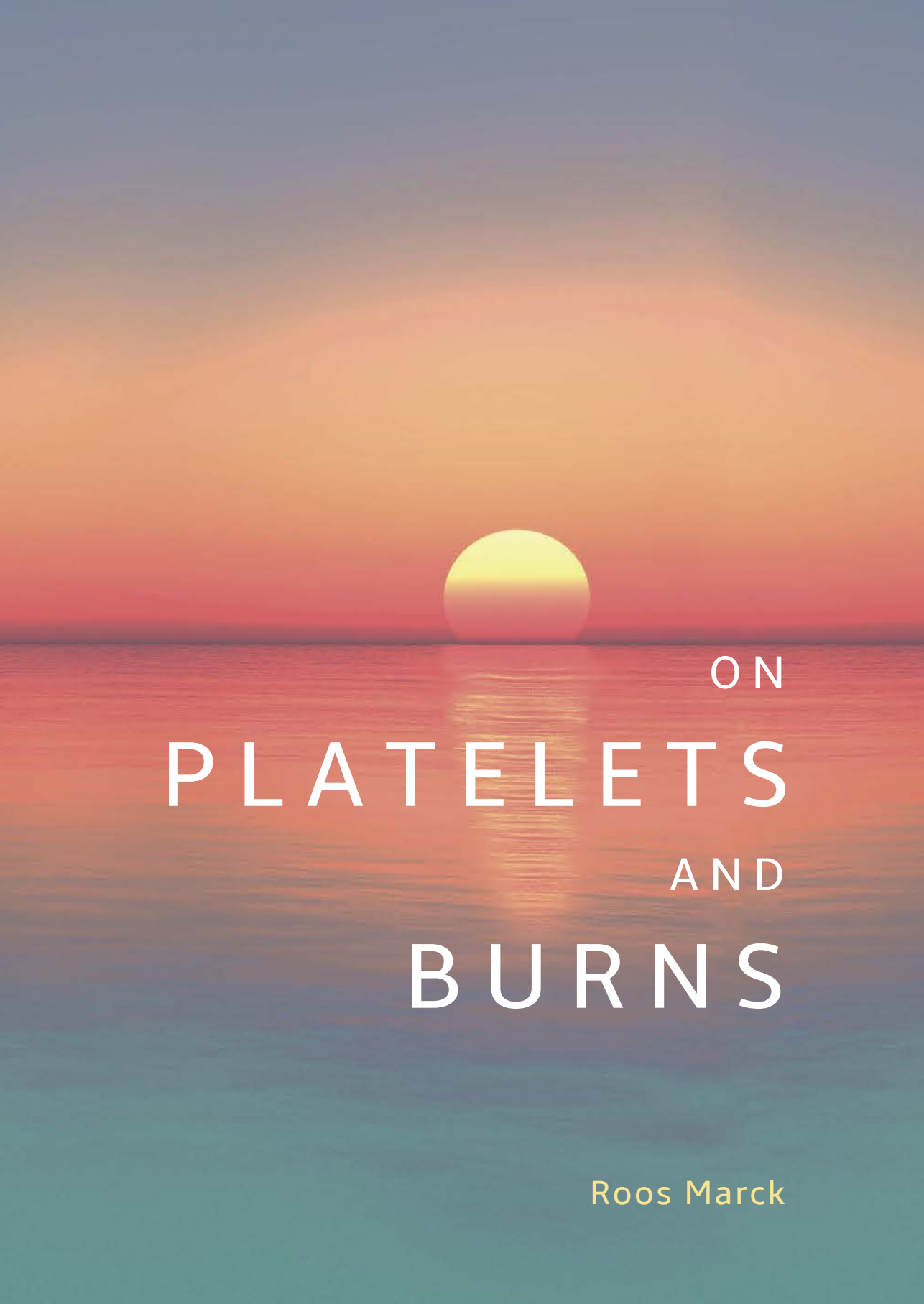
ON
PLATELETS
AND
BURNS
Roos Marck

ON PLATELETS AND BURNS

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Cover, layout and print: Off Page, Amsterdam
ISBN: 978-94-6182-922-1

This PhD thesis was embedded within Amsterdam Movement Sciences research institute, at the Department of Plastic, Reconstructive and Hand Surgery, Amsterdam UMC, Vrije Universiteit Amsterdam, the Netherlands.

Printing of this thesis was financially supported by: Stichting Brandwonden Research Instituut/HUMECA, Stichting Kortjakje, de Nederlands Brandwondenstichting, Nederlandse Vereniging voor Plastische Chirurgie, Junior Vereniging voor Plastische Chirurgie, het Rode Kruis Ziekenhuis, Zorg van de Zaak, Chirurgen Noordwest, Chipsoft, Van Wijngaarden Medical, ABN AMRO, BAP medical, Vakgroep Plastisch Chirurgen Rode Kruis Ziekenhuis.

VRIJE UNIVERSITEIT

ON PLATELETS AND BURNS

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad Doctor of Philosophy
aan de Vrije Universiteit Amsterdam,
op gezag van de rector magnificus
prof.dr. V. Subramaniam,
in het openbaar te verdedigen
ten overstaan van de promotiecommissie
van de Faculteit der Geneeskunde
op vrijdag 14 december 2018 om 13.45 uur
in de aula van de universiteit,
De Boelelaan 1105

door

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geboren te Groningen

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Introduction

INTRODUCTION

Historical perspective of wound healing

Life has existed on Earth for approximately 4 billion years.[1] It first consisted of single-cell organisms such as bacteria. Eukaryotic organisms, complex cells containing organelles, developed almost 1.9 billion years ago, and around 1.7 billion years ago multicellular organisms with differentiated cells with special functions[2,3] began to develop. Around 500 million years ago a large diversity of life forms began to appear, and only 2.5 million years ago the genus Homo developed as part of the order of primates, and the species *Homo Sapiens* developed around only 250 000 years ago.

This timeline demonstrates that life has been present on our planet for around 4 billion years, and has gradually spread widely over its surface, both in water and on land. The essence of any form of life is that it reproduces itself and that its offspring die due to age, disease or injury. The process of reproduction is influenced by external factors. Some of the mutations that occur will enhance the chance of reproduction and others will do just the opposite. This random mechanism lies behind the process of evolution and is called adaptation. Part of evolution is the adaptation of life forms to combat injury caused by other organisms or physical impact. It is likely that such adaptations started quite early, in fact soon after multicellularity began to appear (a billion years ago), given the similarities in immune systems of the multitude of species that exist today.

The first line of defence after when injury or infection occurs is for cells and molecules to go to the site of infection or injury to repair the damage or fight the invader. In most vertebates a second line of defence developed around 450 million years later, whereby lymphocytes, which have memory, react to specific antigens.[4] The process of wound healing probably developed as part of the first line of defence. It has become clear during the last few decades that wound healing is a complex biochemical process in which numerous cell types, growth factors and cytokines play a role. As Mazzucco et al. so clearly state: 'Wound healing is regulated by a well-orchestrated panoply of cytokines, growth factors, and their receptors, leading to a finely tuned symphony of cell responses.'[5] The mutual interaction of all these factors is only partly understood, which consequently makes it a challenge to influence this complicated process of wound healing.

Historical perspective of human attempts to influence wound healing

There is good reason to assume that for a long time mankind has tried to influence wound healing. This is supported by many observations of zoopharmacognosy, which is what appears to be self-medication behaviour of non-human animals. They eat or topically apply selected plants, insects, soil and psychoactive substances to prevent or reduce the effects of toxins and pathogens.[6] It is likely that *Homo sapiens* started to use herbs to treat wounds in prehistoric times. Perhaps the oldest proof of the use of natural products to treat disease is the presence of the fruit of the birch fungus *Piptoporus betulinus* among the belongings of ice-man Ötzi (who lived around 3 300 BC), who used it to treat the parasitic whipworm *Trichuris trichiura* present in his colon. The first descriptions of wound care date from 2 200 BC. On Mesopotamian clay tablets it can be read that wounds were treated at that time by washing, plastering and frequent bandaging with wound dressings.[7] Whether classical Graeco-Roman medicine improved wound care is contestable. Hippocrates recorded that

Greek and Roman doctors used rendered pig fat, resin and bitumen to treat burns.[8] Furthermore, they advocated suppuration as the way to healing.[9] This classical adage of *pus bonum et laudabile* was to hold sway until the end of the 19th century, when the idea of antisepsis developed within a short period of time, while the discovery of microorganisms and sterilisation as a method to eliminate them led to asepsis. This changed surgery significantly, making *sanatio per primam intentionem* a common outcome of surgery instead of a rare one.

The introduction of antimicrobial drugs such as sulfa drugs in 1935 and penicillin in 1942, together with the development of insights into wound debridement, led to a new major step in the treatment of wounds, including burn wounds. This made surgical wound care, if needed in combination with reconstructive surgery, a rather successful and utile activity.

Burn care

Burn care has since evolved continuously, and although improved resuscitation protocols are responsible for the survival of most patients nowadays, burn injuries nevertheless still cause disfiguring scars.[10] This continues to be a burden for burn survivors, as it impacts all aspects of their lives, both physical as well as psychological.[11] Herein lies one of the most important continuing challenges of burn care, to improve the outcome of burn scars.

The quality of burn scars depends on several factors, such as the depth of the burn wound, treatment protocols and patient factors, such as nutritional status.[12] Superficial burn wounds normally heal with no or limited scars; however, deeper burn wounds are prone to scar formation, partly due to the increased time of healing.[12,13] Deitch et al found that burn wounds healing within 3 weeks had a low risk of hypertrophic scarring, whereas burns taking longer than 3 weeks had a high risk for developing a hypertrophic scar.[14] This relation between time to healing and scar formation was recently confirmed in a large paediatric population.[15] Furthermore, an in vivo scratch model showed that scarring appears to occur when injury reaches a critical depth of wounding.[16] Deeper burn wounds are therefore normally treated by excision of the eschar and covered with a split skin graft (SSG). Nevertheless, this still leads to considerable scar formation. Split skin grafts only provide superficial epidermal and partial dermal layers of skin, and heal with a tendency to constrict, often causing scar contractures. Furthermore, because they often have to be meshed to cover larger wound areas, the interstices lead to a typical scarring pattern (*figure1*).

Wound healing in general occurs in phases that ultimately result in the formation of a scar: inflammation, proliferation and remodelling, moderated by the array of cytokines and growth factors as mentioned above. Modulation of these phases can either allow the wound to heal with limited scars or result in excessive fibrosis: when the acute inflammatory phase persists or wound healing is delayed, pathologic scars form.[13] Hence accelerating burn wound healing could decrease scar formation and improve the the quality of scars.[17] This paradigm shift from not just closing wounds for survival, but actually to accelerate wound closure by influencing wound healing on a cellular level, aiming to modify a billion years of bioengineering, reflects human being's continuous striving for progress as well as human hubris. With this ambitious objective in mind, researchers are continuously working on on new treatment opportunities.

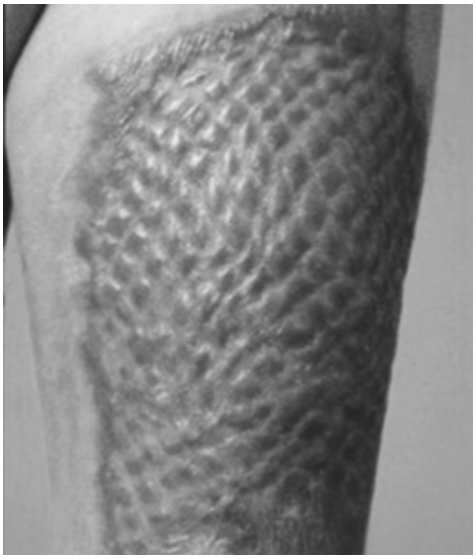

Figure 1. Typical scarring pattern of meshed split skin graft.

Platelets

Platelets are anucleated cell fragments that result from fractionation of bone marrow megakaryocytes. In non-mammalian vertebrates they are called thrombocytes because they still have a nucleus. It is uncertain when platelets were first discovered. Antoni van Leeuwenhoek made precise microscopic observations of platelets around 1675[18]. At the end of the 18th century they were described as "discoid corpuscles"; however, their function remained unclear.[19] The first person to acknowledge their role in haemostasis was the Italian doctor Bizzozero in 1881, who called them: "le piastrine del sangue." He discovered their role in the coagulation process and in thrombosis in vitro and in vivo.[20]

When platelets were discovered in the 19th century, they were believed to be only sticky assistants of haemostasis.[18] However, nowadays they are increasingly recognised to play pivotal roles in wound healing and in the immune system through growth factors and cytokines stored in their α- and δ-granules.[18,21] These growth factors are involved in many ways in all phases of wound healing (inflammation, proliferation and remodelling), such as promoting chemotaxis, cell adhesion, mitogenesis, proliferation and angiogenesis. The most studied of these factors are platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), transforming growth factor β (TGFβ), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF) and insulin-like growth factor (IGF).[5]

In the late 1970s the first publications appeared on the application of "platelet-derived wound healing factors" and more soon followed.[22] From then on, interest in platelet concentrates, alias platelet-rich plasma (PRP), grew exponentially. Platelet-rich plasma is defined as blood plasma with a platelet count above baseline. It has shown favourable results in wound healing and is being applied

in a wide range of different fields of medicine.[21] Since the adjunct of PRP in several fields of wound care showed positive results, and PRP seemed to be a relatively easy and safe treatment option, and, as stated above, in the on-going quest for optimal burn wound healing with less scarring, the idea came about to investigate whether the healing of acute burn wounds could be improved with this new promising treatment of PRP.[23]

AIMS OF THE THESIS

The aim of this thesis is to explore whether PRP may enhance burn wound healing, with the ultimate goal of the improvement of the quality of burn scars. To achieve this, we first explored the working mechanisms and the composition of PRP. Next, we reflected on the current evidence for the different applications of PRP to better define our hypothesis of the potential benefits of PRP for burn wound healing. We then systematically narrowed this search down to the existing evidence of the use of PRP in burn wound treatment, finally tapering down to the potential theoretical grounds for the application of autologous PRP in burn wounds.

However, this search brought us to an unavoidable sub-question. PRP has mostly been studied in relatively healthy populations, yet a burn patient cannot be seen as healthy, since burn injury has, besides disruption of the skin, a substantial systemic effect on patients' interior physiology.[24] Considering the autologous character of PRP studied in this thesis, the effects of burn injury on the platelets themselves must inevitably be studied. We therefore first performed quantitative analyses to determine how platelet count kinetics behaves post-burn injury. However, platelet count alone does not provide information on the qualitative status of platelets post-burn injury, which is why we aimed to study platelet quality by assessing platelet activation and function and growth factor content for relatively extensive periods of post-burn injury. This study consequently enters the realm of coagulation, a very interesting and complicated field on its own.

Moving back towards the main goal of this thesis, however with our sub-question on the systemic effects of burn injury on platelets in mind, we questioned whether the content of autologous PRP obtained from burn patients would be comparable to that of healthy volunteers. We therefore performed research on growth factor quantification of the autologous PRP of burn patients and compared the results with those of the PRP of healthy volunteers.

Finally, to answer the main question of this thesis, we performed a randomized, double-blind, intra-patient controlled study on the application of autologous PRP in the treatment of deep dermal burns, in which we looked at the effects of PRP on primary wound healing by evaluating the take rate and re-epithelialization rates of acute burns treated with split skin grafts (SSG) in combination with PRP compared to SSGs only. Lastly, we evaluated the effect of PRP on scar quality until 12 months after intervention, which we assessed using the objective scar assessment tools we have in our arsenal, such as POSAS questionnaires, DermoSpectroMeter and Cutometer measurements.

The main question of this thesis is: what is the attributive value of PRP for burn wound healing and consequently the quality of burn scars?

We attempt to answer this question by studying the following sub-questions:

1. What is the current evidence from the literature on PRP and specifically on PRP in burns?
2. What is the effect of burns on the quantity, function and quality of platelets in burn patients?
3. What is the effect of burn injury on the quality of autologous PRP?
4. What is the clinical effect of PRP on burn wound healing and outcome of burn wounds?

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2

Considerations on the use of platelet rich plasma, specifically for burn treatment

R.E. Marck, E. Middelkoop, R. Breederveld

Journal of burn care & research May-Jun 2014;35(3):219-227

ABSTRACT

Platelet rich plasma (PRP) is a fraction of blood plasma with a platelet concentration above baseline. After activation of the platelets, growth factors are released, which are involved in wound healing processes. Application of a multitude of growth factors seems to boost the healing process. In this review we provide a comprehensive overview of the many different aspects of PRP, followed by a short outline of the evidence for a wide range of applications and finally narrowing down to a more in-depth analysis of the literature on the potential use of PRP in burn treatment.

We performed an extensive search on PRP and the different biological, as well as practical aspects for the different applications. Furthermore we performed a systematic search on PRP in the treatment of burn wounds.

A high variety exists in PRP products, procedures and content. This makes interpretation and comparison of the evidence difficult. PRP has been reported to have beneficial effects on wound healing in different fields of surgery and in the treatment of acute, chronic and diabetic wounds. Literature on the use of PRP in burns is scarce. Separate growth factors have shown beneficial results in the treatment of burns. Furthermore an animal study, and several case reports showed improved burn wound healing time after the application of PRP. A deep dermal burn could benefit from PRP through its haemostatic anti-microbial abilities and the positive effects seen in wound healing. However, burn patients have an altered physiological state and it is unknown how this may affect platelet function and quality. Furthermore, the effect of PRP on scarring has not been evaluated properly. Future research is needed to elucidate the role of PRP in the treatment of burns.

INTRODUCTION

Thrombocytes, or platelets, are most commonly known for their primary function in the initial phase of wound healing: the haemostasis. However, they are also involved in all the consecutive phases of wound healing.[1] Platelets become activated after aggregation at a disruption of a vessel wall and release many growth factors and other substances.[1,2] This secretion begins within 10 minutes after activation, with more than 95% of the growth factors secreted within one hour.[3,4] For the next 5-10 days, activated thrombocytes continue to release additional proteins. The most studied of these growth factors are platelet derived growth factor (PDGF), fibroblast growth factor (FGF), transforming growth factor β (TGFβ), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), and insulin-like growth factor (IGF), all of which participate in wound healing in numerous ways, such as by promoting chemotaxis, cell adhesion, mitogenesis, proliferation and angiogenesis.[3,5-7] Furthermore platelets have been attributed anti-microbial effects, as well as pain-relieving qualities.[1,8] All of these features make platelets of potential therapeutic use in the treatment of difficult to heal wounds in the form of platelet rich plasma (PRP), plasma with an enriched amount of non-activated platelets. After activation of the PRP, it forms a clot, supporting haemostasis, and releases its growth factors, which boosts the healing process.

In the last decade, numerous studies have been published on PRP. A wide array of different types of PRP exists and an even higher number of applications, with promising as well as conflicting evidence. In this review we provide a comprehensive overview of the many different aspects of PRP, followed by a outline of the evidence for a wide range of applications and finally narrowing down to an in-depth analysis of the literature on the potential benefits of use of PRP in burn treatment. In order to facilitate and inspire researchers and clinicians who are considering the use of PRP in the treatment of burns as well as in the preparation and design of appropriate future studies on the use of PRP in burns.

METHODS

We performed an extensive search on PubMed and Ovid Embase on platelet rich plasma and the different biological, as well as practical aspects of the different applications. Furthermore we performed a systematic search on PubMed and Ovid Embase with the words “platelet” or “growth factor” and “burn” or “thermal injury”, limited to English, French, German or Dutch language.

DIFFERENT ASPECTS OF PRP

Terminology

There are many different names for PRP in use and the terminology is under constant debate (Table 1).[9-13] The term platelet rich plasma (PRP) is most commonly used and historically taken from the transfusion hematology. PRP is a general term, since it does not specify its content or structure. Other names have been suggested, such as pure-PRP, leukocyte PRP (L-PRP), platelet concentrate, platelet-derived wound healing factors and plasma enriched with platelets. Activated PRP has been called platelet-gel, L-PRP-gel or PRP releasate. This ‘jungle’ of terms, as it has been described,[10] makes interpretation of the literature difficult. Obviously, it is important to realize

Table 1. An explanatory overview of different names, types and PRP-like products.

Platelet Rich Plasma (PRP)	**non activated plasma with amount of platelets above baseline**
Platelet Rich Fibrin (PRF)	platelet rich product with 3D structure
Platelet concentrate	platelet rich plasma
Plasma Rich in Growth Factors	type of pure PRP, no leukocytes
Platelet gel	activated PRP
Platelet lysate	activated PRP by lyses, e.g. by freeze-thawing or Triton-X
Platelet releasate	activated PRP by thrombin and calcium chloride

what kind of product is used and for which application it is used for. In this review we will use the general term PRP.

Preparation procedures

PRP is obtained by centrifugation of whole blood, after which the platelets are separated from the red blood cells with a small amount of the plasma. There are numerous methods to create PRP. Most frequently, it is made from autologous blood, which is an advantage since this reduces possible safety issues in clinical application. However, allogeneic PRP might be an alternative worth considering, since safety of the transfusion system nowadays has reached a high level. If it is undesirable to draw extra blood for PRP preparation, such as in acute trauma, septic or elderly patients, allogeneic PRP might be an option.[14] PRP can be prepared, either by blood bank technology, by a manual procedure in a local laboratory or by one of the many bedside devices on the market. These last 'point-of-care' devices, are either manual methods, which can be quite time consuming, or fully automated, which are more costly.

In all procedures, after centrifugation, the PRP has to be separated from the erythrocytes at the bottom and the platelet poor plasma at the top (Figure 1). Some systems use a two-step method, where in the first step the erythrocytes are removed and after a second spin the platelet poor plasma is removed.

An overview of the yield of some of the different systems is shown in Table 2. These different systems produce variable amounts of PRP from variable amounts of blood by variable procedures and even within one methods yields may vary.[2,10,14-19]

Activation techniques

Before application of the PRP, it needs be activated to release the growth factors contained in the a-granules of the thrombocytes. There are several activation techniques, of which thrombin, either bovine or autologous, and calcium chloride are most frequently used. Here it should be noted that thrombin acts as a potent fibroblast mitogen itself and has hemostatic properties of its own.[20-22] Furthermore, freeze-thawing cycles and Triton-X-100 are techniques to lyse platelets in order to release their growth factors.[5,16,23,24] Another form of activation uses batroxobin, a thrombin-like proteolytic enzyme, which starts clot formation, however platelets themselves are insensitive to

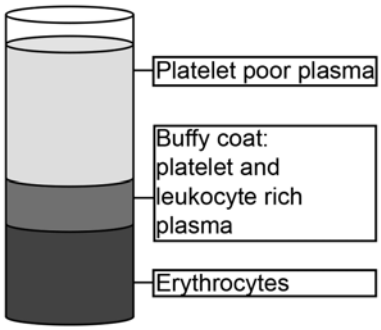

Figure 1. Whole blood after centrifugation: the different layers that appear are shown schematically.

batroxobin. They become activated by the clot that is formed, consequently the release of growth factors from within this clot is slow, prolonging its availability.[24]

These different mechanisms of activation result in different yields of growth factors, and one should be aware that in some cases not all growth factors are completely released by the different techniques, such as clot formation or freeze-thawing cycli.[2,5]

COMPOSITION

Leukocytes

Most PRP-products contain leukocytes because they are derived from the 'buffy coat',[9,10,25,26] the layer of blood plasma which, after centrifugation, contains both platelets and white blood cells (Figure 1). Little is known on what the effect of leukocytes in PRP is. It has been suggested that they have antimicrobial properties.[25,27] Furthermore, leukocytes also produce growth factors involved in wound healing.[5,18,28] Some authors do advocate a strictly platelet pure PRP,[5,12] however so far there is no solid evidence that leukocytes in PRP have unwanted side effects, although theoretically they may have catabolic effects, which could influence wound healing negatively.[29]

Fibrin

Another aspect in which PRP products can differ is the fibrin content. The difference between PRP and platelet rich fibrin (PRF) is that the latter has a more three dimensional fibrin network, where PRP is more fluid. The fibrin network of PRF seems more similar to the natural one, which results in a slow release of the growth factors.[30] An example of PRF with leukocytes is 'Choukrons' PRF which is made with a technique which requires neither anti-coagulant nor any activating agent.[31] Blood is drawn in a glass tube, which immediately needs to be centrifuged at 3000rpm for 10 minutes. Due to the absence of anti-coagulant the platelets become activated by contact with the tube walls, setting off the coagulation cascade. In the middle of the tube a clot is formed and platelets are theoretically trapped in the fibrin meshes of the clot. An example of PRF without leukocytes is Fibrinet PRFM (Cascade Medical, USA). However, despite its apparent ease of preparation, a PRF-clot might not be practical in certain applications, such as injection, where a liquid or gel is more suitable.

Table. 2 An overview of the different yields from some manual procedures and several commercial available systems.

Procedure	Required whole blood (ml)	Amount of PRP (ml)	Platelet count in whole blood (x10*9/ L)	Platelet count in PRP x10*9/ L)	Concentration factor	Platelet Capture Efficiency %	Reference
Manual procedure	9	1	192	1166	6.1	67.48	Cho. 2011 [39]
Manual procedure	5	1.9	263	1196	4.5	172.81	Mazzucco. 2009 [16]
Arthrex	10	3	142.7	378.3	2.7	79.53	Mazzocca 2012 [106]
	10	3	183	361	2.0	59.18	Sundman 2011 [29]
GPS III. Biomet	27	3	142.7	873.8	6.1	68.04	Mazzocca 2012 [106]
	53	6	219	569	2.6	29.41	Everts. 2006 [26]
	27	3	183	701	3.8	42.56	Sundman 2011 [29]
	55	6	273.8	566.2	2.1	22.56	Castillo 2010 [18]
	55	6	197	1603	8.1	88.77	Eppley 2003 [36]
PCCS platelet concentrate collection system. Biomet3i	54	7	210	910	4.3	56.17	Bertrand 2010 [107]
	50	8.5	296	1353	4.6	77.71	Leitner. 2006 [19]
RegenPRP-Kit. RegenLAB	8	5	263	430	1.6	102.19	Mazzucco. 2009 [16]
Fibrinet. Cascade Medical Enterprises	7	1.2	263	1358	5.2	88.52	Mazzucco. 2009 [16]
	9	4.3	296	399	1.3	64.40	Leitner. 2006 [19]
	18	7.5	273.8	443.8	1.6	67.54	Castillo 2010 [18]
Plateltex	6	2	263	1160	4.4	147.02	Mazzucco. 2009 [16]
	16	9.24	246.05	338.7	1.4	79.50	Mazzucco. 2008 [108]
Harvest	50	10	296	1127	3.8	76.15	Leitner. 2006 [19]
Vivostat	120	5	281	1138	4.0	16.87	Leitner. 2006 [19]
Magellan	26	6	273.8	780.2	2.8	65.76	Castillo 2010 [18]

Platelet concentration ratio and growth factor quantification

Platelet concentration factors have been reported to be between 2-fold to 8,5- fold [32] (Table 2). However, to analyze the platelet counts in PRP it is important to resuspensd the PRP completely before analysis[33], since platelets precipitate rapidly in this suspension. This is a critical procedure in the isolation process. It is unclear at present if all studies that present data on platelet counts in PRP perform this resuspension, since this procedure is often not described.

There is some consensus on how many platelets are required in PRP, it is advised by several authors to obtain concentrations of platelet counts of a minimum of 0.8-1x 10^6 / µl.[13,24,34] The idea that more platelets might be better seems incorrect, since a highly concentrated PRP could also have an inhibitory effect on wound healing, as has been shown in a rat study of intestinal anastomosis.[35] The literature is scarce on this subject, so this should be further examined for the various applications.

Next to differing baseline platelet values, great variability exists in growth factor content of platelets. Quantification of growth factors in PRP has been performed in numerous studies and a substantial variation in growth factor content was found.[4,5,18,23,25,30,34,36-40] The level of growth factors in PRP depends on several factors.[32] Firstly, it depends on growth factor concentrations in the alpha-granules of the platelets, which is a patient variable. Next, processing techniques vary, which results in different platelet concentration ratios and extent of platelet activation as well as fragmentation during preparation.[5] Furthermore, it depends on the leukocyte concentration in the PRP, since leukocytes also produce growth factors.[28] And finally, it depends on the completeness of platelet activation before measurement, since not all growth factors are released from the platelets during activation.[5,36,38] No strict correlation was found between growth factor amount and platelet count in the baseline blood or PRP [5,24,25,36,38]; nor with donor age and sex.[38] Despite this variability, growth factor quantification still is the best kind of quality control of PRP since no suitable alternative exists.[6,17]

In summary, a large heterogeneity in PRP-products exists, originating from different preparation procedures, with different PRP content (leukocytes, fibrin structure), different activation techniques and an inter-patient variability of baseline platelet values and growth factor content (Table 2). The only unambiguous factor is that PRP has an increased amount of platelets which are involved in wound healing by their growth factors and cytokines, which are released after activation.[2,39]

APPLICATIONS

In clinical applications, PRP has been used in several fields of surgery and wound care. Many case reports and patient series have been published, however randomized controlled trials are scarce.

In **maxillo-facial surgery** the use of PRP is fairly common, since some of the pioneers in PRP are from this field of surgery.[15,41] Recent systematic reviews suggest beneficial effects of PRP in the treatment of periodontal and intra bony defects, however the use of PRP was not found to enhance soft tissue healing and regeneration in the treatment of gingival recession.[42,43] Another recent systematic review on the adjunctive use of PRP in the therapy of periodontal intraosseous defects found significant additive effects in certain cases, for example in combination with demineralized freezedried bone allograft, but no such effects in other cases, like in combination with bioactive glass. These conflicting results were explained by the heterogeneity of the procedures under study [44]

PRP is widely used in **sports medicine and orthopaedic surgery**, with indications such as rotator cuff repair, anterior cruciate knee-ligament reconstruction and Achilles tendon repair. A recent meta analysis on PRP for orthopedic indications found that of the 33 trials, 9 showed a significant functional benefit for platelet-rich plasma, 21 showed no difference between platelet-rich plasma and the control, and two studies actually found a significant functional benefit for the control.[45] The authors concluded that a lack of standardization in both PRP-procedures and outcome measures made the current evidence insufficient to determine if PRP has clinical effects in the treatment of orthopaedic conditions. This is in concordance with other recent systematic reviews in this field.[46,47]

In **cardiac surgery**, PRP was shown to significantly reduce rates of chest wound infection, chest wound drainage and leg wound drainage.[48-50] On the other hand, a recent randomized controlled trial reported no added value of PRP in wound healing of vein harvest wounds, these authors found no reduction of infection rate and no improved cosmetic outcome in 140 patients.[51] **In gynaecological and general surgery** there are few randomized controlled studies on the use of PRP, as described in a recent review.[50] Several positive case series have been published for a range of surgical indications, such as perianal fistula and inguinal hernia.[52,53] In an inpatient comparative study on PRP in bilateral toenail surgery it was shown that both recovery time and post-operative pain were not decreased with the use of PRP.[54]

In the field of **plastic surgery,** PRP is often applied in facial surgery with positive results, although controlled studies with sufficient power are lacking.[32,55-57] Another recent development is combining PRP with fat, thereby potentially increasing the survival of fat grafts,[58,59] as well as potentially improving scar quality, by stimulating the mesenchymal stem cells present in adipose tissue.[60] However, a recent study did not find any contributive effect of PRP with fat-grafts, although this could be due to a low concentration of the PRP used in this study.[61] A recent systematic review summarized a substantial beneficial effect of PRP for several indications, such as a better wound healing rate, increased survival of fat grafts and an enhancement of bone graft regeneration.[62] This was based on 15 randomized controlled trials and 25 case-controlled studies, however the available studies had multiple limitations, such as small sample size, use of different concentrations of platelets and different modes of application.[62]

SKIN WOUND HEALING

Several in vitro studies on PRP and skin wound healing models have been published. They showed that PRP stimulated proliferation of fibroblasts. [20,63,64] This was shown to be concentration dependent; activated PRP at a concentration of 5% significantly promoted proliferation of dermal fibroblasts,[24,63,65,66] whereas 20% PRP did not.[63] In a clinical setting it is obviously more difficult to regulate concentrations than it is in an in-vitro set up, which could account for the variety of results seen in clinical studies. There are some clinical studies which show positive results of PRP in skin wound healing, which demonstrated enhanced wound closure in acute, chronic and diabetic wounds.[67-70] Nevertheless, there are also studies, which did not show this adjuvant effect of PRP in wound healing. A controlled study with autologous platelet rich fibrin (PRF) on donor sites and meshed split skin grafts in 20 leg ulcers, showed epithelialization was not significantly

influenced by PRF, furthermore no significant difference was found in bacterial flora or pain.[71] These contradicting outcomes of PRP in wound healing have been recently summarized in several systematic reviews, which show that in both chronic wounds and acute wounds (burns were not included) wound healing was significantly improved with the treatment of PRP.[72-75] In a recent meta analysis infection rates and pain were reduced in both types of wounds. However, the quality of many randomized controlled trials was low and another problem the reviewers encountered were the different definitions used for wound healing.[72] A systematic review on the use of PRP for diabetic ulcers showed favourable outcomes of the use of PRP for this indication.[74] However, the most recent Cochrane review concluded that there is currently not enough evidence to suggest that autologous PRP is of value for treating chronic wounds, based on a small number of randomized controlled trials with a high risk of bias.[76]

Summarizing, PRP in its various forms is being used in many different applications. Published evidence shows positive results as well as no effect of the applied PRP. It indicates that further research is warranted, to come to a final verdict about the true value of PRP for the different clinical applications. An important note is that PRP seems to be a safe product, since no side effects or adverse events have been reported.[1,13,15,44,74] Literature reports mention positive or no effects, but no negative effects of PRP on outcome parameters were reported. This indicates that probably no negative effects were found, although a publication bias which hinders negative results from being published cannot be excluded completely.

PRP IN BURNS

Literature on the use of PRP in burns is limited.[77,78] In an animal (porcine) model, full thickness burn wounds treated with platelet rich gel showed a significant increase in vascular in-growth and fibroblastic proliferation, but no enhanced re-epitheliazation.[79]

Furthermore, we found one clinical prospective controlled study where PRP was applied in acute wounds, of which 11 (19%) friction burns. The results demonstrated a significantly faster healing rate.[68] Next, in a study of 10 ocular burns, a faster healing was obtained after the use of subconjunctival infiltration of autologous platelet concentrate.[80] In a case report of one burn patient with 34 % burned body surface area (TBSA), peri-operative application of PRP and PPP improved haemostasis and may have contributed to a decrease in the time to wound healing without complications.[81]

Single growth factors in burn treatment

Several studies have been published on the effects of single recombinant growth factors on the treatment of burns. A recent review provides an extensive overview.[82] rPDGF-BB, EGF, VEGF, TGF-b2 and rKGF have been shown to accelerate burn wound healing in animal studies.[82,83] Second degree burns treated with bFGF vs. placebo in 153 patients with a follow-up of 1 year showed a significant improved wound healing rate, Vancouver scar-scale assessment and duro-, moist and cutometer results.[84,85] The combination of bFGF with split skin grafts resulted in a better color match in 23 burns after 1 year.[86]

The general conclusion of a recent review is that there is encouraging evidence that growth factor therapy may accelerate and enhance burn wound healing, but that further clinical trials are warranted and growth factor cocktails may prove to be more effective.[82] PRP could be considered as a less expensive and autologous growth factor cocktail.

Considerations on PRP in burns

Despite the paucity of the literature on PRP in burns, in theory a deep dermal burn could benefit from PRP in several ways. First, haemostatic qualities of PRP could decrease peri-operative blood loss, as well as improve the take rate of the skin grafts by decreasing continued bleeding, functioning as a fibrin glue, as well as providing a well-vascularized bed for the meshed skin graft. Furthermore, the positive effects of PRP on wound healing, as seen in reports on PRP in in-vitro models, chronic and acute wounds,[65,66,72] could contribute to faster closure of mesh interstices, since PRP promotes vascular in-growth and fibroblast proliferation, and possibly re-epithelialization.

It is uncertain if results obtained in chronic and acute wounds, could be applicable in burns, since a burn wound has a different physiology than these wounds, such as an increased inflammatory response, both systemic as well as locally; increased edema; and a reduced perfusion secondary to hypercoagulability and microthrombus formation.[87-89] Burn patients are in an altered systemic physiological status [87,89] compared to the healthy subjects in whom PRP mostly has been used and studied so far. An oft–stated advice is to withdraw blood prior to the surgery to avoid activation of the platelets, obviously this is not possible in burn patients, in which platelets are already massively activated.[90] We know that platelets in burn patients show a distinct course in time, with a nadir at post-burn day (PBD) 3 followed by a reactive peak at PBD 15, with a gradual return to normal values around PBD 24.[91-93] This time course is affected by several factors, such as age, TBSA% and sepsis.[91,94,95] Little is known on how burn or other trauma might affect the platelets and their function.[96] In trauma patients it has been shown that platelets were activated at least 72 hours after injury and had an increased functionality in the first 48 hours.[96,97] This might affect the quality of PRP and the timing of its application in burn patients.

Another important consideration is that the long term effect of PRP on scarring in burns has not yet been evaluated. Some of the growth factors released from the platelets and leukocytes in PRP are chemotactic in recruiting inflammatory cells, and a prolonged inflammation could result in hypertrophic scarring.[98] Furthermore the effects of single growth factors in scar formation, which is a complex process, are still being unraveled. Several growth factors, especially TGF-b1, TGF-b2 and PDGF, are involved in hypertrophic and keloid scarring of normal skin wounds as well as in burn wounds.[99-103] How the whole package of growth factors delivered by PRP might influence scar formation remains to be seen. So far there have been no reports of hypertrophic scarring after the use of PRP in wound healing, although these long-term results are not from studies on burn patients.[24,44,104]

Finally, there might be an indication for PRP in the reconstructive aspect of burn treatment, since recently some reports were published with positive results of PRP in combination with adipose cells for scar treatment.[60,105]

CONCLUSION

Platelet rich plasma has many potential advantages, boosting the healing process by applying autologous growth factors, supporting haemostasis and having both antimicrobial and pain relieving capacities. In this review all different aspects of PRP have been clarified and we can conclude that a vast variability exists in PRP preparation methods, content and activation techniques. This makes interpretation and comparison of the evidence on the potential benefits of clinical use of PRP difficult. There are many publications on the clinical use of PRP and many of those show positive results. Several recent systematic reviews conclude positive effects of PRP and these publications are illustrative of the ongoing interest in this product. On the other hand, there are also reports where no effect of PRP was observed. We highlighted that these conflicting results could be due to the variability of PRP products or procedures (e.g. not obtaining an adequate PRP concentration) or poor design of the conducted studies, or simply, because there is a lack of effect of the PRP.

In burn treatment the role of PRP remains to be elucidated. In theory burn patients may benefit from the positive effects of PRP on wound healing. However, burn patients have an altered physiological state, the quality of the platelets and the quantity of growth factors could be different compared to healthy individuals. Based on current literature we recommend the evaluation of PRP content and function in concordance with clinical studies. Furthermore, the effect on scarring should be carefully monitored.

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3

Time course of thrombocytes in burn patients and its predictive value for outcome

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Burns. Jun 2013;39(4):714-722

ABSTRACT

Thrombocytopenia is common in trauma and critically-ill patients and is associated with a poor outcome. The objective of this retrospective study was to investigate the course of thrombocytes in burn patients, the influences of various factors on this course and a possible predictive value of thrombocyte counts on outcome in 244 patients admitted to the our burn centre. Their thrombocyte counts were obtained until 50 days post burn. Data on patient demographics, total percentage burned surface area (%TBSA), sepsis and mortality were collected. Multilevel multivariable analysis was performed to investigate the influence of the variables on the platelet course. Cox regression analysis was performed to analyse the predictive value of the variables for mortality. A distinct pattern of thrombocyte counts was seen, with a nadir at day 3 followed by a top at day 15 and a temporarily thrombocytosis. Percentage TBSA and age ($p<0.05$) influence this course. The mean thrombocyte course of septic and non-surviving patients depict a significant lower nadir. Furthermore higher age, %TBSA and low thrombocyte counts at the peak are predictive for 50 day mortality ($P<0.05$). Platelets follow a distinct course post-burn, influenced by %TBSA, and age. These factors and a low thrombocyte peak count predict mortality.

BACKGROUND

Thrombocytes, or platelets, are anucleated cells in the circulating blood, and their predominant physiological function is to maintain primary and secondary haemostasis. In addition, platelets are involved in wound healing as well as in the immune response through the action of growth factors, chemokines and cytokines.[1,2] Aside from beneficial effects, platelet activation has potential detrimental consequences as they are involved in multiple diseases, such as strokes, heart attacks and malignancies.[1]

Thrombocytopenia is a common finding in critically ill and trauma patients and several studies have shown that it is associated with sepsis, a prolonged hospital stay and increased mortality.[3-5] A similar association with burns seems justified; however research concerning the thrombocyte status of burn patients is scarce. In 1944, MacDonald et al described a drop in platelet counts in 12 burn patients. Since then several animal studies [6,7] and a few case reports and small patient series [6,8-12] have been published. These all describe a similar course for thrombocytes following burns, with an initial decrease during the first 3-4 days, followed by a return to normal values within 10-14 days and a temporary thrombocytosis of varying duration. Several authors suggest postponing surgery until this initial thrombocytopenic period has passed.[6,11] Furthermore, previous studies have shown that persistent or severe thrombocytopenia in burn patients is associated with sepsis and increased mortality.[13-17] Guo et al recently published a study in which they have showed that a decrease in platelet count of 65% during the initial days provides a prognostic significance for 30-day mortality.[17] Recently, Warner et al. published a study on thrombocytopenia in a large pediatric burn population.[16] They found that early development of thrombocytopenia followed by depressed thrombocytosis in pediatric burn patients is associated with an increased mortality risk and is affected by the size of the burn as well as by the presence of sepsis.

In this retrospective observational study, we aimed to clarify the time course of thrombocyte levels in adult and pediatric burn patients and to identify the possible factors influencing this course. Therefore, we collected data on thrombocyte counts in a large population of burn patients and we analyzed the association between the observed thrombocyte time course and age, gender, total burn surface area (%TBSA), sepsis and mortality. In addition, the possible predictive value of several variables and thrombocyte counts at the nadir and the peak for mortality were studied.

METHODS

All patients admitted to the burn centre of the Red Cross hospital in Beverwijk, The Netherlands, from January 2005 to January 2011 were screened for eligibility. Patients were included in this study if at least four thrombocyte counts during admission were available, of which at least one was performed within the first 48 hours after the burn injury. The principles outlined in the Declaration of Helsinki were followed. According to the clinical research legislation ethical approval was not necessary.

The thrombocyte measurements were performed on a Cell Dyn Sapphire (Abbott). Aside from the thrombocyte counts, the following demographic and clinical characteristics of the included

patients were obtained: age, gender, total body surface area burned (% TBSA) and occurrence of sepsis and mortality within 50 days post-burn.

For analytical reasons, we categorized % TBSA into <15 %, 15-30 % and >30 % and age into <18 years, 18-49 years and ≥ 50 years. Thrombocytopenia was defined as a platelet count below 150 x 10^9/ L and thrombocytosis a count above 400 x 10^9/ L. Sepsis was determined as defined by the American Burn Association (18). Time since the burn injury was indicated as post-burn day (PBD), with the day of burn defined as day 0.

Statistical analysis

Continuous data are presented as the mean with standard deviation (SD). Independent Student's t-test, Mann Whitney test, one-way analysis of variance and Kruskall-Wallis test were used to compare continuous data between groups. Categorical data are presented as the number and percentage of subjects in the category. The chi-square test or Fisher's exact test were used to compare categorical data.

The course of the thrombocyte counts was analysed graphically and by repeated measures analysis. Analysis of the course of thrombocyte counts after burn injury over time was performed using a linear mixed model with patient as the random variable, time period as the fixed variable, and thrombocyte count as the dependent variable. This model allows accounting for the dependency of repeated thrombocyte counts within the same patient and for missing data. The time period was modelled using data from the literature[6,8-12] and graphical assessment of the data. The effect of age, sex, and %TBSA on the course of the thrombocyte counts after burn injury was evaluated by including these factors as fixed variables in the mixed model. Sepsis and mortality could not be taken into the mixed model analysis because these are time-dependent variables and are therefore unknown at baseline.

Finally, the predictive values for mortality of the variables sex, age (as continuous variable), % TBSA (as continuous variable), and the nadir and the peak of the thrombocyte counts were studied using Cox-regression analysis. The nadir thrombocyte value was defined as the lowest count on day 2-4 PB, and the peak value was defined the highest count on day 11-17. Both landmarks are based on graphical analysis of the data and values from the literature.[16,17] There were only 10 cases of sepsis, which occurred at variable time points, making it nearly impossible to correctly pinpoint one moment; therefore, this variable was not taken into account for the Cox-regression.

Patients who were not actively treated but received comfort care were excluded from this analysis.

P-values of <0.05 were considered statistically significant. All analyses were performed using SPSS 19.0 (SPSS Inc. Chicago, USA).

RESULTS

Of the 1376 patients with burns admitted to our hospital between January 2005 and January 2011, 244 patients were included in the study. Patient characteristics and univariate associations are presented in tables 1 and 2.

Table 1. Demographic data with results from the univariate analysis between variables

		Mortality within 50 days post burn		Sepsis within 50 days post burn		% TBSA			Age (years)		
	Total	**Yes**	**No**	**Yes**	**No**	**0-14 %**	**15-29 %**	**> 29%**	**0-18**	**19-49**	**> 49**
N	**244**	**22**	222	10	234	**84**	**96**	**64**	**41**	**95**	**108**
Male	**153 (63%)**	10 (46%)	143 (64%)	5 (50 %)	148 (63%)	51 (61%)	59 (62%)	43 (68%)	32 (78%)	65 (69%)	56 (52%)*
Female	**91 (37%)**	12 (55%)	79 (36%)	5 (50 %)	86 (37%)	33 (39%)	37 (38%)	21 (32%)	9 (22%)	30 (32%)	52 (48%)
Age (mean (range))	**45 (0-94)**	¥				°					
< 18 years	**41 (17%)**	0	41 (19%)	1 (10%)	40 (17%)	6 (7%)	23 (24%)	12 (19 %)			
18-49	**95 (39%)**	8 (36%)	87 (39%)	2 (20%)	93 (40%)	26 (31%)	40 (42 %)	29 (45 %)			
> 49	**108 (44%)**	14 (64%)	94 (42%)	7 (70%)	101 (43%)	52 (62 %)	33 (34%)	23 (36 %)			
TBSA (mean (range))	**22.8 % (1.5-90 %)**	¥		¥							
< 15 %	**84 (41%)**	6 (27%)	78 (35%)	0	84 (36%)						
15-29 %	**96 (39%)**	5 (22%)	91 (41%)	3 (30%)	93 (40%)						
>29 %	**64 (26%)**	11 (50%)	53 (24%)	7 (70%)	57 (24 %)						
Sepsis	**10 (4%)**	7 (70%)¿	3 (30%)¿								
Mortality	**22 (9%)**		7 (32%)	15 (68%)							

* In the group > 49 years, % TBSA is well distributed; in the other groups there is a significant difference (p<0,001).

° There is a significant difference within all of age and TBSA % groups (P<0,05)

¥ p< 0,05

¿ P< 0,001

Table 2. Mean thrombocyte counts (x10⁹/L) at the peak and the nadir with confidence intervals (CI) for different groups

		Nadir	CI 95 %	P value	Top	CI 95 %	P value
N= 244		163 (SD 79)			707 (SD 270)		
		Range (31-518)			Range(119-1643)		
Non-Survivor		109 (SD 55)	26 to 93	P< 0.001	541 (SD 260)	66 to 299	P<0.005
Survivor		168 (SD 79)			723(SD 265)		
Septic		111 (SD 61) °	4-103	P< 0.05	705 (SD 274)	-170 to 173	
	Non-septic	164 (SD 79) °			707 (SD 270)		
Age	<18	188 (SD 85) °	¥ 1 to 72	P< 0.05	814 (SD 263) ¥	¥ -46 to 189	
	18-49	151 (SD60) °	¥² -38 to 15		743 (SD273) ¥	¥² 19 to 197	P< 0.05
	> 49	162 (SD 88)	¥³ -59 to 10		634 (SD 250) ¥	¥³ -295 to -63	P<0.001
TBSA %	<15 %	196 (SD 83) ¥	* -3 to 47		600 (SD213)	* -268 to -81	P<0.001
	15-30 %	174 (SD 71) ¥	*² 44 to 98	P<0.001	774 (SD 266)	*² -72 to 129	
	>30 %	102 (SD 43) ¥	*³ -98 to -44	P<0.001	746 (SD 298)	*³ 43 to 250	P<0.005
Sex		157 (SD 75)	-35 to 6		707 (SD 279)	-71 to 70	
	Female	172 (SD 83)			707 (SD 252)		

¥ vs. 18-49 category
¥ 2 vs. > 49 category
¥ 3 vs. < 18 category
* vs. 15-30 % category
*2 vs. > 30 % category
*3 vs. < 15 % category

Within the study period of 50 days post-burn, sepsis occurred in 10 patients (4%), of whom 7 (70%) died. In total, 22 patients (9%) died within the 50 days post-burn period. Sepsis and mortality occurred more frequently with increasing age and increased % TBSA (P<0.05; Table 1).

A general thrombocyte course can be determined from these data, as shown in figure 1.

Immediately after the burn injury, thrombocyte counts dropped, starting from 299.2 x 10^9/L (SD 118,5) on average at 0 PBD to a clear nadir at 3 PBD, with a mean thrombocyte count of 146 x 10^9/L (SD 60,4). In the period between 3-15 PBD, there was an increase in thrombocyte count with a mean peak count of 647 x 10^9/L (SD 286,0) on day 15. Subsequently, the thrombocyte counts returned to normal values on day 24. By repeated measure analysis, a spline model could be made with 4 distinct phases, as shown in figure 2. Figures 3-7 depict the average thrombocyte counts over time for the different variables.

No obvious differences in the course of thrombocyte counts between the sexes were seen (Figure 3). This observation was confirmed by the results for the mixed model (Table 3).

A difference in the course of the thrombocyte counts was seen between the three age categories (Figure 4). At the nadir, the youngest group had significantly higher platelet counts (p<0.05), and at the peak, the oldest group had a significantly lower count (p<0.05) (Table 2). The mixed model showed that the oldest age group had significant lower platelet values over the entire study compared to the other age categories (p<0.001; Table 3).

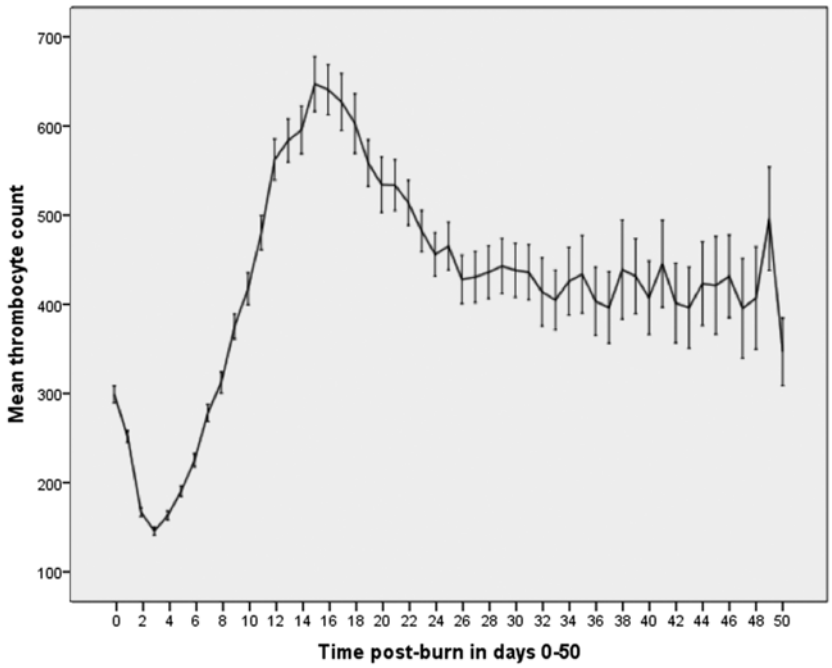

Figure 1. Mean time course for the thrombocyte counts in 244 burn patients with +/ - 1 standard error

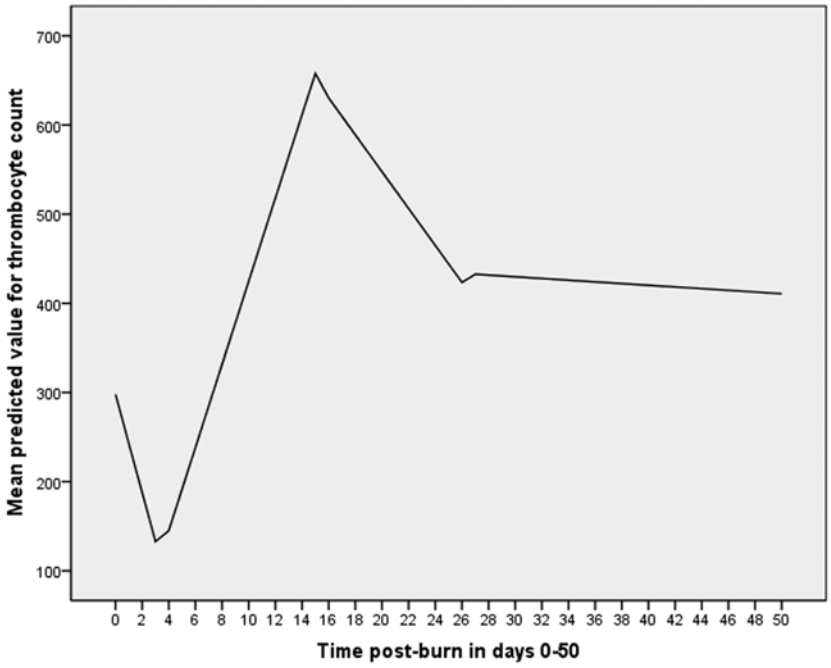

Figure 2. Spline model of the time course for thrombocyte counts in burn patients

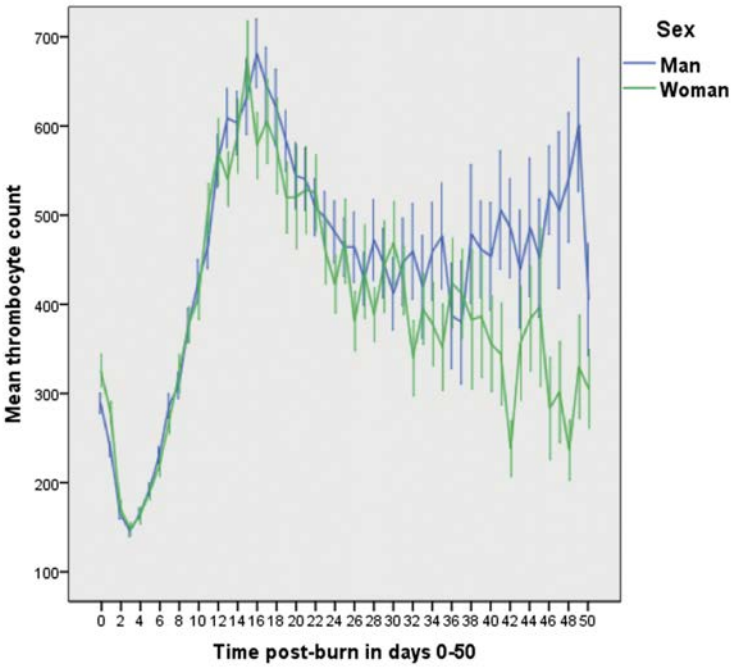

Figure 3. Mean time course of thrombocyte counts in each sex with +/- 1 standard error

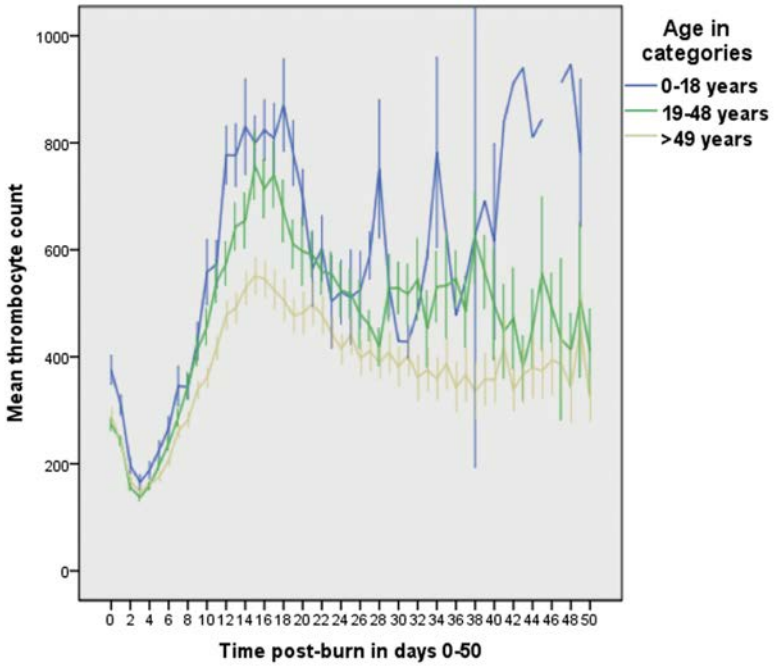

Figure 4. Mean time course of thrombocyte counts for the different age categories with +/ - 1 standard error

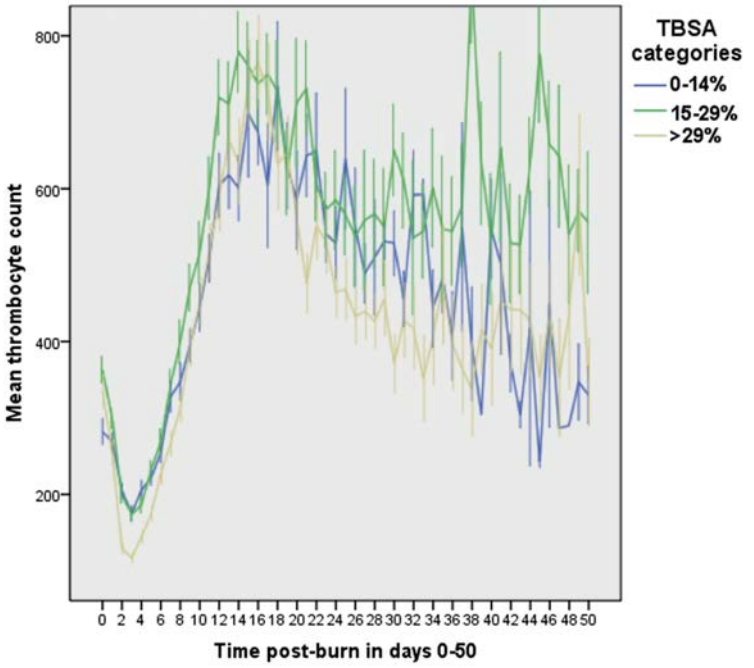

Figure 5. Mean time course of thrombocyte counts for the different TBSA categories with +/ - 1 standard error

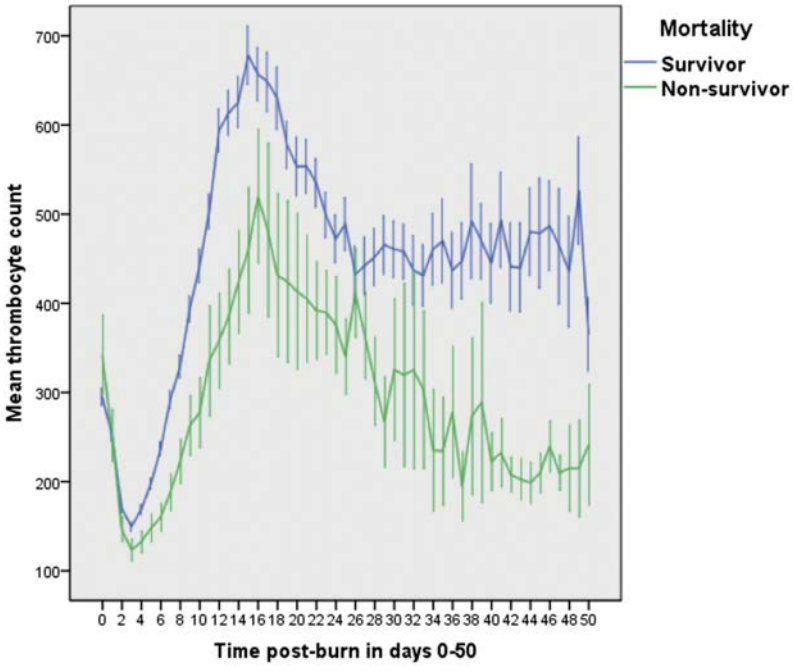

Figure 6. Mean time course of thrombocyte counts for survivors and non-survivors with +/ - 1 standard error

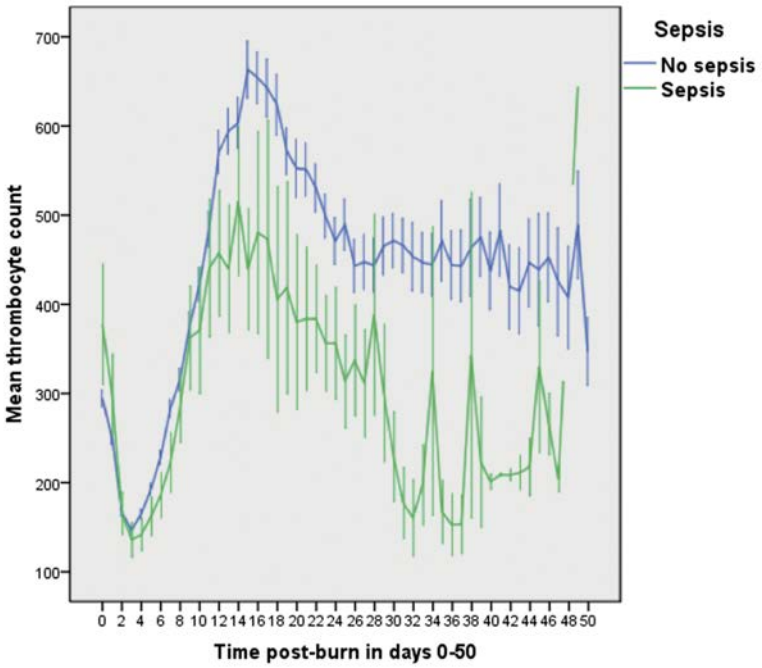

Figure 7. Mean time course of thrombocyte counts for septic and non-septic patients with +/ - 1 standard error

Figure 5 shows the differences between the TBSA categories. The more severely burned patients had a significantly lower nadir ($p<0.001$; Table 2). The less severely burned patients had a significant lower peak thrombocyte count. The mixed model analysis indicated a significant lower platelet count over all periods compared for the TBSA >30% group compared to the other TBSA categories ($p<0.05$; Table 3).

The different thrombocyte time courses for survivors and non-survivors are shown in figure 6. The non-survivors had both a significantly lower nadir and peak ($p<0.001$ and $p<0.005$; Table 2) in the univariate analyses. The influence of septicemia on thrombocyte counts is shown in figure 7. At the nadir, there is a significantly lower count ($p<0.001$; Table 2) in the univariate analyses.

In the Cox-regression analysis, a higher age, higher TBSA % and lower platelet count at the peak are predictive for mortality (Table 4). Sex and a lower nadir were not shown to be predictive for mortality; however, because the mortality rate was low, we performed a univariate Cox-regression, which indicated the nadir as a significant predictor for mortality (Table 4).

DISCUSSION

This retrospective observational study in a large adult and pediatric population showed that the thrombocyte counts have a distinct pattern, with a nadir three days post-burn and a peak at 15 days post-burn, followed by a temporary thrombocytosis, which gradually returns to normal values on day 24. Factors that significantly influence this course are % TBSA and age, while sex does not influences this course. The average thrombocyte courses of both septic and non-surviving

Table 3. Multivariable mixed model analysis of the influence of the different variables on thrombocyte count, with days, periods and interaction between days and periods as time variables.

Parameter	Estimate of fixed effect	P value	95% Confidence Interval
Male	-15.9	.32	-47.7 to 15.7
Female ¥			
< 18 years	137.0	.000	91.6 to 182.5
18-49 years	63.2	.001	29.4 to 97.0
> 49 years ¥			
< 15 %	41.1	.04	2.1 to 80.8
15-30 %	77.5	.000	40.4 to 114.7
> 30 % ¥			

¥ reference category

Table 4. Cox regression analysis for mortality, with the significant predictors for mortality

Multivariate	Hazard Ratio	95 % CI	P value
Age (per 10 years)	1.43	1.07 to 1.93	0.02
TBSA % (continuous)	1.04	1.01 to 1.07	0.005
Peak thrombocytes (per 50 x 10^9/ L)	0.86	0.78 to 0.95	0.02
Univariate			
Nadir thrombocytes (per 50 x 10^9/ L)	0.61	0.40 to 0.90	0.02

patients depict a significant lower nadir in univariate analyses. These findings are in concordance with the literature on burn patients, as well as critically ill and trauma patients.[3-5,9,10,16,17] Furthermore, a higher age, a higher % TBSA and a low thrombocyte count at the peak are predictive for 50-day mortality.

There are several possible causes for the initial thrombocytopenic period. It may be partly due to dilution as a result of resuscitation fluids; however, the lower thrombocyte count persists after fluid replacement has been completed.[11] Therefore, the most likely cause is the consumption of platelets through activation of the coagulation cascade, which occurs in the burn wound as well as at distant sites such as in the kidneys or lungs where micro thrombi can be formed.[12] It can also occur systemically, which is called disseminated intravascular coagulation (DIC).[5,6,10,12] In our study population, no DIC was observed.

Another factor that may contribute to the initial thrombocytopenia is the reduced production of platelets through depression of the bone marrow by circulating inflammatory agents. However, in an autopsy study of 11 burn patients, thrombocytopenia was not associated with fewer megakaryocytes, and in some cases, even higher numbers of megakaryocytes were observed, which suggests that marrow depression is not the mechanism underlying thrombocytopenia.[19]

Finally, drug-induced thrombocytopenia could occur because silver sulphadiazine, heparin, morphine and paracetamol, which are frequently prescribed in burn patients, are known to cause thrombocytopenia.[12,20,21] All of these above-mentioned medications were prescribed in most of our

patients; therefore, it was not possible to include these variables in our analysis. Especially heparin is notorious for causing thrombocytopenia, known as heparin-induced thrombocytopenia (HIT). However, the three-day period of the nadir seems to be to short to develop HIT, so it is an unlikely explanation for the initial thrombocytopenia. Nonetheless, this not to be missed clinical condition should be suspected in the absence of thrombocytosis or in case of persistent thrombocytopenia.[22,24] HIT is paradoxically associated with thrombosis, due to a hypercoagulable state caused by platelet-activating antibodies. It occurs in approximately 2% of all patients receiving unfractionated heparin[23] and incidences from 1.4 to 3.1% have been reported in burn patients.[22,24] In our population, none of the patients developed HIT.

A noteworthy phenomenon in severely burned patients is that the thrombocyte counts can be spurious.[25,26] After an acute burn injury, the red blood cells are damaged and fragmentation occurs. The resulting micro-spherocytes can be misidentified as platelets by the measuring device. It is possible that the real thrombocyte numbers could be even lower in the initial post-burn phase in severely burned patients, although the laboratory staff at our burn center regularly verifies the hematology results in these patients microscopically.

Some authors suggest postponing surgery beyond the thrombopenic phase,[6,11] but this suggestion might be too rigid. Thrombocytopenia is obviously associated with increased bleeding risk, but platelet counts at the nadir are rarely below 50 x 10^9, the threshold below which most guidelines advise transfusions.[3,12,27] The coagulation process is complex, and there are many additional factors involved.[27] Furthermore, Chang et al. studied 23 burn patients and reported a more rapid recovery of platelet counts after surgery compared to non-burn surgical patients. These authors suggested that the increased production of platelets due to the thermal injury prior to the surgery explains this finding.[28] Additionally, it has been suggested that platelet function could be of greater importance than platelet number in coagulation.[29] In trauma patients, it has been shown that platelets are activated at least 72 hours after injury and have an increased functionality in the first 48 hours.[4,29] In burn patients, platelets are activated,[30] but it is unknown how long this activated status continues and how thermal injury affects the functionality of platelets.

A temporary thrombocytosis was observed in all subgroups, in contrast to other studies in which the temporary period of thrombocytosis was not observed in severely burned patients.[9,15,16] The thrombocytosis is most likely caused by a reactive response to the thermal injury or a rebound effect of the bone marrow to its increased consumption and destruction.[5,28] These mechanisms may explain why the least severely burned patients had a significant lower platelet count at the peak.

This study confirmed previous observations that sepsis and thrombocytopenia are linked, although the causality is not yet clear.[11,12,16] Possible factors that may contribute to thrombocytopenia in septic patients include impaired platelet production, due to either bone marrow suppression by septicaemia or by hemophagocytosis; increased peripheral consumption and destruction; or sequestration of thrombocytes by the spleen.[3,12] It has previously been suggested that a persistent thrombocytopenia or low nadir could precede sepsis.[10,15] If true, this relationship would be a very valuable measure for detecting sepsis in burn care, as burn patients are very susceptible to sepsis and common clinical methods for detecting sepsis, such as rising temperature and laboratory infection parameters, are often already high in burn patients, making them unreliable. However, we

did not have enough septic cases to perform a reliable Cox-regression analysis; therefore, we could not assess the predictive value of thrombocytopenia for sepsis.

Patients who did not survive had a lower average platelet count over the time course, when we looked at the individual mortality cases no other trends were noticeable. This result is in agreement with other studies in burn patients.[15-17,31] A higher age, a higher TBSA % and a lower thrombocyte count at the peak at day 15 are predictive for mortality. Although the non-surviving patients in our population did exhibit a lower nadir in the univariate analyses, in the multivariate Cox-regression, the effect of a low thrombocyte count was not significantly predictive for mortality. A recent study found that a decline in platelet count of 65% compared to the admission count on day 3 is a significant predictor for mortality.[17] A reason for these different results may be that our studied population had a lower TBSA % and fewer non-survivors compared to the patient group in the other study.

A limitation of this study is its retrospective design, which resulted in the irregular measurement of thrombocytes. In the beginning, more data were available compared to the end of the study period, which can be seen in the increased oscillation of the lines at the end of the 50-day period. Furthermore, the data at the end were from patients with long hospital stays and were therefore more complex cases. This bias was partly reduced in the mixed model analysis because % TBSA and age were both variables added to the multivariable mixed model. Another limitation is that interventions such as surgery and transfusions were not taken into account. Including these factors was not feasible in this retrospective study design, in which many patients underwent surgery multiple times. Furthermore, Hergt et al. observed no change in thrombocyte counts after most surgical interventions in 13 burn patients.[10]

Statements on the clinical consequences of the nadir and the peak of the thrombocyte counts are difficult, because it remains unknown what the quality and functionality of the platelets is at those time points. Future research should involve thrombocyte activation and function analyses, as thrombocyte function could be of greater importance than platelet number.[29] Furthermore, in addition to the time course for platelet counts described in this study, the functionality of platelets post burn could be essential when considering the use of platelet-rich plasma for treatment of burns.[32]

In conclusion, platelet counts have a distinct time course in all burn patients, which is influenced by % TBSA, age and sepsis and not by sex. High % TBSA, age and a low thrombocyte peak predict mortality, and there are indications that a low nadir may also be associated with mortality. Changes in platelet counts are related to outcome, but this study does not provide absolute numbers. Thrombocyte counts must be evaluated individually in a clinical setting together with all other relevant parameters. Nevertheless, because platelet counts are often readily available, clinicians and researchers in the burn field should pay attention to the change in thrombocyte counts and its predictive value.

CONFLICTS OF INTEREST STATEMENT

No conflicts of interests are present from none of the authors.

No funding was received for this work

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4

Activation, Function and Content of Platelets in Burn Patients

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Platelets 2018:1-7

ABSTRACT

Burn injury has severe impact on the physiologic homeostasis. Platelet counts show a distinct course post-burn injury, with a nadir at day 3 followed by a thrombocytotic period with at peak at day 15, with a gradual return to normal. So far, it is unkown how the functionality and activational status of platelets develop post burn. In this study we investigated if the function, activation and growth factor content of platelets of burn patients are affected and how this evolves in time. Six burn patients with over 15 % total burned surface area were followed during 1 month. Standard hematological and coagulation analyses, thromboelastography (TEG), platelet-function analyser-100 (PFA), several platelet activation parameters (CD62P-CD63, AnnexinV) and growth factors (TGFb1, VEGF, PDGF-AB/BB, EGF, TGFb2, FGF-2, PDGF-AA) analyses were performed. TEG analyses showed procoagulant changes. PFA-100 analyses were nearly all within normal range. CD62P and CD63 and Annexin-V indicated no clear activation of platelets. Growth factor content followed the same course as the platelet count, reflecting a constant growth factor per platelet ratio. Concluding, platelets post burn-injury appear to be functional and not overly activated. However, burn patients seem to remain in a procoagulant state for an extensive period, which may impact their pathology.

INTRODUCTION

Burn injury not only leads to disruption of the integrity of the skin but also severly impacts the physiologic homeostasis of the human body[1]. Platelets are most commonly known for their function in haemostasis, but they also play important roles in wound healing and the immune system. This function is predominantly mediated by growth factors stored in the internal stores of the platelets, which are released after activation.[2] In burn patients platelet count shows a distinct course in time after burn injury, with a nadir at post burn day (PBD) three followed by a reactive peak at PBD 15, with a gradual return to normal values around PBD 24.[3,4] This course is partially associated with the percentage total body surface area burned (TBSA%) and the age of the patient. Furthermore, mortality and sepsis in burn patients are associated with low platelet counts.[3-5]

In trauma (non burn) patients the functionality of platelets is decreased after injury. [6-9] In burn patients there are indications that platelets are activated and show spontaneous aggregration[10,11] Little is known about the platelet function in the time course after a burn injury. Platelet dysfunction could have several clinical implications, such as risk of bleeding, which in turn influences resuscitation protocols as well as timing of surgery. In contrast, hyperreactive platelets affect the risk of developing thrombosis. Burn patients seem to have an increased risk for developing venous thrombosis, although literature is not conclusive with estimations of 0.2 up to 25%[12,13]

Furthermore, platelet dysfunction may even enhance the susceptibility to infection or delay wound healing.[14] Additionally, when considering the use of autologous platelet rich plasma (PRP) as an addition to the treatment of burns it is essential to know if and how platelets are affected by burn injury.[15,16] Growth factor content of platelets is variable between individuals. Several papers have quantified growth factors in PRP, however these were all done in healthy volunteers and never studied in a patient cohort.[15]

Finally, burn injury is associated with systemic coagulopathy, but research on coagulopathy is limited.[12,13] Thromboelastography (TEG), which provides a comprehensive overview of the coagulation reactions, is often performed in trauma populations and it has been suggested that this might be of better predictive value than traditional parameters like prothrombin time (PT) and activated partial thromboplastin time (aPTT) in predicting coagulopathy.[17-19] Several studies have investigated TEG in burn patients.[18,20-24] Two studies concluded that patients are in a hypercoagulable state up to 7 days post-burn injury.[18,22]

In this study we investigated if the function, activation and growth factor content of platelets of burn patients are affected and how this evolves in time after the burn injury. Furthermore, we performed TEG analysis to study the coagulation reactions post-burn.

MATERIALS AND METHODS

The protocol for this prospective study was approved by the local medical ethical committee in Alkmaar, the Netherlands (NH012.050-M012-008). The study was conducted according to the principles of the Declaration of Helsinki (52nd World Medical Association General Assembly, Edinburgh, Scotland, October 2000) and in accordance with the Medical Research Involving Human Subjects Act (WMO).

Blood and sample collection

After informed consent, six burn patients older than 18 years, who were admitted to our burn centre with more than 15% TBSA and no history of recent use of platelet aggregation inhibitors were included between March 2012 until September 2012. With an already planned vena puncture one extra blood sample (4.5 ml, citrate tube) was collected at 5 designated time points post burn: day 0-1, day 3-4, day 8-9, day 12-16, day 20-24, corresponding with respectively the burn day, the nadir, the peak and the return to normal in the platelet count.[3] All patients were treated with prophylactic low-molecular weight heparin.

Blood of healthy volunteers collected in citrate tubes was used as day controls. Whole blood of patients or healthy volunteers was used for PFA-100 and TEG analysis and platelet count was measured on a cell analyser (Sysmex XT2000i, Tokyo, Japan). The remaining whole blood was centrifuged for 15 minutes at 1000 rpm to obtain platelet-rich plasma (PRP) for the flow cytometric analysis of platelet activation markers. The remaining PRP was centrifuged for 4 minutes at 14000 rpm (after addition of 10% Acid-citrate-dextrose solution A (ACD-A) to inhibit platelet activation during centrifugation), to produce platelet-poor plasma for PT, APTT and fibrinogen measurements. The platelet pellet was lysed in sterile water and immediately stored at -80°C until growth factor analysis.

APTT, PT and fibrinogen measurements

Prothrombin time (PT) and activated partial thromboplastin time (APTT) and functional fibrinogen (Clauss method) were measured on the Sysmex CA-7000® analyzer (all from Siemens, Erlangen Germany).

Thromboelastography

Clot formation of whole blood was measured on a TEG 5000 thromboelastograph Hemostasis Analyzer with its accompanying software (Haemoscope Corp, Niles, USA) as described by the manufacturer. Samples were stimulated with citrate-kaolin (CK) (Haemoscope Corp, Niles, USA). The following TEG variables were recorded: the reaction (R) time, which represents time of initial clot formation; the kinetic (K) time, which represents the time from initial clot formation until an amplitude of 20 mm is reached; the angle (alpha), which is measure of the clot growth; the maximum amplitude (MA), which is a measure of the maximum clot strength; and finally, the LY30, which represents the decrease in amplitude 30 minutes after the maximum amplitude is reached and is a measure of spontaneous fibrinolysis. Reference values of TEG results are based on 95% confidence intervals based on 95 whole blood donors as previously described.[25]

Both platelets and fibrinogen contribute to the maximum amplitude. The contribution of platelets and fibrinogen to the maximum amplitude can be calculated with the following formula: MA (CK test) = MAplt + MAfib (CFF test).

The MAfib can be measured with the CFF test and herein the extrinsic coagulation pathway is stimulated with tissue factor, while platelet aggregation is inhibited. Previously, we performed the CFF and CK test with whole blood from 95 donors and measured their fibrinogen concentration.

The correlation between the fibrinogen concentration and MAfib was calculated.[25] In this study we used the fibrinogen concentration from the different time points from our burn patients and calculated MAfib and subsequently the MAplt.

Platelet function

Primary hemostasis was measured using the platelet-function analyzer 100 (PFA-100, Siemens) according to the manufacturer's instructions. The PFA-100 measures the closure time of a standardized 150 µm aperture in a membrane covered with collagen and epinephrine while maintaining constant blood flow.

Platelet activation parameters

Expression of CD62P (using anti-CD62P; Beckmann Coulter, Franklin Lakes, USA A07790), CD63 (using anti-CD63; Beckman Coulter, PN IM1165U), phosphatidyl-serine (with Annexin V ;VPS Diagnostics, Hoeven, The Netherlands, A-705) on platelets was determined by flow cytometry on a FACS Calibur (BD biosciences, San Jose, USA) as described earlier.[26]

CD62P (P-selectin, a marker of alpha-granules) and CD63 (a marker dense granules and lysosomes) are expressed upon platelet activation. Phosphatidyl-serine is expressed on apoptotic platelets. Reference values for the flow cytometry results are based on the mean ±1 standard deviation of the day controls.

Growth factor analysis

Growth factors were analysed with commercial Luminex kits (HCYTOMAG-60K-07, TGFBMAG-64K-03 and HCYTOMAG-60K-02 from EMD Millipore (Burlington, USA) according to the manufacturer's instructions.

Statistical analysis

SPSS statistics 21 (IBM) software was used. For the comparison of means with the respectively maximum or minimum normal values a t-test was performed with a bootstrapping 1000 factor to compensate for the low sample number. For the comparison of the growth factor and platelet relation in time a ratio of growth factor per platelet was calculated and these means were compared with a Friedman test. Significance was set a $p \leq 0.05$. Significant differences are marked in the figures with an asterisk.

RESULTS

Demographic values are shown in table 1. Platelet counts in whole blood are shown in figure 1A. The patients showed an increase in plasma fibrinogen after 3-4 and 8-9 days post-burn, which seemed to normalize at the end of the study period (Figure 1B).

TEG analyses showed during the whole study period a decreased reaction time (R; Figure2A), indicating earlier initiation of clot formation compared to healthy controls.

Table 1. Demographics

N=6	Mean	Range
Age years	46,6	21-64
TBSA%	42	15 -91
Sex	3 male / 3 female	

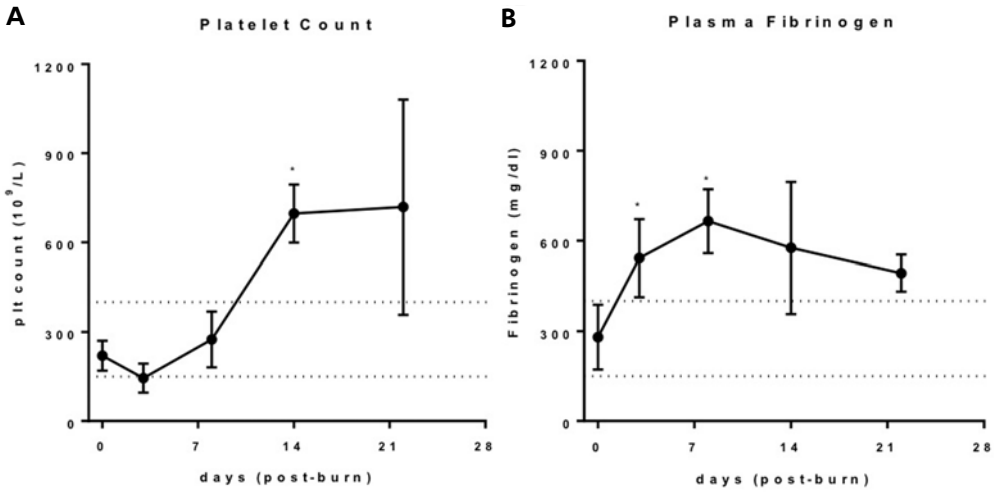


Figure 1. Platelet count and plasma fibrinogen

The kinetic time (K, Figure 2B), reflecting the rate of clot formation, initially showed values in the normal range, but during the study period K-time decreased for all time points post burn-injury compared to the initial values (Figure 2B). The angle (A) initially showed values in the normal range, but increased during the time course post-burn (Figure 2C). Both changes reflect a more rapid clot formation, once started.

The MA immediately after injury was in the normal range, but increased during the course of the study (Figure 2D). This parameter reflects a higher clot strength. MA calculation for either platelet or fibrinogen contribution (Figure 2E, F showed that the increase in clot strength could be attributed to the increased fibrinogen content (Figure 2E).

The Ly30 values were below 5% during the whole study period, except for two patients on PBD 14 (43,7 and 10,3%) and one other, different, patient on PBD 23 (11,1%), suggesting relatively stable clots.

PFA-100 analyses were almost all within normal range of the closure time (Figure 3), indicating no defect in primary hemostasis.-

PT values were within normal limits. APTT values were slightly prolonged (Figure 4 A,B).

The results of the flow cytometric analyses of anti-CD62, anti-CD63 and Annexin V, binding are shown in figures 5A-C. All three depict a high variation, but indicate no clear activation or apoptosis of the platelets.

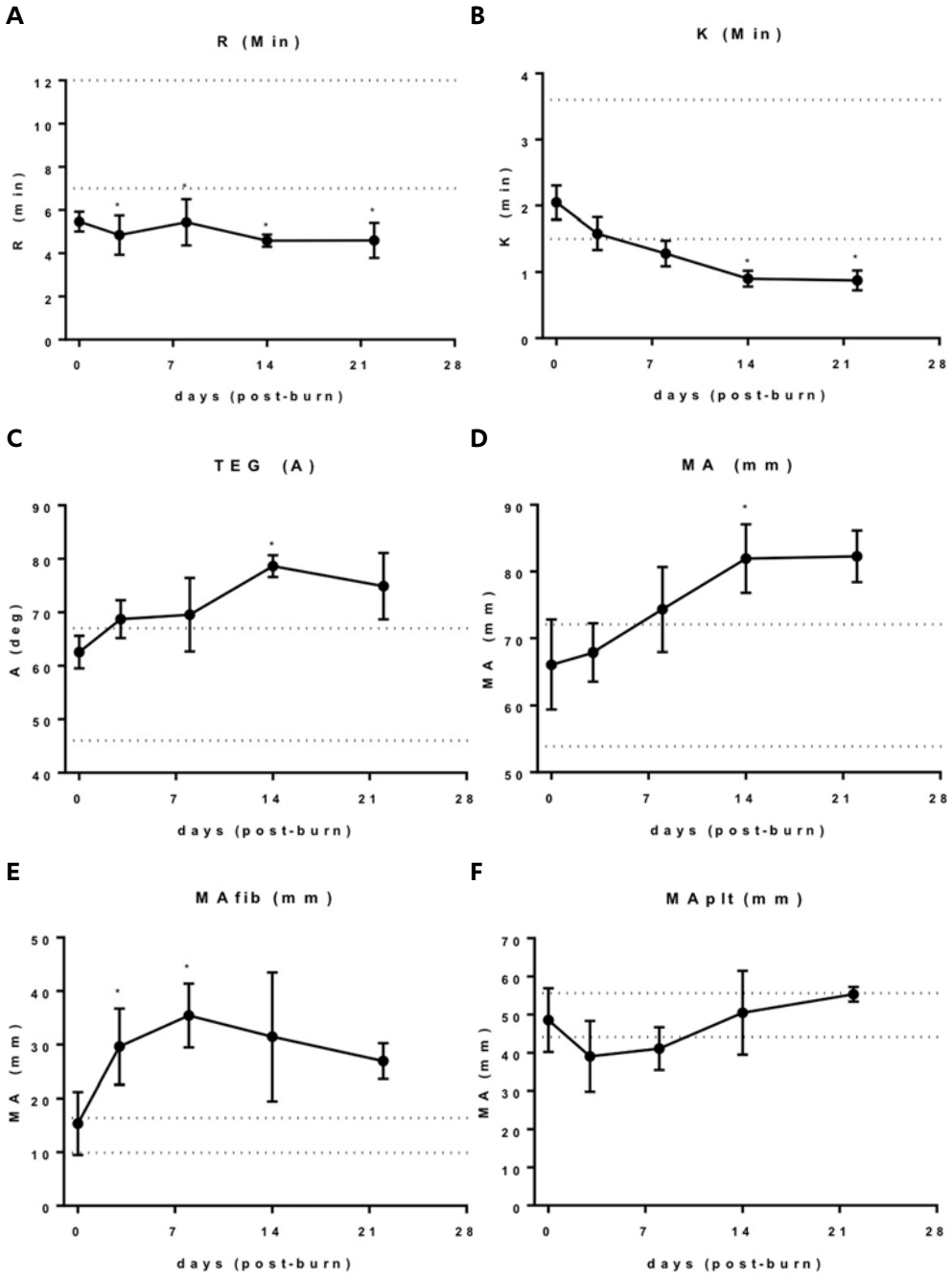


Figure 2a-f. TEG analyses

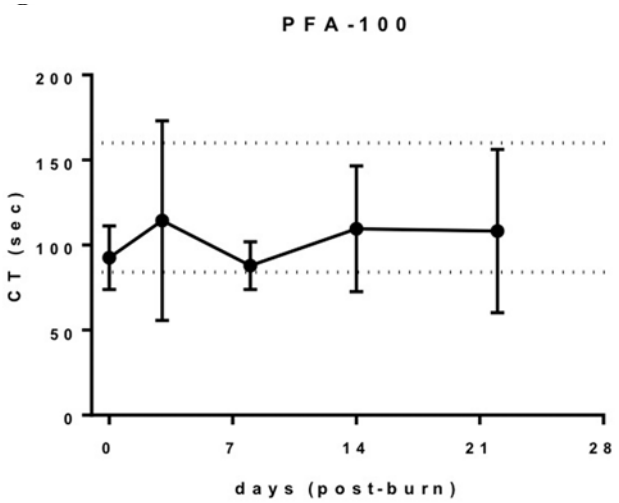


Figure 3. PFA-100 analysis

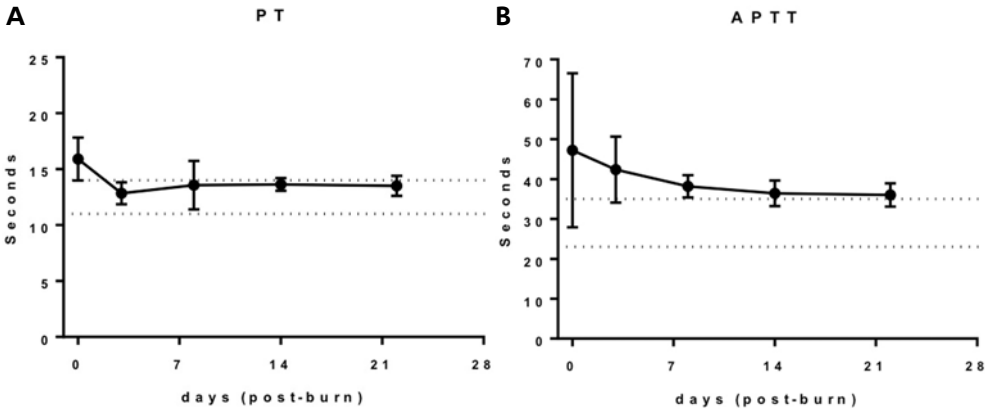


Figure 4. PT and APTT measurements

The growth factor results are depicted in figure 6A-6G. The growth factor content followed the same course as the platelet count. For all the growth factors the amount of growth factor per platelet was rather constant during the total study period as was shown by all non-significant Friedman tests for the comparisons of the mean growth factor per platelet ratio's at all 5 time points (TGFb1: p= 0,7; VEGF: p=0,2; PDGF-AB/BB: p=0,1; EGF: p= 0,2; TGFb2: p=0,7; FGF-2; p= 0,1; PDGF-AA: p=0,1).

DISCUSSION

In patients with burn injury it has been shown that platelets show a distinctive course after the burn injury with a nadir at day 3 and a peak at day 14 and normalization at later time points.[3,4] In the present

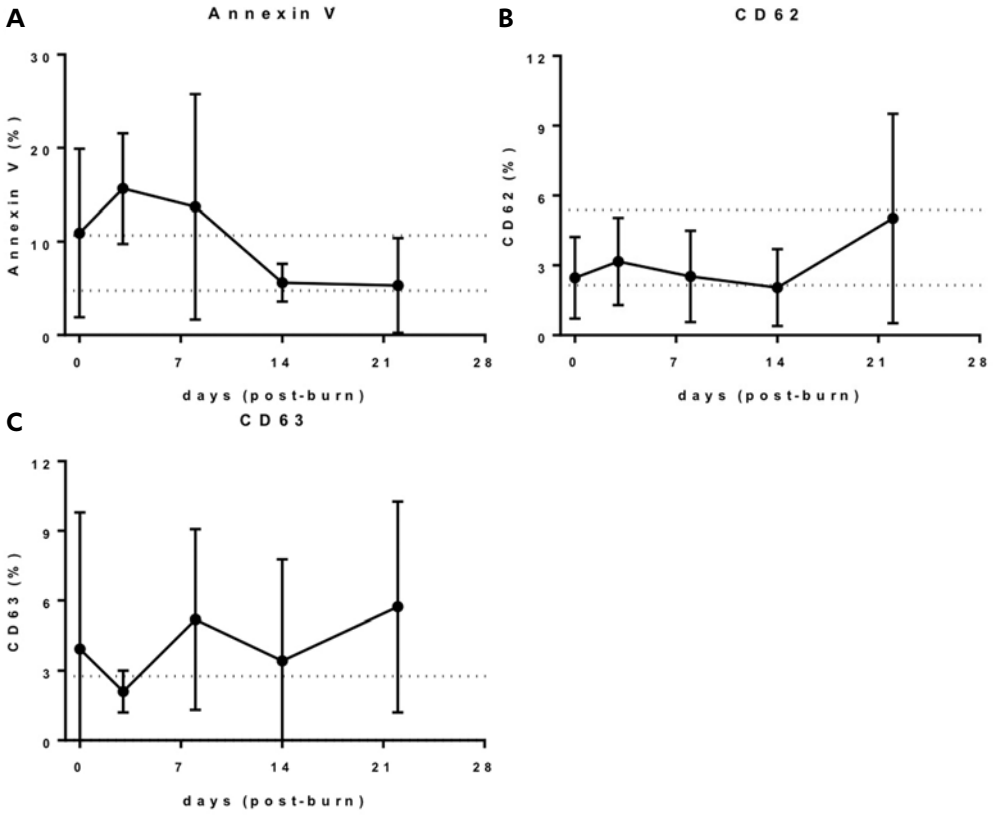


Figure 5. Activation markers

study individual patients were followed for this period of time and the function of their platelets was monitored on time points corresponding with respectively the burn day, the nadir, the peak and the return to normal in the platelet count (Figure 1A). We found that platelets remained functional the entire period as indicated by PFA-100 analysis. Wade et al showed a decreased functionality post burn injury, however this was only measured upon admission and with a different platelet function assay than in the current study. Furthermore, they did not observe decreased function with all their tested platelet agonists.[21]

TEG analysis in current study showed procoagulant changes as depicted in relatively quicker time to clot formation (R) and time till a fixed clot strength (K, amplitude of 20 mm) during TEG analysis. These results of a procoagulant state are in concordance with the few studies on thromboelastography in burn patients.[18,20-24] However, the current study followed individual patients for a longer period (up to 24 days) post burn injury, whereas most other studies only used a maximum of one week follow-up.

In concordance with others, in this study fibrinogen, which is an acute phase protein was increased.[11,18,23] In the initial period following burn injury the increased fibrinogen levels may compensate the decreased levels of platelets.[11,23] Total clot strength (MA) was increased from 8 days

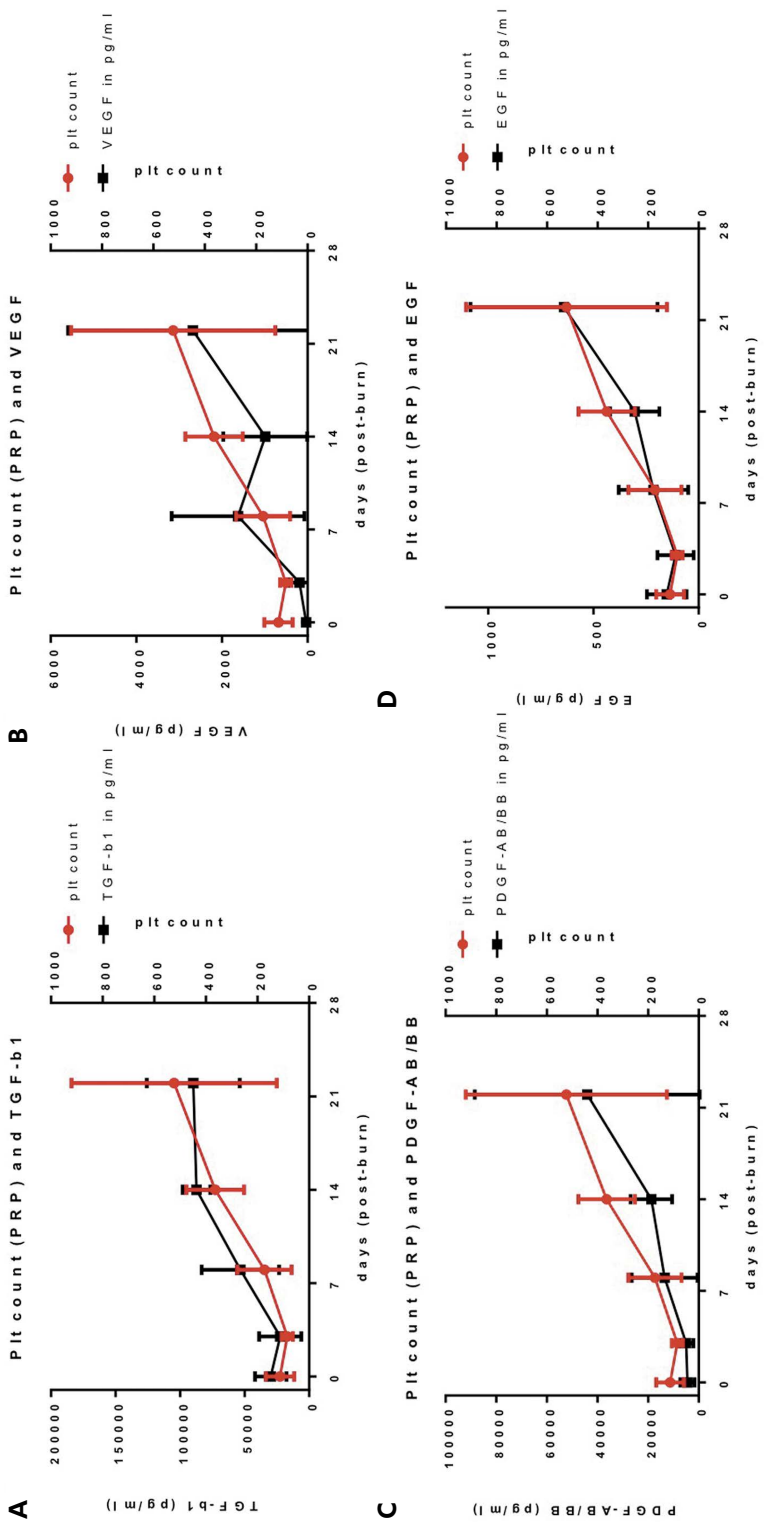
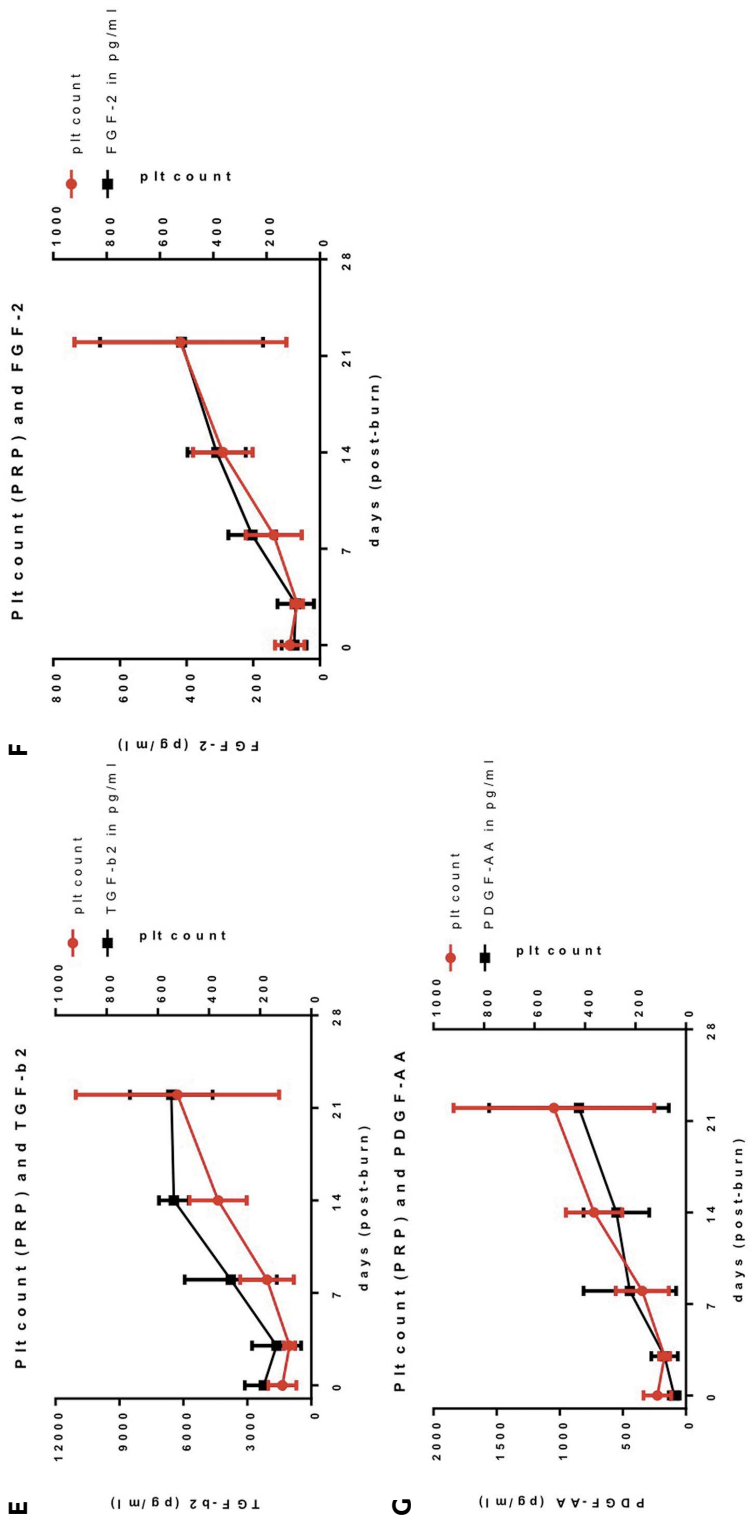


Figure 6. A-G Growth Factor measurements


F
Plt count (PRP) and FGF-2
plt count
FGF-2 in pg/ml
plt count
FGF-2 (pg/ml)
days (post-burn)
E
Plt count (PRP) and TGF-b2
plt count
TGF-b2 in pg/ml
plt count
TGF-b2 (pg/ml)
days (post-burn)
G
Plt count (PRP) and PDGF-AA
plt count
PDGF-AA in pg/ml
plt count
PDGF-AA (pg/ml)
days (post-burn)

post-burn till the end of the study period. This is predominantly explained by the elevated levels of fibrinogen, since the calculated MAfib was increased during the whole study period in contrast to the MAplt.

A shortened R time, increased angle and increased MA indicate a procoagulant status. To get a more complete picture of the coagulation status, ideally also the fibrinolytic potential should be known. We only measured the Ly30, representing the spontaneous clot lysis 30 after the MA is reached, but it has to be kept in mind that Ly30 is not measuring the fibrinolytic potential. The Ly30 was not elevated, except for 2 patients on day 14 and 1 patient on day 23. This is in concordance to other studies in burn patients, and in contrast to trauma patients, in whom Park et al. found an increase in Ly30.[18,27] It is possible that burn patients rebalance their fibrinolytic system during the time course post-burn with the observed procoagulant changes. After burns and inhalation injury, patients show an antifibrinolytic shift 7 days post-burn.[28,29] Since we have found procoagulant changes up to 24 days post-burn, it will be interesting to determine the fibrinolytic status after BD 7.

The whole process of coagulopathy after burns is generally believed to be triggered by endothelial injury and activation of a systemic inflammatory response.[12] Hypercoagulability may be an evolutionary alteration to trauma to prevent bleeding and death, however a negative side effect of this is venous thrombosis and pulmonary embolism.[22] Burn patients are at risk for developing venous thrombosis and although exact incidence is unknown, there are estimations of an incidence from 0.2 up to 25%.[12,13] Due to high heterogeneity in the literature on coagulopathy in burn patients and a wide range of diagnostic criteria and definitions used it is difficult to draw overall conclusions from these studies.[12,17,18,22,30,31]

The activation markers present on the membrane of the platelets show that the platelets after burn injury are not overly activated. The CD62P marker, which indicates the release of the alpha-granules containing the growth factors, indicated no platelet activation. This is in concordance with in the finding that growth factors were still present in the platelets. Analysis of platelet content in terms of growth factors is a novelty in our present study. Over the whole study period, the concentration of various relevant growth factors followed the pattern of platelet count.

There was a large variation in individual concentrations, this could be related to the variation in burn severity in these patients (TBSA 15-91%). Our present data are however insufficient to conduct further subanalyses in this direction, future research in a larger patient cohort could elucidate this. Despite the variation in individual concentrations, there was no difference in growth factor content per platelet. This indicates that there seem to be no "exhausted platelets."[32] This finding is especially important when considering the use of autologous PRP as an adjunct in the treatment of burn wounds, since this product aims for the abundance of growth factor present in a concentrated sample of platelets.[16] Previous quantifications of growth factor content in PRP in the literature have been done with samples from healthy volunteers.[15] In our study we showed that platelets of burn patients are not deprived of growth factor content and hence could be used in an autologous PRP-product for burn patients. However, a recent clinical trial did not show convincing beneficial effects for the addition of autologous PRP in the treatment of burn wounds.[16] Based on our current and previous data we recommend in case of autologous PRP to avoid the initial period post-burn

injury, since in that period both platelet count and subsequently growth factor content of the PRP is reduced.[16] An alternative could be the use of allogenic PRP, since this provides a product of more constant quality.

The current study only describes a small cohort of burn patients. However, all the burn patients in this study depict in general very similar results and therefore these results could be used as a direction for further research. Another limitation is that the clinical course of patients, such as surgical interventions and fluid resuscitation were not taken into account individually. It should be noted that none of the patients from this study developed thrombosis or sepsis, or died. Further research is warranted in a larger patient cohort to clarify the coagulation process in burn victims and how this is related to outcome. This will be a complex task, since coagulopathy is a multifaceted process, especially in a multi-variable group as burn patients with different burn sizes undergoing abundant resuscitation, repetitive surgery, blood and plasma transfusions and with high risks for infection and sepsis.[12,17] However, it will be valuable and will provide essential information to help proceed towards tailored treatment for individual burn patients.

In conclusion, this prospective study shows that platelets after burn injury are functional and not overly activated as we found no signs of degranulation and loss of growth factor content by the platelets. Furthermore, burn patients seemed to be in a procoagulant state for an extended period after burn injury. In the initial period the increase in fibrinogen levels may be compensatory for the lower platelet counts. This study helps to shed a light on the nowadays more recognized role of platelets and the complex process of coagulopathy after burn injury.

ACKNOWLEDGEMENTS

We would like to thank Wim Tuinebreier for his assistance with the statistical analyses. We would like to thank the nursing staff of the Burn Centre in Beverwijk for their help in collecting the blood samples.

DECLARATION OF INTEREST

None of the authors have a conflict of interest or financial disclosure to declare
This research was partially funded by research grants from the Association of Dutch Burn Centres (grantnumber: 12-02) and by a research grant of Sanquin Blood bank (grant number PPOC 14-43).

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5

Growth factor quantification of platelet-rich plasma in burn patients compared to matched healthy volunteers

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Submitted

ABSTRACT

Platelet rich plasma (PRP) is blood plasma with a platelet concentration above baseline. When activated, PRP releases growth factors involved in all stages of wound healing. This could aid wound healing in burns. To expand our knowledge of the effectiveness of PRP, it is crucial to know the content and composition of the PRP product. In this study growth factor quantification measurements of leukocyte PRP from burn patients and gender- and age-matched controls were performed. The PRP of burn patients showed comparable levels of growth factors as those of the PRP of healthy volunteers. Considerable intra-individual variation in growth factor content was found. A clear correlation was found between the platelet count of the PRP and most of the growth factors measured.

INTRODUCTION

Platelet rich plasma (PRP) is produced from blood plasma, and results in a platelet concentration above baseline. When PRP is activated, platelets release growth factors, such as platelet-derived growth factor (PDGF), fibroblast growth factor(FGF), transforming growth factor β(TGF-β), epidermal growth factor(EGF) and vascular endothelial growth factor(VEGF). These growth factors are involved in different stages of wound healing. Improved wound healing qualities have been attributed to PRP by the multitude of growth factors delivered to a wound.[1]

There are numerous types of PRP products and preparation methods. A large number of different application areas exist, from sports medicine and orthopaedics to chronic and acute wounds as well as aesthetic applications. These are described in an extensive body of literature; however, most studies advocating the use of PRP comprise case reports and patient cohorts. Randomized controlled trials are rare, and systematic reviews repeatedly fail to show strong conclusive evidence of the effects of PRP.[1-3] This could be partly due to the great variability of PRP products: variability in preparation protocols; different composition (with/without leukocytes and fibrin content); variability in platelet baseline count and PRP yield, growth factor content per platelet; and finally activation and application methods. Furthermore, for all the different applications it is not known what the most desirable number of platelets and growth factors is.

To expand our knowledge of the effectiveness of PRP, it is essential to know the content and composition of the PRP product. Platelet count and growth factor quantification do not appear to correlate in a constant way.[4,5] Consequently, growth factor quantification still seems the best type of quality control of PRP. A recent systematic review of commercial PRP separation systems showed a large heterogeneity in the concentrations of platelets, leukocytes and growth factors.[6] One of the inclusion criteria of this systematic review was that the studies had to investigate 'healthy volunteers.' This is somewhat curious, because autologous PRP is mostly applied in patients and not healthy volunteers. An extensive search of the literature was performed; however, we could not find studies describing the quantification of PRP content in patients.

In burn patients, the use of PRP has been ascribed potential positive effects on woundhealing.[1,7] The current study was part of a recent randomized trial, which did not to show considerable added value of PRP in acute burns. [8] In addition to the variables in PRP in general described above, a few more are added by burn injury: burn injury has a severe impact on the internal physiology of patients [9], platelet counts show a distinct pattern after burn injury, with a nadir at day 3, a peak around day 14, followed by a gradual return to normal values [10], thus affecting the baseline platelet count from which the PRP is produced. Since platelets are the core ingredient of PRP, it is crucial to know if and how the quality of the PRP could be affected by the burn injury. In a recent study it was found that the platelets in burn patients were not overly activated and remained functional and not deprived of growth factors; however this was tested in platelets in whole blood samples.[11] Current study investigates the quality of leukocyte rich PRP from burn patients, which was produced by a commercially available preparation system, and compared with PRP from age and gender matched healthy controls.

MATERIALS AND METHODS

This study was performed as a sub-study (amendment NL28331.094.09) of a randomized controlled trial (ISRCTN14946762) performed in the burn centre of Beverwijk, the Netherlands, which compared autologous platelet-rich plasma with standard treatment for burn wounds.[8] All ethical committee and institutional permissions were obtained to recruit five consecutive patients, who were already included in the main RCT, after an additional informed consent, between December 2011 and March 2012. The PRP was prepared with the Gravitational Platelet Separation System (GPS-III system, Biomet Merck Biomaterials, Darmstadt, Germany). The instructions of the manufacturer were strictly followed. For details of the trial and preparation methods see previous report.[8] From 27 ml blood from five patients we prepared PRP with an extra GPS-III mini-kit. We matched the patients by gender and age with five healthy volunteers and also prepared PRP with a GPS-III mini-kit.

A small amount of PRP and non-citrated whole blood (WB) was collected in an EDTA (ethylene di-amine tetra-acetic acid) tube, and analysed for baseline measurements, using the Cell-Dyn Sapphire 2 hematology analyzer (Abbott Diagnostics Division, IL, USA). Platelet, erythrocyte and leukocyte count were determined.

PRP was activated with autologous thrombin according to the manufacturer's protocol (i.e. PRP:thrombin = 10:1), and incubated for one hour at room temperature to mimic clinical application as accurately as possible. Activated PRP was centrifuged (10,000x g at 4 °C for 15 minutes), clots were removed, and supernatants were collected and stored at −80 "C until further analysis. The magnetic bead panel Milliplex MAP kits (EMD Millipore, Billerica, USA) were used to analyse FGF-2, EGF, VEGF, TGFβ-1, TGFβ-2, TGFβ-3, PDGF-AA and PDGF-BB. Separate kits were used to analyse TGFβ and PDGF. All kits were used according to the manufacturers protocol. The Milliplex MAP kits were measured using Bio-Plex 200 (Bio-Rad, Hercules, USA) and data were analysed using Bio-Plex manager software.

For statistical analyses SPSS statistics 21 (IBM) software was used. For the comparison of means a Mann Whitney test was used. Correlation was tested with the non-parametric Spearman's rho test. Significance was set at $p \leq 0.05$.

RESULTS

Demographics and haematology results are described in table 1. By chance, the included patients were sampled on consecutive days after the burn injury (Figure1).

The mean ratio of platelet concentration in whole blood platelet count compared to the PRP platelet count was 4.44 (SD 1.04 range: 2.5 to 5.9) (Figure 1a). There was no difference between patients and volunteers, 4,7 vs. 4, 2 respectively (p= 0.78 Mann-Whitney test). No significant difference was found in mean growth factor content between burn patients and matched healthy controls *(*Mann-Whitney tests: TGFβ-1 p= 0.2; TGFβ-2 p= 0.2; TGFβ-3 p=0.9; PDGF-AA p=0.4; PDGF-BB p=0.5; VEGF p=0.8; EGF p=0.7; FGF-2 p=0.8*)*, nor in growth factor per platelet ratio (data not shown).

When one outlier (volunteer 5) was eliminated, there was a clear correlation between platelets in PRP and growth factor concentration, except for VEGF and FGF (Figure 2-6) Spearman's rho correlation tests: TGFβ1 R=0.95, p=0.008; TGFβ-2 R=0.9, p=0.001; TGFβ-3 R=0.8 p=0.02; PDGF-AA R=0.9, p=0.002; PDGF-BB R=0.8, p=0.008; VEGF R=0.3, p=0.4; FGF-2 R=0.1, p=0.7; EGF R=0.7, p=0.03).

Table 1. Demographics and hematology results

	Age	Sex	Post Burn Day	TBSA (%)	Platelet count X 10^3 µl Whole Blood	Platelet count X 10^3 µl PRP	Leukocyte count X 10^3 µl Whole Blood	Leukocyte count X 10^3 µl PRP	Erytrocyte count X 10^3 µl Whole Blood	Erytrocyte count X 10^3 µl PRP
P1	35	F	3	16	212	1141	9.2	29.0	3.0	1.2
P2	67	F	6	9	467	1792	7.5	30.0	3.8	0.5
P3	40	M	10	61	173	1003	8.1	30.0	2.6	1.1
P4	61	F	13	12	524	2500	20.3	94.1	2.6	1.9
P5	72	M	17	5	343	1326	8.10	29.4	4.0	0.5
V1	41	F			282	713	6.3	16.7	4.5	1.5
V2	65	F			311	1248	5.8	24.5	4.4	0.7
V3	42	M			298	1277	7.5	33.2	4.6	0.9
V4	53	F			356	1448	3.8	19.8	4.4	0.7
V5	61	M			284	1678	7.5	48.2	4.7	0.9

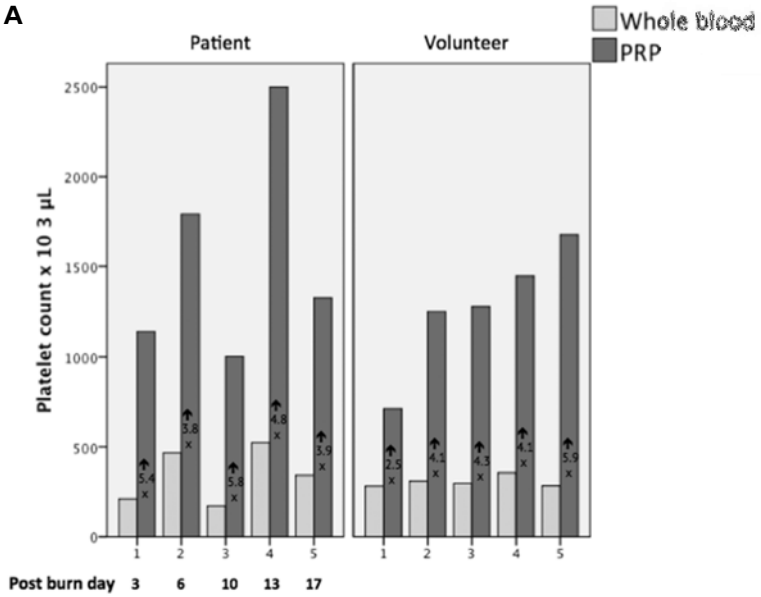

Figure 1a. platelet concentration in whole blood versus in PRP in patient group and in volunteer group; under patient group the post burn day is depicted.

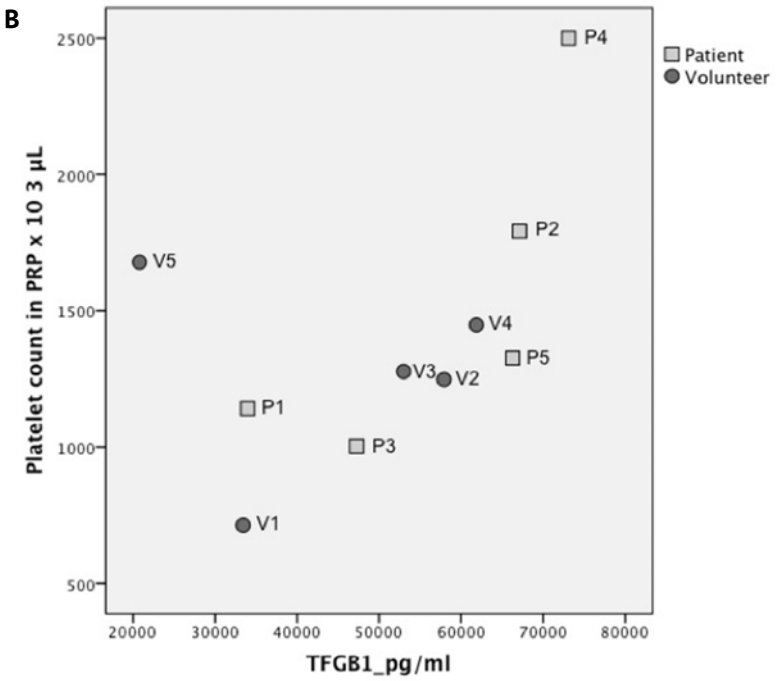

Figure 1b-d. Growth factor quantification per platelet count in PRP for respectively TGFB1, PDGF AA and EGF

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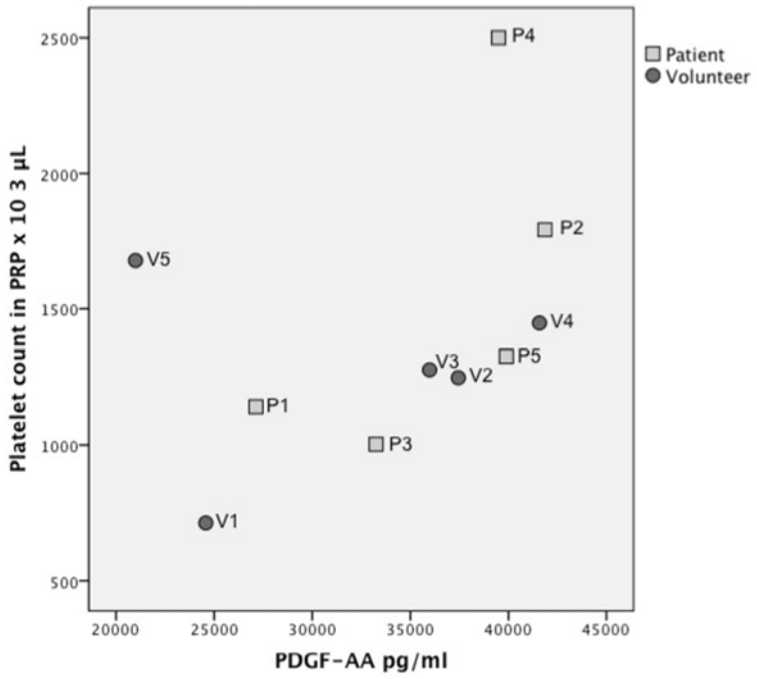
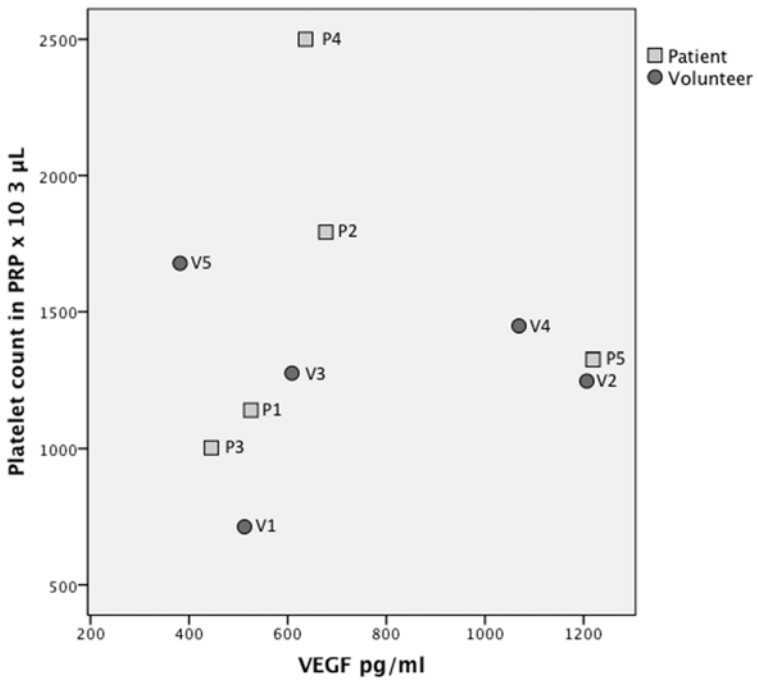

Platelet count in PRP x 10 3 µL
2500
2000
1500
1000
500
20000
25000
30000
35000
40000
45000
PDGF-AA pg/ml
P4
P2
V5
V4
V3
V2
P5
P1
P3
V1
Patient
Volunteer

D


Platelet count in PRP x 10 3 µL
2500
2000
1500
1000
500
200
400
600
800
1000
1200
VEGF pg/ml
P4
P2
V5
V4
P5
V3
V2
P1
P3
V1
Patient
Volunteer

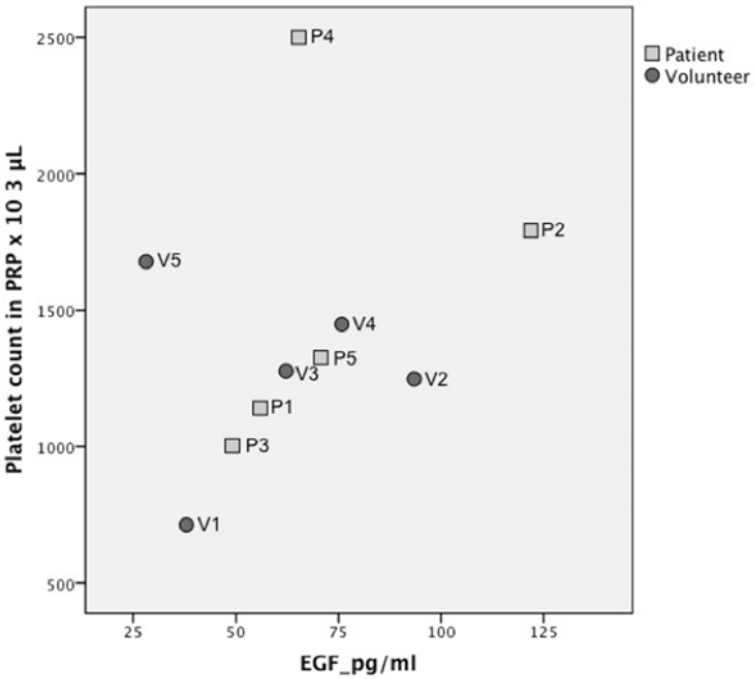


Figure 2-6. Growth factor quantification per platelet count in PRP for respectively VEGF, PDGF BB, TGFB-2, TGFB-3, FGF-2

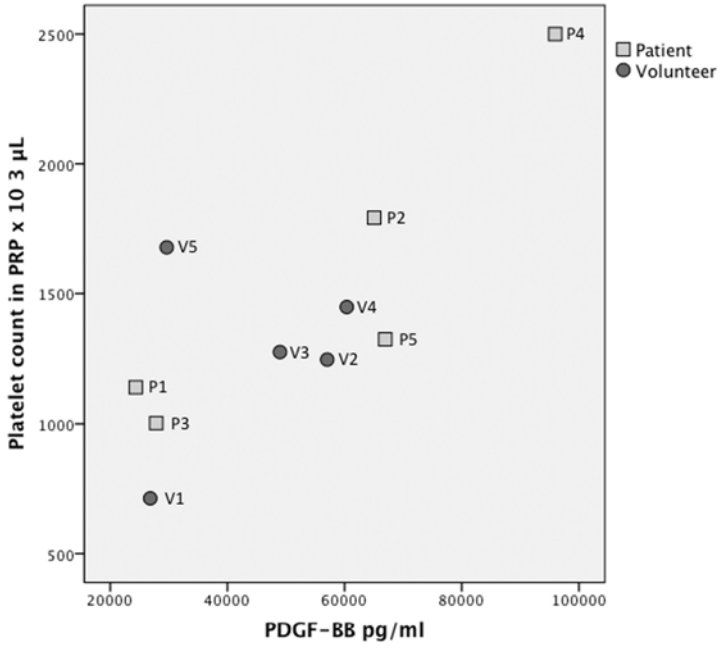


Figure 3.

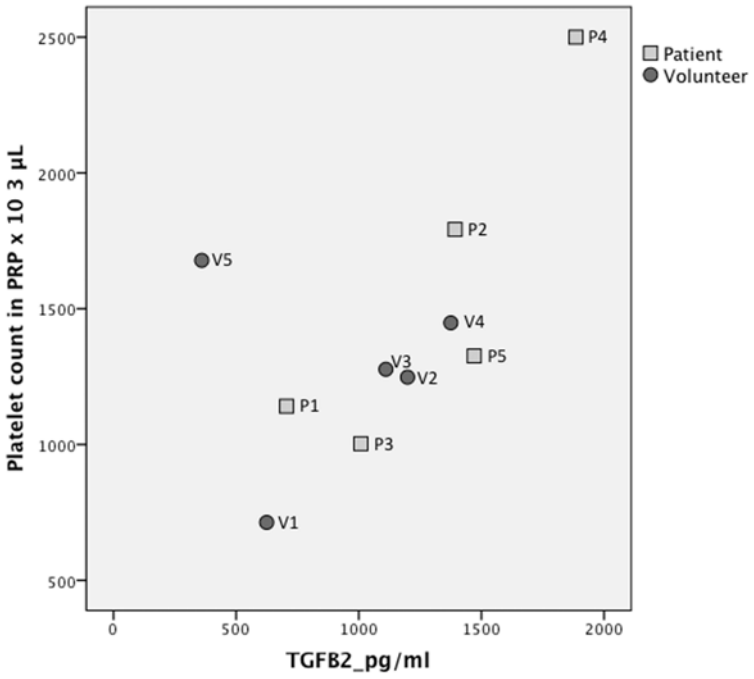


Figure 4.

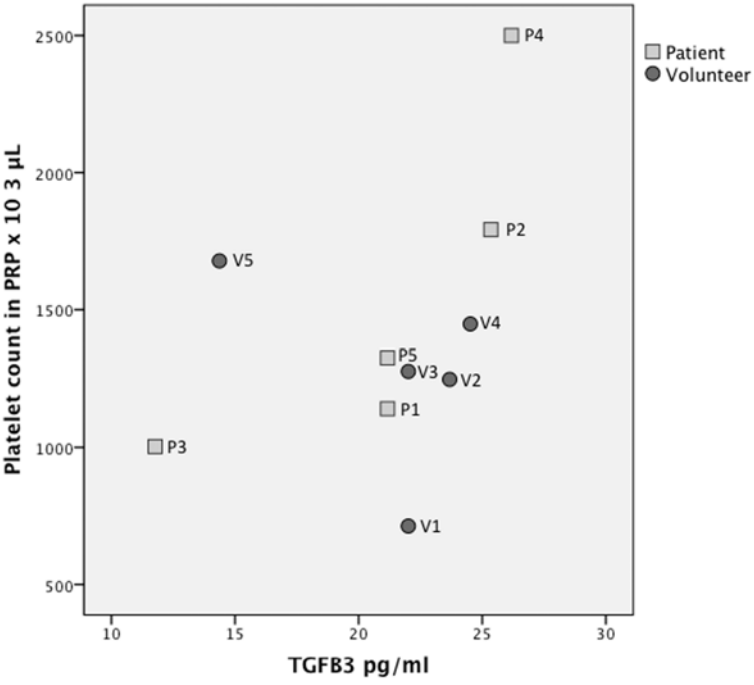


Figure 5.

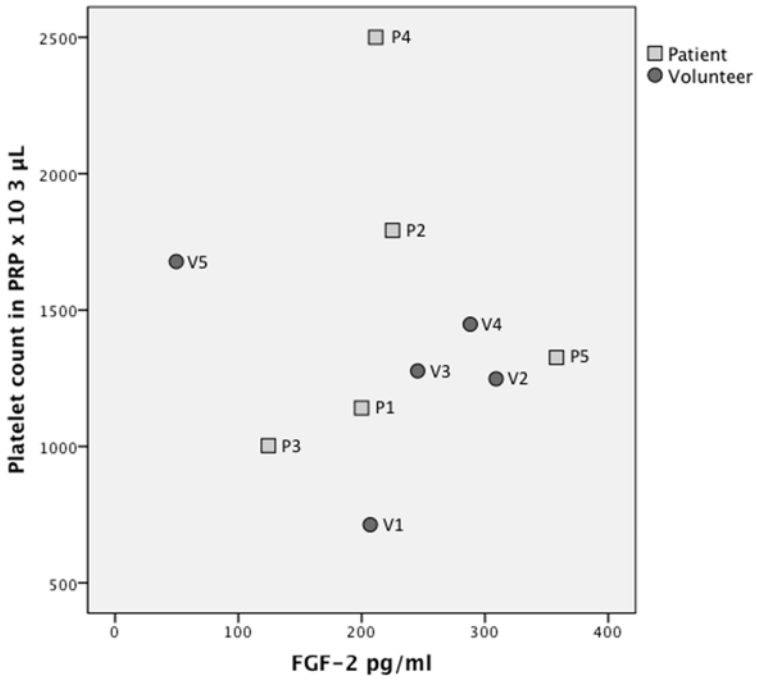


Figure 6.

DISCUSSION

In this study it was shown that PRP of burn patients had comparable levels of growth factors compared to that of the PRP of healthy volunteers. This is despite the systemic effects that burn injury has on the physiology of burn patients. This is relevant additional information to the main RCT of which the current study was part of.[8] The RCT showed that the addition of PRP to the treatment of burn wounds did not result in improved graft take and epithelialization, nor in better scar quality. Only minor beneficial effects in certain subgroups were seen.[8] From the current study it can be concluded that the lack of substantial clinical effect of the PRP in acute burns does not seem to be explained by a general shortage of available growth factors in the PRP of burn patients.

We did find a considerable variation in growth factor concentrations, which is in accordance with literature on PRP-products.[6] More research is required to determine an optimum platelet and growth factor concentration for burns, as well as for other applications. There is some consensus on the minimum platelet counts required in PRP, it is generally advocated that a minimum platelet count of 0.8-1x 10^6/µl should be obtained, however there is no compelling evidence for this. Interestingly enough, we found a clear correlation between the platelet count in PRP and most of the growth factors measured. This has not always been demonstrated in previous studies.[4,5] The platelet count in PRP did not correlate with outcome in subanalyses of the main RCT of which the current study was part of.[8] Nevertheless, platelet count can potentially be used as a quality control parameter for

future research, since it is far more feasible to routinely determine the platelet count in PRP than it is to analyse growth factors. We recommend that further research be done to confirm this finding.

A limitation of this study is that only a small cohort of patients was tested, consequently subgroup analyses were not feasible. The effect of gender and age could not be studied; this may influence the growth factor content, as has recently been suggested.[5] Nor could the effect of TBSA % burned or timing post-burn injury be clarified. It would also have been interesting if we had been able to correlate the growth factor content with the clinical outcome in the RCT; however this was not realistic in this small adjunct study.

In conclusion, burn patients have a comparable platelet growth factor content in PRP compared to matched healthy volunteers. In accordance with the literature, considerable individual variation in growth factor content was found, however a clear correlation between growth factor concentration and platelet count in PRP was seen.

ACKNOWLEDGEMENTS

Biomet (Biomet Europe BV, Dordrecht, the Netherlands) provided the PRP-preparation kits and an unrestricted educational grant which covered some of the remaining costs of this study. Any remaining costs were covered by a research grant from the Association of Dutch Burn Center. There are no other potential financial conflicts of interest for all of the authors.

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6

The application of platelet rich plasma in the treatment of deep dermal burns: a randomized, double blind, intra-patient controlled study

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Wound repair and regeneration, 2016;24(4):712-720

ABSTRACT

Platelet rich plasma (PRP) is a fraction of blood with a platelet concentration above baseline. When platelets get activated, growth factors involved in wound healing are released. The application of PRP has shown good results in wound care, however up to date no substantial research has been performed on the effect of PRP in burn treatment. This randomized double blind intra-patient controlled study investigates the effect of autologous PRP on wound healing in burns that require surgery with a meshed split skin graft (SSG). 52 patients with various areas of deep dermal to full thickness burns, receiving surgery with a SSG were included after informed consent. Comparable study areas A and B (intra patient) were appointed, randomized and either treated with a SSG and PRP or with a SSG alone. At day 5 to 7 post- operative, the epithelialization and graft take rate were assessed. 3, 6 and 12 months post-operative, follow-up measurements were performed in the form of POSAS-questionnaires, DermoSpectroMeter and Cutometer measurements. There was no statistically significant difference between the mean take rate nor the mean epithelialization rate at day 5-7 between the PRP-treated and control areas. However, PRP-treated wound areas showed more often better or equal epithelialization and take rates at day 5-7 than the standard treated areas. Minor effects were also seen in the re-operated and early operated subgroups. At 3, 6 and 12 months post operative, POSAS scores from the patients and the observers, Dermaspectro- and Cutometer measurements did not depict a significant difference between the PRP and standard treated areas. Concluding, the addition of PRP in the treatment of burn wounds did not result in improved graft take and epithelialization, nor could we demonstrate better scar quality. There was, however, a considerable variation in our clinical population.

INTRODUCTION

Platelet rich plasma (PRP) is a fraction of blood plasma with a platelet concentration above baseline. Activated platelets release growth factors, such as platelet derived growth factor (PDGF), fibroblast growth factor (FGF), transforming growth factor β (TGF-β), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), and insulin-like growth factor (IGF). These growth factors all contribute to wound healing in numerous ways, promoting chemotaxis, cell adhesion, mitogenesis, proliferation and angiogenesis.[1] Additionally, platelets have been attributed anti-microbial effects and pain-relieving qualities.[2-4]

Several types and preparation techniques of PRP can be distinguished, at different costs.[5] Various preparation procedures lead to PRP with different content, for example with or without leukocytes, and different structure: fluid, gel or more fibrin-structure.[5] Furthermore, there are different activation methods and PRP can be produced from either autologous or allogeneic blood.[5]

PRP is used in a broad range of clinical healing applications. Beneficial reports on wound healing have been reported in different fields of surgery and in the treatment of acute, chronic and diabetic wounds; however there are also multiple publications, which show no attributive effect of PRP, causing a still ongoing debate on the additional value of PRP. This emphasizes the lack of comparative clinical trials.[5-8] Literature on the use of PRP in burns is particularly scarce.[5,8] A clinical prospective controlled study, where PRP was applied in 59 patients with acute wounds, of which 11 were friction burns, demonstrated a significantly increased healing rate.[9] A recent blinded intra-patient controlled study showed enhanced burn wound healing when wounds were treated daily with allogeneic platelet concentrate in comparison with silver sulfadiazine. But there are some methodological issues in this study, such as unclear randomization method, blinding, allocation of treatment and endpoints; and silver sulfadiazine is known to delay wound healing.[10] Finally, a cohort study in 18 burn patients treated with a split thickness skin graft (SSG) and autologous platelet concentrate, resulted in less pain and earlier discharge compared to institutional controls who did not receive SSG with autologous platelet concentrate. However this control group was described insufficiently.[11]

Long-term follow-up research in this field is even more limited, only one study showed accelerated recovery of viscoelastic properties of full thickness burns treated with autologous platelet concentrate with a SSG, compared to areas treated with a SSG alone, but no improvement in the long-term outcome.[12]

Furthermore, literature on the treatment of burns with single recombinant growth factors showed beneficial results in accelerating and enhancing burn wound healing.[13] PRP could be considered as a less expensive and autologous growth factor cocktail.

Although much progress has been made in burn wound treatment, burns still often result in disfiguring and disabling scars, especially deeper burns that need surgical excision and skin transplantation. A deep dermal burn wound treated with a SSG, could benefit from the addition of PRP in several ways. PRP functions as a fibrin glue with hemostatic qualities, and with the release of the growth factors provides a well nourished site for the SSG and possibly increasing adherence as well as ingrowth of the SSG.[14,15] Furthermore, the interstices of the SSG could heal faster and result in

better scars due to the attributive effect of PRP since it promotes vascular in-growth and fibroblast proliferation, and possibly faster re-epithelialization, as has been shown in *in vitro* models and chronic and acute wounds.[16-19]

In this randomized, double blind, intra-patient controlled study we aimed to test the hypothesis that PRP would increase the rate of wound healing in acute burn wounds and consequently would lead to improved scars. Therefore the effect of autologous leukocyte containing PRP on take rate and epithelialization rate of the SSG in the treatment of deep dermal and full thickness burn wounds was studied. Furthermore we evaluated the effect of PRP on scar quality at 3, 6 and 12 months post-surgery.

MATERIALS AND METHODS

Study design and study population

This study was performed between June 2010 and January 2014 at the Dutch Burn Center of the Red Cross Hospital, in Beverwijk, the Netherlands. The study protocol was approved by the medical ethics committee in Alkmaar, the Netherlands (NL28331.094.09) and registered at www.isrctn.com with number: ISRCTN14946762. The study followed the tenets of the Declaration of Helsinki (52nd World Medical Association General Assembly, Edinburgh, Scotland, October 2000).

Patients 18 years and older admitted at the Dutch Burn Center in Beverwijk with a full thickness or deep dermal burn wound with a surface area of at least 2% total body surface area (TBSA) that received transplantation with a SSG were eligible for this trial. Patients were informed about the study and included after providing signed informed consent. Patients who were temporarily unable to give informed consent (e.g. temporary on mechanical ventilation) were included after acquiring informed consent from their legal representative and requested for informed consent as soon as possible. Patients with insufficient proficiency in the Dutch language or expected to be non-compliant to the study protocol, according to the judgment of the attending physician, were excluded from participation.

Patient, burn-related and treatment characteristics were documented, including gender, age, etiology of the burn and % TBSA burned.

Materials

For this study autologous buffy-coat PRP was used, which contains leukocytes as well as platelets. The PRP was prepared with the Gravitational Platelet Separation System (GPS-III system, Biomet Biologics LLC, Warsaw, IN, USA). The instructions of the manufacturer were strictly followed. In short, prior to surgery blood was drawn by a venous puncture using a disposable system. Respectively 54 and 11 ml was used for the PRP and the preparation of autologous clotting factors, both were mixed with citrate, 6 and 1 ml correspondingly to prevent clotting. Then, the mixtures were transferred to GPS system tubes. The PRP tube was centrifuged for 15 minutes at 3200 rpm. Following centrifugation, the platelet-poor plasma (PPP) was discarded through the side port. The concentrated platelets, on top of the floating buoy, were re-suspended to form PRP and withdrawn from a designated side port.

Autologous clotting factors were prepared by infusing the 12 ml citrated blood in the Clotalyst system and placing it in a heater at 25 °C for 25 minutes. Next, it was shaken to dissolve the formed gel and centrifuged for 5 minutes at 3200 rpm. It was removed vertically from the centrifuge and the top layer, 3-5 ml, which contained the autologous clotting factors was removed through the central port.

Total preparation time is estimated at 45 minutes minimum. The PRP and autologous clotting factors were stored at 4°C only if necessary (maximum of 3 hours) until further use in the operating theatre, where the PRP and the thrombin were drawn up into two syringes supplied with the spray applicator kit. They were then fitted into the 'two syringe assembly component', so that both syringes could be depressed simultaneously, ensuring that the PRP and autologous clotting factors were mixed in the correct 10:1 ratio.

Hematologic analyses were performed on whole blood and the PRP. The PRP was gently shaken for 10 minutes to ensure a representative distribution.[20]

Methods and randomization

During the operation, the burn wounds were cleaned, excised surgically or with hydrosurgery (Versajet, Smith & Nephew) and sufficient wound haemostasis was obtained. In each patient, two comparable wound areas were selected, with respect to the anatomical location and burn depth, of at least 1% TBSA, and assigned A or B. If the sites were adjacent, an area of 1 cm in width between both sites was left out of the assessments. Allocation of A (left, lateral, proximal) or B (right, medial, distal) to the PRP treatment was performed by means of a sealed envelope. Photographs were taken to facilitate localization of the experimental and control area during follow-up. Randomization and further treatment of the patient were performed in absence of the clinical researcher to ensure blinding. The PRP was re-suspended and gently applied directly on the wound using the dual syringe-system as described above, before application of the meshed SSG, of any size, or Meek wall grafts,[21] (Figure 1). All transplanted wounds were covered identically with a non-adhesive wound dressing (Cuticell® or Adaptic ®), with which was left in situ for 5-7 days.

Outcome parameters

Primary early outcomes: graft take and epithelialization

Five to seven days after surgery, the dressings were removed. The wound areas were photographed and two experienced burn clinicians blinded to the treatment code assessed the graft take and epithelialization. Moreover, the post-operative photographs were assessed by two additional blinded researchers, experienced in burn wound care, for a second assessment of the graft take and epithelialization.[22] The means of all these assessments were used for further analyses.

The definition of graft take was the percentage of the graft that was vital and showed good adherence to the wound bed. Epithelialization was defined as the percentage of the wound closure by either skin graft or outgrowth from graft or wound edges.[23] The evaluation closest to day 7 post-surgery was used for further analysis.

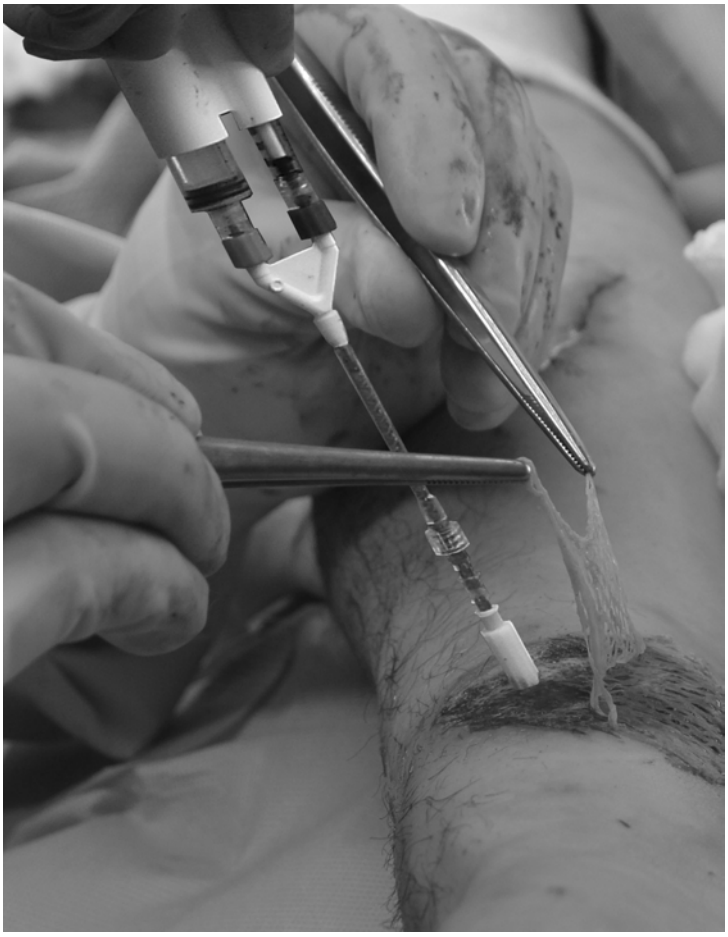

Figure 1. Example of application of PRP and thrombin with the dual syringe-system before application of a meshed SSG.

Secondary early outcomes

The presence of complications, such as graft loss, hematoma, and the necessity of re-grafting were registered. Also, during the admission period of the patients, daily background pain and itch scores were measured using the Visual Analogue Thermometer (VAT) score.[24] Mean scores of the first post-operative week were used for further analyses. Furthermore, wound cultures were taken by swabs at the first dressing change post-operatively and thereafter twice a week until complete wound closure according to the standard care protocol.

Long-term outcomes: scar quality

Scar quality was assessed by the use of several objective and subjective measurement instruments at 3, 6 and 12 months post-operatively in the outpatient clinic. First, the Patient and Observer Scar

Assessment Scale (POSAS) was used. The POSAS is a reliable and validated subjective scar assessment scale, that consists of two numeric scales: the Patient Scar Assessment Scale (patient scale) and the Observer Scar Assessment Scale (observer scale).[25] The six parameters of the observer scale are: vascularization, pigmentation, thickness, relief, pliability and general impression. The six parameters of the patient scale are: pain, itching, colour, stiffness, thickness and surface irregularity. Responses are scored from 1 to 10 for each parameter. For both scales, the mean outcome range was calculated. Secondly, scar elasticity was measured with the Cutometer (Courage&Khazaka GmbH, Cologne, Germany).[26] The vertical deformation of the skin is measured in millimeters when the skin is pulled by means of a controlled vacuum into a defined circular opening. The values were calculated as a ratio versus the values of normal skin. Lastly, to measure the scar color and pigmentation, the DermaSpectrometer® (Cortex Technology, Hadsund, Denmark) was used. This validated instrument measures scar redness (erythema) and pigmentation (melanin) by a narrow-band simple reflectance meter.[27] Data were analyzed as absolute difference between values of the test area and normal skin.

Statistics

Sample-size calculation

At the start of this study, no information was available on the treatment of PRP in burns. Calculation of the required sample size was therefore based on the best evidence available at that time, namely, the assumption that the mean healing rate in days of the SSG in the PRP treated wound areas would be 10% less than in the wound areas with only standard treatment.[28] The mean healing rate in the PRP group would be 18 days compared to 20 days in the standard treated group (SD=5). A two-sided test with an α level of 0.05 and a β level of 0.8 required 52 wound areas in every group. However, since the parameter 'days until 100 % epithelialization' was found to be unreliable in daily practice for several logistic reasons, such as premature discharge, or adherent dressings that obscured assessment of wound healing, we decided to operationalize the parameters take rate and epithelialization rate at day 5-7 post-surgery.

An interim analysis on safety was performed on 26 randomized patients by an independent researcher. It was concluded that is was safe to continue.

Statistical analyses (i.e. Chi-square test, Wilcoxon signed-rank test, paired T-test and Mann Whitney test) were performed using SPSS statistics version 21.0 (SPSS Inc., Chicago, IL). The standard deviation, 95% confidence interval, and p-values were given where appropriate. The significance criterion was set at 0.05.

Subgroup analysis was performed to determine whether certain patient groups or circumstances would benefit more from a treatment with PRP. The following subgroups were evaluated: timing of surgery (post-burn day), size of study area treated with PRP, % TBSA, thrombocyte count in the PRP, age in years and mesh size. Firstly, univariate association analyses were performed by ANOVA or linear regression analyses. Significant variables were entered in a multivariate analysis. The outcome parameter used, was the difference between take rate and epithelialization of the PRP treated area versus the standard care areas; a positive value means superiority of the PRP treatment.

RESULTS

A total of 52 patients were included (Figure 2) of which baseline and treatment characteristics are shown in Table 1. In table 2 data of hematological results are depicted.

Primary early outcomes.

Graft take and epithelialization

There was no statistically significant difference between the mean take rate nor between the mean epithelialization rate at day 5-7 between the PRP-treated and standard care areas (Wilcoxon test p= 0.23; p= 0.1 respectively) (Table 3).

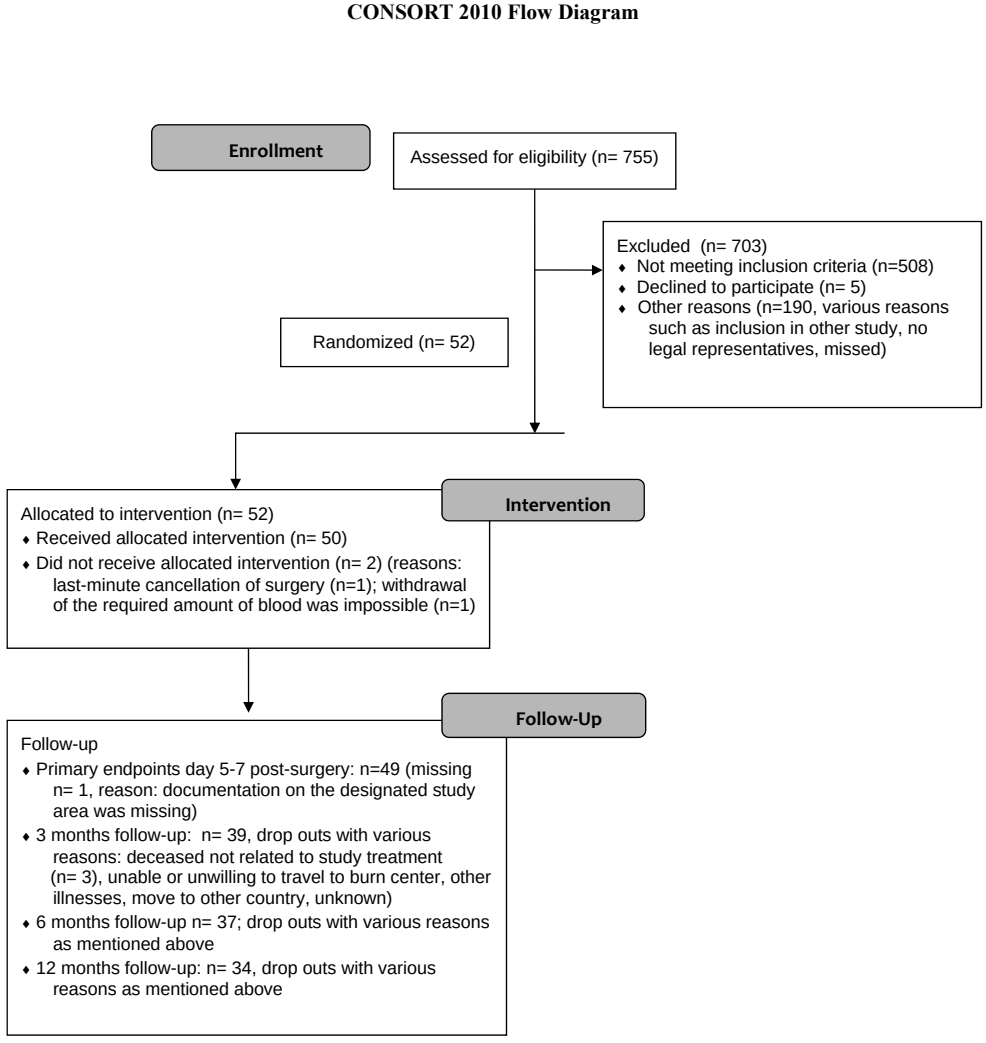

Figure 2. Study Flow Diagram

Table 1. Baseline and treatment characteristics of the included patients

Characteristics	Value
Gender	
Male	31 (60%)
Female	21 (40%)
Age (years)	
Mean (SD)	51.2 (18.6)
Minimum	20
Maximum	90
TBSA (%)	
Mean (SD)	15.6 (14.5)
Minimum	2
Maximum	76
Full-thickness (%)	
Mean (SD)	7.8 (10.8)
Minimum	2
Maximum	59
Surgery at post burn day	
Mean (SD)	11.4 (4.7)
Minimum	2
Maximum	22
Wound bed preparation (n, (%))	
Tangential excision	28 (53.8)
Hydrojet (Versajet)	19 (36.5)
Avulsion	3 (5.8)
Mesh of the SSG (n, (%)	
Mesh 1:1	5 (9.6)
Mesh 1:1.5	18 (34.6)
Mesh 1:2	11 (21.2)
Mesh 1:3	9 (17.3)
Mesh 1:4	1 (1.9)
Meek Wall 1:3	2 (3.8)
Meek Wall 1:4	1 (1.9)
Meek Wall 1:6	2 (3.8)
Meek Wall 1:9	1 (1.9)
Percentage Wound Surface Treated with PRP (n, (%))	
1 %	10 (19.2)
1 % -1.5 %	13 (25)
1.5 %- 2%	15 (28.8)
2 % -2.5 %	7 (13.5)
> 2.5 %	5 (9.6)

Subgroup analyses

When take rate and epithelialization were categorized as equal, better or worse, PRP-treated wound areas showed significantly more often "equal or better" take rates and epithelialization rates at day 5-7 compared to the standard care area's (Chi-square tests p=0.007 for take; p=0.02 for epithelialization).

Table 2. Hematology characterization of whole blood and PRP. These analyses were performed only in the last 44 patients.

N=44	Mean	SD	Minimum	Maximum
Trombocytes baseline Whole blood (x 10^9/l)	519.6	214.3	130	1252
Trombocytes PRP (x 10^9/l)	2139.3	1401.6	156	6500
Ratio Trombocytes PRP/ Baseline Whole Blood	3.9	1.8	1.2	7.5
White Blood Cell Count Whole Blood (x 10^9/l)	11.8	3.9	8.2	22.9
White Blood Cell Count PRP (x 10^9/l)	51.4	17.4	5.4	83.5
Ratio White Blood Cell Count PRP/ Whole Blood	4.5	1.4	1.3	7.3

Univariate analyses showed that for take rate only the early operated patients (surgery ≤ 7 days post burn, n=11), had significantly better take rates in the PRP-treated group than in the standard care group with a mean difference of 12.7% (t-test, p= 0.036, 95% CI 1.0-24.3). For the epithelialization rate we also found one significant predictor: surgery ≤ 7 days post burn, with a mean difference of 9.3 % (t-test, p= 0.033, 95% CI 0.8-17.8). The ≤ 7 days group did not show a difference versus the group that was operated > 7 days with respect to age, % TBSA burned, platelet count or gender.

Secondary early results

Pain and itch

No significant difference was observed in mean VAT-scores of pain and itching between wounds treated with or without PRP (Table 3).

Complications

No significant differences were found in bacterial colonization rates between PRP treated areas and the standard care areas. No severe complications, such as allergic reaction, sepsis or death occurred. However eight (16%) patients needed a re-operation. The need for re-operation was significantly higher in patients that were initially operated at an early stage (surgery ≤ 7 days post burn)(Mann Whitney Test, p= 0.011). This was not influenced by higher TBSA% or larger mesh size (Mann Whitney test, p= 0.28, resp 0.3). In all eight cases, both study areas required re-transplantation, nevertheless the PRP treated areas that required a re-operation were significantly smaller in comparison with their standard treated areas, respectively 45 % vs 58 % (p= 0.049, Wilcoxontest) (Table 4).

Long-term outcomes: scar quality

There were no statistically significant differences between PRP and standard treated areas in POSAS scores (patients/ observers), DermaSpectrometer scores and Cutometer scores at 3, 6 and 12 months (Tables 5-7).

Subgroup analyses with the parameters: timing of surgery (post-burn day); size of study area treated with PRP; %TBSA; thrombocyte count in the PRP; age in years and mesh size used, did not show any significant differences. However, in the early-operated group (≤ 7 days) the POSAS scores and the cutometer scores were more frequently in favor of PRP treated areas.

Table 3. Wound healing characteristics at 5-7 days post-surgery and pain and itch scores the first post-operative week

	PRP	Standard	p-value
Graft take (n=49) (mean % (SD)	80.9 % (25.5)	78.9 % (25.1)	0.25
Epithelialization (n=49) (mean % (SD)	69.4 % (29.3)	67.0 % (29.1)	0.14
VAT (n=27) (mean (SD)	2.0 (2.0)	2.2 (2.2)	0.06
Itch (n=26) (mean (SD)	1.4 (1.5)	1.3 (1.3)	0.39

SD: standard deviation.
Statistics: Wilcoxon- signed rank Test

Table 4. Reoperations: percentage of re-operated areas (as % of study area)

N=8	Mean	Std. Deviation	Std. Error Mean	p-value
PRP treated Area Re-operation Percentage	45.1	34.2	12.1	0.049*
Control Area Re-operation Percentage	57.5	35.8	12.6	

Statistics: Wilcoxon- signed rank Test

Table 5. Mean specific items of the POSAS score.

	Mean specific items of the POSAS score			
	PRP (SD)	Standard (SD)	Improvement (%)	p-value
Observer scale - 3 months (n= 36)				
Redness	4.46 (1.66)	4.42 (1.59)	-0.9	0.61
Pigmentation	3.81 (1.22)	3.81 (1.29)	0.0	0.92
Thickness	2.82 (1.26)	2.88 (1.30)	2.1	0.62
Pliability	3.47 (1.41)	3.50 (1.50)	0.9	0.77
Relief	3.64 (2.41)	3.08 (1.13)	-18.2	0.67
General	3.84 (1.18)	3.76 (1.26)	-2.1	0.94
Total score	3.62 (1.08)	3.58 (1.09)	-1.1	0.61
Observer scale - 6 months (n= 34)				
Redness	3.48 (1.09)	3.47 (1.36)	-0.3	0.78
Pigmentation	3.52 (1.17)	3.61 (1.20)	2.4	0.53
Thickness	2.40 (1.18)	2.51 (1.34)	4.5	0.65
Pliability	2.55 (1.27)	2.76 (1.49)	7.6	0.59
Relief	2.93 (1.46)	2.89 (1.46)	-1.5	0.56
General	3.13 (1.19)	3.21 (1.19)	2.3	0.95
Total score	3.00 (1.10)	3.08 (1.10)	2.3	0.97
Observer scale - 12 months (n=31)				
Redness	2.58 (0.90)	2.57 (1.07)	0.4	0.87
Pigmentation	3.29 (1.17)	3.15 (1.04)	-4.4	0.37
Thickness	1.92 (0.99)	2.16 (1.21)	10.9	0.21

Table 5. continued

	Mean specific items of the POSAS score			
	PRP (SD)	**Standard (SD)**	**Improvement (%)**	**p-value**
Pliability	2.44 (1.08)	2.54 (1.19)	3.8	0.82
Relief	2.41 (1.40)	2.27 (1.03)	-6.1	0.57
General	2.59 (1.04)	2.62 (0.91)	1.4	0.71
Total score	2.54 (0.91)	2.55 (0.91)	0.5	0.92
Patient scale - 3 months (n= 32)				
Pain	2.41 (2.03)	2.13 (1.83)	-13.2	0.47
Itch	4.00 (2.65)	4.32 (2.88)	7.5	0.28
Color	5.48 (1.98)	5.55 (1.94)	1.2	0.80
Pliability	4.29 (2.13)	3.87 (2.42)	-10.8	0.19
Thickness	3.71 (1.99)	3.58 (2.03)	-3.6	0.61
Relief	3.83 (2.09)	3.57 (2.05)	-7.5	0.37
General	4.70 (2.15)	4.27 (2.39)	-10.2	0.07
Total score	4.04 (1.37)	3.94 (1.74)	-2.7	0.77
Patient scale - 6 months (n=33)				
Pain	2.12 (1.95)	2.48 (2.25)	14.6	0.36
Itch	2.65 (2.36)	3.32 (2.09)	20.4	0.08
Color	5.06 (1.95)	4.90 (2.44)	-3.3	0.58
Pliability	3.47 (2.27)	3.69 (2.71)	5.9	0.43
Thickness	3.00 (1.86)	2.94 (2.02)	-2.2	0.87
Relief	3.59 (2.24)	3.63 (2.28)	0.9	0.96
General	4.83 (2.19)	4.69 (2.35)	-2.9	0.88
Total score	3.25 (1.53)	3.50 (2.76)	7.1	0.44
Patient scale - 12 months (n=29)				
Pain	2.14 (2.18)	2.28 (2.37)	6.1	0.90
Itch	1.54 (1.60)	1.79 (1.62)	14.0	0.26
Color	4.15 (2.30)	3.96 (2.44)	-4.7	0.91
Pliability	2.93 (2.12)	2.93 (1.96)	0.0	0.96
Thickness	2.81 (2.22)	3.04 (2.34)	7.3	0.47
Relief	3.33 (2.22)	3.70 (2.13)	10.0	0.23
General	3.81 (2.58)	3.69 (2.53)	-3.1	0.80
Total score	2.76 (1.44)	3.05 (1.50)	9.5	0.46

POSAS: Patient and Observer Scar Assessment Scale. Different items are scored on a scale 1-10. with 1 representing normal skin and 10 representing worst imaginable scar.

SD: standard deviation.

Improvement percentage: a positive value means supremacy of PRP on standard care.

Statistics: Wilcoxon- signed rank Test

Table 6. Scar color and pigmentation; DermaSpectrometer results.

	Scar color and pigmentation; DermaSpectrometer results			
	PRP	**Standard**	**p-value**	**CI**
Erythema				
3 months (n = 34)	7.82	7.14	0.25	-0.49 to 1.83
6 months (n = 34)	5.47	5.70	0.62	-1.11 to 0.67
12 months (n= 31)	3.94	3.78	0.68	-0.62 to 0.94
Melanin				
3 months (n= 34)	8.81	8.40	0.48	-0.76 to 1.59
6 months (n = 34)	6.42	7.00	0.35	-1.80 to 0.65
12 months (n= 31)	4.61	4.38	0.52	-0.50 to 0.97

CI: confidence interval.
Means are calculated as absolute difference between scar tissue and the non-affected skin)
Statistics: Paired samples T-test

Table 7. Scar elasticity; Cutometer results.

	Scar elasticity; Cutometer results			
	PRP	**Standard**	**p-value**	**CI**
3 months (n =33)				
Uf	0.54	0.56	0.40	-0.06 to 0.03
Ua	0.52	0.53	0.71	-0.06 to 0.04
Ue	0.54	0.57	0.23	-0.07 to 0.02
Ur	0.55	0.55	0.82	-0.06 to 0.05
Uv	0.57	0.58	0.60	-0.06 to 0.04
6 months (n = 32)				
Uf	0.83 (0.80)	0.81 (0.75)	0.66	-.05 to 0.08
Ua	0.70	0.66	0.31	-0.04 to 0.11
Ue	0.85	0.84	0.79	-0.07 to 0.09
Ur	0.81	0.74	0.15	-0.03 to 0.16
Uv	0.80	0.74	0.39	-0.07 to 0.19
12 months (n= 29)				
Uf	0.80	0.80	0.81	-0.07 to 0.06
Ua	0.79	0.80	0.94	-0.08 to 0.08
Ue	0.82	0.85	0.37	-0.09 to 0.04
Ur	0.76	0.77	0.72	-0.11 to 0.07
Uv	0.80	0.77	0.34	-0.04 to 0.10

CI: confidence interval.
Uf: Extension. Ua: Pliability. Ue: Elasticity. Ur: Retraction.
Uv: Visco-elasticity
Values represent the mean ratio between scar tissue and non-affected skin.
Statistics: Paired samples T-test

DISCUSSION

This randomized, double blind, intra-patient controlled study showed that the addition of PRP to a SSG in the surgical treatment of deep dermal to full thickness burn wounds did not result in improved take and epithelialization rate compared to control wounds that were treated with a SSG only. Minor beneficial effects of PRP were seen in a categorized and non pre-defined analysis which showed more frequently an equal or higher take rate and epithelialization rate with the addition of PRP. Furthermore, a non-powered sub group-analysis showed that the effect of the PRP on take rate and epithelialization, was especially higher in the group that received early surgery (≤ 7 days after the burn injury). Finally, the addition of PRP seemed to decrease the size of the area that required surgery in a small patient group that needed an extra operation in the same areas. There were no significant differences in pain and itch scores, as well as in colonization rates. Long-term follow-up results did not show significant differences in scar quality.

We did not encounter any severe complications or side effects of the PRP as is in concordance with the literature.[5] The encountered complications, such as the need for a re-operation, shift of the SSG or a hematoma, were equally distributed in both study areas. Interestingly, the addition of PRP even seemed to have a beneficial effect on re-operated areas as stated above.

Platelets and PRP have been attributed pain relieving qualities.[2,3] A trend was found towards lower pain scores for the PRP-treated areas. Unfortunately, not all patients were able to provide VAT scores, because of reasons such as intubation or severe burn sickness.

No differences in bacterial colonization rate between PRP-treated areas and the control areas were found, nor did we encounter any severe wound infections. It is therefore not possible to draw conclusions on the possible protective function of leukocytes in the PRP, which has been described in several papers.[4] However, others claim that leukocytes in PRP have undesirable effects, such as an increased inflammatory reaction[29], which theoretically could affect the final scar quality. Certain growth factors, released from the platelets and leukocytes in buffy coat PRP, as used in the current study, are chemotactic in recruiting inflammatory cells and a prolonged inflammation could result in hypertrophic scarring.[30] Several growth factors, especially TGF-β1, TGF-β-2 and PDGF, are involved in hypertrophic and keloid scarring in both normal skin wounds and burn wounds.[31] So far there have been no reports of hypertrophic scarring after the use of PRP in wound healing.[32,33] Only one study in burn patients described the long-term effects, unfortunately they did not mention the quality of scarring, except improved elastic properties in areas treated with SSG with the addition of autologous platelet concentrate.[12] In the present study we did not find any differences in scar quality between the PRP and standard treated burns. Also importantly, scars were not worse in the PRP treated areas compared to standard treated burns. A beneficial trend was shown towards PRP treatment in subgroup analyses of patients in the early-operated (≤ 7 days) group. However, this group was rather small, which possibly explains the lack of significant results on connected parameters such as scarring.

Previous literature stated that it is important to measure platelet concentration in the PRP[6] and some literature states that PRP platelet concentration needs to exceed 1000 ($x10^3/\mu l$).[33,34] In the present study, platelet counts exceeded 1000($x10^3/\mu l$) in 32/42 (73 %) of the PRP samples.

Nevertheless, platelet concentration in the PRP did not affect the outcomes in the sub analyses of this study. Thus, next to quantity, the quality and content of the platelets seem important. PRP is mostly used and studied in healthy subjects, whereas burn patients have an altered systemic physiological status. [35] An oft–stated advice is to withdraw blood prior to the surgery in order to avoid platelet activation. Obviously, this is not possible in burn patients, where platelets are already massively activated.[36] We know that platelets in burn patients follow a distinct path in time, with a nadir at post burn day (PBD) 3 followed by a reactive peak at PBD 15, with a gradual return to normal values around PBD 24 (Figure 3).[37] This time course is affected by several factors, such as age, % TBSA and presence of sepsis.[37]

It is yet unknown how burn injury may affect the content and quality of the platelets. Burn patients remain in a hypercoagulable state with activated platelets at least one week post burn injury.[38] This might influence the quality of PRP and hence the timing of its application in burn patients. Our study showed significantly lower take rates and epithelialization in both study areas for patients in the early operated group (≤ 7 days) compared to patients operated > 7 days after injury, and especially in this apparently vulnerable group PRP was shown to have a significant positive effect on graft take rate and epithelialization. Further research into the quality and composition of platelets post-burn injury is warranted.

Furthermore, an alternative might be the use of allogeneic PRP, since this could be a standardized product, widely available and not affected by a patient's systemic physiological status after the burn injury.[33] Additionally, there would be no need for blood withdrawal from afflicted patients. However, this is not an autologous product, so biocompatibility issues remain, as well as the risk

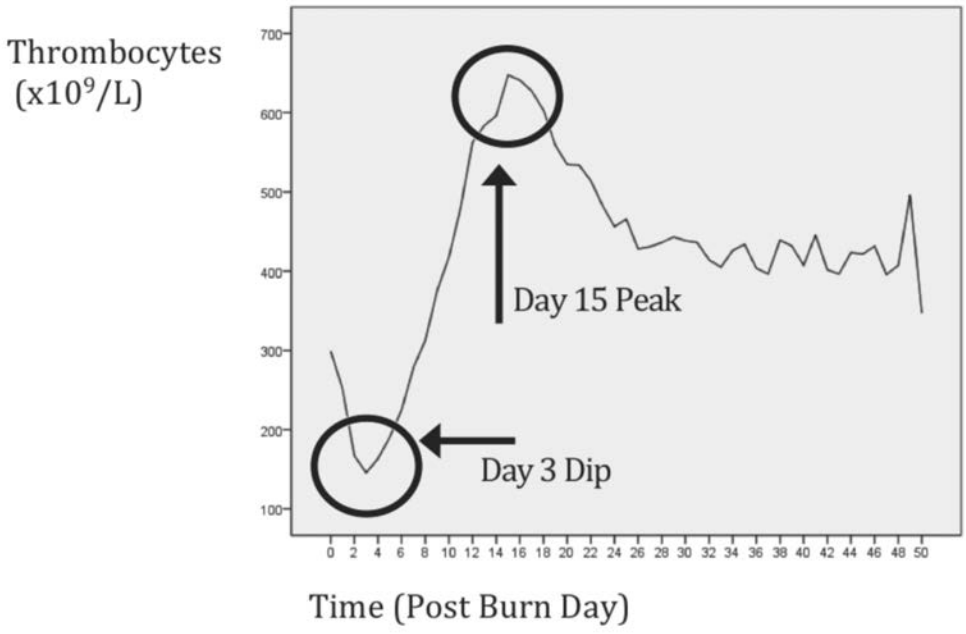

Figure 3. Mean platelet count in whole blood post-burn injury. Adapted from Marck et al.[37]

of transmission of unknown infections and allergic reactions, as can occur after systemic platelet transfusions. There are some case series on the use of allogeneic PRP in wound care.[39,40] None of those reports encountered side effects, so this remains scarcely described and further research on this is needed.

The study suffered from a considerable drop-out rate in the follow-up assessments. This could mostly be explained by an increased patient-hospital distance, since our facility is one of the national burn centers. This could underestimate rare adverse effects, however patients with severe scar problems would have been likely to return to our facility to seek consultation. Furthermore it could have affected the long-term results, though since this is an intra-patient controlled study it affects both studied groups simultaneously.

The studied patient group in the current study is a proper clinical sample reflecting the whole spectrum of burn patients treated in a national burn center, however this also causes the patient group to be rather heterogenic in several ways. There were differences in size of studied areas; level of expansion of meshed skin grafts; timing of surgery and baseline platelet counts in whole blood, as well as in the final PRP. In the data analysis, we noted that there was a high variability in the primary outcome, which could be related to the heterogeneity as described above. The reduction of the data to a dichotomous variable was intended to reduce this variability and highlight any effects PRP treatment might have in these wounds.

The lack of beneficial effects of the addition of PRP to the treatment of burns raises several more questions. Firstly, the PRP of burn patients could be of poor quality due to, as mentioned above, affected and/or already activated platelets. Secondly, the platelet concentration rate in the PRP could be inadequate, because of low systemic platelet counts or large burn areas where the PRP was applied. However, so far it is unknown what an optimal concentration ratio should be, especially in this patient group. Another suggestion could be that PRP needs to be applied more than once, as proposed by Picard et al.[6]

In the current literature there is much debate on the evidence of effects of the addition of PRP on wound healing treatment. These conflicting results could be due to the variability of PRP products and preparation procedures, or simply, because there is a lack of effect of PRP.

CONCLUSION

From this prospective, randomized controlled, intra-patient study it can be concluded that PRP does not seem to improve wound healing or scar formation in acute burns considerably. This should be explored in further research in a more narrowly defined burn patient cohort with a more standardized PRP product. Based on our current data the standard application of PRP to all surgically treated burn patients is not indicated.

ACKNOWLEDGEMENTS

We would like to thank the staff of our burn center: dr Jos Vloemans, dr. Fenike Tempelman and all the nursing staff for elaborating with this study. Henk Huijgen, Sandra Wilpe, Dorien Wenting and Patty Huijpen from the Clincal Laboratory for their assistance in the hematology analyses.

SOURCE OF FUNDING

Biomet (Biomet Europe BV, Dordrecht, the Netherlands) provided the necessary equipment for the duration of the study, PRP-preparation kits and an unrestricted educational grant which covered some of the remaining costs of this study. Any remaining costs were covered by a research grant of the Association of Dutch Burn Centers.

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7

Summary and general discussion

This thesis explores the effect of platelet-rich plasma (PRP) on the healing of acute burns and the burn scar quality, and conversely the effects of burn injury on platelets. This final chapter summarizes the findings of this thesis, puts them in context with up-to-date literature, and reflects on clinical implications and future perspectives.

The main question of this thesis was: what is the attributive value of PRP for burn wound healing and consequently the quality of burn scars?

We tried to answer this question by studying the following sub-questions:

1. What is the current evidence from the literature on PRP and specifically on PRP in burns?
2. What is the effect of burns on the quantity, function and quality of platelets in burn patients?
3. What is the effect of burn injury on the quality of autologous PRP?
4. What is the clinical effect of PRP on burn wound healing and outcome of burn wounds?

CURRENT EVIDENCE FROM THE LITERATURE ON PRP

Background to PRP

Platelet-rich plasma (PRP) is a derivative of whole blood that contains a supra-physiological concentration of platelets. Platelets store growth factors in their alpha granules, which are released after activation.[1] These growth factors are involved in the phases of wound healing (inflammation, proliferation and remodelling) in many ways, such as by promoting chemotaxis, cell adhesion, mitogenesis, proliferation and angiogenesis. The most studied of these factors are platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), transforming growth factor -(TGF-), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF) and insulin-like growth factor (IGF).[2] The delivery of an abundance of these growth factors has shown positive effects on wound healing in in vitro analyses.[3] There are also many studies advocating positive effects of PRP on wounds in many different forms. However, our review in chapter 2 highlights some of the difficulties in this field: variables exist in the preparation and application of PRP, making comparison and interpretation of the evidence far from easy.

Firstly, there are many different types of platelet-rich products with a broad nomenclature. There are not only different names for one similar product, but the products are actually often very different, with different preparation procedures and consequently different content, and they vary in fibrin composition and the presence of leukocytes and/or erythrocytes.

Especially the presence of leukocytes is the subject of on-going debate. Some authors advocate the use PRP without leukocytes because of their potential negative pro-inflammatory effects, and others claim antimicrobial effects of the leukocytes in PRP. A recent review of the subject summarized existing evidence and concluded that despite a number of studies showing that preparations that include leukocytes have antimicrobial properties, there is not enough evidence to attribute all these bactericidal effects to the presence of leukocytes alone. PRP preparations, with or without leukocytes, showed bacteriostatic characteristics against the majority of the bacterial strains tested.[4] On the other hand, proper evidence of harmful effects of leukocytes on wound healing is lacking as well.

Apart from different names and content, there are also different activation and application methods. Finally, there are an inter-patient variable platelet baseline count and platelet yield

rate after PRP production, which also result in variable amounts of growth factors. In brief, a vast heterogeneity exists in PRP products, and the only unequivocal factor is that PRP has an increased number of platelets, which could effect the wound healing process due to its growth factors and cytokines being released after activation.

In January 2016, the Platelet Physiology Subcommittee of the International Society on Thrombosis and Haemostasis (SSC/ISTH) formed a working party of 10 experts (including the author of this thesis), whose task it was to produce a series of consensus recommendations for standardising the use of platelets in regenerative medicine. The working party used a formal consensus method (the RAND method) to develop final recommendations and produced an invited guideline entitled 'Guidance on the use of platelet-derived biomaterials in regenerative medicine and proposal for a new classification system: a consensus of the working party from the platelet physiology subcommittee of SSC/ISTH.' [5]

This guideline proposes a new classification system based on the following: content (presence of leukocytes and erythrocytes, and fibrin composition); activation (use of activation, without activator, frozen-thawed preparations); platelet concentration (subdivided into three categories based on the platelet count (< 900 x 10^3 /µl, 900 – 1 700 x 10^3 /µl and > 1700 x 10^3/µl); and finally the preparation methods (gravitational centrifugation techniques, standard cell separators, autologous selective filtration technology (plateletpheresis) (Table 1). Development of these standardization and evidence-based guidelines and following the recommendations by researchers in the field are essential to the progress of PRP research.

Evidence from the literature on PRP in different applications

Chapter 2 summarizes the literature on the application of PRP in a number of different fields of medicine, such as maxillofacial surgery, orthopaedics and sports medicine, chronic wounds and aesthetic surgery. The overall conclusions were that there are positive as well as conflicting reports based on low-quality studies with many uncertainties on the quality and application of PRP, making general and definite conclusions virtually impossible. As Borzini stated in 2007: 'From a scientific viewpoint, a vast majority of positive papers are not proofs for platelet gel to be considered fully effective, science is not democracy.'[6]

Some systematic reviews and new trials have recently been published. A recent Cochrane review on the application of PRP in the wound care of chronic wounds included 10 randomised clinical trials, with a total of 442 participants (mean age 61 years and 42% women). Autologous PRP increased the healing of foot ulcers in people with diabetes compared with standard care, but it was unclear if autologous PRP had an effect on other types of chronic wounds. These findings are based on low-quality evidence due to the small number of studies and patients included, and their poor methodological quality.[7]

In aesthetic medicine PRP applications have greatly taken flight, especially infiltrating PRP in the face, the so-called 'vampire facelift'. However, again robust evidence of effects is scant. A recent randomized, double-blind, placebo-controlled study on the addition of PRP to lipofilling in the face showed that it significantly reduced postoperative recovery time but did not improve patient

Table 1. is the proposed new platelet-rich plasma (PRP) classification system table from recent publication Harrison et al. [5]

Class	Leukocytes + = > 1%	Red Cells + = > 10%	Fibrinogen/ Fibrin	Activation	Platelet concentration	Preparation category
PRP		-	Low	I	A	1
				II	B	2
Red-PRP		+		III	C	3
L-PRP	+	-	Low	I	A	1
				II	B	2
Red-L-PRP		+		III	C	3
PRF	-	-	High	I	A	1
				II	B	2
Red-PRF		+		III	C	3
L-PRF	+	-	High	I	A	1
				II	B	2
Red-L-PRF		+		III	C	3

Activation is divided into three subcategories:

I for the use of activation

II for PRP application without activator

III for use of frozen-thawed preparations

Platelet concentrates are subdivided into three categories (A, B and C) based on the platelet count range in the samples. The three categories are:

A Platelet count < 900 x10^3 /µl

B Platelet count 900 – 1700 x10^3 /µl

C Platelet count > 1700 x10^3 /µl

The preparation methods are classified into three categories:

1 The Gravitational centrifugation techniques

2 Standard cell separators

3 Autologous selective filtration technology (plateletpheresis).

Abbreviations:

L-PRF, leukocyte-rich platelet-rich fibrin; L-PRP, leukocyte-rich PRP; PRF, platelet-rich fibrin; Red-L-PRF, red blood cell-rich and leukocyte rich platelet-rich fibrin; Red-L-PRP, red blood cell-rich and leukocyte-rich PRP; Red-PRF, red blood cell-rich platelet-rich fibrin; Red-PRP, red blood cell-rich PRP.

+ or _ defines whether (+) or not (-) there are leukocytes equal or greater than (=>) 1% and/or Red cells equal or greater than (=>) 10% of the total cell counts (including leukocytes, platelets and Red cells) in the PRP.

outcome in terms of skin elasticity, improvement of the nasolabial fold, or patient satisfaction.[8] The authors did not report any information on the quality of the PRP itself.

A summary of all recent relevant evidence in sports and orthopaedics falls beyond the scope of this thesis; however, the general conclusion can be drawn that there are positive and inconclusive reports, with a general lack of strength of evidence.[9] For example, Grassi et al. performed a thorough meta-analysis on the application of PRP in acute muscle injuries. They concluded that although PRP treatment was a safe procedure as the studies reported only negligible adverse effects, in terms of efficacy the current literature did not support its use for muscle injuries.[10] However, the lack of robust

evidence has not stopped the great increase in the use of PRP for musculoskeletal indications, which is partially explained by the promising in vitro results, low regulatory issues and popularity fuelled by celebrity testimonials from athletes such as Tiger Woods and Rafael Nadal.[11] As a consequence many clinical trials have been started, unfortunately often without full understanding of all aspects of PRP, possibly leading to disappointing results. As Murray outlines so well: 'PRP may ultimately be considered a hype, however there is also a danger that a potentially beneficial treatment is dismissed as non-effective simply because suboptimised preparations were used in these studies. We will only truly know if PRP can be of therapeutic benefit if the scientific/clinical community accepts that shortcuts cannot be taken, and adopts a comprehensive 'back to basics' approach.'[11]

Evidence for the application of PRP in burns

The literature on the use of PRP in burns was systematically searched in chapter 2. We concluded that the evidence was very limited, ranging from some animal studies to case reports to small patient cohorts. Long-term outcome in this field is even more limited: only one study showed accelerated recovery of the viscoelastic properties of full-thickness burns treated with autologous platelet concentrate with a SSG, compared with areas treated with a SSG alone, but no improvement in the long-term outcome was shown.[12]

Theoretically, a deep dermal burn could benefit from PRP in several ways. First, the haemostatic qualities of PRP could increase the take rate of the skin grafts by decreasing continued bleeding, working as a fibrin glue, as well as providing a well-vascularised bed for the meshed skin graft. Next, PRP could exert a stimulating effect on wound healing by a growth factor-mediated contribution to faster wound closure of mesh interstices. However, a burn injury has a different physiology than chronic or acute injuries, and moreover burn patients are in an altered systemic physiological state, where platelets already seem to be activated.[13] This could influence the quality of the platelets in PRP compared to the healthy subjects in whom PRP was mostly used. It was established in chapter 2 that no information could be retrieved on exactly how burn injury affects platelets and how this could influence the effects of PRP used in burn patients.

GENERAL CONCLUSION AND FUTURE PERSPECTIVES ON CLINICAL USE OF PRP IN GENERAL

To return to the aims set out at the beginning of this thesis, we investigated what PRP is and explored its mechanisms of action. Furthermore we reflected on the current evidence for the different indications in which PRP is used. We established that a huge variation in PRP products and effectiveness exists. We advocate (in accordance with the working party of the platelet physiology subcommittee of the SSC/ISTH in which the author of this thesis is a participant) that future research should focus on assessing the functionality of PRP in a standardised way and that in future trials the quality and content of the PRP should be taken into account and this should be correlated with clinical outcome.

Next we evaluated the existing evidence on the use of PRP in burn injury treatment and established that the evidence is limited; however, we can assume that in theory a burn injury could benefit from treatment with PRP. We realised that the systemic effects of the burn injury could affect

platelets in burn patients. This brings us to the important sub-question of this thesis: what effect does burn injury have on platelets?

THE EFFECT OF BURNS ON THE QUANTITY, FUNCTION AND QUALITY OF PLATELETS IN BURN PATIENTS

Platelet quantity post-burn injury

In chapter 3 we performed retrospective quantitative analyses in 244 burn patients and found that platelet counts show a distinct time course post-burn injury with a nadir at day 3 and a peak at day 14, followed by a general return to normal. This seemingly simple observation has, however, never previously been confirmed in a large patient cohort. Furthermore, it was found that this time course is influenced by the percentage of total body surface area (%TBSA), age and sepsis and not by gender.

However, beyond this plain platelet count, it was found that a high %TBSA, higher age and low thrombocyte peak predicted mortality, and there were indications that a low nadir could also be associated with mortality. The incidence of sepsis was too small in our study group to perform a reliable Cox regression analysis; therefore we could not assess the predictive value of thrombocytopenia for sepsis. This would be very interesting, since platelets are, besides being involved in haemostasis, also important modulators of the host response during infection, serving as sentinels in our circulatory system.[14,15] Whether thrombocytopenia represents platelet activation and consumption as a primary pathologic event or simply serves as an indicator of disease severity is unknown.[14]

Since then, several recent studies have elaborated on our findings. In a larger subgroup of 145 burn patients with a TBSA greater than 20%, Cato et al. confirmed our findings on the pattern of platelet counts post-burn injury.[16] Moreover, the platelet count at the nadir (day 3) combined with %TBSA had a modest association with sepsis.[16] Low peak platelet count showed a strong predictive power for mortality in a multivariable model with TBSA, age and the revised Baux score.[16] Dinsdale et al. continued with this in a recent scientific report in *Nature*, where they analysed 39 burned patients (TBSA 15–39%) with the Sysmex XN-1000 measuring three methods of platelet counting in parallel (namely impedance, optical and the more accurate fluorescent count). They wanted to establish if thermal injury caused direct damage to circulating red blood cells that can result in red cell fragmentation, with the appearance of large numbers of microspherocytes causing overestimation of platelet counts by commonly used impedance analysers.[17,18] In the Burn Centre of Beverwijk, the laboratory staff regularly verifies haematology results microscopically and did not see cases of spurious platelet counts. Dinsdale et al. confirmed this and did not see a difference between impedance, optical and fluorescent platelet counts.[17] They corroborated the finding that platelet counts had a nadir at day 3, followed by a rebound thrombocytosis at day 21. Furthermore, they showed nadir values to be significantly lower in septic patients.[17] Qiu et al. found in another recent retrospective analysis of a series of 610 burn patients that the red blood cell distribution (RDW) to platelet count (PLT) ratio (RDW-to-PLT ratio, which they call RPR) values on the 3rd and 7th days were significantly associated with the mortality rates of severe burn patients ($P < 0.01$). They

claim that the RDW-to-PLT ratio could serve as an independent and novel marker for mortality rate prediction in severe burn patients.[19]

Function and quality of platelets post-burn injury

To evaluate the effects of burn injury on platelets, the activation, function and content of platelets was studied in chapter 4. In six burn patients with more than 15% TBSA burned, blood samples were collected at five designated time points post-burn: day 0–1, day 3–4, day 8–9, day 12–16, and day 20–24, corresponding respectively to the burn day, the nadir, the rise, the peak and the return to normal of the platelet count. Several platelet function, activation and growth factor quantification analyses were performed. It was found that platelets post-burn injury appeared to be functional and not overly activated. However, the burn patients seemed to remain in a procoagulant state for an extensive period of time post-burn.

This chapter engaged with another interesting and complex field on its own: the coagulation process, in which platelets play an important role. Coagulopathy is not strictly defined and a high heterogeneity exists in the literature on coagulopathy in burns. A wide range of diagnostic criteria for, and definitions of, coagulopathy have been used, which makes it difficult to draw overall conclusions. However, with knowledge evolving and the emergence of new tests, there is nowadays more insight into the coagulation process in burn patients. Conventional coagulation assays, such as prothrombin time (PT) and activated partial thromboplastin time (aPTT) are often elevated, indicating a tendency to bleed; however, these assessments only reflect a part of the coagulation process. More advanced viscoelastic coagulation assays (such as the TEG used in chapter 4) mirror the clotting process in vivo, where all cellular blood components involved (besides the endothelial factor) contribute to the clotting process.[20] In trauma patients fibrinogen deficit and hyperfibrinolysis are the leading pathomechanisms of bleeding; however, this is not the case in burn victims, as shown in Chapter 4, where fibrinogen was found to be increased. This is in accordance with other studies, which was confirmed for the first time for a period longer than one week post-burn injury.[20] Potentially, burn patients rebalance their fibrinolytic system during the post-burn time course, since it was shown in another study that after burn and inhalation injury, patients showed an antifibrinolytic shift seven days post-burn.[21,22] Since we have found procoagulant changes up to 24 days post-burn, it will be interesting to determine the fibrinolytic status for this extended period post-burn, which will allow us to obtain a more comprehensive understanding of the full coagulation process post-burn injury and highlight potential clinical implications.

Furthermore, we established in this chapter that growth factor content followed the same course as the platelet count, reflecting a constant growth factor per platelet ratio.

This implies that for the sake of the production of PRP and the wish for a multitude of growth factors, it would be advisable to produce autologous PRP beyond the nadir period to increase the yield of platelets and hence growth factors.

Conclusions and future perspectives on platelets post-burn injury

In view of our initial aim to gain more information on the effect of burn injury on platelet quality, quantity and function set out at the beginning of this thesis, we can conclude that the time course

of platelet count post-burn injury brought to light by our research in chapter 3 now seems well established and has been confirmed by numerous other authors. As an interesting aside, it was found that platelet counts offer predictive value of mortality and sepsis. The question is how to incorporate this in daily care. Although no rigid cut-off values are offered just yet, since platelet counts are routinely available they can be interpreted. A very low thrombocytopenia around day 3, or a persistent thrombocytic period, or a decreased thrombocytic period could raise awareness among burn clinicians that the patient is at risk for sepsis or mortality. Future research in larger cohorts could throw more light on the relationship between platelet count and sepsis. Since sepsis is always a risk for burn patients, it would be very valuable to have a proper prognostic marker for this – platelets have been shown to play an essential role in this process.[14]

Regarding the qualitative effects of burn injury on platelets, we found that platelets post-burn injury appear to be functional, not overly activated and not deprived of growth factors. However, burn patients seem to remain in a procoagulant state for an extensive period after the burn injury has been inflicted. Further research is necessary in a larger patient cohort to clarify the whole coagulation process in burn patients, including the potential fibrinolytic process. This will be a complicated undertaking, since coagulation is such a multifaceted process, especially in a multivariable group such as burn patients with different burn sizes who are undergoing resuscitation protocols, repetitive surgery, and blood and plasma transfusions and who are at high risk of infection and sepsis. However, it will be very valuable and will add essential information to our understanding of coagulation post-burn and how to treat patients optimally, balancing pro- and anti-thrombotic management objectives.

THE EFFECT OF BURN INJURY ON THE QUALITY OF AUTOLOGOUS PRP

The quality of PRP of burn patients

Returning to the main goal of this thesis with our sub-question on the systemic effects of burn injury on platelets in mind, we asked whether the quality of autologous PRP obtained in burn patients was satisfactory. We therefore performed growth factor quantification of the autologous PRP of burn patients and compared this to the autologous PRP of healthy volunteers. For this study we used a commercial 'point of care' system (GPS III, Biomet), which is often used both in daily practice as well as in several clinical studies. Chapter 5 describes the results of growth factor quantification analyses of the leukocyte-rich PRP of five burn patients, compared to the same type of PRP from five gender and age-matched healthy volunteers.

It was discovered that the PRP of burn patients has comparable levels of growth factors compared to the PRP of healthy volunteers. However, a considerable intra-individual variation in growth factor content was noted, which is in agreement with findings in the literature. A clear correlation was found between platelet count in PRP and most of the growth factors measured. This implies that measuring the platelet count in the PRP is an adequate and far more feasible quality check of PRP in daily practice as well as in a study setup. This is also advised by the working party of the platelet physiology subcommittee of the ISTH mentioned above.[5]

Conclusions and future perspectives on PRP quality

An important point to be made is that the ideal platelet concentration (and hence the optimum quantity of growth factors) is not known for any indication. Most authors state that at least 0.8–1 x 10^6/µl platelets should be present in PRP; however, there is no proper evidence for this, and this is probably the most persistent and worst statement referred to in the PRP literature. We may refer to this statement as 'parrot proof'', meaning that authors are merely mimicking each other as parrots do. If this statement is tracked to its source it leads back to an abstract from a poster presentation from 2002, and cannot be considered reliable evidence for compulsatory platelet concentration.[23] However, this number of at least 0.8–1 x 10^6/µl platelets is now broadly used. It might also be incorrect to strive for a maximum of platelets in PRP, since a few in vitro analyses have indicated that too many platelets could have negative effects.[24-26] Further in vitro analyses could provide us with more information on required concentrations; however, in vitro systems will always be a limited reflection of the complex processes in vivo. In vivo data is scarce. Recently Louis et al. performed a randomized, double-blind, non-inferiority trial, in which they compared PRP injections with hyaluronic acid (HA) in knee osteoarthritis, and correlated clinical outcome with growth factor analyses.[27] They found comparable effects of HA and PRP at 3 months, but interestingly, they found a significant correlation between the doses of TGF-b1 and PDGF-AB and the worsening of pain scores at 3 months, indicating a negative effect of too high doses of growth factors. Ideally, future studies should routinely state their platelet counts or perform growth factor quantification of the applied PRP, which could give greater clarification on which concentrations may be suitable for different indications.

THE EFFECT OF PRP ON BURN WOUND HEALING AND SCAR QUALITY

Clinical trial data

Finally, to answer the main question of this thesis in chapter 6, a randomized, double-blind, intra-patient controlled study on the application of platelet-rich plasma in the treatment of deep dermal burns was described. This trial investigated the effect of autologous (leukocyte-rich) PRP on primary wound healing by evaluating the take rates and re-epithelialization rates of acute burns treated with split skin grafts (SSG) in combination with PRP compared to SSGs only. Lastly, we evaluated the effect of PRP on scar quality until 12 months after intervention.

We did not find a statistically significant difference between the mean take rate nor the mean epithelialization rate at day 5–7 between the PRP-treated and standard treated areas. However, since our patient group was a representative clinical sample of burn patients treated in a burn centre, there was a wide range between primary outcomes, and we therefore dichotomised the outcomes. We then found that PRP-treated wound areas more often showed better or equal epithelialisation and take rates at day 5–7 than the standard treated areas. Minor positive effects of PRP were also seen in the re-operated and in the early operated (≤7 days) subgroups.

This last finding seems somewhat contradictory, since we discovered earlier that in this period in particular platelet counts are low and thus growth factor amounts are low as well. However, in

sub-analyses we did not find a correlation between the platelet count in PRP and outcome in take rate or epithelialisation rate, not even if we corrected for area treated with PRP, in other words concentration. An explanation for this finding could be that in this group (≤7 days) in general the take and epithelialization rates were lower compared to the patients who were operated on later, so especially in this seemingly vulnerable group PRP was shown to have a positive effect on graft take rate and epithelialization rate.

Since this RCT was done, no other studies have been published on PRP and burn wounds. Hersant et al. investigated whether SSG with autologous PRP could accelerate and improve wound healing after cutaneous reconstruction for tissue loss secondary to soft tissue infections (no burns). Twenty-seven patients were randomized into two groups. In the PRP group, patients had a significant improved take rate of the SSG of 90% vs. 77% in the control group. Time until complete healing was also significantly shorter in the PRP group compared to the control group: 37.9 days vs. 73.7 days. However, this study was small with no blinding or objective outcome parameters, and no quality control of the PRP was done.[28]

An important feature of our RCT was the long follow-up, providing evidence of the scar quality of burn wounds treated with PRP. At 3, 6, and 12 months postoperatively, the POSAS scores of the patients from the observers' measurements with the Dermaspectrometer and Cutometer did not show a significant difference between wounds treated with PRP and those treated with the standard method. This also means that the scars of PRP-treated wounds were no worse than those of standard treated burns. This is also an important finding, since some growth factors present in PRP (TGF-ß1, TGF-ß2, PDGF) are involved in hypertrophic and keloid scarring of both normal skin wounds and burn wounds. This finding is in accordance with the literature, where there are no reports of increased scarring in PRP-treated wounds; however, long follow-ups are scarce.

Conclusions on the effect of PRP on burn wound healing and scar quality

Does this RCT provide adequate answers to our primary question as to whether PRP aids burn wound healing, leading to less disfiguring scars? The answer is ambiguous. Although the RCT was performed thoroughly, in hindsight there were quite a few variables added to an already variable PRP study setup as mentioned in chapter 2. There were different sizes of burns treated with PRP, with different mesh sizes, at different times post-burn injury. Although we studied the effect of these variables in sub-analyses, it is hard to draw firm conclusions from them, since these sub-analyses are obviously not adequately powered. Although the study reflects a proper cross-section of a clinical burn patient population, it is possible that a positive effect in a sub-group was concealed by the considerable variation caused by all the variables, as could be indicated by the minor positive effects we found.

In retrospect it would have been better to construct a more standardised setup, in which correlation of platelet count in PRP with a standardised area would already rule out several variables and provide more information on optimal platelet count. Nonetheless, if PRP had made an actual substantial difference to wound healing and consequently scar quality, we would probably have been able to measure this. So in the setting of acute burns, the addition of autologous PRP to a burn wound with a SSG does not seem to be justified. On the other hand, PRP appears to have some

effect, although it seems to be minor. Therefore the idea of adding platelets to burn wounds should not be altogether discarded.

FUTURE PERSPECTIVES ON THE EFFECT OF PRP ON BURN WOUND HEALING AND SCAR QUALITY

To summarise, we know that platelets post-burn injury are functional, not overly activated, and not deprived of growth factors in whole blood nor in a PRP product; and we learned that the application of autologous PRP in acute burn wounds does not significantly improve the take rate and epithelialization rate of SSG or burn scars, although it does seem to have a slight beneficial effect on the wound healing of acute burns.

How to proceed from the main findings of this thesis?

In order to reach progress in PRP research more standardization is essential. Nevertheless, patient variables, such as baseline platelet count and growth factor content, are difficult to control. Therefore it seems worthwhile to explore a shift towards allogeneic PRP. This has several theoretical advantages. Preparing allogeneic PRP would eliminate the burden of drawing blood from a patient, sometimes more than 60 ml for a single application of 6 ml PRP. In blood banks, platelets are stored in abundance, since platelets are a side-product of blood donations. Donor blood is mostly used for its erythrocytes, given to patients in packed cell transfusions. Blood donors are thoroughly tested and blood transfusions are considered to be relatively safe, although allergic reactions and transfusion-transmitted infections cannot be completely ruled out. Not much is known about the safety of topical application of blood products; however, it can be expected that the risk for potential reactions would be lower than it would be after systemic administration. A recent study analysed the local immunological effects of allogeneic PRP in in vitro models.[29] The study found that allogeneic PRP can promote the differentiation of monocytes to a regulatory anti-inflammatory population, possibly favouring wound healing. However, it was not clear if allogeneic PRP either matched or mis-matched for AB0 and Rh antigens could lead to different responses. Nevertheless, the authors suggested that the drawback of an immune response typical of allogeneic molecules used in a therapeutic setting would be either not relevant or even negligible.[29]

In the current literature several case reports and case series have been published on the use of allogeneic PRP in wound care.[30] None of them have reported adverse effects. An Italian group reported the outcomes of 115 patients with soft tissue loss in the fingers after trauma who were treated with allogeneic platelet gel twice weekly. The authors stated that they had used his type of gel for years in all sorts of wounds.[31] Furthermore, there is one trial from Iran of 50 burn patients treated with allogeneic PRP compared to treatment with silversulphadiazine in an intra-patient controlled manner, with positive effects of allogeneic PRP on epithelialisation and no adverse events; however, the methodological quality of this report should be regarded with reservation.[32]

Another advantage of allogeneic PRP is that it offers more standardization and can be custom made, thus eliminating many of the variables described in chapter 2. Its wide availability also makes it easier to test repeated applications of PRP.

Another new development is to produce PRP as a powder by means of lyophilisation (freeze-drying). A recent study found consistent growth factor concentrations in a PRP powder made by pooling and processing allogeneic platelet concentrates, after which resuspension with saline chloride provided approximately 1 000 identical ''classic'' PRP applications of 3 mL each, which could be stored for a year.[33] A lyophilised PRP powder has even been tested in burn wounds in a recent trial in Taiwan. Lyophilised PRP powder was applied to deep second-degree burn wounds every 4 days and compared with an unspecified placebo. The authors found significant increased wound closure after 3 weeks of 93% in the PRP powder group compared to 85% in the control group. [34] However, there are serious methodological issues: the placebo was not specified, the study was not blinded, the applications were performed on so-called deep second-degree burns, which are usually treated with excision and split skin graft. Importantly, there was no mention of the allogeneic nature of this product, nor were adverse effects encountered or safety issues reported.

In conclusion, this thesis is a journey through the world of platelet-rich plasma, wound healing and in particular of platelets post-burn injury. The more you know, the more you find you don't know: the deeper you dive into the subject, the more you realise how incredibly complex the human body is. It seems somewhat presumptuous to think that with a little blob of PRP we can actually accelerate wound healing, which is a multifaceted orchestra at cellular level developed over several billions of years of evolution. However, should this overwhelming feeling of humility daunt us and prevent us from trying? No, we should proceed, in a systematic, careful and critical way, to unravel step-by-step the way to improve burn wound healing, ultimately leading to less or scarless healing.

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8

Nederlandse samenvatting

Dit proefschrift gaat over brandwonden en bloedplaatjes. Het beschrijft onderzoek naar het effect van bloedplaatjesrijk plasma (PRP) op de genezing van patiënten met acute brandwonden en de kwaliteit van de resterende littekens, en daarnaast ook, vice versa, onderzoek naar het effect van brandwonden op de bloedplaatjes van brandwondpatiënten.

Het genezingsproces van brandwonden hangt samen met de diepte van de brandwond. Eerstegraads verbranding, zoals de roodheid van zonnebrand, geneest restloos. Oppervlakkig tweedegraadsbrandwonden genezen zonder operatieve behandeling, en veroorzaken meestal geen zichtbare littekens. Diep tweedegraadsbrandwonden en derdegraadsbrandwonden, waarbij de huid grotendeels respectievelijk geheel is verbrand, worden doorgaans geopereerd. Hierbij wordt de verbrande huid weggesneden waarna de wond wordt bedekt met een huidtransplantaat van elders op het lichaam. Het betreft meestal een huidtransplantaat van gedeeltelijke dikte dat met behulp van een zogenaamd 'mesh'-apparaat netvormig is vergroot. De genezing hiervan gaat altijd gepaard met littekenvorming. Een voorbeeld hiervan toont figuur 1 op pagina 11 waarin het netvormige patroon van het huidtransplantaat nog goed herkenbaar aanwezig is. Brandwondlittekens kunnen de kwaliteit van leven, zowel op fysiek, psychisch en sociaal gebied, nadelig beïnvloeden. Dit beweegt, al heel lang, brandwondartsen en onderzoekers om onderzoek te doen naar behandel- en operatiemethoden die de wondgenezing na huidtransplantatie bevorderen en nadelige littekenvorming tegengaan.

Bloedplaatjes worden in het beenmerg gevormd door megakaryocyten (reuscellen) die, elk op zich, enige duizenden celkernloze bloedplaatjes vormen. Deze zijn betrokken bij de stolling van bloed. Zodra ergens een wond ontstaat worden ter plaatse snel bloedplaatjes aangetrokken die samen met stollingseiwitten een bloedstolsel vormen. Bloedplaatjes laten bij dit proces diverse groeifactoren los die een rol spelen bij de hierop volgende fases van de wondgenezing. Daarnaast hebben bloedplaatjes ook een rol in de afweer. Kortom bloedplaatjes zijn belangrijk bij wondgenezing.

Plaatjesrijk plasma (PRP) is bloedplasma met een verhoogd gehalte aan bloedplaatjes. PRP wordt gemaakt door bloed van patiënten zo te bewerken, onder andere door centrifugering, dat er bloedplasma wordt verkregen met een hoog gehalte aan bloedplaatjes. Deze gel-achtige substantie wordt op of in een wond aangebracht met het idee dat dit concentraat van bloedplaatjes de hoeveelheid aan groeifactoren zodanig doet toenemen dat dit de wondgenezing verbetert. Afgelopen decennia zijn er talloze publicaties verschenen over de mogelijk genezing bevorderende werking van PRP. Deze nieuwe ontwikkeling is reden geweest om te onderzoeken of PRP ook bij acute brandwonden een mogelijk gunstig effect heeft.

In **hoofdstuk twee** wordt een literatuuroverzicht gegeven over PRP. Hierin komt naar voren dat er veel verschillende manieren en apparaten zijn om PRP te produceren. Ook varieert de samenstelling en worden verschillende benamingen door elkaar heen gebruikt. Ze hebben wel alle gemeen dat ze een verhoogd aantal bloedplaatjes hebben en door dit aan te brengen op een wond een verhoogde hoeveelheid groeifactoren afgeven.

Vanuit vele uiteenlopende disciplines is gepubliceerd over de toepassing van PRP. Bijvoorbeeld, in de orthopedie wordt PRP gebruikt bij het operatief herstel van pezen en ligamenten (Achillespees, kruisbanden, rotator-cuff). In de hartchirurgie bij de behandeling van infecties

in de borstholte, en in de maxillofaciale chirurgie en de traumachirurgie ter bevordering van de ingroei van bottransplantaten. In de plastische chirurgie bestaat een veelheid aan toepassingen, o.a. bij acute en chronische wonden, faceliftchirurgie en vettransplantatie. De meeste publicaties vermelden een gunstig effect van PRP, een gering aantal geen effect. Complicaties van PRP zijn niet beschreven. De wetenschappelijke bewijskracht van al deze publicaties is doorgaans gering. Ook omdat er zoveel verschillen tussen alle PRP-achtige producten en toepassingen zijn, is het moeilijk om meerdere onderzoeken met elkaar te vergelijken en overkoepelende conclusies te trekken.

Publicaties over de toepassing van PRP bij brandwonden bleken schaars te zijn. Enkele dierexperimenten vermeldden gunstige effecten van groeifactoren op de genezing van brandwonden. Tevens waren er enkele case-reports met gunstige resultaten bij acute brandwonden en was er één klinisch vergelijkend onderzoek: geopereerde brandwonden werden bedekt met alleen een huidtransplantaat of met een combinatie van PRP en huidtransplantaat. Na korte follow-up bleek in de PRP-groep de kwaliteit van de littekens iets beter, maar na een langere follow-up werd geen verschil gezien.

De slotsom van dit overzicht is dat patiënten met diepe brandwonden in theorie voordeel kunnen hebben van het gebruik van PRP tijdens de operatieve behandeling. Dit zou dan berusten op de antimicrobiële , lijmende en genezing bevorderende werking van PRP. Maar tegelijkertijd drong na dit overzicht ook een prangende vraag zich naar voren, die in de literatuur onvoldoende was beantwoord. In tegenstelling tot de vele onderzoeken naar het effect van PRP in relatief gezonde patiënten, gaat het bij acute brandwondchirurgie om patiënten die recent een brandwond hebben opgelopen, hetgeen naast een evidente beschadiging van de huid ook invloed heeft op het 'milieu interieur' van een patiënt. Dit beïnvloedt mogelijk de kwantiteit en de kwaliteit van bloedplaatjes. Onderzoek hiernaar was geboden, waarover wordt bericht in de volgende hoofdstukken.

Hoofdstuk drie gaat over het onderzoek naar de kwantiteit van bloedplaatjes bij brandwondpatiënten. Het betreft een retrospectief onderzoek bij 244 patiënten. Bij alle patiënten daalde het aantal bloedplaatjes na het ongeval met een dip op de derde dag na de verbranding. Twee weken na de verbranding werd een piek gezien in het aantal bloedplaatjes, waarna het aantal bloedplaatjes zich in de daarop volgende weken normaliseerde.

Hiernaast vonden we dat dit beloop werd beïnvloed door de grootte van de brandwond, de leeftijd en of patiënten een sepsis (bloedvergiftiging) ontwikkelden. Ook vonden we dat een lage bloedplaatjespiek, samen met een hoge leeftijd en een hoog percentage verbrand lichaamsoppervlak, voorspellend is voor overlijden. Naast dit beloop wisten we nog niet hoe de bloedplaatjes functioneren na brandwonden. Dit hebben we onderzocht in een volgend onderzoek dat hieronder wordt beschreven.

Hoofdstuk vier betreft het onderzoek naar de kwaliteit van de bloedplaatjes van brandwondpatiënten na het oplopen van de brandwond. Bloedplaatjes bleken functioneel te zijn en niet overmatig geactiveerd. Wel kwam naar voren dat brandwondpatiënten vanaf de verbranding tot aan de genezing in een verhoogde stollingsstaat verkeren. De klinische betekenis hiervan is onduidelijk en vergt nader onderzoek, in het bijzonder naar de stollingsfactoren in het bloed.

Ook werd de inhoud van de bloedplaatjes onderzocht door de hoeveelheid groeifactoren te meten in de plaatjes tijdens dit beloop na de brandwond. Hieruit bleek dat er gedurende dit gehele beloop dezelfde hoeveelheid groeifactoren per bloedplaatje nauwelijks varieerde.

In **Hoofdstuk vijf** is de kwaliteit van PRP van brandwondpatiënten vergeleken met die van gezonde vrijwilligers, door de hoeveelheid groeifactoren te meten in de PRP's. Hierbij vonden we dat de hoeveelheid groeifactoren in de PRP van brandwondpatiënten vergelijkbaar is met de PRP van gezonde vrijwilligers. Wel zagen we dat er redelijk veel variatie in de hoeveelheid groeifactoren tussen de patiënten en ook in de gezonde vrijwilligers groep onderling zat. Ook zagen we dat voor de meeste groeifactoren de hoeveelheid redelijk correleerde met het aantal bloedplaatjes, hetgeen suggereert dat je mogelijk in de toekomst niet meer de hoeveelheid groeifactoren zou hoeven meten, maar dat het veel simpeler te verkrijgen aantal bloedplaatjes mogelijk al genoeg informatie verschaft. Feit is wel dat voor veel indicaties van PRP en ook voor brandwonden het niet duidelijk is hoeveel bloedplaatjes en groeifactoren nodig zijn en of een bepaald aantal mogelijk wellicht averechts kan werken.

Tenslotte wordt in **hoofdstuk zes** een gerandomiseerd en geblindeerd onderzoek naar de effecten van PRP op het behandelresultaat van brandwondchirurgie beschreven. De opzet was eenvoudig: bij iedere patiënt werd een deel van de brandwond op de gewone manier behandeld (excisie van verbrande huid en bedekken van de wond met een huidtransplantaat) en een ander vergelijkbaar deel van de brandwond op de experimentele manier (excisie van verbrande huid, het aanbrengen van PRP op de operatiewond en het bedekken hiervan met een huidtransplantaat). Voor zowel de patiënt als de onderzoekers was niet duidelijk welk gebied behandeld was met PRP. Vastgelegd werd hoe snel en goed de huidtransplantaten ingroeiden en welke complicaties optraden. Vervolgens werden de patiënten gedurende een jaar gevolgd, in welke periode de littekens zijn onderzocht met een aantal objectieve meetinstrumenten.

De resultaten waren als volgt. De ingroei van de huidtransplantaten verliep in beide groepen hetzelfde. Ook de littekenvorming, een jaar lang gevolgd, verschilde niet. Wel werd in een subgroep, patiënten die binnen 7 dagen na verbranding zijn geopereerd, een klein verschil in de mate van ingroei van het huidtransplantaat en in de snelheid van reëpthelialisatie gevonden ten gunste van de PRP-groep. Negatieve effecten die toegeschreven konden worden aan het gebruik van PRP werden niet gezien. Kortom, we zagen geen tot weinig effect van PRP bij de operatieve behandeling van acute brandwonden.

Uit de in dit proefschrift beschreven onderzoeken kunnen verschillende **conclusies** worden getrokken. Het aantal bloedplaatjes in het bloed bij brandwondpatiënten daalt de eerste drie dagen gevolgd door een tijdelijke piek. De bloedplaatjes van brandwondpatiënten functioneren normaal en bevatten constante hoeveelheden groeifactoren. Ook is de kwaliteit van PRP van brandwondenpatiënten vergelijkbaar met die van gezonde vrijwilligers. Maar uiteindelijk lijkt PRP als toevoeging bij een huidtransplantaat bij de behandeling van diepere brandwonden geen significant betere littekens te geven.

Verder onderzoek moet uitwijzen of PRP mogelijk op een andere manier kan worden toegepast bij brandwonden. Bijvoorbeeld door het te maken van bloedplaatjes van bloed van donoren van de bloedbank in plaats van patiënten zelf, of door het bijvoorbeeld niet eenmalig maar vaker aan te brengen. Daarnaast moet er meer informatie komen over hoeveel plaatjes of hoeveel groeifactoren er nodig zijn in de PRP bij brandwonden. Dit alles met als uiteindelijk doel de littekens van brandwondenslachtoffers te verminderen en daarmee hun levenskwaliteit te verbeteren.

9

Dankwoord

Allereerst dank aan alle brandwondenpatiënten en hun familieleden die wilden meewerken aan de onderzoeken.

Professor Middelkoop, lieve Esther, dank voor alles. Dank voor je super analytische vermogen, zelfs al weet je (nog) niets van het onderwerp, dan nog haal je uit feilloos de belangrijkste dingen eruit. Ik heb veel van je geleerd en het was altijd gezellig waar we elkaar ook troffen in het kot, vu of langs de snelweg!

Professor Breederveld, Beste Roelf, dank dat je mij ooit aannam, ook al was ik zwanger..! Dank voor je praktisch meedenken tijdens mijn onderzoeken. Volgens mij dacht je op gegeven moment dat het niet meer ging gebeuren, maar het is af gekomen!

Geachte leden van mijn leescommisie prof. C.M.A.M. van der Horst, prof P. van Zuijlen, dr D. De Korte, prof. B. Van der Lei en prof C. Boer, dank voor het kritisch lezen van mijn proefschrift.

Lieve Jos, Fenike, Paul, Toine, Annebeth, Dorothka, Anita, Allete, Marinke, Patricia en Angelique en alle lieve verpleegkundigen van het BWC. Dank voor de hele fijne tijd op het BWC, het had van mij nog veel langer mogen duren, maar mijn loopbaan denderde door. Dank voor alle koffie-, bloemen- en appeltaartmomenten en al jullie inzet en hulp tijdens de studies. En aan Wim Tuinebreijer dank voor alle uurtjes statistiek met uitzicht op het aquarium dan wel de zee.

Alle lieve oud en nieuwe burn-squad-kotters: Martijn, Pauline, Monica, Carlijn, Kim, Marielle, Zjir, Kelly, Michelle, Dominique, Matthea en Anouk. Het is alweer een tijd geleden dat ik bij jullie begon. Eerst zonder computer en protocol aan de kopse kant bij Jos en Fenike, toen naar het altijd sfeervolle kot op het dak. Dank voor de goede tijden toen en tijdens alle congressen nog lang daarna.

Mijn lieve paranimfen: Carlijntje, color-blockend in Den Haag en zoveel andere mooie momenten, de zoektocht naar Russen altijd eindigend bij de Italianen. Ik was liever nog paar jaar bij je in het kot gebleven, maar gelukkig blijven we elkaar zien en wie weet wat de toekomst ons nog brengt!

En lieve Plo, wij kennen elkaar inmiddels al zo lang. We begonnen op het huis; medisch kruisten onze paden elkaar even bij Hoekie en Rottie, via eindeloos veel bakkies, tripjes, dipjes en hoogtepunten naar nu en tot altijd!

Dank aan mijn opleiders in het AMC, RKZ, Spaarne en Avl; dank voor alles wat jullie mij hebben geleerd en nog steeds elke dag leren. Mijn fantastische collega's eerst in het SLAZ en nu in het AMC dank voor de altijd fijne sfeer.

Lieve schoonfamilie en met name oma Carolien, dank voor alles wat je voor ons doet.

Mijn lieve vrienden, heerlijke biertjes, zonnige vakanties, en fijne niet meer zo goed bewaarde geheimen en trouwe bellen, oude haaien en nieuwe vrienden: dank voor alles!

Mijn lieve ouders en broers, dank voor mijn heerlijke jeugd, dank dat jullie er nog altijd altijd altijd voor mij en voor Jacob en de kinderen klaarstaan.

Mijn liefste Jaki, Willempie, Sammy en Tesseltje op naar onze volgend avontuur in Vancouver. J, dank voor alles wat je doet, waardoor ik kan doen wat ik doe.

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About the author

Roos Marck was born 9 april 1982 in Groningen. She grew up in Goutum, a small village near Leeuwarden, together with her three brothers and parents. After the local Wiardaskoalle, she attended the Stedelijk Gymnasium in Leeuwarden. After graduating in 2000 she moved to Italy; first for three months to Florence, followed by six months of semi-professional field hockey playing in Sardegna.

The next two years she studied both Law and History at the University of Amsterdam. In 2003 she switched to Medicine at the Academical Medical Center (AMC). She got her first experience with research at the department of Vascular Medicine, however during her internships she developed an interest for plastic surgery. In 2008 and in 2009 she joined English/ French teams on several surgical missions to Ethiopia where she performed research on the quality of care of humanitarian surgical missions.

In 2009 she did her final rotations at the department of plastic surgery of the Antoni van Leeuwenhoek hospital (AvL) and the AMC. After receiving her medical degree she worked in 2010 as a surgical resident (anios) at the St. Lucas Andreas hospital in Amsterdam. A year later she started a fulltime research position at the Burn Center in the Red Cross hospital (RKZ) in Beverwijk on the subjects of this thesis under supervison of prof E. Middelkoop and prof R. Breederveld.

Halfway 2012 she started her speciality training for plastic surgery in the AMC under supervision of professor C. van der Horst and dr. M. Obdeijn, with first two years of general surgery in the St. Lucas Andreas hospital, followed by plastic surgery rotations in the AMC, AvL, RKZ and Spaarne Gasthuis. In June of 2019 she will complete her training after which she and her family will move to Vancouver for a one year fellowship (breast reconstructive and cranio-facial). She lives with Jacob and their three children Willem, Sam en Tessel in Amsterdam.

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