Currently known risk factors for hypertrophic skin scarring: a review
Abstract

Hypertrophic skin scarring remains a problem in medicine and causes considerable morbidity. Despite extensive research on this topic, the precise mechanism of excessive scarring is still unknown. Also, an overview of possible risk factors in the development of hypertrophic scars is lacking in current literature.

In order to provide an overview of risk factors for hypertrophic scarring, PubMed searches were performed on risk factors for hypertrophic scar formation. Eleven studies suggesting nine factors associated with hypertrophic scar formation were found. Studies concerning chemotherapy, age, stretch, infection and smoking provide moderate to high strength of evidence, but some other factors haven’t been studied in a convincing manner or are still disputed. Risk factors for hypertrophic scar formation are young age, bacterial colonization and skin subjected to stretch. Chemotherapy, statins and smoking seem to be protective in hypertrophic scar formation.

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Pending revision
Introduction

When skin trauma occurs, rapid repair of the defect is necessary to prevent blood loss and infection in order to assure survival. Human skin achieves quick wound closure through fibrosis and contraction rather than regeneration, which results in scar formation. In a considerable number of burn as well as post-surgical wounds hypertrophic scar (HTS) formation is a complex problem, causing both esthetical and physical difficulties. According to literature about 35% of surgical skin wounds result in hypertrophic scars after one year. HTSs are the result of an abnormal wound healing process where an excessive amount of collagen is deposited within the wound area, causing the scar to become raised above skin surface. Next to that, HTSs often appear red and shiny, cause pain, itch and sometimes even restriction of motion when positioned above a joint, causing significant morbidity. The exact mechanism underlying HTS formation still remains unknown and a limited amount of research has been performed to identify conditions or risk factors associated with hypertrophic scarring. This is in contrast to the extensive literature about keloids. Like HTS, keloids arise as a result of a derailment of the normal wound healing process where an abundance of collagen is produced and deposited in the scar, but their clinical appearance and behaviour differ substantially from the appearance and behaviour of HTS. Where HTS is defined as excessive scar tissue that stays within the boundaries of the original lesion, keloids expand beyond the wound margins. Up to now, no comprehensive overview of possible risk factors for excessive scarring (HTS and keloid) is available the in literature.

The normal wound healing process comprises three successive and overlapping phases: haemostasis & inflammation, proliferation and remodeling. Immediately after trauma the clotting cascade is activated to achieve haemostasis and temporarily seal the defect with a clot. This clot attracts inflammatory and repair cells and secretes cytokines to switch on inflammation. Inflammatory cells cleanse the wound bed to provide a viable environment for tissue repair. They also secrete factors to activate fibroblasts, keratinocytes and endothelial cells which is necessary to start the proliferation phase. During the proliferation phase, which commences 2 to 3 days after trauma, granulation tissue is formed: blood vessels grow into the wound, re-epithelialisation occurs and a temporary extracellular matrix (ECM) is deposited. Also, fibroblasts differentiate into myofibroblasts to attain wound contraction. Completion of re-epithelialisation induces apoptosis of myofibroblasts. During the remodeling phase the ECM is reorganized: immature collagen in the granulation tissue is replaced by thicker and better organized mature collagen fibres and cross-linking occurs. HTS is thought to be caused by a prolonged inflammatory phase and a delayed onset of epithelialisation, which interferes with the resolution of granulation tissue as reflected by the higher amount of myofibroblasts and collagen present in hypertrophic scars.
Also, remodeling is impaired in excessive scar formation, reflected by a higher amount of immature type collagen.\textsuperscript{14}

**Methods**

In order to identify a collection of possible risk factors for HTS, a literature search on factors influencing wound healing was performed in PubMed. Search terms included “Cicatrix, Hypertrophic”\textsuperscript{[Mesh]}, “Wound Healing”\textsuperscript{[Mesh]} and “Risk” \textsuperscript{[Mesh]}. No time limit was instituted. All articles were examined. Articles that examined or described factors other than therapies for HTS associated with hypertrophic scar formation in humans, animals or in vitro were selected. Also, articles cited in the reference sections of the review articles found with the above mentioned PubMed searches were examined. Relevant articles were selected from the lists of cited articles in order to find more evidence for possible risk factors for excessive scarring. Additional literature was collected to clarify the effect of each risk factor on the various phases of wound healing.

In evidence based medicine the strength of evidence of a study depends largely on its study design. There are several guidelines for determining the level of evidence\textsuperscript{17}. Although the number of (sub) classifications varies a bit amongst the different guidelines, the strength of evidence assigned to different types of study designs is very similar. For this review the American Society of Plastic Surgeons (ASPS) Rating Levels of Evidence and Grading Recommendations: Evidence Rating Scale for Prognostic/Risk Studies were used\textsuperscript{18}. Large prospective cohort studies for example are relatively strong evidence (level I), while expert opinions are classified as weak evidence (level V).

Because of their week levels of evidence, case reports and expert opinions were excluded from the study.

Research in animals and in vitro research are not considered part of evidence based medicine; these study designs are considered weaker evidence than expert opinions (< level V)\textsuperscript{18,19}. However, some of the risk factors were supported only by in vitro and/or animal studies. Therefore these types of studies were also included in this review.

**Results and discussion**

In total, 110 relevant articles were found that suggested over 20 different risk factors for abnormal wound healing. Some of these risk factors co-exist or influence each other, for example malignant disease and chemotherapy. Nine possible risk factors for HTS formation were identified. Evidence for these risk factors consisted of 11 studies, which comprised nine clinical studies, one animal study and one in vitro study.
Risk factors for HTS formation suggested in the articles comprised genetic background, young age, allergy, bacterial colonization of the wound and skin subjected to stretch\textsuperscript{2,7,20-22}. Chemotherapy, smoking and local application of statins are protective for HTS formation\textsuperscript{2,21,24}.

No reliable evidence was found on the association of dark skin with HTS formation. Despite of this, the subject is discussed at the end of this section, since dark skin is a generally accepted a risk factor for HTS formation.

Table 1 presents an overview of factors associated with HTS described by other authors up to now. This table includes references and their levels of evidence.

The risk factors for HTS formation and their effects on the different phases of wound healing are discussed below. References concerning the effects of individual risk factors on the different wound healing phases are mentioned in the results section, but are not presented in the table.

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Association with scar formation</th>
<th>References</th>
<th>Type of study - Level of evidence (ASPS guidelines)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetic background</td>
<td>No association</td>
<td>Bayat 2003\textsuperscript{7}</td>
<td>Case-control - III</td>
</tr>
<tr>
<td>Age</td>
<td>↓ HTS</td>
<td>Mahdavian 2012\textsuperscript{2}</td>
<td>Cohort – II</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ketchum 1974\textsuperscript{29}</td>
<td>Case-series – IV</td>
</tr>
<tr>
<td>Allergy</td>
<td>↑ HTS no association (mast cells)</td>
<td>Smith 1987\textsuperscript{20}</td>
<td>Case-control – III</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Niessen 2004\textsuperscript{1}</td>
<td>Cohort – II</td>
</tr>
<tr>
<td>Bacterial colonization</td>
<td>↑ HTS in burns</td>
<td>Baker 2007\textsuperscript{21}</td>
<td>Retrospective cohort – II</td>
</tr>
<tr>
<td>Stretch</td>
<td>↑ HTS</td>
<td>Qautresooz 2006\textsuperscript{22}</td>
<td>Cross-sectional – III</td>
</tr>
<tr>
<td>Chemo therapy</td>
<td>↓ HTS</td>
<td>Lee 2013\textsuperscript{23}</td>
<td>Cohort – II</td>
</tr>
<tr>
<td>Smoking</td>
<td>↓ HTS</td>
<td>Mahdavian Delavary 2012\textsuperscript{2}</td>
<td>Cohort - II</td>
</tr>
<tr>
<td>Statins</td>
<td>↓ HTS</td>
<td>Ko 2012\textsuperscript{25}</td>
<td>Animal study – &lt;V</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Broek 2012\textsuperscript{24}</td>
<td>In vitro – &lt;V</td>
</tr>
</tbody>
</table>

\textit{Table 1: risk factors suggested in literature} \( HTS = \) hypertrophic scarring. \( \downarrow = \) negative association with scar formation, \( \uparrow = \) positive association with scar formation (for example: stretch is positively associated with HTS formation).

**Genetic background**

The aetiology of keloid formation is at least partially genetic, since individuals with a positive family history of keloids are at higher risk to form keloids themselves\textsuperscript{25-27}. Bayat and
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colleagues have suggested that HTS also has a genetic component. However, no convincing evidence is available for a familial pattern in patients who suffer from hypertrophic scars\textsuperscript{7,28}. In their systematic review, Brown and colleagues did not find any pedigree studies on HTS\textsuperscript{28}. Hypothetically, a genetic predisposition could lead to changes in the different phases of wound healing, thereby contributing to HTS formation. TGF-\(\beta\), for example, is involved in inflammation and plays an important role in extracellular matrix formation during the granulation phase as well as contraction\textsuperscript{9}. However, Bayat and colleagues demonstrated there is no association of HTS with changes in TGF-\(\beta\) related genes, which does not support the idea that HTS has genetic causes\textsuperscript{7}.

The latter study is level III evidence, but its results do not provide sufficient evidence to reject the involvement of a genetic predisposition all together.

\textit{Age}

Excessive scars develop mostly in younger patients aged between 11 and 30 years\textsuperscript{25,29}. In addition, Mahdavian Delavary and colleagues found a trend suggesting a reversed association of age with HTS formation\textsuperscript{2}. The inflammatory response decreases with age\textsuperscript{30}. Also, epidermal turnover is slower in elderly individuals, the epidermis contains fewer cells and there is dermal atrophy because of diminished proliferation and lower collagen content\textsuperscript{30,31}. Proliferation and re-epithelialisation are reduced during wound healing in the aged individual\textsuperscript{30}. The remodeling phase is also different in the elderly: collagen I/III ratios are decreased and collagen type I bundles are more disorganized compared to younger individuals\textsuperscript{30}.

Moderate strength of evidence (level II) supports the negative association of age with HTS.

\textit{Allergy}

Smith and colleagues discovered that immunologic hypersensitivity or allergy is associated with the formation of hypertrophic scars\textsuperscript{20}. They hypothesize that the increased degranulation of mast cells observed in allergic individuals supports HTS formation, because mast cell products (histamine and heparin) stimulate collagen synthesis and angiogenesis. Mast cells also produce several inflammatory mediators\textsuperscript{9}. Given the fact that HTS formation is believed to result from excessive inflammation and proliferation, mast cell numbers might be elevated in hypertrophic- compared to normal scars. However, Niessen and colleagues did not find a significant difference in mast cell numbers in HTS versus normal scar tissue\textsuperscript{1}. The authors do not describe the numbers and sizes of the granules within the mast cells. Since an association of increased granule size and/or increased degranulation of mast cells with HTS has not been studied, the precise effect of allergy on HTS formation remains unclear.
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The relation of allergy with HTS formation is supported by level III evidence.

**Bacterial colonization**

Infection is a known risk factor for HTS, primarily based on expert opinions, but mere colonization of the wound with bacteria might also stimulate HTS formation. Baker and colleagues found that colonization of burn wounds with bacteria is associated with HTS. They suggest that HTS formation is the result of bacterial products or increased inflammation caused by colonization. Indeed, bacterial toxins stimulate and prolong the inflammatory phase of wound healing, stimulating hypertrophic scarring. Another possible explanation for the association of bacterial colonization with HTS is the fact that colonization without infection can cause increased granulation tissue formation. As with colonization, infection (resulting from severe colonization) prolongs the inflammatory phase, which can result in HTS formation. Next to that, infection delays the onset and duration of epithelialisation, which is associated with HTS as well. However, the direct effects of colonization and infection on the remodeling phase are not known.

The evidence for the relation of bacterial colonisation with HTS formation has level II strength.

**Stretch**

Skin subjected to stretch is more susceptible for HTS formation. Hypertrophic scars display increased tension compared to atrophic scars and normal skin. Moreover, skin of individuals who develop HTS is subjected to more stretch than skin of individuals who develop atrophic scars. Tension in the dermis results from stretching of collagen fibres. Since collagen fibres are not deposited in the wound defect during the inflammatory phase, theoretically, stretch in the wound does not occur during this phase and therefore cannot influence inflammation. The opposite is true for the proliferative phase. Animal wound model studies and in vitro studies on burn scar tissue indicated that stretch not only stimulates proliferation and angiogenesis, but also decreases apoptosis. The positive effect of stretch on the proliferative phase of wound healing is mediated through the TGF-β/Smad signalling pathway. In addition, in burn scar tissue in vitro, stretch induces the transition of fibroblasts into myofibroblasts, which are typically observed in HTS, but not in mature normal scars. The fact that stretch stimulates the proliferative phase as well as myofibroblast differentiation can explain its role in HTS formation. No literature was found on the effects of stretch on the inflammation and remodeling phase.

The evidence for a relation between stretch and HTS formation is of moderate strength (level III).
**Chemotherapy**

Patients suffering from malignant disease often receive chemotherapy. It is administered systemically, but its effects are transient. Chemotherapy reduces inflammation and damages replicating cells, amongst which tissue-repair cells. Chemotherapy has a negative effect on the proliferation phase of wound healing by suppressing cell proliferation and collagen deposition. No literature was found on scar outcome in relation to chemotherapy administered during the inflammation and proliferation phase. In contrast, Lee and colleagues observed that chemotherapy administered during the remodeling phase reduces HTS formation at the donor site of the TRAM (Transverse Rectus Abdominis Muscle flap) procedure. Since reduced maturation of collagen is associated with HTS and chemotherapy during the remodeling phase reduces HTS formation, chemotherapy is likely to stimulate maturation.

However, other wound healing modulating factors are present in cancer patients as well. First, immune systems of cancer patients are suppressed. Second, poor nutrition occurs in many cancer patients because of a loss of appetite caused by chemotherapy, radiation in the head/neck area or by the disease itself. Poor nutritional status can interfere with normal wound healing. It is unclear to what extent each of these factors contribute to alterations in the process of wound healing.

The inverse relation of chemotherapy with HTS is supported by one study of moderate strength (level II), but it is difficult to determine its individual effect on hypertrophic scarring, since this factor often co-exists with other factors that can influence scar outcome.

**Smoking**

Mahdavian Delavary and colleagues discovered that smoking reduces the risk of hypertrophic scar formation. Sorensen and colleagues saw that the cellular inflammatory response during wound healing is delayed in smokers. Nicotine might impair inflammation through the inhibition of monocyte migration and macrophage proliferation. Although circulating neutrophil numbers are increased in smokers, Sorensen and colleagues found a reduction in neutrophil migration to the wound. Smoking promotes thrombus formation via increased platelet aggregation, but clot composition is altered: there is a reduction of pro-inflammatory cytokines. Jorgensen demonstrated the negative effect of smoking on collagen deposition during wound healing in a prospective case-control study. In smokers, reactive oxygen species cause alterations in immune pathways, decreasing collagen synthesis. Collagen production is further inhibited by smoking induced reduction of serum Vitamin C levels. Also, nicotine can induce hypoxia through vasoconstriction, inhibited erythrocyte production, formation of carbon monoxide and hydrogen cyanide. Hypoxia negatively influences collagen synthesis. Next to that, smoking reduces epi-
theelialisation, angiogenesis and fibroblast proliferation. On the other hand, smoking also seems to stimulate wound contraction. Smoking reduces deposition of mature type I collagen and the formation of collagen cross-links, which results in reduced wound tensile strength. Just as reduced collagen synthesis, defective cross-link synthesis probably results from smoking induced tissue hypoxia. Although smoking inhibits epithelialisation and remodeling, apparently the inhibitory effects on inflammation and proliferation are of such an influence that the net effect of smoking reduces HTS formation.

Moderate strength of evidence (level II) supports the protective effects of smoking for HTS formation.

**Statins**

Intralesional statins reduce HTS formation in animals and in an in vitro HTS scar model. In a rabbit ear wound model, statins reduced scar elevation as compared to wounds treated with vehicle alone (control). Van den Broek and colleagues developed a tissue engineered human HTS model that is similar to HTS that developed in vivo. They demonstrated that statins reduce epidermal- and dermal thickness and normalize chemokine production in the HTS model. Statins reduce inflammation, for example by influencing inflammatory cytokine production. The anti-inflammatory properties of statins have been demonstrated in several conditions, including animal models of inflammatory skin disease. The anti-inflammatory properties of statins could play a role in their favourable effects on HTS. The effect of statins on the rabbit ear wound model was shown to be based on connective tissue growth factor (CTGF) inhibition. CTGF stimulates angiogenesis, fibroblast proliferation, ECM deposition and wound contraction. Consequently, inhibition of CTGF by statins can reduce granulation tissue formation and thereby HTS formation.

Since the association of statins with excessive scarring has been studied in vitro and in animals, this evidence is considered very weak (< level V).

**Dark skin**

In their 1974 review, Ketchum and colleagues reported a higher incidence of HTS in dark skinned individuals compared with Caucasians. Fibroblasts from African skin produce more pro-inflammatory chemokine CCL2 compared to Caucasian. Also, dark skin mast cells contain larger granules. In addition, dark skin has a higher transepidermal water loss (TEWL), which increases inflammation. Woolery-Lloyd and colleagues propose a possible hyper proliferative state in dark skinned patients. Indeed, dark skin contains more and bigger fibroblasts that deposit more collagen. Next to that, the increased TEWL in dark skin enhances proliferation.

On the other hand, African skin keratinocytes may proliferate more than keratinocytes of Caucasian skin, which could accelerate re-epithelialization.
No literature was found on alterations of the remodeling phase in dark skin. The relation of dark skin with HTS is mainly based on expert opinions, which are considered weak evidence (level V).

**Recommendations**

Evidence for some of the risk factors discussed in this article is of weak or moderate strength. Risk factors that should be studied more extensively to provide stronger evidence comprise dark skin and statins.

An important pitfall in reviewing studies on HTS formation is the fact that a reliable, easily applicable and generally accepted method for defining HTS and keloid and differentiating between those two forms of excessive scarring is lacking. Not all authors describe the definition of HTS they used for their studies accurately. Some authors do not describe the criteria at all. As a result, when reading articles on HTS or keloid one cannot be sure that the investigators haven’t been mistaking hypertrophic scars for keloids. Publication of photographs in articles on fibro-proliferative scarring and detailed descriptions of the criteria used to define HTS could partly overcome this problem. However, developing a reliable tool for defining and thereby differentiating HTS and keloid is necessary in order to provide a definitive and robust means to compare studies on excessive scarring.

**Conclusions**

In conclusion, young age, bacterial colonization/infection and stretch are risk factors for HTS formation, while chemotherapy and smoking are protective of HTS formation. Possible risk factors that deserve more extensive scientific study comprise dark skin and statins. Future research on possible risk factors for excessive scar formation preferably has prospective and at least case-controlled research designs and large research populations. Also, a reliable clinical tool for defining and differentiating between HTS and keloid is needed.
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Burcht; fortress of Leiden