Chapter 8

Summary and general discussion
Introduction and aims

Wound healing is a complex process that consists of the haemostasis and inflammation phase, the proliferation phase and the remodeling phase\(^1\). These phases occur sequentially, but also overlap each other. Derailment of the wound healing cascade results in deposition of excess scar tissue of which hypertrophic scars are comprised. Hypertrophic scars (HTS) are raised, red and rigid and cause co-morbidity such as pain, itch and diminished range of motion when positioned over a joint\(^2\). The current conception is that HTS results from excessive inflammation, overabundant proliferation and abnormal remodeling\(^1,3\).

Although numerous studies have been conducted on HTS formation, the exact mechanism as well as the moment of derailment remains unknown. The aim of this thesis was to provide more insight in both local and systemic factors influencing HTS formation as well as the time of onset of hypertrophic wound healing.

Summary of the results

1. Systemic factors and influences

1.1. Predisposing systemic factors - risk factors for HTS formation

In chapter 2 currently known risk factors for HTS formation were studied in the literature. Many risk factors have been reported in the literature, but most are supported by weak evidence. A few risk factors are supported by moderate to strong evidence: young age, bacterial colonization and stretch, which are risk factors for HTS formation; chemotherapy and smoking, on the other hand, protect against HTS formation. Another problem is the fact that there is no international consensus about the definition of HTS and Keloid. As a consequence it is not always clear what was studied (HTS or Keloid or both), which makes it rather difficult to reliably compare these studies. Development of a globally used clinical definition of HTS would resolve this problem. For our study, we defined HTS as scar tissue that is raised at least 1 mm above skin level, but does not exceed the boundaries of the original wound\(^4\).

Risk factors associated with HTS formation were examined in sternotomy patients in chapter 3 and comprised body mass index and ethnic background. In addition, scar discomfort (pain and itch) at four months post-surgery was predictive for hypertrophic scar outcome at one year post-surgery. Anti-hypertensive therapeutics and factors influencing erythropoiesis were found to be protective for HTS formation. All of these factors are able to influence one or more phases of the wound healing process and probably influence the final outcome of the scar.
1.2. Systemic inflammatory response and HTS formation

Surgery as well as extra corporal circulation (ECC) induces a systemic inflammatory response\(^5\). This systemic inflammatory response may affect wound outcome\(^6,7\). Roughly two types of ECC exist: conventional ECC (CECC) and minimal ECC (MECC). MECC has been shown to induce a diminished systemic inflammatory response as compared to CECC\(^8\). Chapter 4 studied the influence of CECC versus MECC on scar outcome. No differences were observed, suggesting that alterations of the systemic inflammatory response do not significantly affect scar outcome. However, it was difficult to draw definitive conclusions, because of the necessity for corticosteroid administration (which suppresses inflammation) in patients who received CECC.

1.3. HTS formation as a systemic phenomenon – association with coronary artery stenosis

Fibrosis is a repair mechanism that can occur in a large variety of tissues\(^9\). In order to examine whether excessive fibrosis can occur simultaneously in different tissues, the association of HTS with coronary artery bypass graft failure and coronary sclerosis was examined in chapter 5. Graft failure and coronary sclerosis are forms of fibrosis that have several features in common with HTS formation such as excessive secretion of inflammatory mediators and altered immune cell numbers\(^3,10,11\). However, an association of symptoms of graft failure/coronary sclerosis with HTS could not be demonstrated. This suggests that patients who develop excessive skin fibrosis do not necessarily develop excessive fibrosis in all other tissues.

2. Local factors

2.1. Local factors and time of onset - early hypertrophic versus early normal wound healing

The local early inflammatory response (three hours after wounding) in hypertrophic wound healing versus normal wound healing was examined in chapter 6. The novel strategy of this paper to examine the very early inflammatory phase of hypertrophic wound healing versus normal wound healing has not been performed before to our knowledge. In contrast to the current opinion that HTS results from excessive inflammation, early hypertrophic wound healing displayed a reduced local inflammatory response, exhibiting lower concentrations of inflammatory proteins IL-6, IL-8 and CCL2 as compared to normal wound healing at three hours post-injury (figure 1)\(^1\). The reduced early inflammatory response might result in a subsequent inflammatory overshoot that fails to stop at the appropriate time. This prolonged inflammatory phase could cause the overabundant granulation tissue- and extracellular matrix (ECM) formation associated with HTS by prolonged secretion.
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of mediators involved in granulation tissue- and ECM deposition\textsuperscript{12}. In addition, a trend of pre-operatively increased numbers of alternatively activated macrophages (M2) was observed in the HTS group, suggesting that resident macrophage population might predispose for HTS formation. Indeed, M2 have been associated with fibrosis before\textsuperscript{13}.

Figure 1: early inflammatory response in normal and hypertrophic wound healing  
NTS = normal scar, HTS = hypertrophic scar, Pre-operative = start of surgery, post-operative = end of surgery (3 hours post-wounding). Pre-operatively the number of M2 macrophages is higher in skin that develops HTS, but reduces post-operatively to the number of M2 macrophages in the NTS group. The amount of inflammatory proteins increases in both the NTS- and HTS group during surgery, but the increase is significantly larger in the NTS- compared to the HTS group.

2.2. Predisposing local factors - keloid predilection sites versus control sites

Chapter 7 provides data of differences with respect to ECM composition and macrophage population in skin of different anatomical sites. Moreover, differences in the mentioned modalities were observed in skin sites susceptible for keloid formation as compared to control sites: keloid predilection sites contained higher concentrations of collagen and decreased numbers of classically activated (M1) macrophages. The reduced M1 numbers suggest a reduced inflammatory state in keloid predilection sites. This might cause the reduced inflammatory phase during wound healing associated with excessive scar formation\textsuperscript{14}. The results suggest that ECM composition and resident immune cell population facilitate keloid formation in predilection sites.

Potential predisposing histological properties for HTS were not examined, since no
reliable large studies are available on HTS-prone anatomical sites and literature is inconclusive on the subject.

General discussion

Of the above described results, the most important finding in this thesis is the reduced local early inflammatory response in hypertrophic wound healing compared to normal wound healing (chapter 6). These data seem to be in conflict with the current conception that excessive inflammation results in HTS formation as proposed by Wang and Liu amongst others. But, previous research performed by our research group does support the new finding of a reduced early inflammatory response in relation to HTS formation. Also, a delayed inflammatory response has been connected to fibrosis in other tissues, which further supports our observations.

At three hours following injury we observed that concentrations of several inflammatory proteins were significantly lower in skin of HTS forming individuals as compared to individuals who developed normal scars. The trend of pre-operatively increased alternatively activated macrophages (M2) in the HTS group could have caused the post-operatively decreased inflammatory protein concentrations, since M2 macrophages can influence the local milieu with respect to the inflammatory status.

However, HTS formation is a dynamic process. Thus in order to elucidate the mechanism leading to hypertrophic scar formation, the entire process, starting within hours after wounding, has to be studied. The process of hypertrophic wound healing starts with a decreased early inflammatory response as described in this thesis. Other authors observed that later stages of hypertrophic wound healing display increased rather than reduced inflammation. Our observation that pain and itch at four months post-surgery were predictors for hypertrophic scar outcome at one year post-surgery (chapter 3) might suggest that increased concentrations of prostaglandins (inflammatory mediators) are present in hypertrophic wound healing at four months post-surgery. At this moment, four months following injury, hypertrophic scars have become clinically visible in the form of erythema and thickening of the scar. This expansive phenomenon is caused by increased micro vessel numbers and vasodilation. Hypervascularization associated with fibrosis also occurs in other tissues, for example in the peritoneum after long term peritoneal dialysis. This hypervascularity is probably caused by activated endothelium in reaction to chronic or repeated damage. Activation of endothelial cells could also cause hypervascularity in HTS formation. In the initially hypervascular and hypercellular HTS, cell number decrease to that of normal skin during maturation, but myofibroblasts continue to exist in the scar. At this stage, there is a shift towards a constrictive form of fibrosis. This transition has also been observed in other forms of fibrosis: in lung fibrosis and scleroderma, endothelial
cell activation in reaction to endothelial damage causes hypervascularity, that eventually progresses into hypovascular fibrosis\textsuperscript{26,27}.

A common feature in all of these fibrogenic processes seems to be endothelial cell activation. The hypothesis that endothelial cell activation could play a role in HTS formation is supported by the fact that the endothelium activating factor VEGF converts scarless healing into scar formation, as shown by Wilgus and colleagues\textsuperscript{28}. Wilgus et al. propose that VEGF can stimulate fibroblasts and that VEGF-induced angiogenesis might stimulate secretion of pro-fibrotic factors, resulting in fibrosis. Endothelial activation occurs in response to inflammatory mediators\textsuperscript{29}. The decreased concentrations of inflammatory proteins we observed in HTS formation might fail to generate the stop signal necessary to end the inflammatory phase at the appropriate time. Hypothetically, this may result in a prolonged secretion of inflammatory mediators, that activate endothelium, stimulate angiogenesis and extracellular matrix (ECM) production, causing erythema and thickening of the scar\textsuperscript{2,3,12}.

The local inflammatory response can also influence the systemic milieu. In burn patients, the extensive skin trauma can give rise to an anti-inflammatory state, leaving these patients very vulnerable to infections\textsuperscript{30,31}. Since the local milieu is able to influence the systemic milieu, the opposite might also be possible: the systemic inflammatory response might influence the local milieu and thereby scar formation\textsuperscript{6,7}. In burn wounds, scientific data on the influence of the systemic milieu on the local milieu are scarce\textsuperscript{32-35}. For example, Yoshida and colleagues observed that the systemic inflammatory milieu might inhibit the local response, but most studies focused solely on the systemic response. In this thesis higher pre-operative systemic CCL-2 concentrations were measured in the HTS group as compared to the normal scar (NTS) group, but the systemic response did not correlate to the local response (\textit{chapters 4 and 6}). This is in agreement with the fact that in some other inflammatory skin conditions and fibrotic diseases the systemic and local immune status do not seem to be related either\textsuperscript{36,37}.

Regarding therapy for HTS formation, the results described in this thesis suggest that future treatment modalities for HTS should be tailor made for the individual patient (depending on the risk factor profile) and targeted at specific time points in the course of hypertrophic wound healing. In the early stage of hypertrophic wound healing the local inflammatory response needs to be stimulated, in order to generate a normal (early) inflammatory response. But at later time points inhibition of the inflammatory response is necessary, because the inflammatory phase in HTS formation fails to shut down automatically. With these treatment regimes, it might be possible to turn the process of hypertrophic wound healing into normal wound healing.
Conclusions and future perspectives

The results presented in this thesis confirm the suggestion that the inflammatory response is one of the key players in the process of excessive scarring. Differences between normal- and hypertrophic wound healing regarding the inflammatory response were visible as early as three hours after wounding. We showed that an inhibited or tardy early inflammatory response is associated with hypertrophic scar formation.

Future research on inflammation and hypertrophic wound healing should not focus on one time point alone, but also incorporate the remaining part of the early inflammatory response (two days post-wounding), the late inflammatory response (for example six days), the granulation phase (for example six weeks) and the moment that hypertrophic scars become clinically visible (three months). This will enable exploration of the dynamic aspects of the process of hypertrophic wound healing. In addition to the local inflammatory response, the systemic inflammatory reaction should be studied simultaneously in order to elucidate the interaction between both systems. A generally accepted simple clinical definition of HTS should be developed in order to facilitate the possibility to compare (clinical) studies on HTS formation. The results obtained in this thesis should be confirmed in burn wound patients, since this type of skin trauma often leads to HTS formation. Although the extensive skin trauma has been shown to induce systemic inflammatory changes shortly after burn injury, the early local immune status has not been explored extensively yet. Also, the effect of the systemic situation on the local milieu needs to be further elucidated. The process of hypertrophic wound healing in burn patients needs to be compared to normal wound healing, but also to hypertrophic wound healing in surgical patients, since the trauma mechanisms are different and consequently the local and systemic responses could be dissimilar.
References


18. Daniel LL, Daniels CR, Harirforoosh S, Foster CR, Singh M, Singh K. Deficiency of ataxia


36. Stichterling M, Sautier W, Schröder JM,


Plantsoen, Leiden; recreatie