treatment strategies and the effect of keloid size on intralesional cryotherapy efficacy is needed. However, patients should be adequately informed about treatment outcomes of both cryotherapy and excision with corticosteroid injections, resulting in relatively low patient satisfaction and frequent need for additional treatment. In addition, we believe there are indications for cryotherapy use in specific situations. For example, in young children when brachytherapy is less suitable, or if after keloid excision the resulting defect can only be closed using transposition flaps, resulting in more and larger scars.

Analyses were done for on protocol measurements, because in the intralesional cryotherapy group more than half of the patients requested additional treatment during follow-up. Therefore, considering the high number of measurements off protocol, it was not valid and meaningful to perform intention to treat analyses.

We terminated the trial prematurely, because patients were not satisfied with the results of intralesional cryotherapy. At the time the trial was designed neither a Data Safety Monitoring Board, nor stopping rules were mandatory following IRB protocol. Also an interim analysis was not planned, because we aimed at a higher inclusion rate

and, based on available evidence at that time, we had not anticipated such a high occurrence of dissatisfaction 6 to 9 months prior to final follow-up. Although we included less than 20% of the planned number of patients, prematurely stopping a trial to protect patients from harm caused by unnecessary treatments (delay in adequate treatment, pain and discomfort after treatment) is always justified [26].

We previously reported that keloids causing pain or itch symptoms have a high negative impact on quality of life [1]. The results of the present trial could not completely confirm our previous findings but showed that reduction in scar symptoms can lead to improved quality of life. Reduction in scar symptoms showed higher correlation with disease specific QoL (Skindex-29), and a trend towards general QoL improvement on the EQ-5D-5L, while improvement in aesthetic outcome was not as strongly correlated to QoL.

In conclusion, for resistant keloids intralesional cryotherapy is inferior to keloid excision followed by brachytherapy. Intralesional cryotherapy can improve primary keloids and may be indicated in these patients and specific cases, but further research on the efficacy of intralesional cryotherapy for primary keloids compared to other treatments is warranted.

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Optimal High Dose Rate Brachytherapy Fractionation Scheme after Keloid Excision: A Retrospective Multicenter Comparison of Recurrence Rates and Complications

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Abstract

Background Extralesional keloid excision followed by brachytherapy is currently considered the most effective treatment. However, the optimal brachytherapy dose and fractionation scheme is unknown and radiation may have considerable side effects. Because keloid formation is a benign condition, often in young patients, it is particularly important to minimize these adverse effects. Therefore, it is key to find the optimal radiation fractionation scheme for keloid treatment.

Methods Patient cohorts from three centers treated with keloid excision followed by 2x9 Gy, 3x6 Gy, or 2x6 Gy high dose rate (HDR) brachytherapy were retrospectively compared regarding recurrence (after at least 12 months follow-up) and complications (after at least 1 month follow-up), using logistic regression analyses.

Results A total of 238 keloids were treated. An overall full recurrence rate of 8.3% was found. After correction for confounders (sex, skin color, keloid location, keloid duration) no statistically significant differences in recurrence rates could be discerned between fractionation schemes. There were 12.8% major and 45.6% minor complications. Lower radiation dose resulted in significantly less complications (OR 0.35, p=0.015).

Conclusions After excision of resistant keloids, HDR brachytherapy with a biological equivalent dose around 20 Gy is recommended based on both low recurrence and complication rates.

Introduction

Keloids are benign fibro-proliferative lesions, which may impair quality of life due to itch, pain, and aesthetic disfigurement [1, 2]. Because of substantial therapy resistance and high recurrence rates, many treatment options are available with little consensus on which is best. Intralesional corticosteroids injection is widely used as primary treatment, with response rates of 77-90% and recurrence rates of 9-50% [3-5]. If non-invasive therapy fails, there is little evidence which invasive technique gives maximal efficacy with minimal morbidity. Since 1909, radiation therapy has been successfully used to treat keloids [6]. Currently, its combination with excision is considered the most effective keloid treatment with reported recurrence rates under 5% [7]. However, it is an invasive technique that may have considerable side effects. It seems key to find the optimal balance between maximum efficacy and minimum side effects by determining the optimal radiation fractionation scheme.

In the last century different radiation modalities and doses have been used for keloid treatment. Evaluation of all modalities combined showed that a biological equivalent dose (BED) of at least 30 Gy is needed to achieve recurrence rates under 10% [8]. Previous research on radiation after excision of keloids showed that high dose rate (HDR) brachytherapy gives favorable results compared to external radiation or low dose rate (LDR) brachytherapy [7, 9]. A short interval from surgery to start of radiation and hypofractionation is currently recommended [7, 10, 11]. These recommendations are largely based on results from studies using external beam radiotherapy (EBRT), since there exist only few studies on HDR brachytherapy [7, 8]. Because brachytherapy and EBRT are different techniques, radiation schemes cannot be directly extrapolated and goal BEDs may differ.

HDR brachytherapy radiation schemes that have been reported for keloid treatment vary considerably with total doses of 10-20 Gy in two to six fractions starting within 24 hours after surgery, resulting in recurrence rates of 0-44% [9, 10, 12-17]. This clearly illustrates more knowledge is needed on how to use HDR brachytherapy best in keloid treatment, by identifying the optimal fractionation scheme with high efficacy on recurrence combined with low rates of side effects.

Within a relatively small area in the Netherlands with similar population characteristics, three University Medical Centers provide keloid treatment with HDR brachytherapy after excision. Each center has been using a different radiation scheme for several years, which enabled a comparison of the long-term results of these three HDR brachytherapy schemes.

Methods

All consecutive patients who had been treated with keloid excision followed by HDR brachytherapy at University Medical Center Rotterdam (Erasmus MC) from 2010 to May 2014, University Medical Center Utrecht from 2009 to 2014, and VU Medical Center Amsterdam from 2003 to 2009 were included. The HDR brachytherapy radiation schemes remained unchanged during these periods.

In general, all centers used one standard after-loading catheter with an Ir192 source. At Erasmus MC (center 1) the catheter was implanted subcutaneously at the closed wound of the excised lesion. Subsequently, simulation films or a CT scan were obtained and treatment planning was performed with the most distant localization according to protocol being 1 cm outside the target. After optimization of the implant, 2 fractions of 9 Gy prescribed to the skin and/or a distance of 0.5 cm from the source train were applied. The inter-fraction time interval was minimally six hours.

At University Medical Center Utrecht and VU Medical Center (center 2 and 3) a 6 Gy fraction was applied within 3 hours after surgery. The next day one (center 3) or two (center 2) additional fractions of 6 Gy were given separated by at least six hours. The catheter was fixed between the subcutaneous and intradermal layers of stitches, entering and exiting at the extreme edges of the surgical wound. The fractions were dosed with 100% at 0.5 or 0.6 cm. Only when multiple catheters were used, a planning CT was made. Part of the cohort of center 3 was previously published [16].

The following data were retrospectively collected from patient charts: sex, age, skin type, number of keloids, size and location of treated keloid, pain and itch symptoms, cause and duration of keloid, previous treatments, acute complications after treatment, (partial or full) recurrence of keloid and additional treatments needed after excision combined with brachytherapy. In case follow-up was less than 1 year or important information was missing in the medical file, telephone interviews were performed. If during the course of data collection new information was noted in the medical files, these patients' data were updated.

Primary outcome was keloid recurrence, scored as 'no recurrence', 'partial recurrence' or 'full recurrence'. 'No recurrence' was defined as a scar that was not raised anymore and did not grow, ache or itch. If the scar was raised, but smaller than the original keloid, and pain and itch were still present, it was scored as a 'partial recurrence'. If compared to the original keloid no improvement in size, pain or itch was achieved, it was classified as a 'full recurrence' [18]. The type of any additional treatment given for the same keloid, after excision followed by brachytherapy, in our opinion also indicated the extent of recurrence. When invasive treatments had been used for a recurrence, this was considered suggestive of a fully recurred keloid. The best fitting recurrence classification was made based on postoperative scar size, symptoms and additional treatments. Keloids were

only evaluated on recurrence when follow-up was more than one year to enable an accurate report [19].

Secondary outcomes were complications following treatment. Complications were defined as 'minor' when they were treated conservatively (i.e., dehiscence, wound infection treated with oral antibiotics, radiation dermatitis grade 2, pigmentation differences), and defined as 'major' in case of surgical treatment, re-admittance, or major impact on patients' quality of life (i.e., radiation dermatitis grade 3 or 4, chronic wounds more than 3 months after surgery, pigmentation differences that needed treatment by tattooing or laser therapy).

The study was assessed by the institutional review board (IRB) of the Erasmus MC (MEC-2015-226) and followed national legislation, and the declaration of Helsinki.

Statistical Analysis

Exact tests (categorical data) or ANOVAs (continuous data) were used to compare outcomes between the centers. We performed binominal logistic regression analyses. To decide which covariates had to be included in the first model, we evaluated differences in keloid characteristics between cohorts and knowledge from previous studies [19-23]. Block 1 included the centers and block 2 included sex, age, skin type (light, tinted, dark), keloid location (upper trunk, ear, other), and duration of keloid. We did not correct for previous treatment because these data were not available for all centers. Missing values in characteristics were imputated for primary outcome analysis (max 7%). P-values <0.05 were considered statistically significant. All statistical analyses were performed using IBM SPSS Statistics for Macintosh version 22.0 (IBM Corp. Armonk, NY: IBM Corp.)

			Center 1	Center 2	Center 3
			2x9 Gy	*3x6 Gy*	*2x6 Gy*
Treatments		*Patients*	**43**	**54**	**49**
		Keloids	**86**	**87**	**65**
Complete data after chart review or from publication		*Patients*	**17**	**0**	**14**
		Keloids	**31**	**0**	**22**
Phone Interviews	Completed	*Patients*	**16**	**32**	**19**
		Keloids	**35**	**52**	**21**
	Declined	*Patients*	**2**	**2**	**10**
		Keloids	**2**	**2**	**14**
	Not reached	*Patients*	**8**	**20**	**6**
		Keloids	**18**	**33**	**8**
Keloids included in analysis	Recurrence (fu > 12 mo)	*Keloids*	**77**	**64**	**52**
		% of total	**90**%	**74**%	**80**%
	Complications (fu ≥ 1 mo)	*Keloids*	**86**	**80**	**60**
		% of total	**100**%	**92**%	**92**%

Figure 1. Overview selection of keloids in analyses
fu: follow-up, mo: months

Results

After combining data from the chart review and telephone interviews, we were able to analyze 226 out of 238 keloids (95%) (Figure 1). There existed differences between the three centers in relevant patient and keloid characteristics (Table 1). For example, skin types differed; center 2 treated much more fair skinned keloid patients (40%) than center 1 (9%). Center 1 treated more keloids that were present for a longer period of time than the other centers (15 vs. 7 years). Center 1 treated more patients with multiple keloids instead of a single keloid (1 keloid: center 1, 17%, center 2, 28%; >10 keloids: center 1, 27%, center 2, 17%). Acne (32%), surgery (23%), piercings (21%), and trauma (19%) were the most frequent causes of keloid formation. Center 1 had treated

most posttraumatic keloids (33%), center 2 most keloids caused by acne (44%), and center 3 most by piercings (32%).

Table 1. Patient and keloid characteristics per center.

			Center 1 2x9 Gy	Center 2 3x6 Gy	Center 3 2x6 Gy	p
PATIENTS			n=43	n=54	n=49	
Male		n (%)	24 (55.8)	26 (47.3)	19 (38.8)	0.263
Age	years	mean (SD)	36.4 (14.3)	32.9 (15.0)	34.4 (14.7)	0.509[e]
Previous keloid treatment	non-operative[a]	n (%)	6 (14.0)	13 (25.0)	17 (37.8)	0.087
	operative[b]	n (%)	34 (79.1)	35 (67.3)	26 (57.8)	
KELOIDS			n=86	n=87	n=65	
Male		n (%)	38 (44.2)	45 (51.7)	26 (40.0)	0.343
Age	years	mean (SD)	39.0 (13.4)	32.2 (13.1)	34.9 (14.7)	0.005[e]
Skin type[c]	I or II	n (%)	8 (9.3)	35 (40.2)[d]	13 (20.0)[d]	<0.001
	III or IV	n (%)	23 (26.7)	28 (32.2)[d]	7 (10.8)[d]	
	V or VI	n (%)	55 (64.0)	12 (13.8)[d]	26 (40.0)[d]	
Location	upper trunk	n (%)	45 (52.3)	36 (41.4)	25 (38.5)	0.180
	ear	n (%)	22 (25.6)	28 (32.2)	28 (43.1)	
	other location	n (%)	19 (22.1)	23 (26.4)	12 (18.5)	
Keloid size	length (cm)	mean (SD) range	5.3 (4.9) 1-26	4.3 (3.5) 0.7-21	5.4 (5.0) 1-26	0.228[e]
	width (cm)	mean (SD) range	2.2 (1.5) 0.5-8	1.8 (1.2) 0.5-7	2.4 (1.6) 0.5-8	0.076[e]
Previous treatment	non-operative[a]	n (%)	12 (14.0)	39 (45.9)[d]	23 (35.4)[d]	<0.001
	operative[b]	n (%)	53 (61.6)	39 (45.9)[d]	30 (46.2)[d]	
Pain present		n (%)	45 (67.2)	43 (58.1)	43 (66.2)	0.461
Itch present		n (%)	52 (80.0)	51 (68.9)	40 (61.5)	0.066
Duration of keloid	years	mean (SD)	14.7 (9.4)[d]	7.3 (6.3)[d]	7.0 (6.6)[d]	<0.001[e]
Follow-up time	months	mean (SD) range	30.9 (15.7) 1-70	43.7 (34.9) 0-109	40.6 (30.5) 0-116	<0.001[e]
Follow-up time >1 year	months	mean (SD) range	34.6 (13.2) 13-70	58.3 (28.9) 17-109	50.1 (26.5) 12-116	<0.001[e]

Characteristics are given with Exact tests to compare the treatment groups (Fisher's or Fisher-Freeman-Halton's) or one-way ANOVA when indicated. [a]Silicone sheets, topical treatments, intralesional corticosteroids injections, lasers. [b]Cryotherapy, Surgery with or without adjuvant treatment. [c]Skin type according to Fitzpatrick. [d]Totals do not add up to group totals due to missing values. [e]One-way ANOVA.

Because these differences between the cohorts could affect treatment outcomes, we corrected for sex, skin type, keloid location and keloid duration. After correction for these confounders, no statistically significant differences in recurrence rates were found between the three centers (Tables 2 and 3). The odds on a full recurrence were higher for males than for females, no significant effect of the other covariates was found.

Table 2. Keloid recurrences and treatment complications per center

	Center 1 2x9 Gy	Center 2 3x6 Gy	Center 3 2x6 Gy	Total
PRIMARY OUTCOME	**n=77**	**n=64**	**n=52**	**n=193**
Full recurrence	7 (9.1)	2 (3.1)	7 (13.5)	16 (8.3)
Partial recurrence	10 (13.0)	13 (20.3)	6 (11.5)	29 (15.0)
No recurrence	60 (77.9)	49 (76.6)	39 (75.0)	148 (76.7)
SECONDARY OUTCOME	**n=86**	**n=80**	**n=60**	**n=226**
Major complication[a]	16 (18.6)	11 (13.8)	2 (3.3)	29 (12.8)
Severe wound dehiscence	1 (1.2)	0 (0.0)	0 (0.0)	
Severe infection	4 (4.7)	0 (0.0)	0 (0.0)	
Dermatitis grade 3/4	0 (0.0)	0 (0.0)	0 (0.0)	
Hyperpigmentation needing treatment	1 (1.2)	1 (1.3)	0 (0.0)	
Hypopigmentation needing treatment	1 (1.2)	4 (5.2)	0 (0.0)	
Chronic wound (>3 months)	12 (14.0)	9 (11.3)	2 (3.3)	
Minor complication[a]	49 (57.0)	32 (40.0)	22 (33.7)	103 (45.6)
Wound dehiscence	19 (22.1)	8 (10.0)	11 (18.3)	
Infection	5 (5.8)	6 (7.5)	4 (6.7)	
Dermatitis grade 2	23 (26.7)	6 (7.5)	0 (0.0)	
Hyperpigmentation	35 (40.7)	11 (14.2)	8 (13.3)	
Hypopigmentation	33 (38.4)	25 (32.5)	3 (5.0)	
No complication	28 (32.6)	41 (51.3)	37 (61.7)	106 (46.9)

All data given are n (%).[a] Wound dehiscence was severe in case of surgical treatment or re-admittance to the hospital, infection was severe in case of re-admittance or surgical treatment, pigmentation differences were severe only when treated (with for example tattooing or laser treatment). Numbers do not add up because some keloids had both minor and major complications.

Before treatment with excision and brachytherapy, keloids caused symptoms of itch (62-80%) and pain (58-67%) in a majority of cases. After treatment, symptoms disappeared or diminished in many patients (no itch 70-79%, less itch 15-25%; no pain 86-89%, less pain 6-8%). Following treatment, only two patients reported more itch and one more pain symptoms.

The occurrence of minor and major complications is shown in Table 2. Most complications, and more severe complications were found for center 1, less in center 2, and even fewer in center 3. After correction for confounders, there were no significant differences between treatment with 2x9 Gy and 3x6 Gy in recurrence rates and complications, but there were significantly less complications following 2x6 Gy compared to 2x9 Gy (Table 3) (OR 0.35, p=0.015). Keloids at the upper trunk had an increased risk of complications (OR 2.5, p=0.032), and ear keloids had the least risk on complications (OR 0.43, p=0.05). A complication that occurred was an increase in pain following treatment. In one patient pain eventually completely disappeared, but another suffered from severe pain and regretted being treated with excision and brachytherapy. Also permanent alopecia was reported twice.

We observed 23 chronic wounds after treatment. However, there was a wide variation in consequences of these wounds. Twenty eventually healed without intervention; for one wound this took over a year and three wounds healed only after surgical debridement. One female patient was treated with 2x9 Gy for a keloid on the back, which had recurred after previous treatment with excision and 2x9 Gy brachytherapy. Subsequently, longstanding crusts became wounds that showed no signs of healing after surgical debridement (two times). Finally, seven months later she was operated again and the area that had been irradiated during both previous treatments was completely excised into healthy skin. After wide undermining, the wound edges could be closed primarily after which the area was treated with brachytherapy for the third time. Again, wound healing was complicated by a long-standing wound dehiscence due to tension, but after four months the wound had completely healed and showed no recurrence.

Table 3. Outcomes of binary logistic regression analyses corrected for sex, skin type, location, and keloid duration

	PRIMARY OUTCOME Full recurrence		95% Confidence interval		SECONDARY OUTCOME Any complication		95% Confidence interval	
	Odds	p-value	Lower	Upper	Odds	p-value	Lower	Upper
Constant	0.037	0.013			1.464	0.570		
Center 2: 3x6 Gy	0.551	0.534	0.084	3.598	0.646	0.321	0.272	1.533
Center 3: 2x6 Gy	3.086	0.106	0.787	12.106	**0.352**	**0.015**	0.151	0.819
Sex	**0.219**	**0.013**	0.066	0.725	0.741	0.341	0.401	1.372
Skin type III-IV	1.845	0.498	0.314	10.835	1.311	0.521	0.573	2.996
Skin type V-VI	2.594	0.290	0.443	15.182	2.109	0.098	0.871	5.106
Upper trunk	0.849	0.836	0.181	3.989	**2.476**	**0.032**	1.082	5.666
Ear	1.071	0.930	0.230	4.982	**0.429**	**0.050**	0.184	0.999
Duration of keloid	1.064	0.089	0.991	1.144	0.982	0.425	0.940	1.026

p-values considered statistically significant if $\alpha<0.05$.

Another female patient who had been previously treated for an earlobe keloid with excision followed by 10 Gy in 2 fractions on separate days with 8 MeV electron beam, presented 21 years later with a basal cell carcinoma at her earlobe. Due to her susceptibility to make keloids and expected deformity with surgical treatment, it was treated with radiotherapy.

Discussion

By retrospectively comparing patient cohorts from three different centers, we found in the present study that after extralesional keloid excision a brachytherapy radiation scheme of 2x6 Gy at 0.5 cm seems to be equally effective preventing recurrences as schemes using higher doses (2x9 Gy or 3x6 Gy), with a lower risk of complications, like infections, chronic wounds and apparent pigmentation differences.

Excision followed by radiation is widely considered the most effective treatment for keloids, and more specifically HDR brachytherapy seems to be the optimal radiation modality [7, 21, 24]. When reviewing existing studies, selection bias and variation in design may hamper strong evidence to support this. However, in our experience excision with brachytherapy is the current golden standard for the treatment of itching or painful resistant keloids [25-27], which was confirmed by the low recurrence rate of 8.3% in the present study. Also important is the good effect of this treatment on the relief of itch and pain, which causes the largest burden in keloid disease [1, 2].

Kal et al.[8] recommended a BED of over 30 Gy. Based on the analysis of results with EBRT and HDR brachytherapy combined, they showed a recurrence rate of <10% with a BED >30 Gy. Our present results show that a similar recurrence risk can be reached with a BED as low as 19 Gy. Guix et al.[13] described a recurrence rate under 10% with even a lower BED of 16 Gy (4x3 Gy). In center 3 three keloids had not received 2x6 Gy after excision as planned, resulting in even a lower radiation dose, and two of them had a recurrence (67%). If these keloids would be excluded the recurrence rate of center 3 would be 10%. This indicates that 2x6 Gy brachytherapy might be the lower limit for effective keloid prevention, which is in line with recent findings of Mankowski et al.[21], who reported a BED of 20 Gy is needed for recurrence rates <10%. Contrary to these findings regarding brachytherapy, recent attempts of EBRT after keloid excision with a BED under 30 Gy failed and these groups increased their dose [19, 20]. Van Leeuwen et al.[7] promote hypofractionation to even a single fraction, however, recent results of a 13 Gy single fraction scheme lead to 24% recurrence, which is inferior to results found with 2 to 4 fractions [28].

For resistant keloids a full recurrence rate under 10% is a good result. Partial recurrences were considered successful treatments if a stable state was reached and pain and itch

were relieved, but it is important to mention partial recurrence when informing the patient about the treatment. Many patients hope and believe that after treatment their scars will disappear and are therefore often disappointed by the aesthetic result. Patients should know that the treatment goal is to improve symptoms and keloid size, but that usually an optimal aesthetic result is not achievable.

In the present study we found much higher complication rates than previously described [7, 8, 21]. This could be explained because we actively searched the patient files for side effects and reported them in detail, while in literature complications are often not even reported. An important finding of the present study is that the lowest dose resulted in fewer complications.

Radiation can have carcinogenic effects that depend on dose, organ and age. Usually carcinogenesis becomes apparent after more than 10 to 20 years, and because the first publication on HDR brachytherapy was in 2001[13], it is hard to give an accurate estimation of the risk. Radiotherapy has been long used in keloid treatment and most cohort studies have reported no carcinogenicity, which gives no certainty when patients are not followed for more than 10-20 years. Some case reports exist on radiation induced malignancies after keloid treatment, but causality cannot be proven [29]. In general, radiation for keloid treatment is considered acceptable. We must be confident of the right indication: keloids that cause symptoms and are therapy resistant. Furthermore, we have to keep patient age in mind and use an appropriately low radiation dose, because radiation induced malignancies definitely occur following treatment of benign diseases [29, 30].

The geography of the Netherlands enabled a unique possibility to compare similar populations treated with different fractionation schemes during similar time-periods. Most studies have compared historical cohorts in periods quite far apart that could cause differences in outcomes due to inevitable improvements in equipment, technique and perioperative care. A drawback of our study design is a possible confounding by indication, caused by different physicians deciding that treatment with excision and brachytherapy was indicated. Excision with brachytherapy is considered a last resort treatment for aggressive, resistant keloids. Some keloids without any previous treatment in our cohorts had been treated straightaway with brachytherapy. However, most of these patients had previously suffered from other keloids that had been treated with many modalities with disappointing results, so they were not willing to go through the range of therapeutic options again.

The retrospective design of our study raises some limitations, due to missing data in the chart reviews and recall bias during interviews. This could have caused an underestimation of the occurrence of complications in the postoperative period. Recurrence mostly occurs in the first year after treatment, but can also occur several years later. Follow-up of at least one year is needed to correctly estimate the recurrence risk. In our analysis

of recurrences we used a minimum period of 1 year after surgery but fortunately the mean follow-up time of all centers was much longer.
In conclusion, a BED of more than 30 Gy has been the standard in radiation planning after keloid excision, but our results show that with HDR brachytherapy a BED lower than 30 Gy performs very well on recurrence as well as complication rates. We recommend using a lower radiation scheme of 2x6 Gy to reduce complications and minimize stochastic effects. For improved patient convenience, low risk on catheter dislocation, easier in house logistics and lower costs, planning both fractions on the day of surgery, with at least six hours in between, is a promising option that should be further analyzed in the future.

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General Discussion

This thesis aimed to improve care for keloid patients. As outlined in the introduction, this was done by evaluating the burden of keloids and by providing evidence on efficacy of keloid treatment. In the present chapter the main findings are discussed in the context of recent literature and clinical experiences, with recommendations for clinical practice and future research.

Part I Burden of keloids

Historically, keloid research has focused on observer ratings or objective measurements. Nowadays, medicine has changed and patient reported health related quality of life (HRQL) has gained attention. High burden was found in several other skin conditions, which makes it essential to measure HRQL in all skin conditions.

Although patient reported outcomes (PROs) and HRQL are becoming increasingly important outcomes in keloid disease, still only a few studies exist that invariably show the large impact of keloid disease [1]. We determined that keloids have a considerable burden on HRQL, specifically on the emotional or mental HRQL components. Assessed with a disease-specific HRQL instrument, patients reported to suffer from keloids as much as from psoriasis, eczema, rosacea, cutaneous lupus erythematosis, or acne vulgaris. Assessed by a generic HRQL instrument, physical functioning was hardly affected, but mental HRQL was clearly diminished even more than with arthritis or congestive heart failure. We found pain and itch scores are the most important predictors of burden on the different HRQL instruments (Chapter 2: *Emotional Quality of Life is Severely Affected by Keloid Disease: Pain and Itch Are the Main Determinants of Burden*).

In order to measure these PROs accurately good and validated instruments are needed. As generic HRQL measures do not include concepts like 'appearance', a disease specific instrument is needed. Mundy et al. looked into disease specific tools specifically for scars and found four options of which the Patient-Reported Impact of Scars Measure (PRISM) met most of the validation criteria [2]. We used another disease specific HRQL instrument: the Skindex-29, developed for psoriasis, but validated and used in many other skin diseases. It includes questions on symptoms and appearance of the skin condition. When evaluating longitudinal data, PROs responsiveness is very important, sometimes needing a very specific tool to detect clinically relevant changes. For example, Guy et al. developed a HRQL tool for head and neck keloids because they did not find the expected high burden on a generic or 'total body' keloid instrument [3]. The Skindex-29 has shown responsiveness (although not for keloids/scars) and the ability to use it in more than one condition, enabling comparison between diseases, which is impossible if the tool would have been designed for one condition only.

The importance of pain and itch in keloid burden has been confirmed by others [3,4], even in a group of visible keloids only. Of these facial keloid patients, 48% sought medical treatment because of their pain symptoms, and physical symptoms were considered to have the greatest effect on HRQL. Unfortunately, the effect of pain and itch reduction on the burden of keloids has not been addressed yet.

The majority of keloids are painful, but burn scars can also be painful, however, not much is known about the prevalence of painful normal scars. After review of the literature, we found only one study addressing pain in scars in general (not a subgroup of pathologic scars) in a proper way, finding pain in 9.7% of scars after cutaneous surgery, which in 1.7% was moderate or severe (Numeric Rating Scale >3) [5].

Pathologic and burn scars have the highest pain intensity (up to 6/10). Pain symptoms did not correlate with overall nerve fiber density consistently, while a correlation with an increased number of peptidergic fibers, either absolute or relative, might be present (Chapter 3: *A Systematic Review on Prevalence, Etiology and Pathophysiology of Intrinsic Pain in Dermal Scar Tissue*). In pain perception different types of fibers transmit the signals. We analyzed pain in keloids more in-depth to evaluate its nociceptive and neuropathic components to find possible therapeutic options. However, sensitive characteristics differed substantially between patients, making it impossible to assign pain to a specific type of fiber. About half of the patients were classified with neuropathic pain. In contrast to the heterogeneity of the sensitive characteristics of keloids, their dermal and epidermal nerve fiber density was uniformly decreased compared to normal skin, independent of their sensation profile (Chapter 4: *Sensory Perception and Nerve Fiber Innervation in Patients with Keloid Scars*). In contrast to what we expected we could not correlate nerve fiber density to the patients' pain symptoms.

A recent review on neuropathic pruritic and pain symptoms stated that dysfunction of unmyelinated C-fibers (warmth detection, heat pain and cold pain thresholds) and thinly myelinated Aδ-fibers (mechanical pain, heat pain and cold detection threshold) are of special interest [6]. Intra-epidermal nerve fiber density (IENFD) is often decreased in conditions with neuropathic numbness (negative symptom) or neuropathic pruritus or pain (positive symptom), a value below the 5th percentile is indicative of small fiber neuropathy [6]. Because only the standard (age and sex dependent) IENFD of the lower leg is known, in our pilot we could not calculate the percentiles and whether these corresponded to the results of the DN4 or Pain Detect questionnaires or QST results. Another study by Springer et al. [7] evaluated post-thoracotomy pain with objective measures (among others QST and IENFD), like we did for painful keloids in chapter 4. On QST they found changes, but no distinguishing profile. They did not find a correlation of pain with fiber density and claimed this is in line with most recently published findings. Like us, they concluded too much heterogeneity precludes a clear functional or structural profile discriminating patients with pain from those without pain.

Despite our efforts, no further insight in the structural mechanism behind keloid pain could be found. Symptoms and pain characteristic differ widely between patients and some suffer neuropathic pain; this makes evaluation of pain for each patient important to ensure an individualized treatment approach.
I am convinced pain and itch are main determinants of burden in keloid patients and that controlling these symptoms should be the main goal of treatment. In our prematurely terminated randomized controlled trial (RCT; Chapter 7: *Intralesional Cryotherapy versus Excision with Corticosteroids or Brachytherapy for Keloid Treatment: A Randomized Controlled Trial*), we found pain and itch reduction, but could not show a quality of life improvement, probably because the study was underpowered. Others were able to show HRQL improvement after successful keloid treatment (no recurrence) [8, 9], underscoring that successful treatment can relieve the burden of keloid patients.

Recommendations for further research

- For future studies, evaluating keloids incorporating a HRQL instrument is inevitable. More data will provide more opportunities to determine which factors (e.g., pain) cause disease burden, and will inform us how improving these factors (after treatment) improves HRQL.
- As we deem pain and itch important factors in HRQL of keloid patients, relief of these symptoms should be the main treatment goal. Most recent studies only looked at symptom reduction as a secondary outcome, while we would advocate making this (or an instrument including this) the primary outcome.
- Hardly any literature on epidemiology of keloids or scar pain in general is available, while it could help us understand the size of the problem better. In this century only Sun et al. [10] and Kipora et al. [11] have studied keloid prevalence, showing a prevalence of 0.3% and 8.3% in a Taiwanese and Kenyan population, respectively. No recent numbers are available for Caucasian, Indian, Arabic or Hispanic patients.
- At our institution we only evaluate keloid patients' pain routinely with a numeric rating scale from 1 to 10. If pain is a patient's main symptom we should additionally determine whether the pain has neuropathic features. We should start evaluating if symptomatic care with common topical medical treatments for neuropathic pain (i.e., capsaicin or lidocaine cream, Botulinium toxin injections) or even systemic neuromodulators (i.e., amitryptalin, pregabalin) are effective in treating keloid pain, can control these symptoms and improve HRQL.

Part II Treatment of keloids

Keloid treatment is a challenge for both patients and physicians due to high therapy resistance, recurrences and several treatment side effects. A large variety of treatments is available. Several treatment algorithms have been described, but there is no overall consensus on keloid treatment. In the next paragraphs my findings are discussed and recommendations are given based on my experience and the studies described in this thesis.

Importance of information

During the work on this thesis, I regularly evaluated keloid patients, of whom many had not been well informed about their condition prior to their consultation and consequently had no idea of the treatment options and difficulties. Some of them had been previously treated, but they had only been informed about the provided treatment at that time, and other options had not been discussed. With better patient education and counseling, like in many other conditions [12], patients can feel more in control of their treatment. Control is what patients may be looking for when bothered with a scar that is 'out of control'. Only after proper education and acknowledgement of the severity of their condition, patients felt at ease and a good patient-physician relation could be build.

For other conditions and treatments it has been shown that patient satisfaction with treatment is dependent on patient satisfaction with information before treatment and the quality of the physician-patient-relation [13]. Also in keloid treatment, with often poor to moderate aesthetic outcomes, which is not desired by patients, treatment satisfaction may rise with good patient education, managing expectations about outcomes. On the other hand, knowledge about available treatments may also encourage patients to press for these treatments. Even in case their keloid is actually not suitable for it, and demanding referral to other departments or hospitals.

If medical conditions are benign, not life-threatening, and severity can be judged by the patient; HRQL improvement is the main treatment goal. When many treatment options exist that all suffer from some disadvantages to patients, patient and doctor together should make a decision about the treatment plan. To do right to patient autonomy, built trust and improve understanding (therefore it is important to have one physician doing follow-up), leading to better treatment adherence and maybe even better outcomes [12, 14]. In summary, adequate patient information, a good physician-patient relation (follow-up by same physician), and shared decision-making will add to HRQL improvement.

Stepped care approach

Among experts there is agreement on a stepped care approach in keloid treatment options, although the specific steps sometimes differ [15-21]. Due to treatment side effects, stepped care approaches should be standardized, with each step losing successfully treated patients, exposing a decreasing number of patients with therapy resistant keloids to more invasive treatments.

Mostly three steps are described moving from non-invasive (e.g. silicones, topical corticosteroids, pressure), to minimally invasive (e.g. corticosteroids injections, other injectables, lasers) to invasive treatment with surgery and adjuvant treatment (e.g. corticosteroids injections, brachytherapy). In our experience silicones and topical treatment can be beneficial to other treatments but are not sufficient as a stand-alone keloid treatment. Also, for the last invasive step a distinction between surgery with adjuvant treatment and surgery with radiotherapy could be made to differentiate this last resort treatment, with long-term risks due to ionizing radiation, from other treatments. Finally, different steps should be made for primary and recurrent keloids (Figure 1; Chapter 7: *Intralesional Cryotherapy versus Excision with Corticosteroids or Brachytherapy for Keloid Treatment: A Randomized Controlled Trial*).

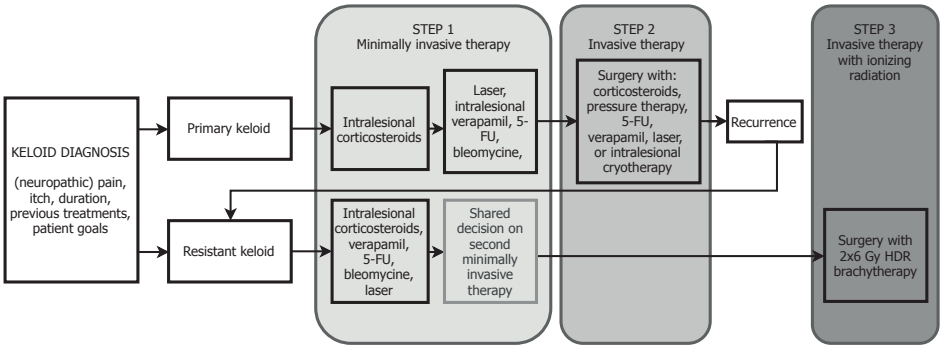

Figure 1 Proposed treatment algorithm
5-FU: 5-fluorouracil

Unfortunately, options within different steps can be limited. In part due to local practice (availability of lasers, medications or instrument makers) and treatment availability (registered medications, use of chemotherapeutic protocols). But also, because evidence comparing different treatments is not up to current standards of evidence-based medicine, which makes it harder to convince institutions of the added value of a certain treatment modality in patient-care and change institutions practices and treatment availability.

In general, it is recommended to use conservative treatments whenever possible, at the moment the main conservative treatment modality available is corticosteroid

injections. Unfortunately, not all keloids respond to corticosteroids, which leaves many physicians without any other option than surgery. While the efficacy of many other treatments has been shown, no definite statement can be made on the comparison of corticosteroid injections with other conservative treatments. In chapter 5 we compared the effect of 5-fluorouracil (5-FU) injections with corticosteroid injections and found that 5-FU alone is not more effective than triamcinolone acetonide (TAC) injections. However, a combination therapy may be more effective, and might have a positive effect in keloids that do not respond to corticosteroids only.

At the same time, others were also trying to answer this exact question with a review of available evidence [22-24]. Carroll and Patel [22] performed a non-systematic review, including 5 papers of which 3 RCTs, and they suggested that although more studies are needed, combination therapy is likely to give optimal results. Shin and Kim [24] only investigated the additional use of 5-FU or TAC after keloid excision; they included 2 and 4 studies, respectively, without providing inclusion criteria. The 2 included studies on additional 5-FU use were the most favorable. In addition, also a small arbitrary selection of available papers on surgery in combination with corticosteroid injections was included and they compared it to all kinds of controls (e.g. excision with radiotherapy). They concluded that the additional use of 5-FU is highly effective and that TAC has no effect on recurrence reduction after excision. However, these conclusions were based on incomplete data and flawed analysis. Shah et al. [23] published a systematic review one year after ours was published; they included additional case-reports and a double publication. Conclusions similar to ours were drawn, but they considered there exists enough evidence to routinely start using 5-FU/TAC combination therapy in keloid treatment. Recently, one case report [25] showed good results with 5-FU/TAC, but no comparison to TAC alone has been published. Furthermore, no new comparative studies on 5-FU efficacy, specifically in combination with TAC, have been published since our review was published.

To our opinion, our findings still need further research. The group of Dr. Niessen in the VUmc (Amsterdam, Netherlands) wanted to provide this evidence, but current Dutch regulations for chemotherapeutic use made the treatment so costly that a trial could not be funded and consequently, 5-FU injections for keloids are probably not feasible as standard of care in the Netherlands.

Intralesional cryotherapy and excision followed by brachytherapy

Brachytherapy has been used for years in keloid treatment and current literature still regards it as the most effective treatment option preventing recurrences [26, 27]. Over time different ways of radiation delivery have been used, and new methods are being explored with the goal to lower patient discomfort and radiation dose on healthy tissue. Recently, a topical application of a radiation source was very effective in a small

series [28]. The reach of only 11 mm depth can be a limitation for bigger keloids. But an advantage of topical application is that no new wound healing process with an inflammatory phase can excite keloid growth. It could also be a solution for keloids covering such large areas that wound closure, let alone tension free closure, is impossible.

Another more controversial approach for severely affected keloid patients was suggested by Ogawa et al. and Komatsu et al. who used transposition flaps to close the big defects created after excision of large keloid areas and delivering 20 Gy radiation at both flap recipient and donor incisions [29, 30]. While using skin transpositions in keloid surgery is usually considered a risk because lengthening the incision lines can give rise to recurrence of a bigger keloid, they averted this by prophylactic radiation treatment of the donor site incisions, exposing a much larger area to ionizing radiation. Whether these techniques will evolve to be beneficial to other ways of application should be confirmed.

However, ionizing radiation does come at a cost. Reports of malignancies in radiated tissue after keloid treatment do exist [31, 32] and we also found a patient with a basal cell carcinoma on the earlobe years after keloid excision and brachytherapy of the same area (Chapter 8: *Optimal High Dose Rate Brachytherapy Fractionation Scheme after Keloid Excision. A Retrospective Multicenter Comparison of Recurrence Rates and Complications*). In general, we can state that when needed radiation is justified, but in a dose 'As Low As Reasonably Achievable' (ALARA principle). Beside serious stochastic effects, there are also acute radiation effects that can be problematic, specifically wound healing problems. Therefore, the priority is to use as little ionizing radiation as possible.

We tried to decrease our ionizing radiation dose in keloid treatment in two ways. First, we investigated whether intralesional cryotherapy, a new promising treatment, could reduce the number of patients needing brachytherapy (Chapters 6 and 7: *Intralesional Cryotherapy versus Excision with Corticosteroids or Brachytherapy for Keloid Treatment: A Randomized Controlled Trial*). No doubt, intralesional cryotherapy has been proven superior to topical cryotherapy [33, 34]. Unfortunately, our RCT had to be terminated prematurely, because too many patients were not satisfied with intralesional cryotherapy results. On the other hand, our study with limited statistical power was able to show that for resistant keloids, excision with brachytherapy is superior to intralesional cryotherapy. In the small group of primary keloids, cryotherapy performed better than in resistant keloids and excision with corticosteroids had higher recurrence rates than excision with brachytherapy (the first was performed in primary keloids and the latter in resistant keloids). However, group sizes were too small to determine how intralesional cryotherapy performs compared to excision with corticosteroids. A recent review of intralesional cryotherapy effects on keloids showed slightly more volume reduction compared to our results in primary keloids; just like us they also found no complete keloid eradication and similar recurrence rates [35].

Second, we studied whether a lower radiation dose could be used. Currently, we cannot replace excision with brachytherapy with intralesional cryotherapy, but we found a benefit of a lower radiation dose on acute radiation effects (Chapter 8: *Optimal High Dose Rate Brachytherapy Fractionation Scheme after Keloid Excision. A Retrospective Multicenter Comparison of Recurrence Rates and Complications)*. After correction for confounders (sex, skin color, keloid location, keloid duration), no statistically significant differences in recurrence rates could be discerned between fractionation schemes with 2x9 Gy, 3x6 Gy and 2x6 Gy, but significantly less minor and major complications as infections, wound dehiscence and chronic wounds were found. This shows a BED of 20 Gy is sufficient to control keloid recurrence. Subsequently we use 2x6 Gy at our center, instead of one fraction per day we give both fractions on the operative day to minimize the risk of overnight catheter dislocation, reduce hospital admission costs and to meet patients' preferences.

Need for a keloid treatment guideline

In case a patient is referred to our outpatient clinic with an earlobe keloid we usually start conservatively with serial triamcinolone acetonide injections. If this patient would visit another hospital, it could well be that a different treatment would be prescribed. For example; 5-FU with or without TAC injections are often used in Asia; in other regions dermatologists have more experience and availability of lasers; some centers have very competent medical instrument makers that can make good pressure devices; a radiotherapist works with ionizing radiation and might suggest brachytherapy as an option. Occasionally, patients are treated for many years without good effect, while paradoxically sometimes a patient is referred for radiation as a primary treatment.

Due to above mentioned undesired variation in clinical practice, it seems like every keloid patient follows a different path through our healthcare system. In the Netherlands, care for keloid patients could be improved if keloid patients would be offered an evidence-based, standardized health care path (stepped care) with identical therapeutic options, including shared-decision making. A multidisciplinary guideline, including general practitioners, dermatologists, plastic surgeons, radiotherapists and general surgeons, could facilitate this [36].

So what could a guideline offer? It would lead to awareness of treatment difficulties among general practitioners and stimulate standardized referrals. In my opinion, dermatologists could best provide the conservative and minimally invasive treatments, because they often have experience with and access to topical and intralesional medical treatments, but also lasers and cryotherapy. They regularly perform serial treatments including long-term follow-up of patients and their outpatient clinics are suitable to build on a long-term patient-physician relation. If after set periods of time the first treatment steps are not successful, patients are referred for surgical treatment. These latter

patients are preferably assessed at a joint outpatient clinic, to decide what the surgical plan will be.
A guideline can also aid physicians in informing patients on treatments outside of their own experience and inform them what centers do provide this treatment if doctor and patient decide on the patient's best option. A guideline also can suggest what appropriate treatment periods are for the particular treatments. In times of expanding health-care costs and limited resources, cost reduction can be made by reducing practice of 'lower value care' by providing a 'best practice' guideline [14, 37].

Recommendations for further research

- Future research should focus on finding good conservative or minimally invasive treatments as alternative to serial intralesional corticosteroid injections for patients that show poor results with corticosteroids injections and that are not suitable for surgery or wish to stay with conservative treatment.
- Further research is needed to establish the place of intralesional cryotherapy (if any) within the treatment steps (stepped care). Whether it should be a step between minimally invasive treatment and surgery, whether it belongs to the same step as excision followed by corticosteroids, or whether it could completely replace excision followed by corticosteroids. Another topic of interest would be to investigate whether primary keloids all react well to intralesional cryotherapy, or whether certain keloid and patient characteristics have impact on the response (e.g., younger age, smaller keloids) predicting treatment effect.
- The outcomes of our (Erasmus MC Rotterdam) new radiation protocol after keloid excision (2x6 Gy HDR Ir192 brachytherapy on day of surgery with at least 6 hours apart) should be prospectively evaluated and maybe compared to the 2-day schedule (VUmc Amsterdam).
- The feasibility of large flap surgery with prophylactic brachytherapy and non-invasive radiation skin patches for keloids covering large body areas should be further explored.

Future research on keloids outside the scope of the present thesis

Etiology

Research on the etiology of keloids was not part of the present thesis. Keloids are fibrous tumors, accompanied by an aggravated inflammatory response of the reticular dermis. Although over the years groups have tried to find out what exactly causes keloids, it seems to be a multifactorial condition. No proven or universally accepted pathway has been identified. The theory of Ogawa's group is that after trauma or inflammation the

inflammatory response is prolonged and aggravated by mechanical tension on the skin (local factor), and hypertension or increased systemic inflammatory factors (due to large wounds or allergic reactions) in genetically prone people (several chromosome loci and SNPs have been identified) [38].

It has been extensively shown that in keloids inflammatory factors like IL-1, IL-6, TNF-α, and TGFβ are increased. Authors showing that an inflammatory factor is elevated in keloids often have proposed that it could be the target of new treatments. But, keloids only exist in humans and no animal or laboratory models completely cover the biological behavior of keloids, prohibiting translational research. Currently, the step from bench to patient in therapeutic options is too big; a good keloid model would mean large steps forward could be made in collecting evidence on therapeutic options. Progress in that area is being made [39], but no animal models with intact immune reactions have scars showing keloid behavior. Building a good pre-clinical model is probably only possible when the causative factors of keloids have been elucidated. Although much progress has been made, we do not know when an optimal keloid model will become widely available.

Revision of study design hierarchy

During my training as a medical doctor, I was taught the golden standard in evidence-based medicine is a randomized controlled trial (RCT). This is also the current opinion advocated by major medical journals and the Dutch ministry of health, wellbeing and sports (VWS), which requires evidence from RCTs to support whether treatment is effective or not. At the start of my research period when I was writing a research proposal to compare treatment effects, we therefore did not hesitate to design a randomized controlled trial. However, during my research period doubts set in when inclusion proved to be extremely difficult due to strong treatment preferences of patients, consequently not consenting with randomization. This problem often occurs, more in surgical trials compared to medical trials, because patients have stronger preferences regarding surgical options than between one pill and another [40, 41].

In hindsight, with a non-randomized design we could have collected much more data on our outcomes. The question rose: When does large group size win over randomization? More sophisticated designs are available to tackle this problem, like the 'patient preference trial' [42]. Using this design, patients that refuse randomization are still followed and outcome collection is the same as for randomized groups. This makes it possible to analyze whether the randomized arms and parallel arms differ on baseline characteristics, and outcomes can be analyzed for all patients (where they should be considered a cohort) or for randomized patients only. If we would have anticipated on high refusal of informed consent, this would have been a preferable design.

There is no doubt on the value of RCTs, however, because methodology of cohort studies has improved, various forms of bias have been reduced. It has been shown that a single RCT can be as flawed as a single cohort, and that predictions from multiple cohorts do not differ from predictions based on RCTs [43]. One is not always superior to another and level of evidence should be judged per paper. This has been shown more than a decade ago, but still we hold on to the hierarchy in study designs. Furthermore, RCTs are expensive and might not be the best use of limited research funds, specifically if they turn out unsuccessful.

For future (keloid) research on treatments that are both available outside of study context, I recommend to anticipate on low patient consent with randomization and take this into account when choosing a study design. Less well-known designs like the 'patient preference trial' may be the preferred option!

Collective data collection

Even better than an innovative study design would be continuously monitoring outcomes in clinical quality and research registries that eventually will improve our insight in keloid treatment effects and practice even more, because all patients that are nationally treated are included annually. What data should be registered, and whether that will be detailed enough to be valuable, without taking up too much time, is a challenge. If every physician would collect outcomes the same way, effect of treatments could be compared. That is what is needed, because case reports or case series in a heterogeneous condition without any reference to other treatments is almost without value. Specifically, with the amount of variation within the condition, keloid disease and usual small groups sizes in studies, it would be ideal to follow all patients treated in the Netherlands.

In more rare and severe diseases, the urge to do this is more prominent and proved to be of value, enabling several studies that would not have been possible without centralized data collection [44]. Baugh et al. internationally collected data on a specific brain tumor, with clinical data, images, biopsy tissues and molecular DNA and RNA data. This resulted in a more intensive collaboration between clinical, translational and basic scientists, enabling projects on disease-model development, epidemiology, identifying in what patient treatments works and when it does not. This comes with a time and costs investment, a national registry collecting this much data is very costly. However, registries enable major improvements in medical care and simultaneously reduce costs making it worthwhile [44, 45].

Value based healthcare

Some of the topics discussed above are part of a greater development in healthcare: the shift from volume driven care to outcomes driven care, also known as value based

healthcare. In 2009, Michael Porter shared his opinions on how healthcare should change [46]. He believes a completely different approach on health will improve care while reducing costs. It includes preventive care, accessible health insurance, measuring health value and how treatment improves value, and promoting reimbursement by added value instead of number of treatments. Healthcare providers' role in this transition is changing how health or value is measured, in objective measures, patient reported outcome measures (PROMs) and patient reported experience measures (PREMs), proving treatments add to the value that matters most to patients (Figure 2). Finding the right measures is not an easy task. The instrument should measure the right dimensions and cover all concepts of interest [2], but should also be easy and quick in use to have patients take it every visit. Internationally, the importance of measuring outcomes in a proper way is growing, groups of experts together with patients are carefully selecting outcome measures based on what is most important for them [47]. Subsequently, standard outcome sets have been defined for a number of conditions. So far, no outcome set has been developed for keloids or scars, or even one skin disease, but it is a matter of time before they will be available.

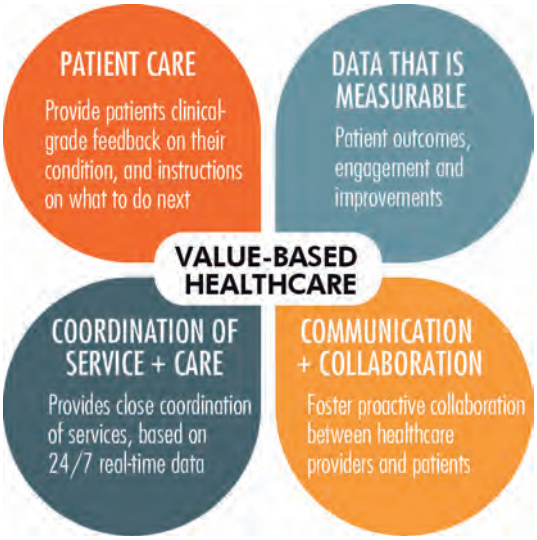

Figure 2. Diagram illustrating value based healthcare

When these standardized outcomes are measured, caregivers should discuss the outcomes with their patients to personalize treatments to the individual values of the patients. But they can also be used for comparison to other practices and learn from colleagues that are top performers [14, 45]. You can evaluate whether changes in health care have affected outcomes; see whether an improvement has paid off or whether cost-savings have impacted on value gain. And eventually, it might also be used by in-

surance companies to implement value-based payment. So, with measuring outcome values correctly, we can see where we are, where we need to go, and what we need to do to get there at the best rate.

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Summary

This thesis aims to improve care for keloid patients. Keloids are fibroproliferative lesions that can develop after an exaggerated inflammatory response to a trauma of the dermis.

In **Chapter 1** skin anatomy, normal wound healing and different types of disturbed wound healing are described. Within pathologic scarring two types exist: hypertrophic scars and keloids. Their main differences in clinical behavior are highlighted. Several factors that can provoke keloid scarring are given. The sparse existing literature on patient burden of keloids is discussed, as well as the challenges in keloid treatment, and evidence-based treatments.

Part I Burden of keloids

First, the burden of keloid disease was assessed using validated disease specific and general health related quality of life (HRQL) questionnaires, afterwards, factors which add to the disease burden were identified. The identification of the factors that cause the greatest HRQL impairment, enables us to target treatments accordingly.

Many keloid patients report pain as their main symptom, however, often no treatment is prescribed to relief pain. To explore symptomatic therapy options, a systematic review on the etiology of pain in scars and a pilot study investigating several sensible characteristics of keloids were performed.

Main findings

Chapter 2

- Having keloid disease was associated with a considerable impairment of emotional wellbeing, with most impairment on emotional and mental HRQL.
- Pain and itch were the strongest predictors of HRQL impairment in keloid patients.
- Treatment should aim at alleviating physical symptoms of keloids.

Chapter 3

- Burn and pathologic scars often lead to high intensity pain symptoms.
- Scar pain has many characteristics of neuropathic pain that could be caused by an imbalance of C-fibers subtypes.
- Scar tissue itself may alter the nerve fiber distribution; the imbalance results in ongoing neuro-inflammation and pain symptoms.

Chapter 4

- Somatosensory differences in keloid scars can show aspects of nociceptive or neuropathic pain and are extremely heterogenic.
- No specific subgroup of nerve fibers that may be affected in keloid disease could be identified.
- No correlation could be shown between keloid symptoms and epidermal and upper dermal nerve fiber density.

In part I, the importance of pain and itch symptoms on quality of life was shown, which has been confirmed by other groups. Unfortunately, our (prematurely terminated) trial did not show an improvement in quality of life after successful keloid treatment, defined as pain and itch reduction, most likely due to statistical under-powering. However, other groups were able to show quality of life improvement after successful keloid treatment. Due to their high impact on disease burden, reduction of pain and itch symptoms should have a more prominent role in keloid treatment and research. During the initial patient consultation, the frequency and impact of pain should be clarified, differentiating between nociceptive and neuropathic pain. If neuropathic pain is the main symptom, local symptomatic therapy (e.g. capsaicin patches or botox) should be tried in future studies to find out whether symptoms are relieved and quality of life is improved.

Part II Treatment of keloids

At our center, we were searching for ways to reduce the use of post-surgical brachytherapy in keloid treatment after we had encountered some complications with this treatment. When the new, promising treatment with intralesional cryotherapy was promoted we hypothesized that use of ionizing radiation could be decreased if other successful treatment options became available. Therefore, a trial was designed to evaluate the efficacy of intralesional cryotherapy compared to surgical excision with either adjuvant corticosteroid injections or brachytherapy. In the Netherlands, several different brachytherapy schemes and doses are currently in use, but there is no evidence on which scheme performs best. Our aim to reduce the use of brachytherapy in keloid patients stimulated us to investigate whether other treatments could be effectively used as well and whether radiation doses could be reduced. In this way we wanted to provide scientific evidence guiding clinicians in their choice of treatment, mainly based on patient reported outcome measures.

Main findings

Chapter 5

- Serial intralesional 5-fluorouracil injections were effective in 45–96% of keloid patients.
- Combination therapy with triamcinolone acetonide (a corticosteroid) and 5-fluorouracil may perform better than triamcinolone acetonide alone.

Chapter 6 and 7

- Intralesional cryotherapy is inferior to keloid excision followed by brachytherapy for resistant keloids.
- In primary keloids, intralesional cryotherapy resulted in some keloid improvement and therefore may be used in these patients and specific cases.

Chapter 8

- A recurrence rate of 8.3% was found after keloid excision followed by postoperative high dose rate (HDR) brachytherapy, independent of 2x9 Gy, 3x6 Gy or 2x6 Gy radiation schemes.
- In 12.8% of patients major complications occurred, e.g. chronic wounds and infections. Complications were more often seen with increasing radiation doses.
- After excision of resistant keloids, high dose rate (HDR) brachytherapy with a biological equivalent dose of 20 Gy is recommended based on both low recurrence and complication rates.

In the general discussion (**Chapter 9**) the effects of patient information, physician-patient-relation, and shared decision making on outcomes are discussed. These concepts should be incorporated more in keloid patient consultations to achieve higher treatment satisfaction rates. Consequently, the rationale behind a stepped care approach in keloid treatment can be explained and a treatment plan can be made together with the patient. If required, this plan will be adjusted based on the treatment response.

The beneficial effects of radiation on keloids is further explored, new less and more invasive techniques are described. However, we should not forget about stochastic effects in the young keloid patient population. We also reported a patient with a basal cell carcinoma after keloid treatment, and more malignancies after radiation to treat a keloid have occurred. The indication should be carefully made and the dose should be as low as reasonably achievable. Unfortunately, intralesional cryotherapy is not an effective alternative treatment option for resistant keloids, and consequently cannot decrease brachytherapy indications.

A more uniform referral and treatment plan could further improve care for keloid patients as well as reduce costs. This could be reached by developing a multidisciplinary guideline. It will improve coordinated care and reduce low value care.
Finally, improvements in basic research on keloid etiology are discussed, including the necessary animal model that enables translational research. In addition, recommendations are given how future research projects could be more profitable, choosing the right patient reported outcomes, choosing the most fitting study design or building on national registries for collection of scientific data and outcomes. When taking this all together (patient information, patient perspective, shared decision-making, outcome collection, adjusting treatment plans based on outcome) a value-based approach to keloid care is provided. Only when we will succeed in creating these conditions needed for value-based keloid care, we will be able to truly improve our care for keloid patients.

Nederlandse samenvatting

Het onderzoek beschreven in dit proefschrift had als doel de zorg voor keloïdpatiënten te verbeteren. Keloïden zijn overmatig woekerend littekenweefsel ofwel fibroproliferatieve, posttraumatisch laesies van de dermis die ontstaan door een ongecontroleerde inflammatoire reactie.

In **Hoofdstuk 1** worden ter introductie de anatomie van de huid, de normale wondgenezing en de verschillende soorten verstoorde wondgenezing beschreven. Zowel hypertrofische littekens als keloïden worden onder pathologische littekens gerekend. De belangrijkste verschillen in klinisch gedrag tussen deze twee typen littekens worden uitgelegd. Daarbij is het van belang onderscheid te maken tussen de twee typen in onderzoek en bij behandeling. De diverse factoren geassocieerd met het ontstaan van keloïden worden beschreven. Bovendien wordt een overzicht gegeven van de spaarzame literatuur over de invloed van keloïden op de kwaliteit van leven, de moeilijkheden bij onderzoek naar keloïdbehandeling en de verschillende behandelopties.

Deel I Ziektelast van keloïden

Om de grote invloed van de aandoening te illustreren, hebben we de ziektelast van keloïden bepaald en gekeken welke factoren het meeste aan de ziektelast bijdragen. Wanneer de factoren met de grootste impact op kwaliteit van het leven bekend zijn, kunnen behandelingen specifiek worden gericht op het verbeteren van die factoren die de kwaliteit van het leven het meest kunnen verbeteren. Veel keloïdpatiënten rapporteren pijnklachten als een groot probleem, maar behandelingen richten zich zelden op deze pijnklachten. Om symptomatische behandelopties te verkennen, hebben we zowel een literatuurstudie gedaan naar pijn in littekens alsook een pilotstudie naar de pijnsensaties ten gevolge van keloïden.

Voornaamste bevindingen

Hoofdstuk 2

- Keloïdpatienten hebben een aanzienlijk verminderde kwaliteit van leven, met de meeste beperking op het emotionele en mentale welbevinden.
- Pijn en jeuk hadden de sterkste invloed op kwaliteit van leven bij keloïdpatiënten.
- Behandeling zou zich moeten richten op het verlichten van fysieke symptomen van keloïden.

Hoofdstuk 3

- Brandwondlittekens en pathologische littekens leiden vaak tot een hoge pijnintensiteit.
- Pijn in littekens heeft veel kenmerken van neuropathische pijn die kan worden veroorzaakt door een disbalans van C-vezelsubtypes.
- Het littekenweefsel kan de zenuwvezeldichtheid veranderen; een disbalans resulteert in een voortdurend neuro-inflammatoir proces dat pijn veroorzaakt.

Hoofdstuk 4

- Somatosensorische afwijkingen in keloïdlittekens, tonen aspecten van nociceptieve en van neuropathische pijn, maar deze afwijkingen zijn extreem heterogeen tussen de patiënten.
- Er kan geen specifieke subgroep van zenuwvezels worden aangewezen die zijn aangedaan bij keloïden.
- Er kon geen correlatie worden aangetoond tussen klachten en zenuwvezeldichtheid in de epidermis of oppervlakkige papillaire dermis.

In deel I toonden we het belang van pijn- en jeuk-symptomen op de kwaliteit van leven van keloïdpatiënten aan; dit is ook door andere onderzoekers bevestigd. In de beschreven voortijdig gestopte studie (hoofdstuk 6 en 7) verminderden pijn en jeuk na een succesvolle keloïdbehandeling, maar zagen we geen verbetering van de kwaliteit van leven na deze behandeling. Andere studies lieten wel een verbetering in kwaliteit van leven na een succesvolle keloïdbehandeling zien; waarschijnlijk kwam dit in onze studie niet naar voren door te kleine onderzoeksgroepen.
Vanwege de grote invloed op de ziektelast van keloïdpatiënten zouden pijn en jeuk symptomen een meer prominente rol moeten krijgen in de spreekkamer en in keloïdonderzoek. Tijdens de anamnese moeten de frequentie en invloed van pijn worden besproken en dient onderscheid te worden gemaakt tussen nociceptieve en neuropathische pijn. Wanneer neuropathische pijn de belangrijkste klacht is, zouden we kunnen onderzoeken of lokale symptomatische therapie (bijvoorbeeld capsaïcinepleisters of botox injecties) pijn en jeuk vermindert en de kwaliteit van leven verbetert.

Deel II Behandeling van keloïden

In het Erasmus MC waren we op zoek naar manieren om het gebruik van postoperatieve brachytherapie voor keloïdbehandeling te verminderen vanwege het optreden van complicaties. We veronderstelden dat het gebruik van ioniserende straling kan worden verminderd wanneer er andere succesvolle behandelingsopties beschikbaar

zouden zijn. Toen de nieuwe, veelbelovende behandeling met intralesionale cryotherapie werd geïntroduceerd, werd dan ook een onderzoek gestart. Er werd een klinische studie opgezet om de werkzaamheid van intralesionale cryotherapie te vergelijken met keloïdbehandeling door operatieve verwijdering en aanvullende corticosteroid injecties of bestraling door middel van brachytherapie. In deze studie hebben we patiënt-gerapporteerde uitkomstmaten gebruikt.
Daarnaast worden op dit moment in Nederland verschillende doseringen bestraling met brachytherapie gehanteerd ter voorkoming van recidief keloïdvorming, maar het is niet duidelijk welke dosis optimaal is. We wilden de resultaten van de verschillende behandelingen naast elkaar leggen om wetenschappelijk bewijs te bieden voor de best werkzame dosis.

Voornaamste bevindingen

Hoofdstuk 5

- Seriële intralesionale 5-fluorouracil injecties waren effectief bij 45-96% van de keloidpatiënten.
- Alleen combinatietherapie met triamcinolone acetonide (een corticosteroïd) en 5-fluorouracil is mogelijk effectiever dan alleen triamcinolone acetonide.

Hoofdstuk 6 en 7

- Intralesionele cryotherapie is inferieur aan excisie gevolgd door brachytherapie als behandeling voor resistente keloïden.
- Voor primaire keloïden verbeterde intralesionele cryotherapie de keloïden, daarom zou bij deze patiënten en in specifieke gevallen voor intralesionale cryotherapie gekozen kunnen worden.

Hoofdstuk 8

- We vonden een recidiefkans van 8,3% na keloïd-excisie met postoperatieve brachytherapie, onafhankelijk van het gebruikte stralingsschema (2x9 Gy, 3x6 Gy of 2x6 Gy).
- Bij 12,8% van de patiënten traden ernstige complicaties op, waaronder chronische wonden en infecties. Complicaties werden vaker gezien bij een hogere stralingsdosis.
- Na excisie van resistente keloïden wordt brachytherapie aanbevolen met een biologische equivalent dosis (BED) van 20 Gy, vanwege de lage kans op recidieven en complicaties.

In de beschouwing (**hoofdstuk 9**) wordt de invloed van informatievoorziening aan de patiënt, de arts-patiëntrelatie en gedeelde besluitvorming op de behandeluitkomsten besproken. Deze concepten zouden nog meer moeten worden toegepast in de zorg voor keloïdpatiënten om patiënttevredenheid na behandeling te verbeteren. De redenen voor een trapsgewijze opbouw van de behandelingen kunnen worden uitgelegd en gezamenlijk kan een behandelplan worden opgesteld. Zo nodig kan dit plan worden aangepast op basis van resultaten gedurende de behandeling.
Onderzoek naar nieuwe technieken voor keloïdbehandeling met bestraling worden besproken, zowel meer als minder invasieve opties. Toch moeten we niet voorbijgaan aan de stochastische effecten van ioniserende straling in deze jonge patiëntengroep. In dit proefschrift wordt bijvoorbeeld een patiënt gerapporteerd met een basaalcel carcinoom na keloïdbehandeling met bestraling. Ook in de literatuur zijn beschrijvingen van maligniteiten na bestraling van een keloïd te vinden. De indicatie voor het gebruik van brachytherapie moet zorgvuldig worden gemaakt en de dosis moet zo laag mogelijk zijn.
Helaas is intralesionele cryotherapie niet effectief gebleken als alternatieve behandeloptie voor resistente keloïden en kan deze behandeling de brachytherapie indicaties niet verminderen. Wat verder de zorg voor keloïdpatiënten kan verbeteren en kan resulteren in lagere behandelkosten, zijn meer uniforme verwijzingen en behandelplannen. Dit kan bereikt worden door een multidisciplinaire richtlijn te ontwikkelen. Het zal de gecoördineerde zorg verbeteren en gebruik van minder effectieve behandelingen verminderen.

Tot slot wordt aandacht besteed aan de vorderingen in basaal onderzoek naar het ontstaan van keloïden en het ontwikkelen van een diermodel dat translatieonderzoek mogelijk moet maken. Tevens wordt geadviseerd hoe toekomstige onderzoeksprojecten meer kunnen opleveren door de juiste uitkomstmaten en de meest geschikte onderzoeksopzet te kiezen, als ook door het opzetten van nationale registraties die behandeluitkomsten of wetenschappelijke gegevens verzamelen. Patiëntinformatie, patiëntperspectief, gedeelde besluitvorming, uitkomstverzameling, aanpassing van behandelingsplannen op basis van uitkomst; tezamen vormen deze kenmerken de onderdelen van 'waardegedreven' gezondheidszorg. Pas wanneer we erin slagen de voorwaarden te creëren die nodig zijn om 'waardegedreven' keloïdzorg te bieden, zullen we de zorg voor keloïdpatiënten echt kunnen verbeteren.

Appendices

List of Publications
PhD Portfolio
Dankwoord
Curriculum Vitae

List of Publications

Bijlard E, Uiterwaal L, Mureau MAM, Hovius SER, Huygen FJ. A systematic review on prevalence, etiology and pathophysiology of intrinsic pain in dermal scar tissue. Pain Physician. 2017 Feb;20(2):1-13.

Bijlard E, Kouwenberg CAE, Timman R, Busschbach JJV, Mureau MAM. Burden of Keloid Disease: A Cross-sectional Health-related Quality of Life Assessment. Acta Derm Venereol. 2017 Feb 8;97(2):225-229.

Abstract Bijlard E, Verduijn GM, Harmeling JX, Dehnad H, Mureau MAM. Results of Keloid Treatment with Excision followed by Brachytherapy. Plast Reconstr Surg - Global Open. 2016;4(9S):87-88.

Harmeling JX, Kouwenberg CAE, Bijlard E, Burger C, De Jager A, Mureau MAM. The effect of immediate breast reconstruction on the timing of adjuvant chemotherapy: a systematic review. Breast Cancer Res Treat. 2015;153(2):241-51.

Abstract Bijlard E, Timman R, Verduijn G, Niessen FB, van Neck JW, Busschbac JJV, Hovius SER, Mureau MAM. Intralesional cryotherapy versus excision with corticosteroids or brachytherapy for keloid treatment: preliminary results of a randomized controlled trial. Plast Reconstr Surg. 2015;136(4S):149-50.

Abstract Kouwenberg CAE, Bijlard E, Timman R, Busschbach JJV, Mureau MAM. Emotional quality of life is severely affected by keloid disease: pain and itch are the main determinants of burden. Plast Reconstr Surg. 2015;136(4S):150-1.

Bijlard E, Steltenpool S, Niessen FB. Intralesional 5-fluorouracil in keloid treatment: A systematic review. Acta Dermat Venerol. 2015;95(7):778-82

Bijlard E, Timman R, Verduijn GM, Niessen FB, Van Neck JW, Busschbach JJV, Mureau MAM. Intralesional cryotherapy versus excision with corticosteroids or brachytherapy for keloid treatment: study protocol for a randomized controlled trial. Trials. 2013;14:439.

Bijlard E, Holman F. Late complicatie van LAGB: intraluminale migratie
met obstructie bij de ileo-coecale overgang. NTvH. 2011;20:184-186.

Den Dunnen WF, Brouwer WH, Bijlard E, Kamphuis J, Van Linschoten K, Eggens-Meijer E, Holstege G. No disease in the brain of a 115-year-old woman. Neurobiol Aging. 2008;29(8):1127-32.

Publications without peer-review

Horbach S, Bijlard E, Debeij J. Een landelijk onderzoeksnetwerk voor de plastische chirurgie. Nederlands Tijdschrift voor Plastische Chirurgie. 2017;8(1):22-23.

Bijlard E, Debeij J. De kracht van observationeel onderzoek: RCT is niet altijd het beste design. Nederlands Tijdschrift voor Plastische Chirurgie. 2016;7(4):155-156.

PhD Portfolio

Summary of PhD training and teaching

Name PhD student: E Bijlard
Erasmus MC Department: Plastic and Reconstructive surgery, and Hand surgery
Research School: NIHES

PhD period: March 2012 – December 2017
Promotor(s): Prof.dr. S.E.R. Hovius
Supervisor: Dr. M.A.M. Mureau

1. PhD training

	Year	Workload (ECTS)
General academic skills		
- BROK/GCP Refresher course	2016	0.2
- Research Integrity Congress, Erasmus MC	2015	0.2
- Medical Business Masterclass, ABN-Amro Amsterdam	2014	0.3
- Didactic skills. Deel BKO. Teach the Teacher 1, workshop Omgaan met groepen, workshop individuele begeleiding. Desiderius School Erasmus MC	2014	2
- Science in transition. Symposium. Erasmus MC	2014	0.2
- Research Integrity training for PhD students 9, 16, 23 april 2013, Medische Ethiek Erasmus MC	2013	2
- Biomedical English Writing and Communication Course. Erasmus MC	2013	4
- Good Clinical Practice	2011	
Specific medical courses		
- Stralingshygiëne 4A/M voor medisch specialisten. *Leiden*	2016	1
- Microsurgery training. Skillslab Erasmus MC. 4 hours/week, start May 2012 – June 2015	2012-2015	14
- Workshop Tendon reconstruction. Skillslab Erasmus MC	2013/2014	0.2
- Workshop Nerve reconstruction. Skillslab Erasmus MC	2013/2015	0.2
- Workshop local transposition flaps. Skillslab Erasmus MC	2013/2015	0.2
- LifeCell Masterclass Breast Reconstruction. AMC, Amsterdam	2013	0.2

Presentations

- ASPS meeting. "Long-term Results of Excision followed by Brachytherapy for Keloid Treatment" *Los Angeles, USA*	2016	1
- Voorjaarsvergadering NVPC. "Langetermijnresultaten van keloïdbehandeling met excisie gevolgd door brachytherapie in het Erasmus MC" *Eindhoven*.	2016	0.5
- ASPS meeting. "Intralesional Cryotherapy versus Excision with Corticosteroids or Brachytherapy for Keloid Treatment" *Boston, USA*.	2015	1
- Najaarsvergadering NVPC. "Een vergelijking tussen intralesionale cryotherapy, excisie met corticosteroïden of met brachytherapie in de behandeling van keloïden" *Amsterdam*.	2015	0.5
- European Academy for Dermatology and Venereology Spring Meeting. E-poster presentations: Burden of keloid disease; 5-FU in keloid treatment: A systematic Review. *Valencia, Spain*.	2015	1
- Presentation of my research (department of dermatology Erasmus MC, Esser foundation, department of plastic surgery VU medical center)	2012-2013	0.3

Conferences attendance

- NVPC Scientific Meeting, Multiple locations April 2012, October 2012, April 2013, October 2013, April 2014, October 2014, October 2015, May 2016, October 2016	2012-2015	2
- Kortjakje Zondagschool voor Plastische Chirurgie Kasteel Kerckebosch, Zeist November 2012, November 2013, March 2014, March 2015	2012-2015	0.3
- 25th Esser Course. Oncoplastic breast reconstruction	2017	0.3
Esser Course. Farewell professor Hovius. *Rotterdam*.	2016	0.3
World congenital Hand Congress. *Rotterdam*.	2015	0.6
24th Esser Course. Ins and outs of nose reconstruction	2014	0.3
22nd Esser Course. What's new in breast reconstruction.	2014	0.3
20th Esser Course. Masterclass Neuropathic Pain	2013	0.3
- Wound Congress. *Rotterdam*.	2016	0.3
- Reconstructive Surgery Trial Network day. *London, UK*.	2016	0.3
- NVSCA meeting	2014	0.2

2. Teaching and Lecturing

	Year	Workload (ECTS)
Lecturing		
- Anatomie van de hand 2h 2012 2e jaars studenten. Snijzaal Erasmus MC	2012	0.3
- Introductie plastische chirurgie 1 h januari 2013 2e jaars keuze onderwijs. Onderwijscentrum Erasmus MC	2013/2015	0.3
Supervising practicals and excursions, Tutoring		
- Tutor 1st year medical students	2014-15	1
- Microsurgery course November 2012, January 2013, November 2014, April 2015. Skillslab Erasmus MC	2013	2
- Practical course Minor curriculum: Tendon reconstruction 2012. Skillslab Erasmus MC	2012	0.3

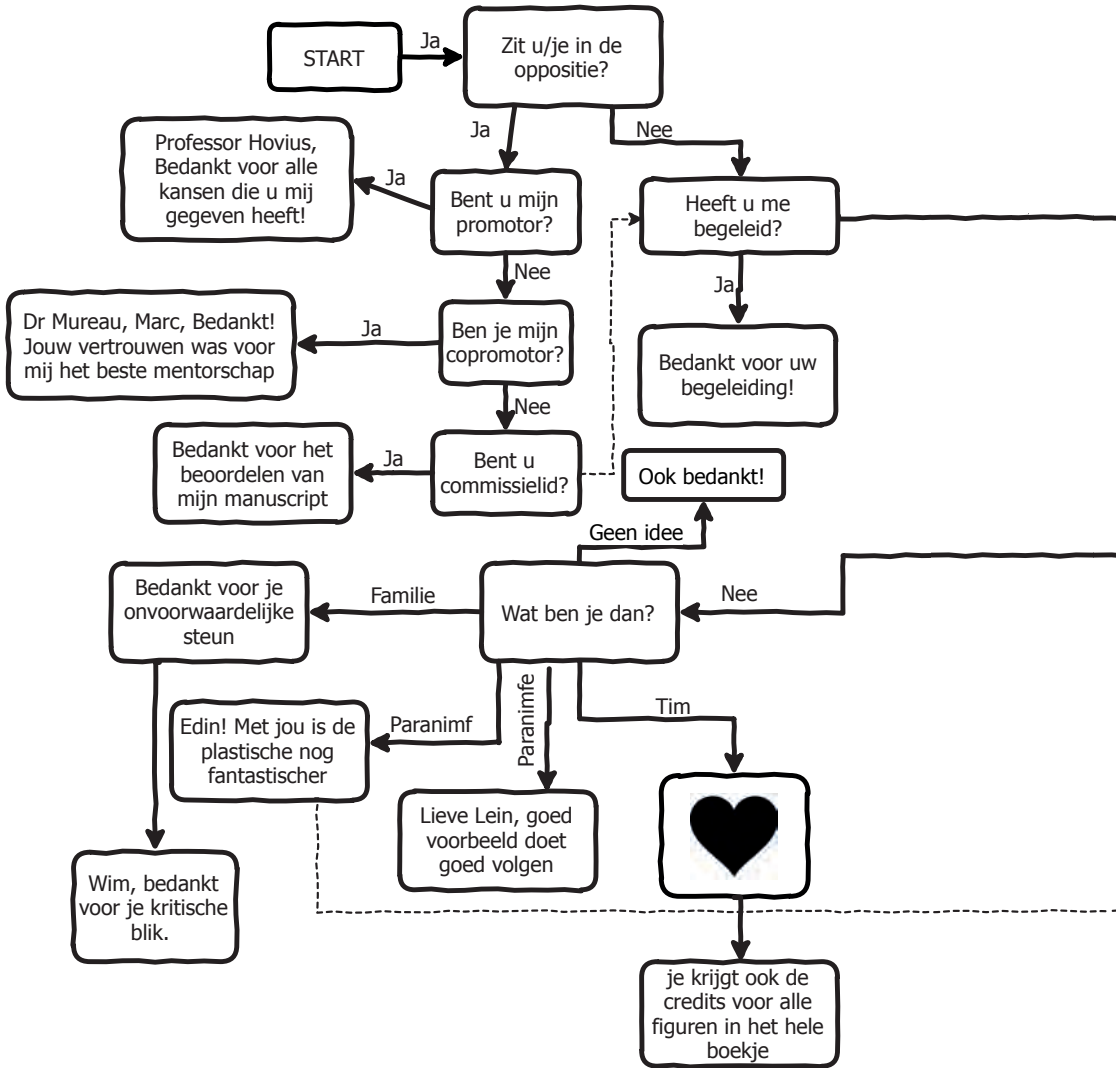
Supervising Master's theses

- Lisa Uiterwaal: Systematic Review: Causes of pain in scars 18-03-2013 to 16-08-2013	2013	3
- Elise Bijlard: Optimizing Immunohistochemistry protocols 22-04-2013 to 2-08-2013	2013	3
- Casimir Kouwenberg: Burden of Keloid Disease 02-12-2013 to 18-07-2014	2014	3
- Frank de Jongh: Influence of Keloid characteristics in treatment decisions 01-04-2014 to 02-10-2014	2014	3
- Xavier Harmeling: Systemic oncologic treatment and breast reconstruction – A meta-analysis 01-04-2014 to 19-12-2014	2014	3

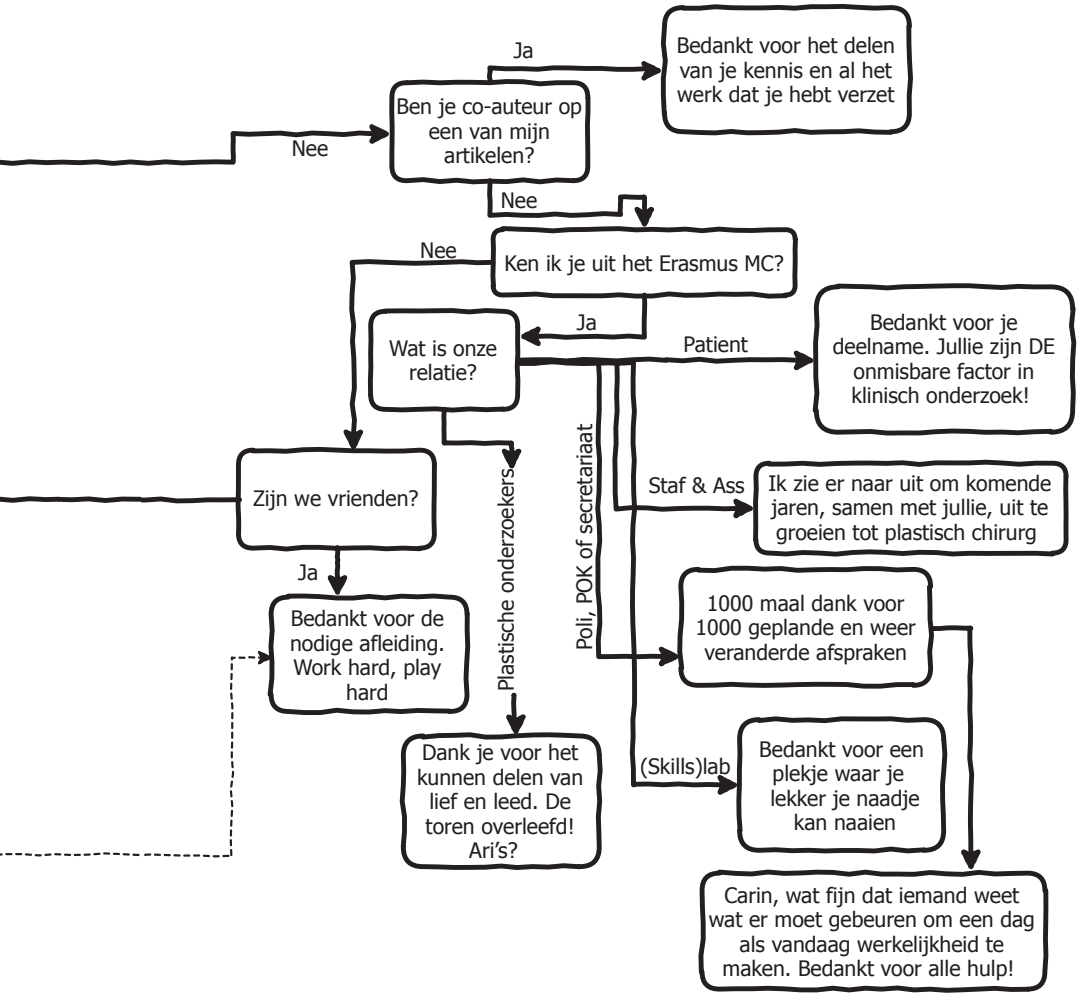
3. Other

	Year	**Workload (ECTS)**
- Chair of the Resident representation of Zaans Medical Center		
- Member Wetenschappelijke Koepel NVPC	2016-2017	3
- Website editor 'Heelkunde region 1'	2016-2017	3
- Organizing annual social program of the department of plastic and reconstructive surgery and hand surgery	2016 2012-2014	1 1
- Organizing 19th Esser Course To the Base of the Thumb: Sponsoring		
- Organizing 21st Esser Course Wide Awake: Sponsoring	2013	2.5
- Organizing 23rd Esser Course. On your nerves: Promotie en drukwerk		
	2013	2.5
	2014	2.5

Dankwoord



Figuur 1. Stroomdiagram ter bepaling van dankwoord voor de gegeven ondersteuning gedurende de totstandkoming van dit proefschrift


Ja
Bedankt voor het delen van je kennis en al het werk dat je hebt verzet
Nee
Ben je co-auteur op een van mijn artikelen?
Nee
Nee
Ken ik je uit het Erasmus MC?
Ja
Wat is onze relatie?
Patient
Bedankt voor je deelname. Jullie zijn DE onmisbare factor in klinisch onderzoek!
Zijn we vrienden?
Plastische onderzoekers
Poli, POK of secretariaat
Staf & Ass
Ik zie er naar uit om komende jaren, samen met jullie, uit te groeien tot plastisch chirurg
Ja
Bedankt voor de nodige afleiding. Work hard, play hard
1000 maal dank voor 1000 geplande en weer veranderde afspraken
Dank je voor het kunnen delen van lief en leed. De toren overleefd! Ari's?
(Skills)lab
Bedankt voor een plekje waar je lekker je naadje kan naaien
Carin, wat fijn dat iemand weet wat er moet gebeuren om een dag als vandaag werkelijkheid te maken. Bedankt voor alle hulp!

Curriculum Vitae

Eveline Bijlard was born on September 10th 1984 in Apeldoorn, The Netherlands. She attended secondary school at 'Veluws College Walterbosch' in Apeldoorn, from which she graduated in 2002. In that same year she started the Studies of Movement at the Free University Amsterdam (VU). After completing her first year there, she moved to Groningen and started medical school at the University of Groningen, where she graduated with a 'cum laude' master's degree in 2009. Her master's education included internships at the University of Southern California (Los Angeles, USA scientific intern), Deventer Ziekenhuis, Erasmus MC, and Coupure Center for plastic surgery (Ghent, Belgium).

Foto door Jelle Pieter de Boer

She started as resident not in training in general surgery in Tilburg (Tweesteden Ziekenhuis, Dr. M.S. Ibelings) in 2010, followed by a position in plastic surgery in Enschede (Medisch Spectrum Twente, Dr. O.T. Zophel). During these years she worked on a research proposal with Dr. M.A.M. Mureau (Erasmus MC). This research proposal was granted by NutsOhra in 2012 and made it possible for Eveline to start as a full-time PhD student at the Erasmus MC, University Medical Center Rotterdam (supervisors Dr. M.A.M. Mureau and Prof. dr. S.E.R. Hovius, department of plastic and reconstructive surgery, and hand surgery). During her PhD research period, Eveline also completed the NIHES research master Clinical Epidemiology and worked as a resident not in training from May 2014 till August 2014.

Eveline started her residency in plastic surgery at Zaans Medisch Centrum Zaandam general surgery department (Dr. F.C. den Boer) in June 2015, and she continued her residency at the department of plastic and reconstructive surgery, and hand surgery at Erasmus MC, University Medical Center Rotterdam starting July 2017 (Prof. dr. I.J.M. Mathijssen, Dr. A.J.M. Luijsterburg and Dr. X. Smit).

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