Maggot debridement therapy in surgery

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Citaat

‘No: they will clean it,

wait and see’

(Djimon Hounsou in the movie ‘Gladiator’ 2000)

‘Doctors!

Go to the wounded!

Do not wait for them to come to you.’

(Norman Bethune[1], 1890-1939)

Dr. Norman Bethune 1922

Contents

Chapter

1

Introduction

A long history of maggot therapy

Maggot-therapy is a medical curiosity that has had little influence on the course of modern medicine.[2] This statement might have been true as late as 1988,[3] but now with more than 100 papers published on the subject in the past decade alone, it's no longer true. Maggot debridement Therapy (MDT) has been used in many cultures and has been used in wound healing for centuries.[4,5] There are reports of the successful intentional use of maggots by Ngemba-tribes in Australia[6], in the province of Yunan in China[7] and the Mayan Indians.[8] The oldest known written record in which myasis (human infested with maggots) is described, is the Old Testament. The first European medical reference to maggots appeared in the Hortus Sanitatus in 1491.[5] This book was probably written by its printer, Jacob Meydenbach. It is a collection of herbal knowledge drawn from medieval and classical authors, such as Galen, Albertus Magnus and Dioscorides.

Ambroise Paré (1509-1590) is credited as the father of modern MDT.[9] He was the first to observe the beneficial effects of fly larvae on wounds. He described a patient who, against all odds, recovered. He believed however, that the observed 'wurms' were the result of 'Generatio Spontane' (this theory introduced by Aristotle, states that from an individual of one species a total different species could develop). In literature, there is no evidence that Paré intentionally used maggots as a method to clean or heal wounds. The only reference is the often-cited case that occurred in 1557 at the battle of St. Quentin, when Paré observed soldiers whose wounds were covered in maggots. He mainly described the negative effects of the maggots and above all did not know the relationship between flies, eggs and maggots.[10-11]

Baron Larey (1766-1842), a famous surgeon in the army of Napoleon Bonaparte, wrote about soldiers who had maggot infested wounds, but was frustrated that it was difficult to persuade his patients to leave the maggots in place. He believed that "maggots promoted healing without leaving any damage".[9]

During the American Civil War a group of imprisoned Confederate medical officers were forced to leave the wounds of their patients undressed, as they were denied bandages. They found that many of the larva-infested wounds cleared up quickly, while many of the Union wounded died.[12] Zacharias, a Confederate surgeon was the first to intentionally apply maggots to the wounds of soldiers, in order to clean and debride them.[13]

A famous quote of Zacharias: *'During my service in the hospital in Danville, Virginia, I first used maggots to remove the decayed tissue in hospital gangrene and with eminent satisfaction. In a single day they would clean a wound much better than any agents we had at our command..... I am sure I saved many lives by their use, escaped septicaemia, and had rapid recoveries.'*

The first surgeon to use MDT on patients in hospital was the orthopedic surgeon William Baer. In the 1920s he was faced with a group of untreatable patients with severe osteomyelitis (antibiotics had not yet been discovered). He successfully treated many patients by means of maggots, and because of his success the therapy became regularly used in the United States.[13] Dr Baer however, experienced problems with sterility, with subsequent tetanus developing in some of his patients. By 1934 more than 1,000 surgeons were using maggot therapy. Surgical maggots were commercially available from Lederle Corporation.[14] With the introduction of antibiotics in the 1940s, the use of maggots declined. MDT fell into oblivion due to the fact that antibiotics such as

sulphonamides and penicillin were industrially fabricated. In the years to come, MDT was (Chapter 14) largely abandoned, with some case reports being published in the mean time. In 1989 Dr. Ronald Sherman rediscovered MDT. He acknowledged that despite modern wound treatment, not all wounds healed. He started rearing larvae and used them successfully in a controlled trial on decubital ulces.[15] Another factor might have been the appearance of antibiotic-resistant bacteria (eg, methicillinresistant Staphyloccocus Aureus), in which case MDT seemed to work very efficiently.[16]

At the same time in England Dr. John Church[17;18] and Steven Thomas[19;20] were involved in MDT. The Biosurgical Research Unit at SMTL commercially rears maggots; Biomonde does so in Germany.

MDT in the Netherlands

Dr. Gerrolt Jukema was the surgeon who introduced maggot therapy in the Leiden University Medical Center in 1999.[21] A patient with a severe crush injury of both feet was treated with maggots in order to salvage at least one of his limbs. Against all odds, both wounds healed with good functional and cosmetic result.[22] Maggot therapy has been known in the Netherlands for a longer period, as can been seen, for instance, in the fact that Military Services in the Netherlands equipped its soldiers who were going to Korea (in the 1950's) with the basic knowledge regarding maggot therapy, how to apply it in order to treat wounded soldiers awaiting pick-up by the helicopters (which at that time could sometimes be a couple of days).[23] Unfortunately, the Dutch Institute for Military History could not find any records on this.[24]

MDT was introduced at the Rijnland Wound Center in 2002. The first few patients were treated with maggots derived from Leiden University Medical Center. The first patient treated was a patient who suffered from a severely infected below-knee amputation, which in the surgeon's opinion needed to be converted to an above-knee amputation. The patient however, persuaded the surgeons to try a period of maggot therapy about which he had read in a lay Dutch newspaper. After treatment of the first few (5) patients, we held our first presentations for general physicians[25] and discussed our results with doctors and nurses of our hospital.[26] This led to publications in the lay press[27], leading to more patients coming to our hospital with the question whether or not their wounds could be treated with MDT.

We have argued that maggot therapy should not only be reserved for the wounds that are difficult to heal, but could also be used as a first-line treatment.[28] However, with the start of our Rijnland Wound Center there were many unanswered questions and these have become the basis of this thesis. With maggot therapy we were able to get a lot of wounds in the granulating phase. However, we found that sometimes it is actually more difficult to close the cleaned wounds for which we have proposed several options, like Topical Negative Pressure Therapy[29], Oasis Wound Matrix[30] and many others, this eventually cumulating in our first organised wound symposium of the Rijnland Hospital on 11th September 2006 and the treatment of our 150th patient (with MDT) in February 2007.

Revival of MDT

Maggot therapy has seen a real revival, in the period 2004-2006, 60 papers were published on MDT, and on Pubmed almost 200 articles can be found. MDT has been approved by the FDA (Food and Drug Administration) and is now a medical device (issue 510(k)33391). Dr. H. Wolff, from Sweden, wrote her thesis on 'Studies of Chronic Ulcers & Larval Therapy' in 2004. The International Society of Biotherapy was founded in 1996, to investigate and develop the use of living organisms, or their products, in tissue repair.

Their 7th meeting was held in Seoul Korea in June 2007.[31] Currently 300 centers in the United States and about 1,000 centers in Europe are using MDT.[32] In these days of evidence based medicine, we must conclude that there is no evidence from multiple, large, randomised studies, simply because there have not been any, although one is currently on its way in England. It is a trial on the effectiveness of MDT in chronic venous leg ulcers, including a total of 600 patients.[33] We believe however, that randomised studies can only be performed if some of the basic questions are answered, such as which factors influence the effectiveness of MDT. The group of Petherick et al. questioned themselves, for example, about patient acceptability, which in our opinion is a very important question.[34] If a randomised study is undertaken without reference to factors influencing the outcome, these studies will have confounding factors. In this thesis, I will answer some of these basic questions.

MDT is a form of debridement; a biological debridment. Some wounds can better be treated with surgical debridement, others perhaps with chemical debridement. Debridement in its different forms will now be outlined.

Debridement

The term "debridement" comes from the French desbrider, meaning "to unbridle". Debridement refers to the removal of dead, damaged, or infected tissue in order to improve the healing potential of the remaining tissue.[35] Debridement as a medical term was probably first used by surgeons working in war zones, who recognised that grossly contaminated soft tissue wounds had a better chance of healing (and the soldier of surviving) if the affected tissue was surgically removed to reveal a healthy bleeding wound surface.[36] It seems that the chronic wound does not progress through the stages of wound healing (hemostatis, inflammation, proliferation and maturation) but the healing progress stagnates in the inflammatory phase. If necrotic tissue is left intact, it is very difficult to maintain a moist wound environment, to keep the wound free from infection, prevent excessive inflammatory response and to ensure the closure of wound edges.[37] If the wound is not debrided the healing process will be impeded. Another point is that if necrotic tissue is not removed, the open wound or ulcer cannot be properly assessed.[36] Other consequences of not debriding a wound is imposition of additional metabolic load, psychological stress, compromising skin restoration, abcess formation, odour, nutritional loss and sub-optimal clinical and cosmetic outcome.[38] Debridement additionally removes senescent cells from the wound bed.[39] Senescent cells are cells that (due to aging) have a reduced growth capacity, are morphologically changed and have an over-expression of certain matrix proteins.[40] By removing necrotic tissue, the increased bacterial burden is reduced (traditionally considered greater than 105 colonies per gram of tissue). It is known from earlier studies that wounds exhibiting increased bacterial burden have reduced healing responses when compared to wounds containing fewer bacteria.[41] Biofilms (communities of bacteria and other organisms that are embedded with an extrapolyosaccharide matrix) have detrimental effects on wound healing; debridement may also help remove these biofilms.[42] Debridement, which results in bleeding, stimulates the production of growth factors. Platelets control bleeding and form a platelet plug. In addition, activated platelets release various growth factors and cytokines.[43] These act as chemoattractants for inflammatory cells and mitogens for fibroblasts and epithelial cells, all crucial components of proper wound healing.[42] There is no level 1 evidence that debridement (in any form) has a beneficial effect on wound healing[44], compared to no debridement. However, international consensus is that debridement is a vital adjunct in the care of patients with chronic wounds.[45;46] There are several methods of

debridement: surgical, mechanical, autolytic, chemical, enzymatic and biological.[47] It's not clear which method of debridement is to be preferred. Each method seems to have its own indications and contra-indications. In a published review on debriding agents for surgical wound healing by secondary intention, it was concluded that there is proof of the effectiveness of autolytic debridement; they could find no studies into the other forms of debridement.[47] In studies on diabetic patients, enzymatic debridement was found effective (3 Randomised controlled trials); there was no proof in favour of surgical debridement (1 RCT) or maggot therapy (1 RCT).[46] It seems that not all necrotic wounds can be addressed with the same debriding method. The different debridement methods will subsequently be discussed, with figures that illustrate each method.

Biological Debridement (Maggot Debridement Therapy)

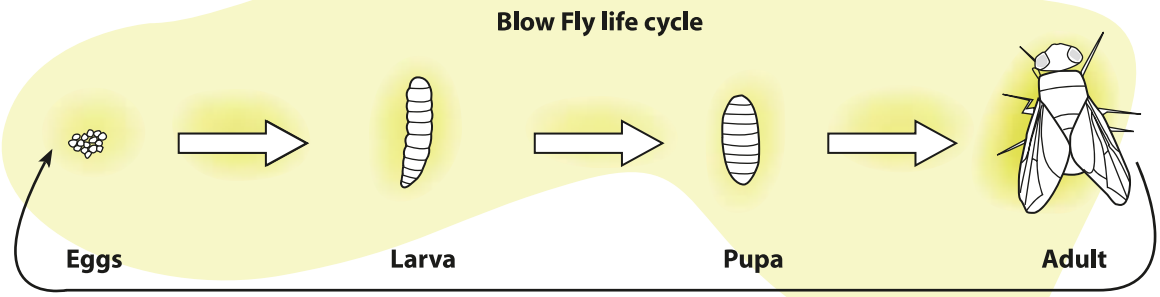

Figure 1: Showing the life cycle of the blow fly.

It is important to realize that the larvae of the blow fly (lucilia sericata) is the stage of the maggot's development in which it can be used for MDT. The larvae are relatively small (<2 mm) when they are applied and can grow up to 1 cm in 2 to 3 days. In order to complete the cycle the larvae will need to pupate (at a lower temperature than the human body temperature). The cycle **(see Figure 1)** takes about 14 days to be complete. The larvae will never stay on the wound for this long, for they are changed 2 to 3 times per week. The maggots are put in place on the wound. There are several application techniques, which will be described in detail in chapter 4. The larvae are sterilized in a specialized production facility. They can be easily obtained from Tuesday till Friday (if ordered the day before). In the Netherlands larvae can be obtained by BiologiQ (Apeldoorn, The Netherlands).

It is still not clear how maggot therapy works. It is probably more complicated than the mere washing out of bacteria by the serous exudate or the simple crawling of the larvae in the wound. 'Maggots move over the surface of the wound, secreting proteolytic enzymes that break down dead tissue, turning it into a soup which they then ingest'.[48] The mechanism for the beneficial effect of maggot therapy is likely their extracorporal digestive system. Maggots produce enzymes such as tryptase, peptidase, and lipase and release these into their environment. This may help break down debris and necrotic tissue, while leaving healthy tissue unharmed. The resulting semiliquid debris is absorbed and digested by the maggots.[49-51] They act as necrophages and destroy bacteria.[48;52] In addition, maggots secrete allantoin, ammonia, and calcium carbonate, which produce an alkaline environment.[53] This acts as a barrier against bacterial colonization and stimulates the growth of granulation tissue.[13] Also, the crawling of

maggots in the wound is thought to create a mechanical stimulus for the growth of granulation tissue.[54] Besides, they produce growth stimulating factors[55], which have been shown to promote the growth of fibroblasts.[49] Nigam et al. recently published an article discussing evidence supporting the potent antibacterial action of maggot secretions. Besides debridement and desinfection, a third important factor of MDT is discussed: enhanced healing.[56]

Figure 2: Production facility of maggots in Germany (Biomonde).

Figure 3: Showing the non-sterile part of maggot rearing. Some maggot larvae are grown into the adult stage (fly) in order to keep the production going.

Figure 4: The eggs intended for use in MDT are sterilized. Cultures are taken, in order to prevent induction of infection in patients. In all maggot treatments performed in the LUMC and Rijnland Wound Center Leiderdorp, this has never occurred.

Surgical Debridement

Surgical debridement refers to the extensive removal of tissue, sharp debridement refers to minor tissue sparing debridement that can be repeated and can be performed at the bedside of the patient or in a procedure (surgical) room.[36] Necrotic tissue is removed, using a scalpel, scissors, forceps or a curette. This is especially indicated if a rapid debridement is needed, it can be done at the patient's bedside. It seems ideal if there is a large quantity of necrosis that needs to be removed.[57] Surgical debridement is the only debriding method if the patient has systemic signs caused by the wound (e.g., sepsis or cellulitis).[42] One should consider bleeding problems in patients with clotting/bleeding disorders or patients on anticoagulant therapy. Another problem is that surgical debridement is not always very selective, for viable tissue lying adjacent to the necrosis can be removed.[58] Surgical debridement requires special training and expert comfort level and anatomic knowledge.[59] Sometimes an operating room or anesthetics are needed for extensive procedures, this limits the possibility of repeated surgical debridements. A new alternative is the use of a laser which both cuts and cauterizes.[42]

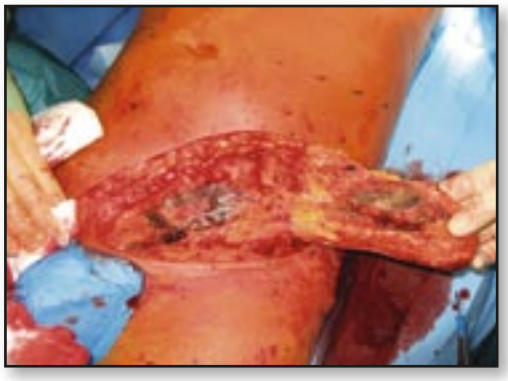

Figure 5: Showing a patient with a necrotizing fasciitis, for which surgical debridement is performed.

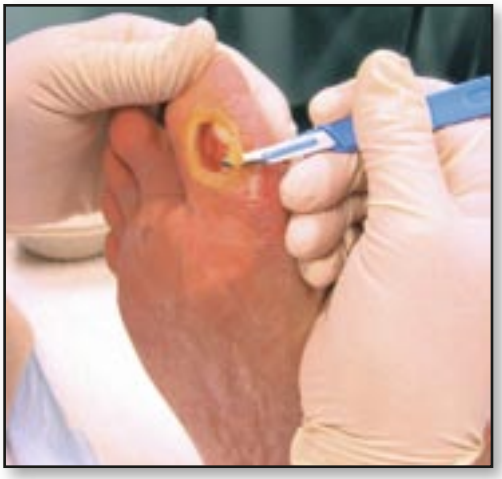

Figure 6: Sharp debridement of a diabetic neuropathic ulcer, using a scalpel.

Mechanical Debridement

In mechanical debridement, non-discriminatory physical forces are used in order to remove necrotic tissue and debris from the wound surface. The traditional wet-to-dry treatment consists of a moist dressing applied to the wound, which is subsequently removed when the dressing has dried out. It seems ideal for larger wounds, and for patients unfit for surgery. The main disadvantage is that it is non-selective and painful. Other problems include the frequent dressing changes, maceration of surrounding skin, and bleeding, while the time-consuming and labor-intensive characteristics of MDT further aggravate the patient's discomfort. Dressing fibers stick to the wound which can cause a foreign-body reaction. This method now seems outdated, with the current availability of other methods.[42] Rehydration can ease the removal of the surface eschar and debris on the surface of the wound. Hydrotherapy or wound irrigation is a relatively slow technique that uses moving water to dislodge loose debris. There is little evidence to support its effectiveness. The danger of cross infection should be taken into account when using hydrotherapy. Health care professionals need personal protective equipment with a view to aerosolization. There is also a theoretical risk of fluid embolism and promotion of infection if irrigation is too vigorous. Other forms of mechanical debridement include high-pressure irrigation and whirlpool baths. In this way, wounds are debrided using water, but these methods may also result in periwound maceration. Other forms of mechanical debridement are ultrasonic therapy and laser therapy.
A relatively new method using Fluidjet Technology (Versajet Hydrosurgery System®, Smith & Nephew, Hull, UK) seems promising for hard-to-heal leg ulcers.[60]

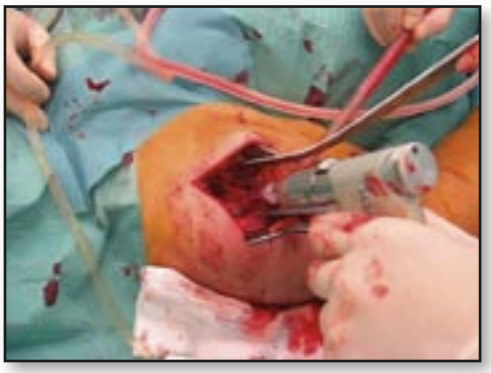

Figure 7: After surgical debridement, hydrotherapy is applied to clean the infected shoulder joint. In this figure the use of a Hydrojet® is demonstrated.

Autolytic Debridement

In autolytic debridement, the body's own enzymes and moistures are used to rehydrate, soften and finally liquefy necrosis and slough. It relies on enhancing the natural process of selective liquefaction, separation and digestion of necrotic tissue and eschar from healthy tissue that occurs in wounds because of macrophage and endogenous proteolytic activity.[59] Autolytic processes are accelerated if there is a moist environment.[61] It is a somewhat selective method, for only necrotic tissue is liquefied. When this therapy is used, occlusive or semi-occlusive dressings are used for lysosomal enzymes to have a better contact with the wound.[62] One of the main disadvantages is the slow speed, the chances of (anaerobic) infection and the chances of maceration of the surrounding skin.

Autolytic debridement is recognised to be effective in the maintenance phase of debridement. Examples of this treatment method are the use of hydrocolloids, hydrogels, alginates and transparent films. This method is selective and causes little or no pain. However, autolytic debridement may be slow.[42]

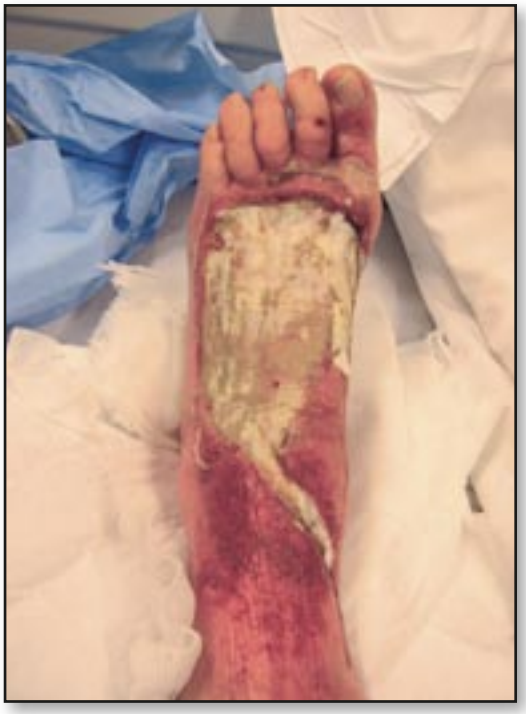

Figure 8: A wound covered in yellow slough is treated with an analginate dressing for further debridement. In this case Kaltostat® (Convatec, The Netherlands) was used.

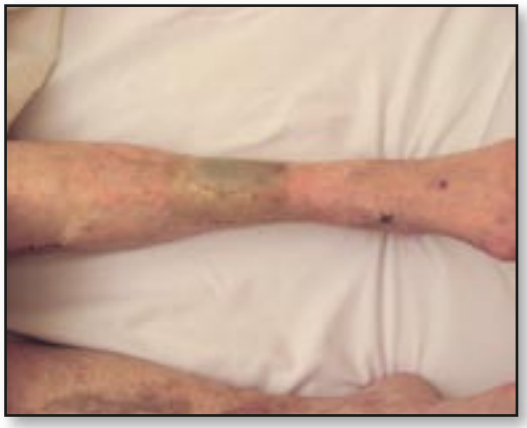

Figure 9: Showing an arterial ulcer of the left lower leg treated with a hydrocolloid dressing (Duoderm, ConvaTec, The Netherlands) in order to achieve autolytic debridement.

Enzymatic Debridement

In enzymatic debridement preparations are used such as streptokinase or streptodornase or bacterial-derived collagenases. Streptokinase and streptodornase aim to break down and rehydrate the necrotic tissue. Despite their availability for more than 30 years, there is little evidence to prefer the use of these to alternative methods. This process relies on the addition of proteolytic and other exogenous enzymes on the wound surface. These enzymes break down necrotic tissue and can be effectively combined with moist wound healing. Bacterial-derived collagenases show great potential and may promote healing.[63] The two most widely used proteolytic enzymes in Europe are fibrinolysin/desoxyribonuclease (Elase®) and collagenase (Novuxol®). In a study on collagenase in decibutal ulcers these seemed more effective than autolytic debridement.[64] In retrospective studies it seems effective for hard-to-heal ulcers.[65] Enzymes are inactivated by heavy metals (silver, zinc), which may be introduced from some wound care products, such as antimicrobial dressings (e.g. Actisorb Silver®, Flammazine®) and detergents present in skin cleansing agents inactivate enzymes. Care must be taken therefore, to use enzymatic debridement agents such as collagenases in the correct care sequence if they are to be maximally effective.[59] The products originate from very different sources, for example Elase® from bovine pancreatic tissue and Novuxol® from Clostridium histolyticum. However, several products are used for enzymatic debridement, ranging from pineapples and papaya to plankton and the newest product is a silicone-based controlled-release device for accelerated proteolytic debridement.[66] Combinations of enzymatic products like crab and krill are also available.[67] It seems we have not seen the end of enzymatic debridement yet, with new preparations and combinations being studied.

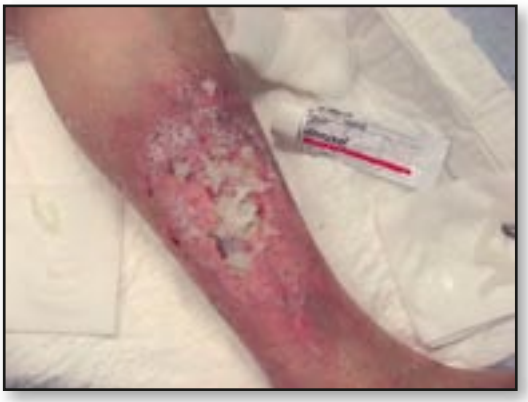

Figure 10: A painful necrotic ulcer treated daily with Novuxol® (Smith and Nephew, The Netherlands).

Chemical Debridement

Chemical debridement is not widely used due to the fact that it causes pain and also because the healthy underlying tissue is damaged during the therapy.[37] Another problem is that its effectivity is debated.[68] It is non-selective. Some authors call all chemical and enzymatic debridement chemical debridement. However, this is not right.[69] Sodium hypochlorite (Dakin's solution), hydrogen peroxide, povidonc-iodine and acetic acid all damage the cells needed for healing, such as fibroblasts. Some clinicians feel that these

antiseptic solutions can be used in case of infected wounds, for the prevention of the spread of infection takes priority over the protection of the few cells needed for healing.[70]

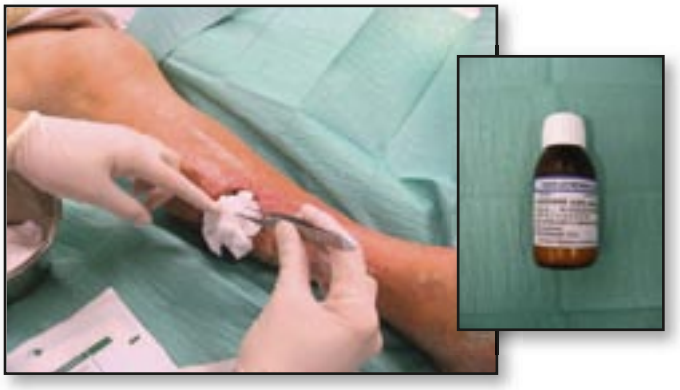

Figure 11: Sodium hypochlorite is applied to the wound. There are different treatment protocols, some prescribing the application for 15 minutes, three times a day.

Outline of the thesis

In chapter 1 of this thesis the history of maggot debridement therapy has been discussed. Starting from the oldest records of maggots known, until more recent history: the introduction of MDT in the Netherlands. There are six forms of debridement, biological debridement (MDT) is one of these methods. All different debridement methods have been briefly discussed.

In chapter 2 basic observations of MDT are described: histopathological, microbiological and laboratory investigations.

In chapter 3 a study is described in which patient-, wound- and therapy factors influencing the outcome of MDT are studied.

In chapter 4 two different application techniques are described and compared.

Chapter 5 consists of case reports and case series, such as MDT in amputation sparing surgery, MDT in breast-conserving therapy, MDT in necrotizing fasciitis, MDT in order to improve the outcome for infected amputation stumps and MDT in a palliative setting.

In chapter 6 adverse effects and safety issues are discussed: in particular the YUK-factor, bleeding complications and pain management.

In chapter 7 two articles are described in which possible contra-indications for MDT are discussed: smoking and chronic limb ischemia.

The general discussion is found in chapter 8, followed by a summary in English and in Dutch. References are published separately as is the publication list and the curriculum vitae.

Chapter

2

Basic observations

2A Histopathological observations

Based on the following article:

International Journal of Dermatology
P. Steenvoorde[1], J.J. Calame[2], J. Oskam[1] from the department of Surgery[1] and Pathology[2], Rijnland Hospital, Leiderdorp, The Netherlands.
Maggot treated wounds follow normal wound healing phases. Int J Dermatol 2006; 45(12): 1477-9.

Maggot debridement therapy (MDT) is used as an approach to help remove necrotic tissue and to prevent the need of disabling amputations of hands or limbs.[71-72] For wounds treated with MDT as an alternative to amputation, the limb salvage rate is reported to be about 50%.[73] It is not exactly clear how MDT works. There are several proposed mechanisms: mechanical effects and tissue growth effects, the direct killing of bacteria in the alimentary tract of maggots and the ability of maggots to produce several antibacterial factors.

We have taken tissue biopsies of four patients who were treated for chronic infected diabetic ulcers of the lower extremity with maggot debridement therapy (see **Table 1** for patient-, wound- and applicationcharacteristics). In three cases it affected the heel of the patient and in one only the big toe. There were two males and two females, average age was 74 years (range 63-88). There were different factors present affecting wound healing, like smoking (n=2), chronic limb ischemia (n=2) and overweight (n=2). In this study a diagnosis of chronic limb ischemia (CLI) was made when both pedal pulses of the involved foot were absent and/or the ankle-brachial pressure index was less than 0.6 and/or the absolute ankle pressure was below 50 mm Hg.[74] Prior to the treatment with maggots, the wounds had existed for 6 months on average (range 1-12 months).

Two wounds were limited to the skin and subcutaneous tissue only, two were deeper and had affected the joint or bone. There are two different MDT-application techniques in MDT: the contained technique and the free-range technique. An average number of 305 maggots were used per patient, in 6.8 applications over a treatment period of 3 weeks on average. The outcome was successful with the wound closed in three cases; in one case it was necessary to perform a partial amputation of the hallux. Unfortunately, two patients (patient one and four) died within one year after MDT, however both unrelated to the therapy or to the wound.

Table 1: Patient-, wound- and applicationcharacteristics of MDT treated patients.

Nr.	Sex	Age	Over-weight	Smoking	*CLI	*DM	Region	Depth	Application Technique	Nr treatments (nr. Of maggots in total)	Outcome
1	M	82	+	-	-	+	Heel	Subcutis	Contained	6 (180)	Closed
2	F	63	-	-	+	+	Heel	Bone	Free-range	6 (420)	Closed
3	F	64	+	+	+	+	Too	Bone	Contained	4 (120)	Minor amp
4	M	88	-	+	-	+	Heel	Subcutis	Free-range	11 (500)	Closed

F = female M = male *CLI = Chronic limb ischemia *DM = Diabetes Mellitus

In all biopsies there were no signs of malignancy. **Table 2** shows the histological findings of the biopsies taken before starting maggot therapy. As would be expected a marked neutrophil granulocyte infiltration is present within the ulcerated surface and within the dermal component. No regenerative changes are detected such as angioneogenesis and fibroblast proliferation. Wound healing occurs in three overlapping phases: the inflammatory phase ('lag phase'), the proliferative phase (tissue formation) and the remodelling phase.[75] The initial reaction to wound healing is the inflammatory phase. The inflammatory phase usually lasts 4 to 6 days. Hemostasis is the beginning of wound healing. The forming clot is composed of a matrix of fibrin, eventually plasmin will dissolve the fibrin cloth. The thrombocytes initiate a complex chain of reactions leading to an influx of white blood cells through the capillary pores to the wound. Within hours leucocytes can be seen on the site of injury. Their numbers are reduced significantly in the following days if no infection occurs. The tissue formation phase usually begins about 4 to 5 days after wounding and will last several weeks. Angiogenesis and the formation of granulation tissue, re-epitheliazation and the formation of an extra-cellular matrix, are the main components. The tissue remodelling phase is the last phase in which collagen type III is replaced by the stabler collagen type I. This phase lasts up to several years and is the actual scar formation phase.

Table 2: Microscopic examination of the wound prior to MDT.

Patient Nr.	Bacteria	Leucocytes	Angiogenesis	Granulation tissue	Fibroblasts
1	-	++	-	-	+
2	+	++	-	-	-
3	+	++	-	-	-
4*	-	++	-	-	-

-	is absent
+	is present
++	is predominantly present
*	from patient 4 only follow-up biopsies during therapy have been taken

Tissue biopsies of the wound were performed of all 4 wounds treated; this was done in the week prior to, and in the week after MDT. Standard haematoxylin and eosin stained slides were performed. None of the wounds had healed at the time of the biopsy, therefore the microscopic examinations only revealed wounds in the inflammation phase or in the tissue formation phase. The inflammation phase is microscopically characterized by the presence of bacteria and the abundant presence of granulocytes. In the tissue granulation phase, there are less bacteria and leucocytes, and more signs of angiogenesis and fibroblasts are present. Therefore, we looked in all biopsies for signs of bacteria, leucocytes, signs of angiogenesis and the presence of fibroblasts (see **table 2** prior to MDT and **table 3** post-MDT).

Table 3 shows the results after maggot debridement therapy of patients 1, 2 and 3. The wounds are clean, necrotic tissue has been cleared. The process of healing has started adequately. There are now sings of angiogenesis, granulation tissue is present, and so are fibroblasts. The wound healing phases of these three patients clearly went

from the inflammatory phase to proliferative phase, as is normal in wounds that heal. In **figure 1**, patient no. 3's histopathological examination of the wound prior to MDT is shown, in **figure 2**, after MDT. The fourth patient however, did not reach the healing phase. Biopsies that were taken during therapy showed very diverse pictures, partly responsive by showing a healing pattern, partly the debris still being present and causing active inflammation. The histological results of the fourth patient could have been biased by different biopsy sites. The wound showed signs of clinical granulation tissue, however this was very fragile. Pathological anatomical examination of wounds treated with MDT show that wound healing occurs in phases, comparable to those normally seen in non-maggot wound healing.

Table 3: Microscopic examination of the wound post MDT.

Patient Nr.	Bacteria	Leucocytes	Angiogenesis	Granulation tissue	Fibroblasts
1	-	-	++	++	+
2	-	+	++	++	++
3	-	++	++	++	++
4*	-	-/++	+/++	+/++	-/++

-	is absent
+	is present
++	is predominantly present
*	from patient 4 only follow-up biopsies during therapy have been taken.

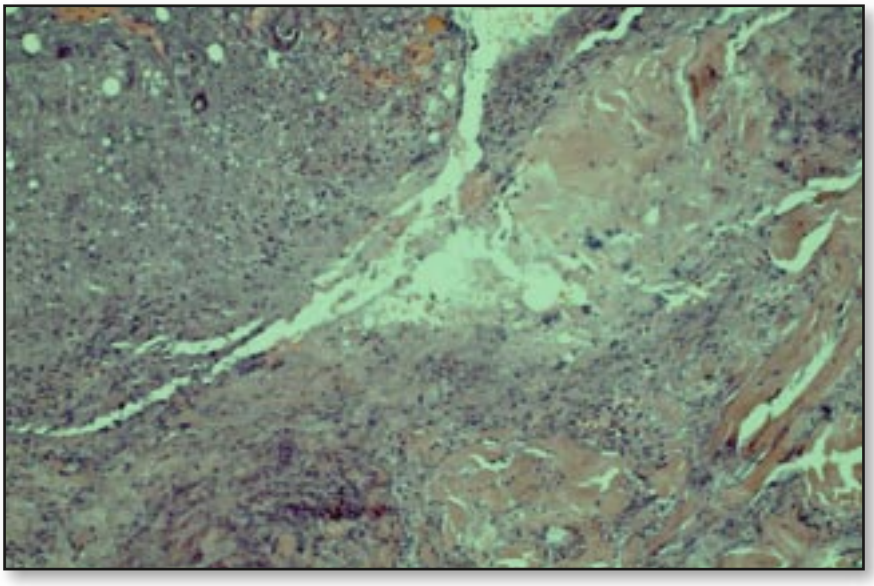

Figure 1: Showing pathological examination of the wound of patient no. 3 prior to MDT; bacteria and leucocytes are predominantly apparent; there is no angiogenesis, nor any sign of fibroblast proliferation.

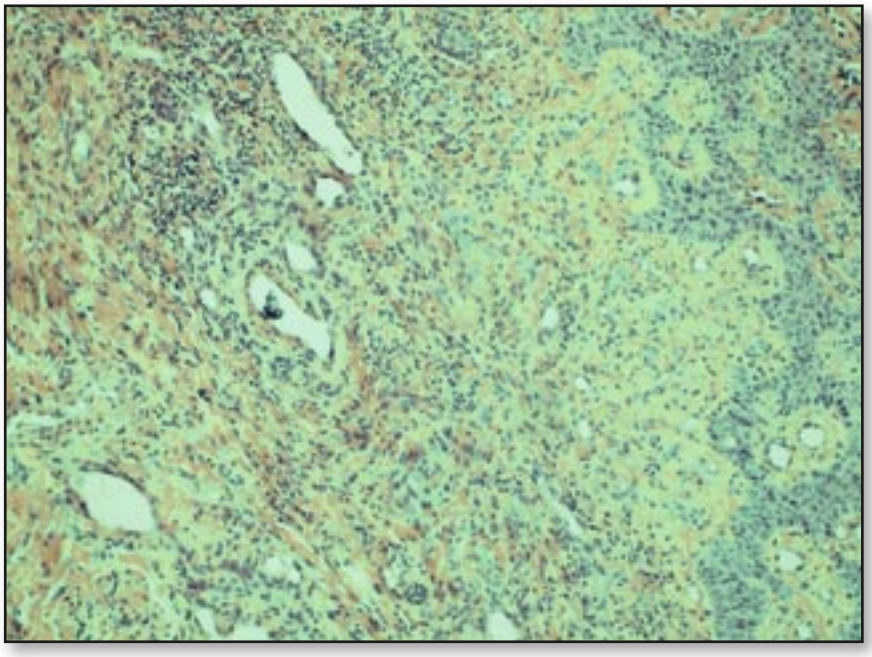

Figure 2: Showing pathological examination of the wound of patient no.3 after MDT; there are no bacteria; leucocytes are still present; but now angiogenesis and fibroblasts are also appearing.

2B Laboratory and microbiological observations

Based on the following articles:

Journal of Woundcare
P. Steenvoorde[1,2], G.N. Jukema[2]
Department of Surgery Rijnland Hospital, Leiderdorp, The Netherlands[1]. Department of Traumatology, Leiden University Medical Centre, Leiden, The Netherlands[2].
Can Laboratory investigations help us to decide when to discontinue larval therapy?
J Wound Care 2004 Jan; 13(1):38-40.

Journal of Tissue Viability
P. Steenvoorde, G.N. Jukema
Department of Traumatology, Leiden University Medical Centre, Leiden, The Netherlands.
The anti-microbial activity of maggots, in vivo-results. J Tissue Viability 2004; 14(3): 97-101.

Introduction

It is often not clear when MDT should be discontinued, in other words when it's time to continue with another form of treatment. One of the statements heard is, MDT is discontinued for 'there is complete debridement' or 'the wound is now fully red and granulating'. Hersh et al.[76] showed that the extent of closure of infected postoperative deep sternal surgical wounds, treated early with topical negative pressure (TNP), is indicated by the level of plasma C-reactive protein (CRP), with a median CRP level at closure of 45mg/l.[77] Guided by these studies, we explored, through a retrospective open-label non-comparative cohort study, whether the clinical decision to discontinue larval therapy can be confirmed by laboratory investigations, particularly significant reductions in leucocyte count, CRP levels and erythrocyte sedimentation rate (ESR).[77] It was questioned wether laboratory investigations correlated with clinical judgement.

Secretions of larvae of the common greenbottle (Lucilia sericata) have, in vitro, been shown to be most effective against Gram-positive bacteria, like streptococcus A and B and Staph. aureus. Gram-negative bacteria, especially Escherichia coli and Proteus spp., and to a lesser extent Pseudomonas spp., are more resistant to maggot secretions.[78-79] It was questioned wether these observations in vitro could be reproduced in-vivo. The in-vivo results of the use of maggots (Lucilia sericata) to treat Gram-positive and Gram-negative infected wounds are presented.

Method

In 1999–2002, 16 patients receid MDT at Leiden University Medical Centre in the Netherlands **(Table 1)**. Patients only received antibiotic therapy if clinical signs of infection were present, such as necrotising fasciitis or meningococcal sepsis. After adequate debridement with DT, most wounds were treated with TNP and split-skin grafting.[80-81] For MDT: average treatment time was 27 days (range: 12–83). An average of seven dressings was used (range: 3–21). Almost 15,000 maggots were used (average per patient: 925 maggots; range: 100–2900). Four patients used the net technique. The rest had Biobags (Polymedics Bioproducts, Peer, Belgium).
Laboratory investigations were performed on the first and last day of treatment **(Table 2)**.

The protocol for maggot treatment in the authors' hospital requires a wound swab of every treated wound on every maggot change. A swab is sent for culture (using Stuart medium) for aerobic and anaerobic organisms. Because all maggots in the hospital are sterile before application to the wound, new emerging bacteria in the wounds do not result from the application of the maggots. Antibiotic therapy is given when there are signs of systemic infection, which is always directed at the cultured micro-organism.

Wound cultures are always taken as a superficial wound swab and never as a deep tissue biopsy culture. Although microbiological assessment of chronic diabetic patients is probably more sensitive[82], the (sometimes small) size of the wounds and the need to sedate non-diabetic patients for deep tissue cultures stopped the authors from using deep tissue biopsies. An analysis of all wound cultures taken 1 month before, during the whole maggot treatment period, and 1 month after treatment with MDT was undertaken. A wound culture can either be sterile, show growth of a Gram-positive or a Gram-negative bacteria, or both. If, for example, before maggot treatment three wound cultures were taken and two of these showed a Gram-positive bacteria, the chance of culturing a Gram-positive bacteria is 0.66 (see **Table 1**, patient 1). These wound cultures were then analysed for Gram-positive **(Table 3)** and Gram-negative bacteria **(Table 4)**.
The data were analysed using Spearman's rho, which is a measure of association between two variables measured on at least an ordinal scale. An association of p=0.05 was considered a significant effect.

Results

In our hospital, the most frequent indication for the therapy is osteomyelitis. It was initiated after surgical debridement and antibiotic therapy had failed. All patients gave informed consent. Of the wounds, 50% had a multivariate aetiology. Main causes and influencing factors were: trauma (50%), Diabetes mellitus (38%), arterial disease (38%), rheumatoid arthritis (13%), steroid use (13%), venous insufficiency (6%) and meningococcal sepsis (6%). Average treatment time with maggots was 27 days (range 12–83 days), with an average of seven dressings applied (range 3–21 dressings). In total almost 15 000 maggots were used (average per patient 925 maggots, range 100–2900). Most patients were treated for osteomyelitis **(Table 1)**. All wounds eventually responded to the therapy and healed within six months. Three patients died: one due to a traffic accident and two of underlying disease (cancer and autoimmune vasculitis).

For CRP and ESR, there was no significant difference between values on the first and last day, although there was a trend towards lower values. However, the Friedman statistical test showed there was a significant reduction in leucocyte count on the last day of treatment: the median leucocyte count at baseline was 10.5 (x 10e9/L) compared with an endpoint of 8.4 (x 10e9/L) ($p<0.05$). After treatment and debridement, the leucocyte level was normal at 8.4. Average laboratory values for all three tests one month before and one month after larval therapy were the same as those recorded on the first and last days of treatment. There was a non-significant reduction in CRP levels and ESR, again with a trend towards lower values following treatment: the average CRP level was 86mg/l one month before treatment and 40mg/l one month after (non-significant) and the average ESR was 70mm/h before and 58mm/h after (non-significant).

In **Table 3** the result for Gram-positive cultures are presented. Gram-positive bacteria are cultured less often after maggot treatment than before. Using Spearman's rho this is a non-significant effect (p=0.07). Gram-negative bacteria **(Table 4)**, on the other hand, are cultured more often after maggot treatment than before (p=0.001).

Discussion

MDT is a very potent form of debridement. In our patients, removal of necrotic tissue or infection from infected, sloughy, necrotic wounds led to lower infectious parameters. The results demonstrated a significant reduction in leucocyte levels one month following discontinuation of larval therapy. In line with a previous study on TNP[77], we expected that CRP would be the best laboratory value for guiding decisions on when to discontinue

larval therapy. However, CRP showed a non-significant trend only. It is still not clear how maggot therapy works. It is probably more complicated than the mere washing out of bacteria by the serous exudate or than the simple crawling of the larvae in the wound. 'Maggots move over the surface of the wound, secreting proteolytic enzymes that break down dead tissue, turning it into a soup, which they then ingest'.[48] Maggots are capable of destroying bacteria in their alimentary tract. They also produce substances with healing properties, such as allantoin and urea. There is also a change in the wound pH, from acidic to alkaline, as a result of the ammonia and calcium carbonate excreted by the maggots.[55] In the 1930s Robinson and Norwood were able to show that Gram-positive bacteria (B-haemolytic Streptococcus and Staph. aureus) are ingested and killed completely as they pass through the gut of the larvae.[83-84] More recently the direct killing of Gram-negative bacteria (E. coli) by maggots was studied. Most of the bacteria were killed, but 17.8% of the hindgut still harboured live bacteria.[85] In vitro, maggot secretions were found to adequately kill Gram-positive bacteria but Gram-negative bacteria were killed less effectively.[79] Gram-negative bacteria appeared to grow faster in the presence of maggots, possibly as a result of an increase in the pH of the wound. This retrospective study showed that the chance of culturing a Gram-positive bacteria is higher before than after treatment with maggot therapy (p=0.07), and found the opposite effect for Gram-negative cultures (p=0.001). Looking at a subgroup of these 16 patients, namely the four patients in which the chance of culturing a Gram-negative bacteria after treatment with maggots increases (patient 1, 4, 9 and 12), shows an interesting effect. The only difference between this subgroup and the other 12 patients is that fewer maggots were applied (645 in the subgroup vs 1020 in the other group). Looking at another subgroup, namely the patients who were treated with a minimum of 1000 maggots in total (patients 2, 3, 11, 14, 15 and 16), the chance of culturing a Gram-negative bacteria decreased after treatment with maggots. The number of maggots needed to debride a wound is estimated at 10 larvae per cm^2 of wound, but there seems to be no maximum number of larvae per cm^2 of wound.[86] Special calculators have been developed to calculate the number of maggots needed to debride a wound, based on size and percentage of wound area covered with slough.[87] In accordance with in-vitro findings[79;83-85], maggot therapy appears to be more effective against Gram-positive bacteria. Reasons for faster growing of Gram-negative bacteria during maggot treatment could be because of a result of an increase in the pH of the growth medium. Another reason could be that endotoxins produced by Gram-negative bacteria are capable of destroying secretions produced by maggots.

Conclusion

The methodological limitations of this cohort study, which was open-label and non-comparative, preclude a definite conclusion on whether laboratory investigations can be used to guide discontinuation of larval therapy. However, we believe that, for our patients, laboratory investigations, especially leucocyte count, can help aid this decision, although they cannot replace clinical judgement. While they did not achieve significant results in this study, in our opinion other laboratory investigations, such as CRP and ESR, also have a value in demonstrating the astounding detoxificating effects of larval therapy.

In this study it was found that, Gram-positive bacteria are digested and killed more easily than Gram-negative bacteria. The authors believe that a higher number of maggots is not only needed for a larger wound, or for a wound covered with a higher percentage of slough, but also for a Gram-negative infected wound. A limitation of the present study was that all patients who were septic or had a severe wound infection were treated with antibiotics directed at the causative agent which would probably have influenced the subsequent cultures.

Table 1: Characteristics of the patients treated with sterile maggots.

Pat. No.	Sex	Age (years)	Diagnosis	Region of therapy	Underlying condition	Period of MDT (days)	Technique: free-range or biobag?	No. of maggots applied	No. of dressing changes
1	M	50	Osteomyelitis	Lower leg	Vascular	32	Free-range	800	9
2	M	60	Osteomyelitis	Knee joint	Vascular/ DM	12	Free-range	1000	4
3	M	41	Osteomyelitis	Both feet	Trauma	28	Free-range	2900	7
4	M	81	Osteomyelitis	Femur	Trauma/ Steroid/ DM/Vascular	28	Biobag	550	8
5	F	62	Osteomyelitis	Lower leg	Trauma/ Vascular	20	Biobag	360	6
6	M	70	Osteomyelitis	Lower leg	Trauma/ DM	25	Biobag	260	6
7	M	33	Osteomyelitis	Lower leg	Trauma	37	Biobag	500	10
8	M	59	Osteomyelitis	Elbow	Trauma	24	Biobag	240	6
9	M	38	Osteomyelitis	Heel	DM	83	Biobag	780	21
10	M	50	Fasciitis necroticans	Neck-head	RA/ Trauma	13	Biobag	560	4
11	M	46	Fasciitis necroticans	Abdomen and perineal region	Scrotal abces	19	Biobag	1200	5
12	F	88	Soft tissue infection	Upper leg	Trauma	27	Biobag	450	8
13	M	51	Soft tissue infection	Upper leg	Trauma/ Vascular	13	Biobag	100	4
14	M	54	Gangrene	Stump lower limb	Vascular/ DM	11	Free-range	2000	3
15	M	16	Gangrene	Both hands and feet	Meningococcal Sepsis	27	Biobag	2100	8
16	M	61	Ulcus cruris	Lower leg	Venous insuf./ DM/ RA/ Steroid	34	Biobag	1000	10
Average:		**54**				**27**		**925**	**7**

abbreviations: F = Female, M = male, DM = Diabetes Mellitus, RA = Rheumatoid Arthritis, Venous insuf. = Venous insufficiency.

Table 2: Laboratory test results for leucocytes (x 10e9/L), CRP (mg/L) and ESR (mm/h) at the first and last day of treatment.

Pat. No.	Leucocytes		CRP		ESR	
	First day	Last day	First day	Last day	First day	Last day
1	14.1	8.4	163	59	58	64
2	13	13.1	26	218	86	91
3	9.7	11.2	29	193	52	98
4	11.1	5.2	47	0	125	37
5	4.2	4.0	32	77	134	138
6	10.3	10.4	5	9	18	34
7	7.3	7	3	2	5	4
8	10.1	6.4	227	26	140	140
9	9.1	6.6	17	5	19	8
10	10.6	7.0	30	6	21	9
11	11.6	10.5	123	26	140	84
12	7.6	6.9	29	24	59	60
13	22.4	8.4	61	19	-	39
14	9.6	8.5	124	68	123	80
15	11.5	11.9	16	36	41	70
16	12.4	9.9	87	42	57	44
Average	10,45	8,4*	31	26	58	64
Range	4.2-22.4	4.0-13.1	3-227	2-218	5-140	4-140

*significant Friedman Test ($p<0.05$)

Table 3: The chance of culturing a gram-positive bacteria.

Patientnr.	Before maggots (1 month)	Maggot-therapy	After maggots (1 month)
1	0.66 (3)	0.62 (13)	0.38 (13)
2	0.8 (5)	1 (2)	1 (1)
3	-	1 (3)	1 (4)
4	0.5 (2)	0.3 (23)	0 (7)
5	0.75 (8)	0 (8)	0.66 (3)
6	0 (1)	0 (3)	-
7	0 (1)	0.2 (10)	0.2 (5)
8	2 (1)	0.5 (4)	0 (1)
9	1 (2)	0.33 (15)	0 (1)
10	0.6 (5)	0.1 (29)	2 (1)
11	0 (4)	0 (9)	-
12	0 (2)	0.17 (6)	1.25 (4)
13	0.55 (11)	0.33 (9)	-
14	0.8 (5)	0.1 (10)	0 (5)
15	1 (2)	1.5 (2)	-
16	0 (4)	0 (13)	0 (2)
Median	**0.66**	**0.20**	**0.20**
Average	**0.54**	**0.36***	**0.41**

* Non-significant (p=0.07)
In between brackets is the number of woundcultures.
\- missing value

Table 4: The chance of culturing a gram-negative bacteria.

Patientnr.	Before maggots (1 month)	Maggot-therapy	After maggots (1 month)
1	1 (3)	1.38 (13)	1.53 (13)
2	0.2 (5)	0.5 (2)	0 (1)
3	-	0 (3)	0 (4)
4	0 (2)	0 (23)	0.14 (7)
5	0.38 (8)	1.25 (8)	0.33 (3)
6	0 (1)	0 (3)	-
7	1 (1)	0.9 (10)	1 (5)
8	0 (1)	0 (4)	0 (1)
9	0 (2)	0.6 (15)	2 (1)
10	0.8 (5)	1.38 (29)	1 (1)
11	0 (4)	0.77 (9)	-
12	0 (2)	0 (6)	0 (4)
13	0 (11)	0.11 (9)	-
14	1 (5)	0.9 (10)	0.4 (5)
15	0 (2)	0 (2)	-
16	0.25 (4)	0.38 (13)	0 (2)
Median	**0.25**	**0.60**	**0.33**
Average	**0.29**	**0.51***	**0.4**

* Significant (p=0.001)
In between brackets is the number of woundcultures.

Chapter

3

MDT and factors influencing outcome

Based on the following article:

Annals of the Royal College of Surgeons of England
Pascal Steenvoorde[1,2], Cathrien E. Jacobi[3], Louk van Doorn[2] and Jacques Oskam[1,2] .
From the departments of Surgery[1] and the Wound Healing Department[2] of the Rijnland Hospital Leiderdorp and from the Department of Medical Decision Making[3], Leiden University Medical Center, Leiden both in the Netherlands.
Maggot Debridement Therapy of infected ulcers: patient- and woundfactors influencing outcome. A study on 101 patients with 117 wounds. Ann of the Royal Coll of Surg England 2007; 89(6): 596-602.

Introduction

Despite antibiotic treatment and other measures many chronic ulcers do not heal. Infection and bacterial colonization is one of the factors delaying wound healing. Based on literature, there seem to be no clear indications or contra-indications for MDT, but patients with open wounds and ulcers that contain gangrenous or necrotic tissue with infection seem suited for MDT.[88] Success-rates of MDT, reported in literature, vary, but seem to be around 80 to 90%.[55;89-90] The present study discusses the observations of MDT in patients with complex and chronic wounds in whom major limb amputation or sepsis was the only alternative, if no MDT would be performed. In total, 101 patients with 117 wounds, seen in our surgical department, were treated. Patient characteristics, wound characteristics and treatment characteristics are described. Moreover, factors are identified that significantly influence MDT-outcome. On the basis of these factors, patient selection for MDT could be improved.

Methods

Study characteristics

Patients: In the period August 2002 and December 2005, all patients who presented at the surgical department of the Rijnland Hospital, Leiderdorp, The Netherlands, with infected wounds with signs of gangrenous or necrotic tissue who seemed suitable for maggot debridement therapy (MDT), were asked whether they would enrol in a prospective case series study regarding MDT. All types of patients were included: patients from the dermatology department sent directly for this therapy, patients with infected diabetic feet, with arterial leg ulcers, with traumatic infected ulcers and with chronic wounds that would not heal despite treatment by the primary physician. Patients were excluded from the study if the treating surgeon believed an urgent amputation could not be postponed (for example in case of severe sepsis) or if life expectancy was shorter than a few weeks. Most patients had wounds of worst-case scenarios, for which the only alternative seemed to be amputation or surgical debridement (in theatre).

Protocol: Standard protocol prescribed patients to be treated in the outpatient department. If patients were too sick or already admitted, treatment was preformed while admitted. All black dry necrotic tissue was removed prior to the therapy. All patients gave informed consent to be treated by MDT. Antibiotic treatment was not a contraindication for MDT. Indications for antibiotic therapy were based on those formulated by the international consensus on diagnosing and treating the infected diabetic foot. These indications are bone or joint infection, extensive cellulites (>2.0 cm) or systemic signs.[91] Antimicrobial therapy was always broad covering staphylococci, streptococci, gram-negative bacilli and anaerobic bacteria. When culture and sensitivity results where

available, more specific therapy was considered. According to literature antibiotic therapy does not influence the effects of maggots.[92]

Technique of maggot application: Patients would come to the clinic twice a week for maggot placement or maggot changes. Maggots arrive early in the morning and can be ordered until 24 hours before the clinic begins (BiologiQ™, Apeldoorn, the Netherlands). Every three to four days, new maggots were placed on the wound until thorough debridement was reached.

Outcome: We defined 8 different outcomes of MDT, based on outcome definition in the literature.[55;88-90;93] and experience with the technique:

Effect of MDT observed (beneficial outcome)

- Wound fully closed by second intervention (for example split skin graft);
- Wound spontaneous fully closed;
- Wound free from infection and <1/3 of original wound size;
- Clean wound (free from infection/necrosis/slough), but same as initial size;

No effect of MDT observed (unsuccessful outcome)

- No difference observed between the pre- and post-MDT-treated wound;
- The wound is worse;
- Minor amputation (for example partial too amputation);
- Major amputation (for example below knee amputation).

Patient and wound characteristics

Patient characteristics: At presentation, the following patient characteristics were recorded: age, sex, weight, height, presence of diabetes mellitus, smoking behaviour, the presence of chronic limb ischemia and other relevant medical history.

Weight and height of the patient were used to calculate the patient's Body Mass Index (BMI), dividing weight (kg) by squared height (m). A BMI between 25 and 30 indicates that the person is overweight, while a BMI of 30 or more is classified as obesity.[94] A patient was recorded overweight accordingly. If a patient's height and weight at the time of MDT were lacking, the patient was scored as overweight if the treating surgeon and the nurse doing the actual maggot changes, thought so. Smoking behaviour was recorded as yes or no. A patient was recorded a non-smoker if non-smoking for more than three months. The diagnosis of lower chronic limb ischemia (CLI) was made if both pedal pulses of the involved foot were absent and/or the ankle-brachial pressure index was less than 0.6 and/or the absolute ankle pressure was below 50 mm Hg. Conservative wound healing usually takes place above the threshold of chronic critical limb ischemia. If the absolute systolic ankle pressure and/or the ankle-brachial index are below this threshold, foot pulses tend to be absent, the extremities are cold and wound pain is common. Wound healing in this group is difficult. The Second European Consensus[74] has outlined the following criteria for a diagnosis of chronic limb ischemia: recalcitrant rest pain or distal necrosis of more than 2 weeks' duration in the presence of (1) a systolic ankle pressure of 50 mm Hg or less, or (2) systolic toe pressure of 30 mm Hg or less, or (3) a transcutaneous oxygen pressure of 10 mm Hg or less. For patients with wounds above the ankle, these data were not recorded.

Wound characteristics: The following characteristics related to the wounds were recorded: ulcer site, presence of chronic venous insufficiency, whether trauma was cause of the wound, whether a fracture accompanied the trauma, depth of the wound, presence

of septic arthritis and the presence of wound infection.
Chronic venous insufficiency was recorded on clinical grounds and standard treatment consisted of three or four layer compression treatment. Depth of the wound was recorded as following: superficial (containing only epidermal and dermal layers) or deep containing bone, joint or tendon. In case of infection near a joint, it was recorded whether there was a septic arthritis. A diagnosis of wound infection was made if there was purulent discharge and/or two local signs present (warmth, erythema, lymphangitis, lymphadenopathy, edema or pain.

Therapy characteristics: Regarding the therapy, the following characteristics were recorded: the total number of maggots needed to reach the outcome, the number of maggot applications and whether or not the patient was admitted during the maggot therapy. Also the application-type was recorded.

Statistical analysis

To find characteristics of patients or wounds that might predict beneficial outcome of MDT, univariate analyses using Chi-square and T-test statistics were performed. If characteristics were showing a statistical trend ($p<0.100$) in the univariate analyses, they were included in the multivariate statistics. Multivariate logistic stepwise regression was performed with the dichotomous outcome (good result vs. bad result) as the dependent variable and the selected patient-, wound-, and treatment characteristics as the independent variables. Results were considered statistically significant if p-values were below 0.05. For inclusion in the multivariate analysis, the worst wound of a patient (if a patient presented with more than one wound) was included. If patients had similar wounds at both sides, one was chosen. If then no choice of wounds had been made, wounds at the heel or infected below knee amputation wounds were selected.

Results

Patient characteristics

From august 2002 until 31 December 2005, 101 patients with 117 wounds were treated with MDT in our hospital. During this period, 1 patient presented with 4 wounds in total (1.0%), 1 patient with 3 wounds (1.0%),11 patients with 2 wounds (10.9%) and 88 patients with 1 wound each (87.1%). The patient group consisted of 56 men (55.4%) and 45 women **(Table 1)**. Their average age was 71.0 years (range: 25-93 years, standard deviation (SD): 14.6 years). Forty-one patients (40.6%) were treated while admitted. Within the study period, 24 patients (23.8%) died. None of the patients died because of postponed amputation or from sepsis occurring at the wound site. One of these patients died during the actual MDT, although this death was not related to the therapy or wound. The patients who died were significantly more often classified in ASA class III or IV at study entry (91.7% vs. 64.9%, P=0.023), and suffered more often from diabetes mellitus (70.8% vs. 37.7%, P=0.009) than the other patients (81.8% versus 43.4%; p=0.047). Moreover, the patients who died seemed somewhat older than the other patients (75.4 years, SD: 12.0; vs. 69.6 years, SD: 15.1; P=0.086). There were two male diabetic patients treated with chronic wounds of the lower extremity who were on dialysis. Both diabetic patients unfortunately required a major amputation. Lower limb amputation in diabetics on dialysis is 14%. The proportion of patients requiring amputation on dialysis is approximately 4% per year.[95]

Wound characteristics

Most wounds (N=110; 94.0%) were lower extremity wounds, of which most were located on the lower leg (N=35; 29.9%) and heel (N=30; 25.6%) **(Table 2)**. The wounds existed on average for 7.2 months before starting with MDT (range 1 week-11 years; SD: 16.1 month). In 56.4% of the wounds (N=66), tendon, muscle or bone was visible.

Therapy characteristics

On average, 2.4 maggot applications (range: 1-11) were used on the wounds, with one (N=43) or two (N=35) applications as the most frequent **(Table 2)**. As one application remains 3 or 4 days on the wound, the treatment ended for most patients within or after one week. In total, 21,740 maggots were used to treat the 117 wounds, indicating an average of 186 maggots per wound (range 20-780).

Therapy results

In this study we defined 8 different outcomes. Of the 117 wounds treated with MDT, for 116 an outcome could be determined: 78 wounds (67.2%) had beneficial outcomes and 38 wounds (32.8%) had unsuccessful outcomes **(Table 3)**. MDT resulted in complete debridement and epithelialization in 37 of the 116 wounds (31.6%), it resulted in complete debridement and closure by secundary intervention in 23 wounds (19.7%), in 12 wounds (10.3%) the wound was free from infection and the wound size was less than one third of the initial wound size, and in 6 wounds (5.1%) the wound was free from infection, necrosis and slough, but remained its initial size.

Factors influencing outcome

All wounds caused by trauma had beneficial outcomes (N=24). All wounds in which there was a septic arthritis, had unsuccessful outcomes (N=13), as the entire joint including a part of the proximal adjacent bone had to be amputated (N=8/9; **Table 2**). These two characteristics are therefore very important as predictors of MDT outcome. The univariate analyses revealed the following characteristics that had a negative impact on successful outcomes of MDT treatment **(Tables 1 and 2)**: older age (P-value=0.033), chronic limb ischemia (P<0.001), non-traumatic origin of the wound (P<0.001), a duration of the wound of 3 months or more prior to MDT (P<0.001), a deep wound (P<0.001), and septic arthritis (P<0.001). Furthermore, the presence of diabetes mellitus (P=0.066) and clinical instead of outpatient treatment (P=0.096) showed a trend significance. The use of a biobag had a significant negative impact on successful outcome in the univariate analysis. (p=0.01)

The multivariate analysis showed that three characteristics additional to non-traumatic origin of the wound and the presence of septic arthritis, had predictive value for MDT outcome. An age of 60 years and older (Odds Ratio (OR): 7.3; 95% Confidence Interval (95% CI): 1.3-40.0), chronic limb ischemia (OR: 7.5; 95% CI: 1.8-31.1), and a wound with visible tendon, muscle or bone (OR: 14.0; 95% CI: 2.8-70.4) negatively influenced good outcome of MDT. These characteristics were adjusted for the other characteristics in the model.

Table 1: Characteristics of patients treated with maggot debridement therapy (N=101).

Characteristics*		Total	Good Result	Bad Result	P-value†
Number of patients		101 (100)	69 (69.0)	31 (31.0)	
Age (yrs)	Mean (SD)	71.0 (14.6)	69.6 (15.9)	74.1 (11.0)	
	< 60	21 (20.8)	19 (27.5)	2 (6.5)	0.033
	≥ 60	80 (79.2)	50 (72.5)	29 (93.5)	
Gender	Male	56 (55.4)	37 (53.6)	19 (61.3)	
	Female	45 (44.6)	32 (46.4)	12(38.7)	
Quetelet Index	≤ 25	62 (61.4)	46 (66.7)	16 (51.6)	
	> 25	39 (38.6)	23 (33.3)	15 (48.4)	
Diabetes Mellitus	No	55 (54.5)	42 (60.9)	12 (38.7)	0.066 (trend)
	Yes	46 (45.5)	27 (39.1)	19 (61.3)	
Current Smoker	No	66 (65.3)	46 (66.7)	19 (61.3)	
	Yes	35 (34.7)	23 (33.3)	12 (38.7)	
Chronic limb ischemia	No	48 (47.5)	44 (63.8)	3 (9.7)	<0.001
	Yes	53 (52.5)	25 (36.2)	28 (90.3)	
Outpatient treatment	No	41 (40.6)	24 (34.8)	17 (54.8)	0.096 (trend)
	Yes	60 (59.4)	45 (65.2)	14 (45.2)	
ASA-class	I	5 (5.0)	5 (7.2)	0 (0.0)	
	II	24 (23.8)	18 (26.1)	6 (19.4)	
	III	48 (47.5)	33 (47.8)	14 (45.2)	
	IV	24 (23.8)	13 (18.8)	11 (35.5)	
Deceased	No	77 (76.2)	14 (20.3)	9 (29.0)	
	Yes	24 (23.8)	55 (79.7)	22 (71.0)	

*: characteristics are displayed in N(%), unless otherwise specified.
†: Univariate results

Table 2: Wound and treatment characteristics (N=117) of 101 patients treated with maggot debridement therapy.

Wound characteristics*		Total	Good Result£	Bad Result£	P-value$
Number of wounds		117 (100)	78 (67.2)	38 (32.8)	
Traumatic origin	No	92 (78.6)	54 (69.2)	38 (100.0)	<0.001
	Yes	25 (21.4)	24 (30.8)	0 (0.0)	
Location	Too	9 (7.7)	5 (6.4)	4 (10.5)	0.030
	Foot	27 (23.1)	16 (20.5)	11 (28.9)	
	Heel	30 (25.6)	19 (24.4)	11 (28.9)	
	Lower leg	35 (29.9)	29 (37.2)	5 (13.2)	
	BKA†	9 (7.7)	3 (3.8)	6 (15.8)	
	Other	7 (6.0)	6 (7.7)	1 (2.6)	
Duration (months)	Mean (SD)	7.2 (16.1)	8.2 (19.4)	5.4 (5.0)	
	< 3	48 (41.0)	41 (52.6)	6 (15.8)	<0.001
	≥ 3	69 (59.0)	37 (47.4)	32 (84.2)	
Depth	Superficial	51 (43.6)	47 (60.3)	4 (10.5)	<0.001
	Deep‡	66 (56.4)	31 (39.7)	34 (89.5)	
Septic arthritis	No	104 (88.9)	78 (100.0)	25 (65.8)	<0.001
	Yes	13 (11.1)	0 (0.0)	13 (34.2)	
Wound diameter >2cm	No	28 (23.9)	60 (76.9)	29 (74.4)	
	Yes	89 (76.1)	18 (23.1)	10 (25.6)	
Biobag application	No	58 (49.6)	46 (59.0)	12 (31.6)	0.010
	Yes	59 (50.4)	32 (41.0)	26 (68.4)	
Outpatient	No	48 (41.0)	28 (35.9)	20 (52.6)	
	Yes	69 (59.0)	50 (64.1)	18 (47.4)	
Number of treatments	Mean (SD)	2.4 (1.8)	2.4 (1.9)	2.4 (1.6)	
	< 3	75 (64.1)	48 (61.5)	26 (68.4)	
	≥ 3	42 (35.9)	30 (38.5)	12 (31.6)	
Total maggots	Mean (SD)	185.8 (135.3)	179.7 (143.9)	200.5 (117.6)	
Maggots per treatment	Mean (SD)	85.1 (48.3)	79.8 (44.6)	95.6 (54.6)	

*: all characteristics are displayed in N(%), unless otherwise specified.
£: One patient died before the wound could be checked; therefore a result could only be given for 116 wounds.
$: Univariate results
†: BKA= below knee amputation
‡: Deep: visible tendon, bone or muscle

Table 3: Results of MDT in 101 patients with 117 wounds†.

	First wounds* (N=100†)	All wounds (N=116†)
	N (%)	N (%)
Good outcome		
1. Wound fully closed by second intervention (for example split skin graft)	23 (22.8)	23 (19.7)
2. Wound spontaneous fully closed	30 (29.7)	37 (31.6)
3. Wound free from infection and <1/3 of original wound size	11 (10.9)	12 (10.3)
4. Clean wound (free from infection/necrosis/slough), but same as initial size	5 (5.0)	6 (5.1)
Bad outcome		
5. There is no difference between before and after MDT	3 (3.0)	5 (4.3)
6. The wound is worse	1 (1.0)	1 (0.9)
7. Minor amputation (for example toe)	5 (5.0)	5 (4.3)
8. Major amputation (below knee amputation or above knee amputation)	22 (21.8)	27 (23.1)

†: One patient died before the wound could be checked; therefore no result could be given.
*: First wounds are the wounds for which the patients were included in the study.

Discussion

In this study we described the results of Maggot Debridement Therapy (MDT) in 101 patients with 117 wounds in total. Of the 117 wounds treated, 78 (67.2%) had beneficial outcomes and 38 (32.8%) had unsuccessful outcomes. It is very difficult to determine meaningful outcomes of MDT. It is even more difficult to compare MDT-results with results of other studies. In this study outcomes were not defined as wound scores[96], but outcomes were based on an intention to salvage limbs. Church and Courtenay suggested the following outcomes for MDT: complete, temporarily complete, relatively complete, significantly beneficial, partially beneficial, economical and failed.[88] These categories are somewhat misleading. A patient for example, that unfortunately, dies before complete wound healing falls in their category failed, but could in our study be placed in outcome category 3.

Wolff et al. reported successful debridement (66-100% of necrosis and slough removed) in 59/74 patients (79%). Their wounds were of mixed aetiology, with 51% arterial leg ulcers, 39% diabetes and 14% venous leg ulcers.[89] According to their definition our categories 1-4 would be defined as successful debridement. Courtenay et al.[90] reported their results of 70 MDT treated patients. Most wounds were leg ulcers.

Arterial insufficiency 22% and diabetes 16% were the mean etiologic factors. In total 50 wounds were fully or partially debrided (85%), 8 remained unchanged (14%) and 1 (2%) showed progression during the therapy. Mumcuoglu et al. reported their results on twenty-five patients suffering mostly from chronic leg ulcers and pressure sores in the lower sacral area. Underlying diseases were mainly venous ulcera (48%) and paraplegia (20%). Complete debridement was achieved in 38 wounds (88.4%).[55] Given the problems with defining outcome and trying to compare patient-groups with mixed aetiology: MDT seems to benefit the patient in about 70-80% of the cases, which is the case in our study.

Of the 117 wounds treated with MDT, 78 (67%) had beneficial outcomes and 38 (33%) had unsuccessful outcomes **(Table 3)**. Some of these wounds, however, were treated with MDT not to prevent a minor amputation, but to prevent a major amputation. Thus for some wounds, the unsuccessful outcome (7= minor amputation) was the only possible outcome (N=4). This unsuccessful outcome may be the best possible outcome, if a patient, for example, presents with a severe osteomyelitis of the toe. MDT is then initiated, and maggots can resolve all necrotic tissue, slough and bacteria, but they are unable to remove infected bone or tendon. This removal needs to be done surgically, thus through amputation of the toe. In such cases, minor amputation may be considered a successful outcome as major amputation has been prevented.

All wounds with a traumatic origin (N=24) healed completely. All wounds with septic arthritis (N=13) failed to heal and led to a unsuccessful outcome. Optimal maggot feeding can only occur when the maggot spiracles are exposed to air, therefore deep joint infections can't be treated with MDT. All septic joint infections described in this study, where small joints (most Metatarsal joints), this factor might therefore be an explanation of these failures. According to a multivariate analysis, wound duration before MDT treatment longer than three months, chronic limb ischemia, and septic arthritis negatively influenced successful outcome of MDT. Previous research showed that ischemia at presentation of diabetic ulcers significantly predicts healing rate.[97]

Outcome was not negatively influenced by sex, diabetes mellitus, smoking, location of the wound, wound size or overweigthness. In literature, wound healing seems to be negatively influenced by age[98], as we also showed in this study. Sex had no effect on the outcome of ulcers, which was comparable to other studies.[97] Ulcer size was a significant predictor in a study on 194 diabetic ulcers for amputation: ulcer size in the healed ulcer group was 1.1 (0.5-2.6) cm^2 and 3.9 (1.4-5.4) cm^2 in the amputation group.[97] In the study of Oyibo et al. the largest ulcers were the deepest and most infected, and were possible confounding factors. In our study, in which 45% of patients were diabetic, ulcer size was defined as smaller or equal to 2 cm in largest diameter or larger than 2 cm. We did not find any association between ulcer size and maggot therapy success. Increasing depth was found to be a major predictor of unsucsessful outcome. In an earlier published study we found that the contained technique significantly reduces it's effectivity, wich was also the case in this larger serie. However in a multi-variate analysis this effect could not be shown.

In conclusion, 78 of 116 wounds (67%) had a successful outcome, of which 53 healed completely and 11 healed almost completely. These results seem to be in line with literature. All wounds with a traumatic origin (N=24) healed completely, whereas all wounds with septic arthritis (N=13) failed to heal. According to a multivariate analysis, chronic limb ischemia (OR: 7.5), the depth of the wound (OR: 14.0), and an age of 60 years or older (OR: 7.3) negatively influenced outcome. Outcome was not influenced by sex, quetelet index, diabetes mellitus, smoking, ASA-classification at presentation,

location of the wound, wound size or wound duration. By carefully selecting patients for MDT could increase MDT-outcomes. This could lead to a reduction in overall-costs, in an improved acceptance of the therapy. Maybe even more importantly, this study seems to be the basis for a randomized study, for patient- treatment and wound-factors influencing outcome are now known.

Chapter

4

Considerations in application technique

Based on the following article:

Advanced Skin and Woundcare
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And the Department of Medical Decision Making[2], Leiden Univerisity Medical Center, The Netherlands.
Maggot Debridement Therapy : Free-range or contained ? An in-vivo study. Adv Skin Wound Care 2005 18(8):430-435.

Introduction

There are two different application techniques for MDT: the free-range technique and the contained technique. There is a debate on which method should be used. This retrospective study describes clinical observations in 64 patients, in order to see which technique is most effective.

Free-range technique

In his work, Baer[13] used a free-range technique in which the maggots were put freely in the wound. A "cage" was then placed around the wound, preventing the maggots from escaping. Sherman[99-101] describes the most widely applied free-range technique used today: Disinfected maggots are applied to the wound surface area, the wound and maggots are covered with a cagelike dressing, and the dressing is topped with nylon chiffon.

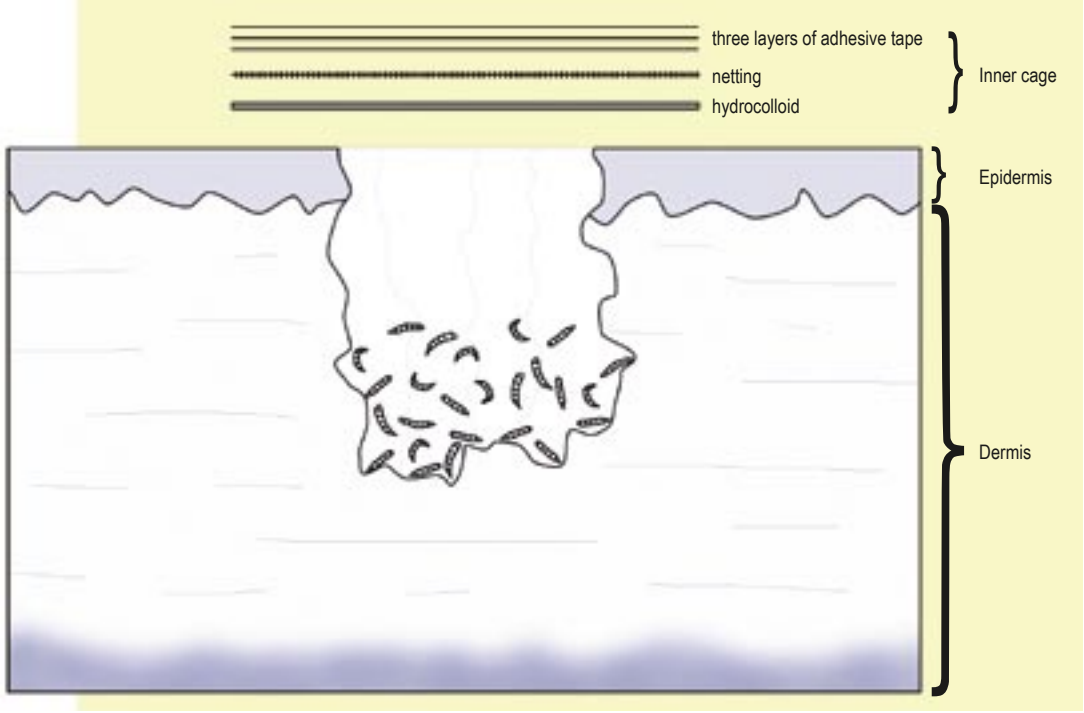

Figure 1: Free-range technique: Maggots are placed freely in the wound. To prevent escape, maggots are covered by an "inner cage."

Contained Technique

Because maggots in the contained technique are placed in the wound in a bag, maggot migration (or escape from the wound) occurs less frequently[102], which is essential for hospital hygiene.[103] Containment, however, can have a significantly negative effect on maggot growth.[104] Although physicians prefer the free-range technique, it is generally believed that patients would be more agreeable to MDT if the contained technique is used.[105] Maggots are not visible with the contained technique, which seems to improve patient acceptance. In a phenomenological study, Kitching[106] showed that the experience of MDT was not as scary as patients had imagined. Steenvoorde et al[107] reported that when patients were well informed, few were deterred by the idea of maggots, and there was a high degree of acceptance of MDT therapy with either application technique.

In addition, a recently introduced contained MDT technique[103] (Biobag; BiologiQ, Apeldoorn, The Netherlands) improves the acceptance of live maggots, facilitates their use,[108] and avoids physical discomfort.

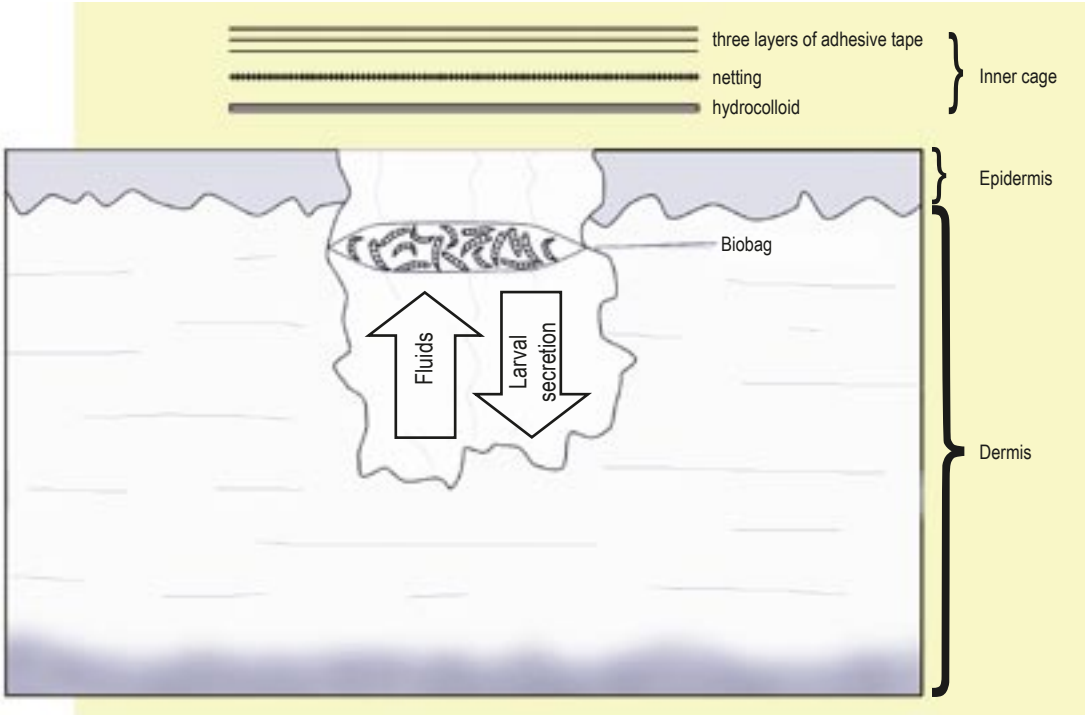

Figure 2: Contained Technique: In the contained technique, the maggots are placed in a bag (either self-fabricated or commercially available).

Methods

Between August 2002 and December 2004, 64 patients were enrolled in a study comparing free-range and contained techniques of MDT; all patients gave informed consent. These patients had presented at the Rijnland Hospital surgical department with 69 chronic wounds that showed signs of gangrenous or necrotic tissue. For this study, chronic wounds were arbitrarily defined as wounds existing for longer than 4 weeks.

In general, a chronic wound is defined as any wound that fails to heal within a reasonable period; there is no clear cutoff point for wound chronicity.[109] Patients were not eligible for the study if the treating surgeon believed an urgent amputation could not be postponed (eg, because of severe sepsis) or if life expectancy was less than a few weeks.

The 3 physicians and 3 nurses involved in the study recorded the following patient characteristics: age, sex, treatment location, and American Society of Anesthesiologists (ASA) classification, which is a physical status classification that serves as a prediction of anesthetic/surgical risks **(Table 1)**. In addition, they recorded the following wound characteristics: duration (in weeks), location (eg, toe, foot, heel, lower leg, below-knee amputation, or other), size (measuring the largest diameter), and depth (superficial, containing only epidermal and dermal layers, and deep, containing bone, joint, or tendon).

Table 1: Anesthesia/surgical risk classification*

Class I	—	healthy patient
Class II	—	patient with mild systemic disease
Class III	—	patient with severe systemic disease
Class IV	—	patient with severe systemic disease that is a constant threat to life
Class V	—	moribund patient; not expected to live longer than 24 hours, irrespective of surgery

* Based on guidelines from the American Society of Anesthesiologists.

Maggot debridement therapy

Because they were not commercially available at the start of the study, maggots were obtained from the nearest university medical center. Maggot application was done on Tuesday and Friday afternoons. Each MDT application remained on the wound for 3 to 4 days; MDT continued until thorough debridement was achieved. At the authors' institution, MDT was introduced with the contained technique, and the first 6 of 69 study wounds (9%) were treated this way. Since then, the standard application technique at the institution has been the free-range technique. However, there were no strict indications for either technique. The choice of application technique was determined by maggot availability, wound dressing difficulty, and physician preference. The following therapy characteristics were recorded: number of maggots needed, number of applications, type of application technique, and whether the patient was admitted to the hospital during MDT. With the free-range technique, maggots were placed freely on the wound **(Figure 1)**.[101] First, a hydrocolloid sheet (DuoDerm Thin; ConvaTec, Skillman, NJ) was taped to the skin surrounding the wound. Nylon netting (BiologiQ) was then taped on the wound edges. The purpose of the adhesive and the covering net (inner cage) was to act as a barrier to reduce maggot migration. The outer cage, consisting of wet gauze and a light bandage, was then wrapped over the net. Because maggots may not thrive if the wound is too dry, the outer cage was changed daily as needed. Laboratory results indicate that diluting maggot excretions with normal saline (0.9% sodium chloride) does not influence the effect of therapy; however, dilution with sterile distilled water causes a considerable drop in bacterial action.[110] Therefore, normal saline was used to wet the gauze in the present study. For the contained technique, maggots were placed in either a polyvinyl alcohol (PVA) or a net bag **(Figure 2)**. With the PVA bag, the maggots were enclosed between

2 thin (0.5 mm) layers of PVA hydrosponge, which were heat-sealed over a small cube of spacer material to form a bag.[103] These bags were either selffabricated or purchased commercially (Biobag). With the net bag, the maggots were placed in nylon netting with a small cube of spacer material; the netting was closed with a suture. The bag containing the maggots was then placed inside the wound. Similar to the free-range technique, nylon net was placed over the bag and taped on the wound edges. Wet gauze and a light bandage were then wrapped over the net. It is debatable, however, if an outer cage is necessary with the contained technique. A simpler application technique is to place the bag in the wound and cover it only with the wet gauze and a light bandage. The number of maggots per bag varied. The self-fabricated PVA bags contained 15 to 20 maggots,[102] commercially available PVA bags contained 100 to 200 maggots, and self-fabricated nylon netting bags contained 50 to 200 maggots.

Eight MDT outcomes were defined according to outcome definitions reported in the literature[55;88-90;93] and the authors' experience with the technique. These include (1) wound fully closed by secondary intervention (eg, split-skin graft), (2) wound fully closed spontaneously, (3) wound free from infection and less than one third the initial size, (4) wound clean (free from infection/ necrosis/slough, but same as initial size), (5) no difference, (6) wound worsened, (7) minor amputation (eg, partial toe amputation), and (8) major amputation (eg, below-knee amputation). Outcomes 1 through 4 were considered beneficial MDT outcomes; outcomes 5 through 8 were considered unsuccessful MDT outcomes. However, because it is difficult to define meaningful outcomes of MDT, and even more difficult to compare MDT results with other studies, the outcomes in the present study were not defined as wound scores.[111] Instead, outcomes were based on an intention to salvage limbs. Church and Courtenay[88] have suggested the following outcomes for MDT: complete, temporarily complete, relatively complete, significantly beneficial, partially beneficial, economical, and failed. These categories are somewhat misleading, however. For example, a patient who dies before complete wound healing would be included in the "failed" category. In the present study, however, the same patient would be placed in outcome category 3.

Descriptive analysis techniques (chi-square and t test) were used to describe the results of MDT using free-range and contained techniques (SPSS 11.5 for Windows; SPSS, Inc, Chicago, IL). Differences were found to be statistically significant if P values were below .05.

Results

Most patients were treated as outpatients, with 25 patients (39.1%) admitted to the hospital. The study included 37 men (57.8%) and 27 women (42.2%), with an average patient age of 68.5 years (SD 15.2). At presentation, most patients were in ASA categories III and IV (n = 39; 60.9%), indicating high anesthetic/surgical risk. Thirty-two patients were diabetic (50%), and 34 patients (53%) met the criteria of chronic limb ischemia. The Second European Consensus[74] criteria for diagnosing chronic limb ischemia are recalcitrant rest pain or distal necrosis of more than 2 weeks' duration in the presence of a systolic ankle pressure of 50 mm Hg or less. These data were not recorded for patients with wounds above the ankle. Of the 69 wounds, 54 (78%) were treated with free-range MDT and 15 (22%) were treated with the contained technique. In the contained technique group, 6 patients received the selffabricated PVA bag,[102] 6 patients received the commercially available PVA bag, and 3 patients received the self-fabricated net bag. Seventeen (25%) wounds were traumatic in origin, and most wounds had existed for more than 3 months before therapy (n = 43; 62%). Wounds were located on the toe

(n = 6), feet (n = 16), heel (n = 18), lower leg (n = 21), or other location (n = 8). Thirty-five wounds (51%) were considered superficial, and 34 (49%) were deep. There were no statistical differences in patient and wound characteristics between the 2 application techniques.

Average number of treatments/maggots

The mean number of maggot applications was 2.8 (range 1-11), indicating an average treatment of 9 days. Of wounds with successful outcomes (n = 50), 15 (30%) needed only a single application of maggots. Another 29 wounds (58%) were fully debrided within 1 week (ie, 1 or 2 maggot applications needed). Overall, about 12,580 maggots were used for 69 wounds, indicating an average of 182 maggots per wound (range 20-500). On average, the contained technique required more maggot applications than the free-range technique (4.3 vs. 2.4 treatments; P = .028) and more maggots to complete the treatment per wound (277 vs. 156 maggots; P < .001) **(Table 3)**. No statistical differences were seen between the techniques regarding the average number of maggots used per application (83 vs. 68 maggots; P = .101). Because more maggot applications were needed with the contained technique than with the free-range technique, the contained technique was also more costly. In addition, commercially contained maggots are more expensive.

Outcomes

Of 69 wounds, 50 (73%) had beneficial outcomes and 19 (27%) had unsuccessful outcomes **(Table 4)**. In 41 cases, the wound fully closed spontaneously or by secondary intervention. Minor amputation occurred in 4 cases (6%), with major amputation in 12 cases (17%). In the contained technique group, 6 of 15 patients eventually needed major amputation, compared with only 6 of 54 patients in the free-range technique group (P < .01). Free-range-treated wounds had more beneficial outcomes than wounds treated with the contained technique (n = 43 [79.6%] vs. n = 7 [46.7%]; P = .028).

Discussion

It is not completely clear why MDT promotes wound healing. Healing may be related to mechanical effects[112] or tissue growth effects[49]; it may be a result of the direct killing of bacteria in the alimentary tract of the maggots[83-85]; or it may be a result of antibacterial factors produced by maggots.[113] Some of these mechanisms seem to work less efficiently with the contained technique of MDT. However, the contained maggots still produce some activity, which supports the "soup" theory of Thomas et al.[48] This theory states that necrosis, wound exudate, and the various substances produced by maggots form a soup, which the maggots then further ingest.

Maggot containment may reduce effectiveness,[104] although in-vivo research has been lacking until now. In the present study, the free-range technique resulted in significantly better outcomes compared with the contained technique (P = .028). The mean number of treatments was also lower with the free-range technique than with the contained technique (P = .028). No differences in wound depth or size were found between the groups. The number of maggots used per treatment was significantly lower in the free-range technique (about 160 maggots) than in the contained technique (about 280 maggots) (P < .001). Caution should be used in interpreting these study results, however; the unequal number of wounds in the groups (free-range 54, contained 15) may have had an impact. Although the contained technique of MDT appears to be less effective than the free-range technique based on the present study, it has its place in wound care.

Patient preference,[114] bleeding complications in patients with natural or pharmacologically induced coagulopathies or exposed vessels or internal organs,[101;115] and fears about hospital hygiene[102] are indications for the contained technique. Additional studies are needed to justify these different indications, however.

Conclusion

Based on clinical observation of 64 patients and earlier published studies, this nonrandomized in vivo study suggests that the contained technique reduces the effectiveness of MDT.

Table 2: Patient and wound characteristics of 64 patients with 69 wounds, treated with MDT.

		Total	Free-range	Contained	P-value
Patient characteristics		64 (100.0)	50 (78.1)	14 (21.9)	
Age	Mean (SD)	68.5 (15.2)	67.8 (15.4)	71.2 (14.8)	P=0.459
	< 60 years, N (%)	15 (23.4)	12 (24.0)	3 (21.4)	P=0.841
	≥ 60 years, N (%)	49 (76.6)	38 (76.0)	11 (78.6)	
Sex	Male, N (%)	37 (57.8)	28 (56.0)	9 (64.3)	P=0.579
	Female, N (%)	27 (42.2)	22 (44.0)	5 (35.7)	
ASA-classification	I or II	25 (39.1)	21 (42.0)	4 (28.6)	P=0.548
	III or IV	39 (60.9)	29 (58.0)	10 (71.4)	
Treatment location	Clinic	25 (39.1)	18 (36.0)	7 (50.0)	P=0.523
	Outpatient clinic	39 (60,9)	32 (64.0)	7 (50.0)	
Wound characteristics		69 (100.0)	54 (78.3)	15 (21.7)	
Size	< 2 cm	20 (29.0)	17 (31.5)	3 (20.0)	P=0.585
	≥ 2 cm	49 (71.0)	37 (68.5)	12 (80.0)	
Depth	Superficial, N (%)	35 (50.7)	29 (53.7)	6 (40.0)	P=0.517
	Deep*, N (%)	34 (49.3)	25 (46.7)	9 (60.0)	
Duration (months)	Mean (SD)	8.3 (19.3)	9.1 (21.6)	5.4 (3.6)	P=0.509
	less than 3, N (%)	26 (37.7)	23 (42.6)	3 (20.0)	P=0.195
	3 and more, N (%)	43 (62.3)	31 (57.4)	12 (80.0)	

Deep*: visible tendon, bone or muscle

Table 3: Technical characteristics of MDT in 64 patients with 69 wounds.

Therapy characteristics		Total	Free-range	Contained	P-value
		69 (100.0)	54 (78.3)	15 (21.7)	
Nr. of applications	Mean (range)	2.8 (1-11)	2.4 (1-6)	4.3 (1-11)	P=0.028
Maggots per treatment	Mean (range)	182 (20-500)	156 (20-500)	277 (100-500)	P<0.001
Maggots per application	Mean (range)	71 (15-200)	68 (15-125)	83 (30-200)	P=0.101

Table 4: Results of MDT in 64 patients with 69 wounds, separated by application technique.

Outcome	Total	Free-range	Contained	P-value
	N (%)	N (%)	N (%)	
1. Wound fully closed by second intervention	21 (30.4)	18 (33.3)	3 (20.0)	P=0.075
2. Wound spontaneous fully closed	20 (29.0)	18 (33.3)	2 (13.3)	
3. Wound free from infection and <1/3 of initial size	7 (10.1)	5 (9.3)	2 (13.3)	
4. Clean wound, but same as initial size	2 (2.9)	2 (3.7)	0 (0.0)	
5. No difference	2 (2.9)	2 (3.7)	0 (0.0)	
6. The wound is worse	1 (1.4)	0 (0.0)	1 (6.7)	
7. Minor amputation	4 (5.8)	3 (5.6)	1 (6.7)	
8. Major amputation	12 (17.4)	6 (11.1)	6 (40.0)	
Total beneficial outcome (outcomes 1-4)	50 (72.5)	43 (79.6)	7 (46.7)	P=0.028
Total unsuccessful outcome (outcomes 5-8)	19 (27.5)	11 (20.4)	8 (53.3)	
Total	**69 (100.0)**	**54 (78.3)**	**15 (21.7)**	

Chapter

5

Case reports and case series

5A Maggots in amputation sparing surgery

Based on the following article:

Clinical Infectious Diseases
G.N. Jukema[1], A.G. Menon[1], A.T. Bernards[2], P. Steenvoorde[1], A. Taheri Rastegar[1], J.T. van Dissel[3]
Section of Traumatology, Department of Surgery[1], Department of Medical Microbiology[2], and Department of Infectious Diseases[3], Leiden University Medical Center, Leiden, The Netherlands
Amputation-sparing surgery by nature : maggots revisited. Clin Infect Dis 2002; 35(12): 1566-71.

Introduction

In these times of high-tech medicine, it can still be efficacious to resort to basic principles that have evolved in nature and that may help the physician combat specific medical problems.[9;116;117] For instance, traumatic wounds that fail to heal because of recurrent infections and underlying pathology, such as vascular insufficiency or diabetes mellitus, often leave physicians no choice but to resect the affected tissue. For minor wounds, this will not compromise the patient's quality of life, but for larger wounds on the extremities, as often occur in patients with vascular insufficiency or diabetes mellitus, amputation of part of a limb can be the only option. In selected cases, use of natural removers of necrotic and infected tissue—maggots (sterile larvae of *Lucilia sericata*)—may result in adequate wound healing and prevent the need to amputate a limb.[13;54]
In the past 3 years, we have applied sterile maggots to help remove infected necrotic tissue in 11 selected patients. We describe 2 of these patients in detail.

Case history 1.

A 16-year-old male patient was admitted to an intensive care unit because of meningococcal sepsis. The diagnosis was made on the basis of culture of skin biopsy samples, which yielded *Neisseria meningitides* serogroup C. The patient received intravenous treatment with ceftriaxone in combination with gentamicin and rifampin; after a few days, therapy was changed to benzylpenicillin G, U iv 12_106 per day.
The patient survived the acute episode of meningococcal sepsis but developed infectious necrosis of the extremities of the hands and feet **(Figure 1)**. The patient was transferred to the trauma unit of our institution (Leiden University Medical Center, Leiden, The Netherlands); at admission, he was still febrile. Open partial borderline amputation of all middle phalanges of the second through the fifth fingers of the left and right hands and a resection of the distal phalanx of the left and right thumbs were done. In addition, Syme's amputation (amputation at the level of the ankle joint) of the right foot was done, as well as extensive soft-tissue debridement of the left foot. Empirical treatment with flucloxacillin, 1 g iv 6 times daily, was administered. *Staphylococcus aureus* susceptible to flucloxacillin were isolated from cultures of swabs of the amputation wounds of the fingers, of the stump from the Syme's amputation, and of the left foot wound. Seven hundred fifty sterile maggots (Polymedics Bioproducts) in 20 porous, polyvinyl alcohol (PVA) bags ("biobags") were placed on the wounds intraoperatively **(Figure 2)**. After 3 days, the patient's clinical situation had improved substantially, and the high fever had subsided. The wounds showed significant improvement: granulating tissue had begun to grow and the amount of necrotic tissue was reduced. Therapy with maggots in biobags

was administered 7 times, and additional surgical debridement was not necessary. After 5 weeks, a superficial soft-tissue defect at the top of the partial amputation of the fifth finger of the right hand and the wound on the left foot were covered with autologous mesh grafts. After 2 months, the patient was discharged from the hospital to a rehabilitation center, and at 5 months all tissue defects had healed. The patient is able to walk with a prosthesis, without the help of crutches, and he is able to use both hands well **(figure 3)**.

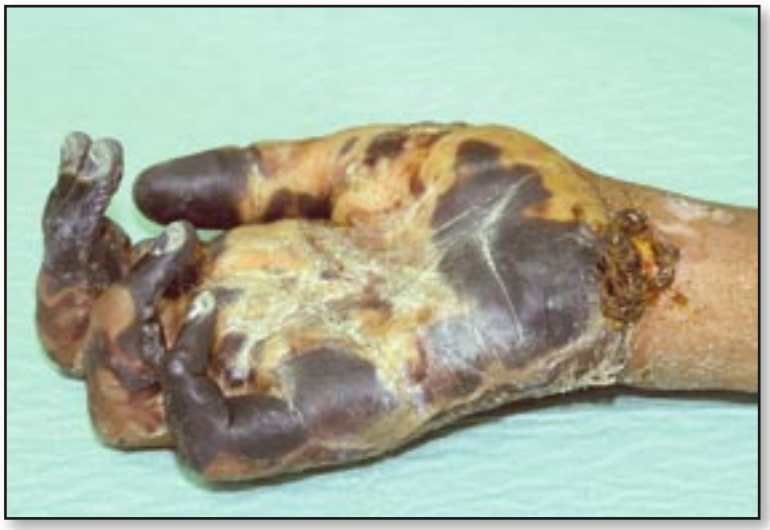

Figure 1: Patient 1. Necrosis of the hand, a sequela of meningococcal sepsis.

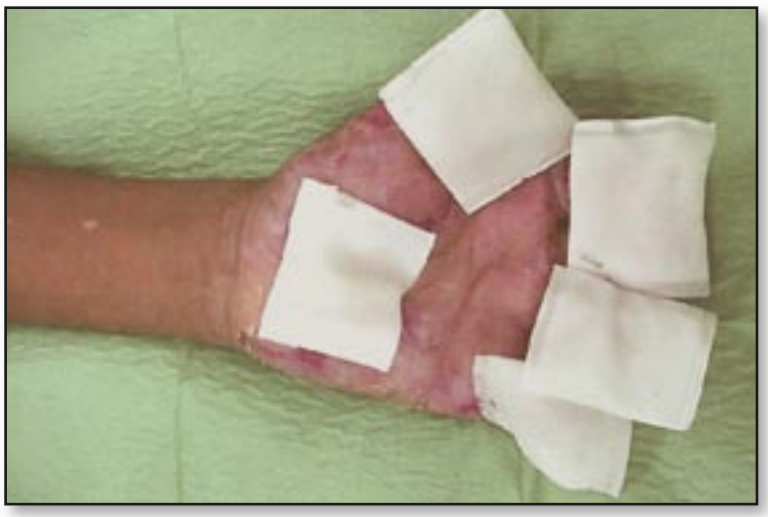

Figure 2: Patient 1. After partial amputation of the second through the fifth fingers, the left hand was covered with 5 "biobags" containing 20–30 maggots each. The porous polyvinyl alcohol membrane of the biobags allows free exchange of secretions and wound debris.

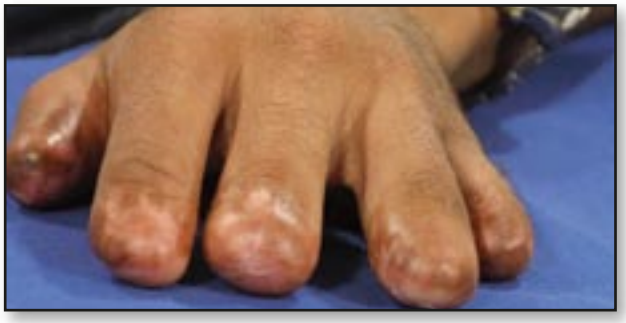

Figure 3: Patient 1. Left hand at the 1-year follow-up examination.

Case history 2.

A 54-year-old man with insulin-dependent diabetes mellitus and a smoking history of 35 pack-years had undergone an amputation of the first (great) toe of his left foot because of a nonhealing small wound. A surgical wound infection with *S. aureus* spread to the lower left leg, and amputation of the lower limb was done. Subsequently, the stump became infected and would have required an extended amputation **(Figure 4)**. At this point, the patient was transferred to our hospital. At admission, he had a severe infection with wet gangrene of the stump, which required immediate surgical debridement and partial resection of the soleus and gastrocnemius muscle. The remaining tissue, however, showed poor vascularization. Postoperatively, maggot therapy was initiated; the patient did not receive systemic antibiotic therapy. The local inflammation rapidly decreased, and the condition of the lower extremity and upper leg improved **(Figure 5)**. Within 1 week, signs of infection were subsiding, and the wound showed signs of granulation. For 2 weeks, the wound was treated with a combination of maggots and PVA foam (Biogard; Polymedics), after which maggot therapy was stopped and vacuum sealing treatment with PVA foam (Vacuseal/VAC Soft-Foam; KCl) was administered for another week. Finally, the wound was covered with a mesh graft transplant. The patient was discharged from the hospital after 5 weeks. Four months after discharge, the patient could walk with a prosthesis. After › 3 years of followup, no signs of infection have occurred **(Figure 6)**.

Figure 4: Patient 2. Gangrenous infection of the lower left leg stump.

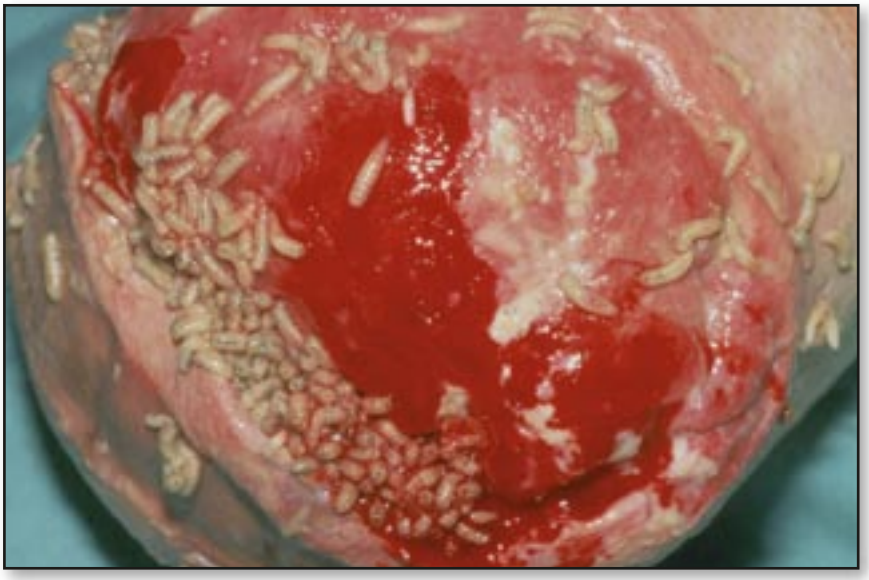

Figure 5: Patient 2. Lower left leg stump: 200–700 maggots were applied directly to the wound surface.

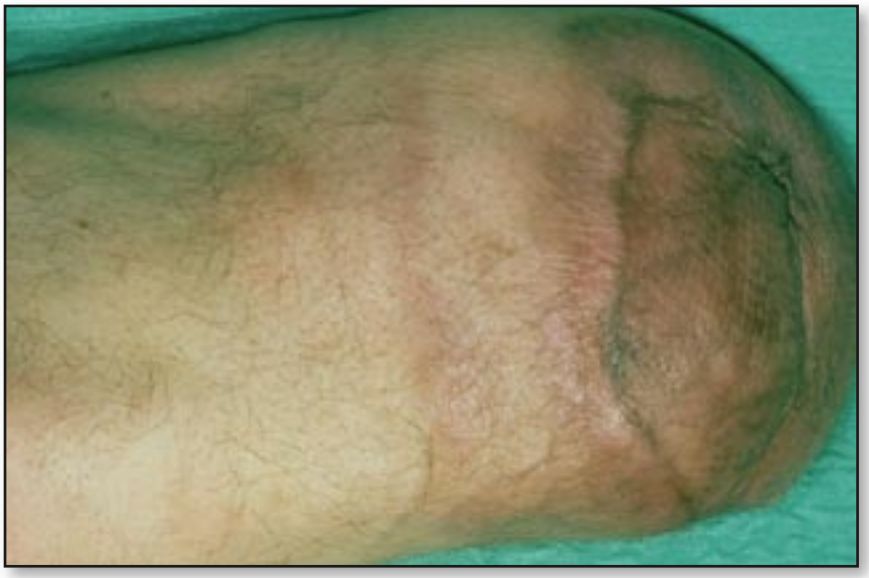

Figure 6: Patient 2. Lower left leg stump at the 1-year follow-up examination.

Discussion

These 2 patients with severe, secondarily infected necrotic wounds were treated with maggots, and this approach apparently helped remove necrotic tissue and prevented the need for disabling amputations of hands or limbs. For patient 1, an open amputation of both upper extremities below the elbow joint and both lower legs would have been necessary. For patient 2, who had diabetes, severe infection of the stump of the lower leg coincided with wet gangrene, a condition that usually necessitates amputation up to the upper leg. In both cases, maggots were applied to remove remaining necrotic tissue, thus helping to prevent the need for disabling amputations. In case of severe infections of a limb, natural "biosurgery" by sterile maggots may prevent the need for amputation and thus preserve the patient's quality of life.[118]

We used 2 methods to apply maggots to the wounds of the 11 patients in our series. For the first 3 patients, sterile maggots were put freely on the wound surface, which was then covered with a loose net dressing (**Table 1**; patients 2, 3, and 6). After 3–4 days, maggots grow to 8–10 mm in length and the wound becomes painful because of their biting and crawling **(Figure 5)**. Usually, large numbers of larvae (e.g., 1100) are applied to the wound surface, and administration of regional anesthesia often becomes necessary to reduce pain. Therefore, more recently, larvae have been incorporated within small "biobags," the size of ordinary tea bags, made of porous PVA membrane. Maggots in biobags are no less active necrophages than are free maggots; they secrete enzymes and absorb wound debris through the permeable bag membrane, but do not cause the painful sensation of biting and crawling larvae directly on the wound **(Figure 2)**. After the maggots have cleaned the wound, the biobags containing the maggots are removed, and rapid growth of granulating tissue may then be stimulated by vacuum sealing of the wound with PVA foam and polyurethane film[80] at a suction pressure of 50–60 kPa.
In the 2 cases we describe here, amputation of extremities could be avoided, despite the serious medical problems of severe infection and vascular insufficiency secondary to smoking and diabetes mellitus. The preservation of the extremities was possible, at least in part, because of application of "surgical" maggots. During the last 3 years, we have used maggots as adjunct treatment for 11 patients **(Table 1)**. The range of underlying diseases in these patients (open osteomyelitis in 5 patients, gangrene in 2, and soft-tissue infection or Charcot's joint in 4) matches the indications mentioned in the sparse literature on the subject: for example, osteomyelitis[119], venous ulcers[100;120], and diabetic foot infection.[118]

Although the methodological limitations of the present open-label, noncomparative cohort study precludes a definite conclusion concerning clinical efficacy, we believe that, for our patients, the local application of maggots, in most cases followed by vacuum sealing with PVA foam, may have helped prevent the need for disabling amputations. Nine of 11 patients recovered fully, and 2 died during follow-up. Deaths were not related to the primary infection for which maggots were applied: 1 patient died because of an accident and the other died months after treatment was finished because of an underlying hematologic disorder. Our experience shows that, even now, there may still be a place for an ancient treatment modality, such as application of "surgical" maggots.

Table 1: Summary of clinical characteristics of 11 patients treated locally with sterile maggots.

	Sex	Age (yr)	Diagnosis	Infected Region	Underlying condition(s)	Duration of Maggot therapy (days)	Dressing or no. of biobags used	Total no. of Maggots applied	No. of times maggots changed
1	M	50	Osteomyelitis	Tibia/fibula	Vascular	32	Net	800	9
2	M	60	Osteomyelitis	Knee joint	Vascular/ DM	12	Net	1000	4
3	M	41	Osteomyelitis	Both feet	Crush trauma both feet	28	Net	2900	7
4	M	81	Osteomyelitis	Femur	Trauma/ Steroid/ DM/Vascular	28	93	550	8
5	F	62	Osteomyelitis	Tibia/fibula	Trauma/ Vascular	20	31	360	6
6	M	54	Gangrene	Stump lower limb	Vascular/ DM	11	88	2000	3
7	M	16	Gangrene	Both hands and feet	Meningococcal Sepsis	27	78	2100	8
8	F	88	Soft Tissue Infection	Femur	Trauma	27	53	450	8
9	M	46	Soft Tissue Infection	Abdomen and perineal region	Fasciitis Necroticans	19	24	1200	5
10	M	51	Soft Tissue Infection	Femur	Trauma/ Vascular insufficiency	13	28	100	4
11	M	63	Ulcus Cruris Charcot feet	Lower leg	Chronic ulcers/ DM/ RA/ Steroid	34	78	1000	10

NOTE. DM = diabetes mellitus; F = female; M = male; RA = rheumatoid arthritis. "Biobags" indicates porous, polyvinyl alcohol bags containing maggots; "net" indicates a loose nylon mesh wound dressing over free maggots.

5B MDT for infection after breast- conserving surgery

Based on the following article:

Journal of Woundcare
P. Steenvoorde, J. Oskam
Department of Surgery Rijnland Hospital, Leiderdorp, The Netherlands.
Use of larval therapy to combat infection after breast-conserving therapy. J Wound Care 2005; 14(5): 212-213.

Introduction

Postoperative infection rates in breast conserving surgery, particularly with axillary clearance, can be as high as 18%.[121] Treatment generally comprises standard wound care: drainage of pus, removal of necrotic tissue and targeted antibiotic therapy. The main indications for MDT are infected ulcers of the extremities[89;102] with and without osteomyelitis, although all wounds with slough or necrosis can be treated with MDT.[122] Rare indications are subacute mastoiditis[123], necrotising fasciitis[124] and even infected malignant wounds.[3;125] Accidental myiasis in the breast has been reported,[126] but recent literature on the application of larvae in an infected breast is scarce. This paper presents a case study of a patient with an infected wound after breast conserving surgery for malignancy. Despite aggressive surgical and antibiotic therapy the wound persisted, healing only after MDT.

Case study

A 59-year-old woman underwent breast conserving surgery for a 4 x 2.5cm (Bloom Richardson grade III) adenocarcinoma of the breast. All lymph nodes were removed and were free of tumour. Adjuvant therapy comprised radiotherapy (66Gy) and chemotherapy. One day after surgery a large, uninfected haematoma was apparent post-surgery, which resolved leaving a fixed swelling. This was aspirated and clear fluid obtained.
No malignant cells were identified and bacterial cultures were negative. The wound produced clear fluid and the breast was red and tender, despite broad spectrum antibiotic therapy. Eight months after initial surgery, when the patient was being treated on an outpatient basis, a fluid-producing fistula was excised in theatre. Initially this gave a good result, but a month later the patient reported high fever and presented at the A&E department with severe mastitis. The wound was opened revealing large quantities of pus, which grew *Staphylococcus aureus* on culture. The patient was re-admitted and given intravenous antibiotics (Floxapen). The wound, measuring 7 x 2cm and 4cm deep **(Figure 1)**, was covered with yellow slough at the base **(Figure 2)**. It was initially managed with alginate dressings and topical negative pressure. Pathological examination revealed no recurrence of tumour. It was decided to treat the wound with MDT, to which the patient willingly agreed. The alternative, in our opinion, was further surgery, which would mean a breast amputation. MDT was administered in the outpatient department.
Some 60–80 maggots were applied twice three days apart. At the third application a

BioBag (BioMonde) was used as some maggots had 'escaped' on the previous occasion. To manage the wound odour, CarboFLEX (ConvaTec) was used, but with very limited effect. There was no alternative, except to change the outer dressing daily. Pain was adequately controlled with morphine (Durogesic 25µg plaster). After the first application the slough at the base of the wound had reduced. After the third (and final) application (10 days' therapy) the wound bed was free of necrosis, pus and slough **(Figure 3)**. An alginate (Kaltostat, ConvaTec) was used after this. The wound eventually healed and was fully closed four months after starting larval therapy. In total, 280 maggots were used.

Discussion

Breast-sparing surgery generally comprises removal of the tumour and ipsilateral sentinel lymph node removal. Standard radiotherapy and, depending on pathological examination, chemotherapy are given. Adjuvant chemotherapy does not produce signifi - cantly more wound infection.[127] Infection rates after breast-sparing surgery are between 10–18%, but axillary clearance seems to be a risk factor for infection.[121]

In our hospital the standard larvae application method is 'free-range' — the maggots are applied directly onto the wound. However, a BioBag is used as indicated by patient preference, and for difficult to-access wounds and uncontrollable pain. In the BioBag, live maggots are enclosed between two 0.5mm layers of polyvinyl alcohol hydrosponge, which are heat sealed over a small cube of spacer material to form a bag.[103] Maggot migration is reduced, as is pain sensation.[102] Application is simple and acceptance of live maggots is improved.[128] The bag containing the maggots is placed inside the wound and covered with a non-sterile nylon net which is taped in place. Wet gauze is applied over the net and covered with a light bandage.

Conclusion

Infection after breast conserving surgery is not uncommon. If the infection does not respond to standard surgical and antibiotic care, maggot therapy seems to be a treatment of last resort. To lower the risk of escaped maggots and increase patient-acceptance, we recommend the use of contained maggots in this specific type of wounds.

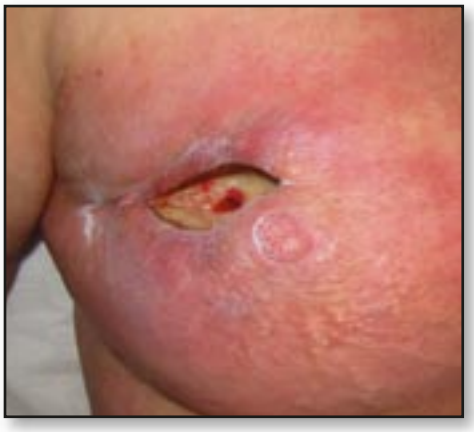

Figure 1: The wound before larval debridement therapy.

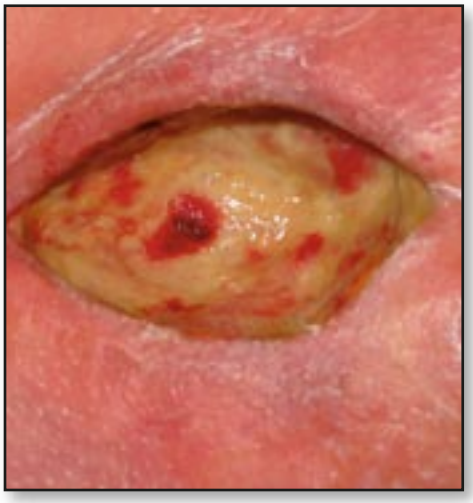

Figure 2: Yellow slough covering the base of the wound.

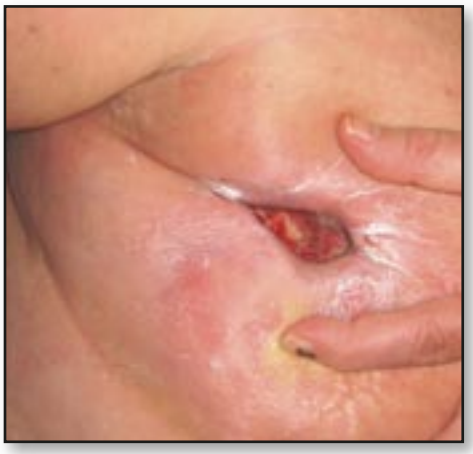

Figure 3: After three applications of MDT, the wound is clean and granulating.

5C MDT in Necrotizing fascitis

Based on the following article:

Wounds
P. Steenvoorde[1], C.E. Jacobi[2], Chun Yu Wong[1], G.N. Jukema[1]
From the Department of Surgery, section Traumatology[1] and Department of Medical Decision Making[2] Leiden University Medical Center, the Netherlands.
Maggot debridement therapy in necrotizing fasciitis reduces the number of surgical debridements. Report on 15 treated patients. Wounds 2007; 19(3): 73-78.

Journal of Woundcare
A.L. Rozeboom[1], P. Steenvoorde[1], H.H. Hartgrink[1], G.N. Jukema[2]
Department of Surgery[1] and Traumatology[2], Leiden University Medical Centre, Leiden, The Netherlands[2].
Necrotizing fascitits following a simple pelvic fracture: case report and literature review. J Wound Care 2006; 15(3):117-120.

Introduction

Necrotizing fasciitis is a rare, but potential lethal, bacterial infection of the fascial and subcutaneous tissues. The aetiology is not yet fully understood. Patients, however, often have a prior history of some sort of (trivial) trauma, like insect bite, scratch or abrasion.[129] Risk factors are associated with immunosuppression, such as advanced age, chronic renal failure, peripheral vascular disease, diabetes mellitus, and drug misuse.[130] Mortality rates of this condition remain high, ranging from 6-76%.[131] Early recognition and improved supportive measures lower mortality rates.[132] Bacterial cultures may show a wide variety of organisms[133], but *Group A Streptococcus (Streptococcus Pyogenus)* is the causative agent in up to 71% of all human cases.[134-135] *Streptococcus Pyogenus* is a Gram-positive, nonmotile, nonsporeforming coccus that occurs in chains or in pairs of cells. The treatment of choice, after the diagnosis necrotizing fasciitis has been made, is urgent radical surgical debridement in combination with broad-spectrum antibiotic therapy.[136] In most cases, repeated debridements are needed.

Maggot Debridement Therapy (MDT) has been proven to be very effective in the treatment of gram-positive bacterial infections[78;83;137-138], and therefore necrotizing fasciitis seems to be a logical indication for MDT. In this study, we report on the results of 15 patients, with necrotizing fasciitis treated with surgical debridement, antibiotic therapy in combination with MDT, in the period from November 2001 until November 2005. To illustrate, two patients will be presented in detail. Patient- and treatment characteristics of all treated patients will be presented and discussed. These characteristics were evaluated in order to provide insight into optimal MDT strategies for this condition.

Case 6 (Table 1)

A 79-year-old woman presented to our emergency department with a stable closed fracture of the left superior and inferior pubic ramus after a fall. Previous medical history included resection of the bladder, uterus and ovaries, and creation of a Bricker deviation (ureter-ileo-cutaneostomy) as a result of bladder cancer 23 years previously. The patient was admitted to the hospital and discharged three days after mobilisation. The fracture needed no special care, and full weight-bearing was allowed. Four days after discharge she returned to the hospital with a red, tender, swollen, left upper leg. She was severely

confused. On admission her vital signs were: Temperature: 35.6°C, Blood pressure: 115/80mmHg, Pulse rate: 95 beats per minute, Leucocytes: 17.5 x 109/l (normal 4.5–10.0 x 109/l) and C-reactive protein: 310mg/l (normal 0–20mg/l). An X-ray of the leg **(Figure 1)** showed free air in the subcutaneous tissue, which is indicative of a gasproducing bacterial infection. In theatre severe infection of the adductor muscles was found, and wide excision of necrosis, pus and a non-viable muscle fascia was performed. Initial Gram-staining of the necrotic tissue revealed Gram-negative and Gram-positive rods and Gram-negative and Gram-positive cocci, suggesting an abdominal focus. Indeed, the abdomen was rigid and distended without apparent peritonitis; computed tomography **(Figure 2)** showed fluid collection in the lower pelvis and free intraperitoneal air, indicating intestinal perforation. There seemed to be a connection between the fluid collection in the abdomen and the upper left leg. Further surgery revealed a perforation of the small bowel **(Figure 3)** in the lower pelvis near the fracture. A small segment of ileum was removed, and a side-to-side anastomosis performed. It was apparent that the perforation of the small bowel was related to the fracture site, where a sharp fracture line could be felt. To prevent a recurrence, a piece of vascularised omentum (fat) was sutured on the sharp edges of the fracture. To the medial side of the lacuna vasorum in the groin, we observed a false route where abdominal pus tracked to the upper leg. This was debrided, and a second debridement of the upper leg wound was undertaken. The femoral artery lay unprotected in the wound **(Figure 4)**, extending from the groin to the knee. The wound was treated with local gentamicin beads and several suction drains. Definitive culture grew Enterococcus faecalis, Hafnia alvei, Klebsiella oxytoca, Clostridium perfringens, Proteus mirabilis and Prevotella species, for which the patient received meropenem intravenously for two weeks. Three days after the first operation, the gentamicin beads and drains were removed. TNP therapy (Vacuum Assisted Closure [VAC], KCI, San Antonio, USA) was applied the following day **(Figure 5)**. Polyvinylalcohol foam (Versa Foam, KCI) covered the base of the wound and the exposed vessels, and was changed once in six days. Continuous pressure of 125mmHg was applied. After six days of TNP therapy, MDT was instigated because only the proximal part of the wound had improved; the distal wound still harboured large quantities of pus and necrosis. Thirteen polyvinylalcohol-biobags (Vitapad, Polymedics, Peer, Belgium), each containing 10 maggots, were placed in the wound at the same time **(Figure 6)**. After eight days of MDT the wound had improved significantly, so VAC Instill Therapy (KCI) was applied. This dual system provides TNP and delivers controlled amounts of topical solutions to the wound, in this case rinsing the foam three times per hour with an antiseptic agent: polyhexamethylbiguanid combined with polyethylene glycol in Ringer's solution (Lavasept 0.2% solution, Fresenius, Bad Homburg, Germany).[139;140] After two sessions (in total six days) the wound had improved considerably, and could be surgically closed over suction drains. Two weeks after the secondary closure the wound showed signs of healing and the patient was sent to a rehabilitation centre for further convalescence. The wound healed fully **(Figure 7)**.

Case 8 (Table 1)

A 46-year old male with no relevant medical history, besides an appendectomy and a perianal-fistula more than 20 years before current presentation, was referred to our hospital with a Fournier's gangrene, after first he was examined on the emergency department of the referring hospital. The patient had a history of smoking and used about 36-56 grams of alcohol daily (3-4 units). The patient presented with a red and tender right scrotum, which, in retrospect, had been present for seven days. He had

been treated, by his general practioner, with oral Ciprofloxacin® in the last 4 days, for a presumed infected sebaceous gland in the right groin. The patient was, after broad-spectrum antibiotic therapy with *netilmycin, amoxicyllin and metronidazol,* taken directly to theatre. An extensive fasciitis was found to be present on (predominantly) the right side of the abdomen, scrotum and perineum. A large part of the abdominal skin (including abdominal fascia) and scrotum had to be excised **(Figure 8)**. Initial gram-staining showed a mixed culture. Definitive cultures showed *Bacteroides species*, *Diphteroids* and a *Enterococcus Faecalis*. In the following 10 days, 6 surgical debridements were performed. Because sepsis persisted and the wound did not show any signs of healing, it was decided to perform MDT. Sterile maggots of the Lucilia Sericata were placed in biobags (containing an average of 20-30 maggots per bag) on the wound **(Figure 9)**. The patient was treated with sterile maggots of the Lucilia Sericata, for a period of 19 days. In total, 1200 maggots were applied. The wound clearly showed signs of granulation, with being able to secondary close the wound partially and perform a mesh graft on the rest of the wound, only 3 days after stopping of MDT and 32 days after the initial presentation to our hospital. Post-operative course was uncomplicated following this last operation. The patient could be discharged from the hospital uneventfully. The patient returned to his work, and remained in a good condition now more than three years after the last operation **(Figure 10)**.

Methods

Patients presented in our hospital with necrotizing fasciitis were treated with a combination of surgical debridement, antibiotic therapy and MDT. Patient- and treatment characteristics were, retrospectively, recorded from the patients' charts. Indications for MDT were necrosis and slough. All MDT-applications where discontinued when the wounds were 100% red and full of granulation tissue. The decision to discontinue the therapy was a clinical one, and was made by the last author in all patients. In an earlier study on patients with MDT, we have shown that leucocyte count were significantly lowered on the stopday of MDT compared to the startday of MDT.[141] In this study all maggot applications where perfomed with the contained technique, using biobags.

In the Biobag-technique (Vitapad®, Polymedics Bioproducts, B.V.B.A. Peer, Belgium), larvae are enclosed between two 0.5-mm-thin layers of polyvinyl alcohol hydrosponge, which are heat sealed over a small cube of spacer material to from a bag.[103] The bag containing the maggots is placed inside the wound. A net is placed over the bag and taped to an adhesive on the wound edges. Over the net, wet gauze and a light bandage are wrapped. Catheters were placed inside the bandages, in order to wet the gauze 3-times daily with normal saline solution (0.9%); to prevent maggot's death by dehydration. Every three to four days new contained maggots were placed on the wound until thorough debridement was reached. Every day the gauze where changed.

Possible differences in patient- and treatment characteristics and outcome were statistically tested using SPSS 12.0.1 for Windows (SPSS, Chicago, IL, USA) and evaluated. For analysis, we did split the patients into 2 groups by the median number of days of starting MDT after diagnosis of the necrotizing fasciitis.

Results

From November 2001 until December 2005, a total of 15 patients with necrotizing fasciitis were treated in our hospital with a combination of surgical debridement, antibiotic therapy and MDT **(Table 1)**. After diagnosis, all patients received broad-

spectrum antibiotic therapy, which was changed according to the antibiogram.

All patients were treated in theatre with a surgical debridement, after a clinical diagnosis of necrotizing fasciitis. There were 10 males (67%) and 5 females treated **(Table 2)**. Their age ranged from 18-79 year, with an average of 51 years. The necrotizing fasciitis was located in the groin area (N=6; 40%), on the upper leg (N=3; 20%), on the arm (N=3; 20%), on the abdomen (N=2; 13%) and in the head and neck region (N=1; 7%). Three patients were diagnosed with Fournier's gangrene (20%). Some patients suffered from conditions that are known to influence necrotizing fasciitis. The most important were cancer (N=4), diabetes mellitus (N=3) and trauma (N=3).

In total, the patients needed 43 surgical debridements in theatre (average 2.9 per patient, range 1-6). In three patients one of the surgical debridements was combined with a laparotomy; in two cases a resection of a part of the jejunum was performed in one case a colostomy was created. In 5 of the 15 patients, *Streptococcus Pyogenus* was the sole causative agent, in 2 patients *Streptococcus Pyogenus* was found in the initial culture combined with another causative agent. Therefore, in almost half of the patients (N=7; 46%) *Streptococcus Pyogenus* was cultured. Three patients did not have to be treated at the ICU. The average number of days at the ICU was 15 days, with a median of 4 days (range 0-135). The total hospital stay ranged from 3 to 135 days (mean 44 days; median 36 days). Two patients died (13%). A 58-year old male patient, with necrotizing fasciitis at the upper leg unfortunately died during MDT of cardiogenic shock in combination with a pneumonia. His death was influenced by his co-morbidity. Another male, 56 years old, with necrotizing fasciitis at the abdomen, right scrotum and upper leg also died, because of extensive liver metastasis of a primary urothelialcell carcinoma. His wound showed signs of granulation and was scheduled for secondary closure, but the condition of the patient deteriorated leading to his death.

For the treatment of all 15 patients, a total of 10160 maggots were used (average 680 per patient; range 90-2000). The maggots were applied in 679 bags; indicating an average of 45 bags per patient (range 9-100 bags). The MDT period was on average 17 days (range 3-38 days). We split the patients into an early treated group (within 9 days after diagnosis; N=8) and a late treated group (more than 9 days after diagnosis; N=7), as the median number of days of MDT start after diagnosis was 9 days. This in order to gain insight if early application of maggots in necrotizing fasciits might improve patient prognosis. Between the early and late-treated group there were no statistical significant differences in outcome, although it seemed that the early treated group had a shorter ICU stay (4 vs. 29 days; P=0.213) and a shorter total hospital stay (30 versus 59 days; P=0.094). The number of surgical debridements was statistically significantly lower in the patients where maggots were applied within 9 days after diagnosis (1.8 versus 4.1 surgical debridement; P=0.001). We could not show a statistical significant difference in other treatment characteristics, i.e. the number of maggots applied, the total MDT treatment time, and duration to wound closure, between the early and late-treated group. This lack of statistical significance, mostly due to small sample size, does not mean that these results do not have any clinical relevance.

In all patients, except the two patients who died, the wounds eventually healed. Of the 13 healed patients, two patients (15%) were treated with mesh graft and secondary closure, 6 patients with secondary closure (46%) and five patients with mesh graft only (39%). The average time until closure of the wound was performed was 12 days (range 0-39 days). Of all secondary closures and mesh grafts there were no failures. Secondary closure was performed on average after 10 days (range 0-21 days) and mesh graft at 19 days (range 0-39 days) after the end of MDT.

Discussion

In this report, 15 patients with necrotizing fasciitis are described, in whom the treatment consisted not only of surgical debridement and antibiotic therapy, but also of treatment with sterile maggots. We showed that this potentially lethal condition was, in most cases, successfully treated with this technique. The first description of necrotizing fasciitis in English literature was by the Confederate Army surgeon Joseph Jones in 1871, which he named 'hospital gangrene'.[142] Hippocrates however, was the first to give a description of the disease.[143] Meleney described an outbreak in Beijing in 1924[144]; it was not until 1952 when Wilson called it necrotizing fasciitis.[145] Other terms historically used include necrotizing erysipelas, haemolytic streptococcal gangrene, non-clostridial cellulites, non-clostridial gas gangrene, synergistic necrotizing cellulites, bacterial synergistic gangrene, necrotizing cellulites and gangrenous erysipelas.[146] Today the preferred term is necrotizing fasciitis.[131] Necrotizing fasciitis can affect any part of the body, but the extremities, the perineum and the truncal areas are the most commonly involved.[132] In this study most patients had a necrotizing fasciitis of the groin region (40%); the extremities were affected in 40% of the cases. Alfred Jean Fournier described necrotizing fasciitis of the perineum and scrotum, which is now referred to as Fournier's gangrene.[147] Fournier's gangrene predominantly occurs in the male population in a ratio of 1:10. Up to 2000, 1726 cases have been described in literature.[148] Fournier's gangrene is mostly due to infection from local skin, urinary tract of colorectal region.[148] Mortality rates for necrotizing fasciitis reported in English literature range from 6-76%; mortality rates are significantly increased if operative debridement is delayed.[131] In our study two patients (13%) died, one of progressive cancer metastasis and the other due to co-morbidity. Failure to recognize and diagnose fasciitis probably contributes to the high mortality rate.[149] We believe in our patients, mortality was not related to late diagnosis, nor was it due to postponed surgical debridement. Diagnosing necrotizing fasciitis is not simple. Pathognomonic for the disease are crepitus (present in 37% of cases) and soft tissue air on plain radiograph (57% of cases).[130] However, diagnosis remains a clinical one; severe pain disproportionate to local findings in association with systemic toxicity should raise the suspicion.[132]

In modern times, MDT has proven to be a valuable treatment option for various indications. In 2000, Wollina et al. described indications for MDT; fasciitis necroticans was not separately mentioned.

In-vitro and in-vivo investigations have shown that sterile maggots (larvae of Lucilia sericata) are especially capable in the treatment of infected wounds with gram-positive bacteria. Necrotizing fasciitis, which is mainly caused by gram-positive bacteria, therefore seems to be a perfect indication for MDT. [78;83;137;138] The treatment of choice after the diagnosis necrotizing fasciitis has been made, is urgent radical surgical debridement in combination with broad-spectrum antibiotic therapy.[136] In most cases, repeated debridements are needed. There have been reports of necrotizing fasciitis treated with maggots; but only in the form of case-reports. In recent literature, successful debridement with MDT of fasciitis of head and neck[124] and Fournier's gangrene[150] has been described by others as well. In literature it's debated that MDT is contraindicated in cases of rapidly advancing infections (like necrotizing fasciitis).[151-152] We disagree, although we would like to stress, that we believe the first debridement in case of necrotizing fasciitis should always be surgical. Only after administration of broad-spectrum antibiotic therapy and surgical debridement, maggots may be placed on the wound, as an additional treatment method, not as the only one. After a few day's when the results of bacterial cultures of the

wound are present, antibiotic treatment can be adjusted to a smaller spectrum if needed. In this patient series, we have shown that relatively early application of maggots reduced the number of performed surgical debridements. In the early treated group the number of surgical debridements was considerably lower compared to the late treated group (1.8 versus 4.1; P=0.001). This means that the use of maggots reduced the necessity to go back to theatre and perform a surgical debridement. We would like to stress that MDT is not the only woundtreatment available for necrotizing fasciitis, after adequate debridement and disinfection others treatments are sometime necessary before wound closure can be achieved. Vacuum assisted closure (VAC®) is a very potent wound therapy to stimulate further granulation tissue. In necrotizing fasciitis VAC has proven its value. [153-155]

We believe that any reduction in surgical debridement could, eventually, lower the high mortality rates associated with necrotizing fasciitis. Furthermore we believe cosmetic and functional outcome might be improved, for the extension of surgical procedures is reduced. This is because maggots are able to discriminate more effectively between viable and non-viable tissue, compared to the surgeon's knife. Caution should be taken to conclude definitively that MDT replaces the surgical debridement altogether, which can not be concluded from a retrospective case-series. We believe however that necrotizing fasciitis is such a dreadful disease with a high mortality and morbidity, that any possible reduction in the number of needed surgical debridements in this sick population might improve prognosis for these patients.

Table 1: Necrotizing Fasciitis: Characteristics of Patients treated with Maggot Debridement Therapy.

Nr	Sex	Age (yr)	Region	Underlying condition	Primary culture	Result (days after stopping MDT)
1	F	36	Lower Arm	Trauma	Pseudomonas	MG and SC (0 days)
2	M	62	Elbow/Arm	DM	Streptococcus pyogenus	SC (21 days)
3	F	30	Abdomen	Infected Lumbar Neurostimulator	Mixed culture	SC (2 days)
4	M	50	Head-Neck Thorax	RA (corticosteroid therapy)	Streptococcus pyogenus Pseudomonas Candida albicans	SC (14 days)
5	M	72	Gluteus/ Upper Leg	CLI, bladder malignancy	Clostridium septiceum	MG (14 days)
6	F	79	Upper leg	Trauma Menigneoma	Enterococcus faecalis, Hafnia alvei, Klebsiella oxytoca, Clostridium perfringens, Proteus mirabilis and Prevotella species	SC (3 days)
7	F	70	Groin	Obese. Incarcerated femoral hernia	Faecal flora	MG (39 days)
8	M	46	Abdomen/ Perineum/ Scrotum	-	Streptococcus pyogenus	MG + SC (3 days)
9	M	56	Groin and Scrotum	Prostatitis DM	Streptococcus pyogenus Klebsiella oxytoca Pseudo-monas	MG (0 days)
10	M	54	Scrotum	Surgery for a Hydrocele	E. Coli	MG (10 days)
11	M	58	Upper Leg	Pneumectomy: Thymoma, Grawitz, Pacemaker, Cardiac-Decompensation	Klebsiella oxytoca	† on day 3
12	M	40	Elbow/Arm	Trauma	Streptococcus pyogenus	SC (0 days)
13	M	30	Upper Leg	DM	Streptococcus pyogenus	SC (20 days)
14	M	56	Abdomen/ Right Sacrum/ Upper Leg/ Perineum	Open abdomen after neobladder for urotheelcarcinoma	Stenotrophomonas maltophilia Enterococcus faecalis.	† Died on day 73
15	F	18	Left Groin	-	Streptococcus pyogenus	MG (30 days)

abbreviations:
DM = Diabetes Mellitus, RA = Rheumatoid Arthritis, CLI = chronic limb ischemia, MG = Mesh Graft, SC = Secondary Closure
Patient nr. 8 has been briefly mentioned in a previous report, however in that article the number of biobags has been erroneously reported as 88 (instead of 64).

Table 2: Summary of Patient- and Treatment Characteristics of 15 Patients who presented with Necrotizing Fasciitis and were treated with Maggot Debridement Therapy.

		Total group	Early MDT (≤ 9 days)	Late MDT (> 9 days)	P-value†
Sex	Male (N;%) Female (N;%)	10 (66.7) 5 (33.3)	6 (75.0) 2 (25.0)	4 (57.1) 3 (42.9)	
Age	Mean (SD)	50.5 (17.2)	51.1 (20.9	49.7 (13.5)	
MDT start (days after diagnosis)	Mean (SD)	9.4 (11.6)	2.3 (3.0)	17.6 (12.6)	‡
Number of Surgical Debridements	Mean (SD)	2.9 (1.6)	1.8 (0.9)	4.1 (1.3)	0.001
Number of maggots	Mean (SD)	713.3 (536.1)	603.8 (581.2)	838.6 (492.2)	
Number of bags (days)	Mean (SD)	45.3 (25.9)	41.9 (27.3)	49.1 (25.8)	
Duration of MDT	Mean (SD)	16.9 (8.9)	16.1 (10.8)	17.7 (7.0)	
ICU stay (days)	Mean (SD)	15.4 (34.0)	3.6 (3.3)	28.9 (47.8)	
Hospital stay (days)	Mean (SD)	43.5 (33.4)	30.0 (22.9)	59.0 (38.3)	0.094
Result	Mesh Graft Sec. Closure MG+SC Granulation Death*	4 (30.8) 6 (46.2) 2 (15.4) 1 (7.7) 2	1 (14.3) 5 (71.4) 0 (0.0) 1 (14.3) 1	3 (50.0) 1 (16.7) 2 (33.3) 0 (0.0) 1	
Time to result (after stopping MDT)	Mean (SD)	15.5 (19.9)	13.1 (10.5)	18.1 (27.9)	

* Death of these patients was not due to MDT.
† As the patient group consisted of so little patients, only P-values ‹ 0.200 are presented.
‡ As the patients were split for this variable, the variable was, of course, statistically significantly different between the groups (P=0.005). **Figures**

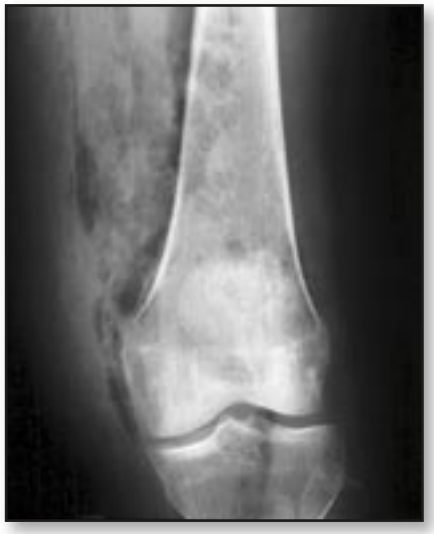

Figure 1: (patient 6). X-ray of the distal left femur showing air in the soft tissues, which is suggestive of necrotising fasciitis.

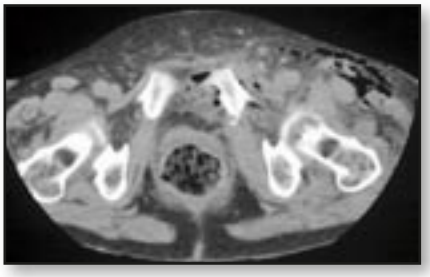

Figure 2: (patient 6). CT scan of the abdomen showing fluid and free intraperitoneal air near the fracture, which is suggestive of a gastrointestinal bowel perforation.

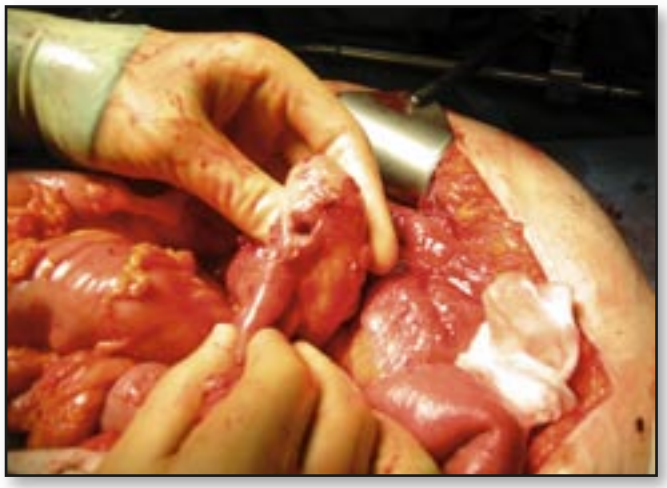

Figure 3: (patient 6). The perforation in the small bowel causing the necrotising fasciitis.

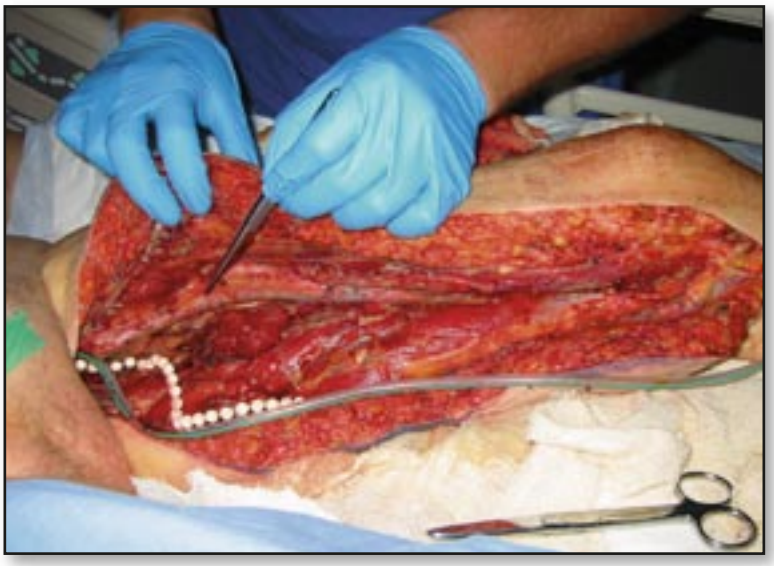

Figure 4: (patient 6). After debridement the wound was left open. Gentamicin beads and a suction drain were placed near the pubic bone. The exposed femoral artery is shown.

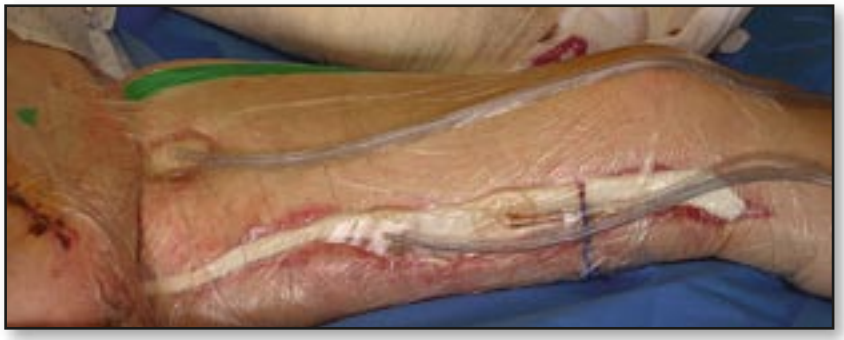

Figure 5: (patient 6). The wound was initially treated with TNP therapy.

Figure 6: (patient 6). A Vitapad® is placed in the wound.

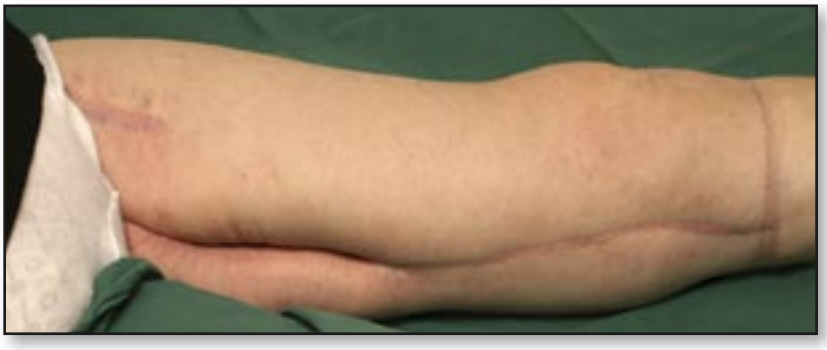

Figure 7: (patient 6). The wound is fully healed.

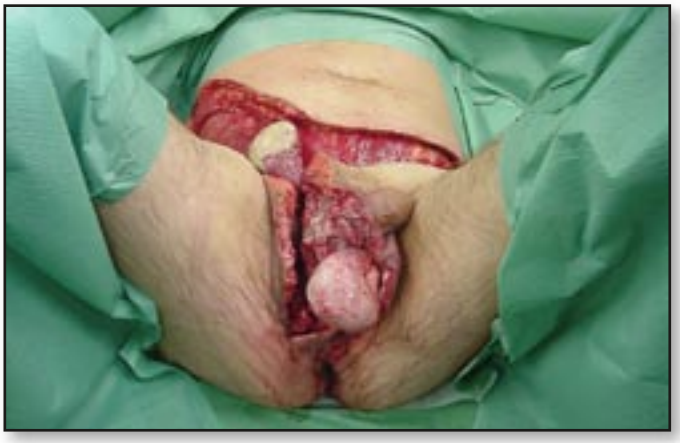

Figure 8: (patient 8). After surgical debridement and fasciectomy of the abdominal fascia, perineum and scrotal fascia.

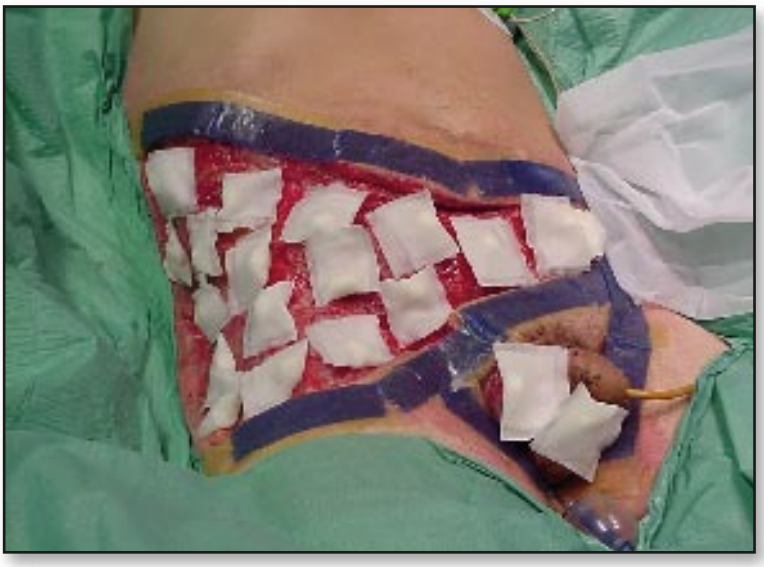

Figure 9: (patient 8). The Vitapads® are placed on the wound. The wound edges are taped with an adhesive tape in order to prevent maggot escapes.

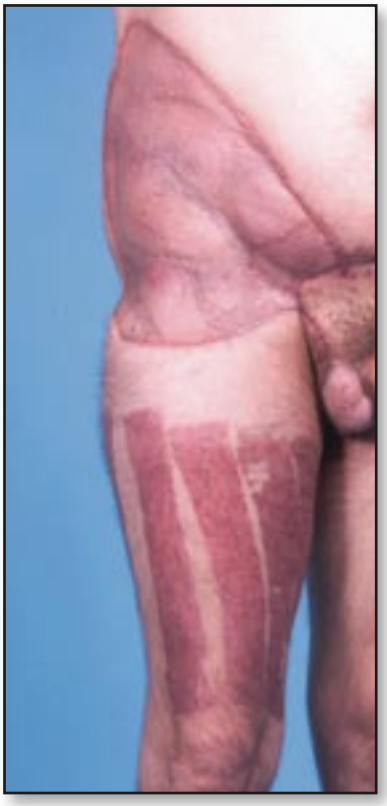

Figure 10: (patient 8). Post-operative end-result after 1 year; the wound fully healed after mesh grafting.

5D MDT in infected amputation wounds

Based on the following article:

Journal of Prosthetics and Orthotics
P. Steenvoorde, J. Oskam
Department of Surgery Rijnland Hospital, Leiderdorp, The Netherlands.
'Modern Wound treatment of infected transtibial amputation: JPO 2006; 18:17-20.

Introduction

One of the most disastrous complications of a transtibial amputation, besides death, is gangrene and opportunistic infection, necessitating a transfemoral amputation. This gives not only a higher mortality rate[156] but also reduced ambulation rates.[157] Wound complications occur in 5% to 22% of lower extremity amputations.[156-158] Conversion rates to transfemoral amputation are estimated to be between 9% and 19%.[156;158] MDT of infected transtibial amputation has been described before, but not in a large series.[102] Since the first report of vacuum-assisted closure (VAC) therapy in 1997, more than 100 articles on the subject have been published.[81] The technique is simple: a subatmospheric pressure is applied to a wound by means of an open-cell foam in the wound connected with a tube to a vacuum source. The fluid is then collected and removed.[159] The technique removes interstitial fluid and potential harmful inflammatory mediators.[160] Furthermore, it lowers interstitial pressure, thereby promoting the expansion of vessels. There is also a presumed reduction of bacterial load, although the latter is debated.[16]

Methods

From August 2002 to December 2004, five patients with infected transtibial wounds were treated with MDT in our hospital **(Table 1)**. All applications were performed in our outpatient department, including our admitted patients. We used two application techniques: the contained technique and the free-range technique.

Case 1

A 59-year-old man with no relevant medical history was treated for a painful ingrown toenail with a complete nail resection. This was complicated by severe infection of the hallux, unresponsive to antibiotic treatment. A stenosis of the superficial femoral artery was successfully treated with radiological percutaneous transluminal angioplasty.
The condition of the patient suddenly deteriorated, necessitating intensive care admission. He needed several laparotomies for a perforation of his colon, necessitating resection of the left colon and the creation of a colostomy. In the same period, a transtibial amputation was performed. The amputation wound deteriorated, with a severe infection unresponsive to antibiotic treatment **(Figure 1)**. We advised the patient to undergo a transfemoral amputation, which the patient refused. He urged us to try maggot debridement therapy. We performed a stump revision, removing the black eschar on removing 1 to 2 cm of the tibia. We started treating the wound with maggots incorporated in a polyvinyl alcohol biobag **(Figure 2)**. In this biobag, the maggots can still act as necrophages. The biobag was placed in the wound, which was subsequently covered with a nylon net and attached to the skin through several adhesive layers to prevent the

maggots from escaping and prevent further damage to the skin. The total maggot treatment time was 4 weeks, with two weekly changes. In total, 240 maggots were used. Eventually, the wound closed secondarily, and the patient is now ambulating with a prosthesis.

Figure 1: Patient 1. Severely infected transtibial amputation, with necrosis and pus draining on the lateral side. Wet gauze is placed in the marrow of the tibia.

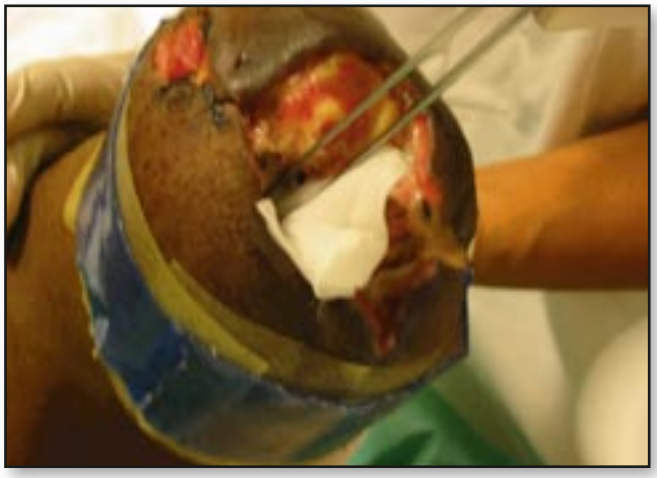

Figure 2: Patient 1. Polyvinyl alcohol bag filled with approximately 20 live maggots is placed in the wound.

Case 2

A 71-year-old man with a history of insulin-dependent diabetes was treated in our hospital for an osteomyelitis of the fourth toe of the left foot. The patient was obese and had a history of hypertension, hypercholesterolemia, and a cerebrovascular accident. Angiography revealed a complete stop of the popliteal artery just proximal to the trifurcation. A femoral- pedal bypass seemed feasible, but the infection progressed and we feared a possible infection of the distal anastomosis. Adequate debridement had to be performed first. The toe was amputated. Despite adequate debridement and appropriate antibiotic therapy, a plantar abscess developed, necessitating a transtibial amputation. Unfortunately, this wound did not heal. There was a wound dehiscence with necrosis and pus, but muscles seemed viable. At this time, MDT was started. The maggots were put freely on the wound, covered only by a net, to prevent the maggots escaping. The patient was treated in the outpatient department. After 1 week of MDT (200 maggots used), the wound was fully clean and VAC was started **(Figure 3)**. VAC was also performed in the outpatient department, and after 2 weeks, the wound **(Figure 4)** could be secondarily closed. After removal of the stitches, the patient started ambulating and is now ambulating well with a prosthesis.

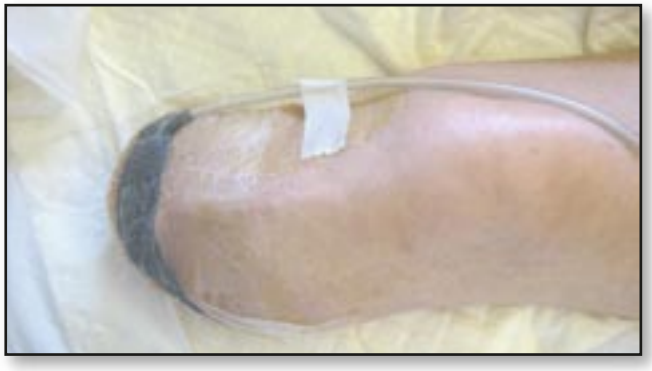

Figure 3: Patient 2. Wound treated with vacuum-assisted closure therapy.

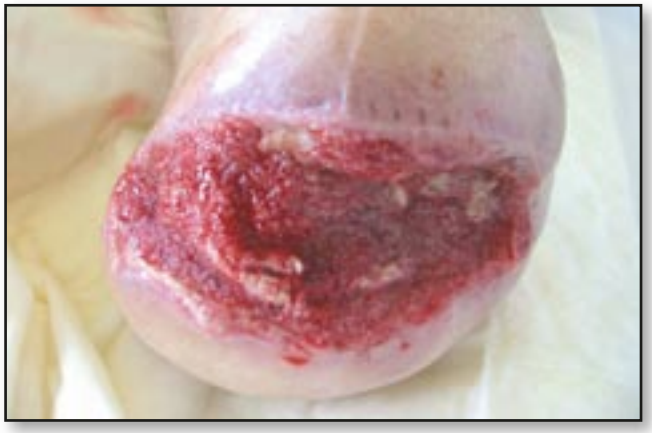

Figure 4: Patient 3. Granulating wound after vacuum-assisted closure therapy. The wound was subsequently successfully secondary closed.

Results

In total there were five patients treated (see **Table 1** for characteristics) with maggots in order to prevent conversion of the amputation level. All patients (3 male, 2 female; mean age, 71 years; range, 59 to 85 years) had vascular insufficiency. None of the patients was on dialysis. Two patients were diabetic. Four patients had a severe infection of the transtibial amputation, with visible bone. Two patients were treated while admitted, two while in a nursing home, and one patient was treated ambulatory. One patient was treated with the biobag technique. Eventually, four wounds healed completely; one needed conversion to a transfemoral amputation. Three patients are ambulating now with a prostheses. In this article we have described five patients with severe infection of transtibial amputations in which the use of MDT and VAC resulted in fewer conversions to transfemoral amputation, with positive effects on mortality and morbidity.

Table 1: Characteristics of patients with infected transtibial amputation treated with maggots

	Sex	Age	DM	Osteomyelitis	Wound duration before MDT (mo)	Setting	No. of Maggot Applica-tions	No. of maggots used	Technique	Outcome
1	M	59	-	+	0.5	Hospital	8	240	Biobag	Ambulating with a prosthesis
2	M	71	+	+	1	Outpatient	2	200	Loose	Ambulating with a prosthesis
3	F	74	+	+	1	Nursing home	3	200	Loose	Ambulating with a prosthesis
4	M	64	-	+	6	Hospital	5	500	Loose	Above knee amputation, wheelchair
5	F	85	-	-	3	Nursing home	3	90	Loose	Wheelchair, wound healed.

5E MDT in palliative medicine

Based on the following article:

American Journal of Hospice & Palliative Medicine
P. Steenvoorde[1], L. P. van Doorn[1], C.E. Jacobi[2], J. Oskam[1]
Department of Surgery Rijnland Hospital[1], Leiderdorp, The Netherlands.
And the Department of Medical Decision Making[2], Leiden Univerisity Medical Center, The Netherlands.
Maggot Debridement Therapy in the palliative setting? Accepted for publication Am J Hosp Palliat Care 2007; 24(4): 308-10.

Introduction

Success-rates of MDT in literature differ but range from 70-80%.[55;89;90;162] Patient selection seems to be a critical factor in predicting success. In previous studies we have shown that traumatic wounds for example, treated with maggots will heal almost always and wounds with open joints on the other hand, will generally lead to an amputation of the affected joint. Wound healing is clearly impaired in older patients with co-morbidity. It's not difficult to predict that an infected traumatic ulcer which is present for 1 month in a 30 year old patient treated with MDT will heal earlier compared to a three-year old foul smelling wound in an ischemic leg in 93-year old female. In this article we would like to argue that wound closure is not always feasible and is not always the aim of the treatment. Sharp debridement of a necrotic or infected ulcer is not always feasible, for sometimes this is too painful. Admittance and performing sharp debridement in theatre is not always possible due to co-morbidity. Maggot debridement therapy does not need admittance of the patient; it can be performed simply in the outpatient clinic. We argue that infection-removal, pain- or odour reduction could also be defined as a succesful outcome in some patients. We would like to present a typical patient, with a reduced life expectancy in whom the goal of MDT was not closure of the wound but infection control, odour and pain control, and moreover prevention of major amputation. We will also discuss MDT results in patients that died within a year after MDT compared to the group still alive, one year after MDT.

Patient

A 94-year old female presented to our wound clinic with a chronic, non-healing wound, after referral from the dermatology department. Previous history revealed a history of Chronic Obstructive Pulmonary Disease (COPD), atrial fibrillation, cardiac decompensation and the patient had a history of a peptic ulcer. A chronic ulcer on the left lower leg, present more than a year, showed no healing tendency under compression therapy and treatment with an alginate dressing. The patient had received several antibiotics without any result. Wound aetiology was not clear; it presented after a minor trauma. Due to the ulcer, the patient was unable to walk, and came to our clinic with the use of a wheelchair. On physical examination we saw a large, foul smelling, ulcer on the left lower leg. There were no arterial pulsations on the lower leg. Ankle/brachial index was 0.4. Angiography showed a single peroneal artery only, there were mulitple stenosis of the popliteal artery. Unfortunatley operative and endovascular intervention, was not feasible. The aetiology of the ulcer was not completely clear, for she had psoriatic problems on other places of here body. We concluded there was a psoriatic mixed arterial/venous ulcer. Pathological examination showed no signs of malignancy.

The base of the wound was covered with thick yellow layer (see **Figure 1**). It was decided to remove the necrosis. This was too painful in the outpatient department. As we believed surgical debridement in theatre not to be without serious risks, due to the co-morbidity of the patient, the patient was treated with an alternative form of debridement: Maggot Debridement Therapy (MDT). From our own experience we did not believe MDT alone could completely heal the ulcer. With the MDT we hoped to remove the infection and hopefully, reduce pain problems. Our secondary goals were to prevent a below knee-amputation which the patient and family feared. We clearly observed a full debridement after three applications (see **Figure 2**), and despite all negative factors influencing wound healing we even observed some healing tendency (see **Figure 3**). Most importantly a reduction in pain, as stated by the patient, and odour, as stated by the patient, her family, and the treating physician, was achieved. The patient eventually died within a year; her death was unrelated to the ischemic ulcer. She died with a stable ulcer, without any pain and without any signs of infection.

Results

From August 2002 until the first of January 2006, a total of 101 patients presenting with 117 wounds were treated in our hospital.[162] On the first of January 2006, 77 patients (76%) were still alive. Patients that died within the study period significantly more often were (like the patient presented in this paper) of ASA III or IV (91.7% vs. 64.5%, p=0.007).

They also more often had diabetes mellitus (70.8% vs. 36.8%, p=0.004). **(Table 1)**. There was no significant difference regarding age, sex, quetelet index, outcome of the wound and smoking. More importantly outcome did not differ between the two groups. In other words even if the wound was completely closed, or there was no effect at all, mortality remained the same.

Discussion

According to Church's outcome classification of MDT[88], the effect of MDT in the present case would have been classified as *Significantly beneficial* (long-term considerable pain relief, without full wound healing) or at least *Partially beneficial* (no full wound healing, but there has been some improvement in the patient's clinical state, with reduction of specific symptoms such as pain, odour, and wound secretion). We believe for our patient, this was the best possible outcome. We used MDT in order to remove infection, reduce odour, reduce pain and eventually prevent a below knee amputation. This succeeded; the pain was diminished, the odour reduced and the wound showed signs of healing. Still the patient died. In maggot literature, as with other wound treatments, outcome is recorded as succesful or as failed; Healed or non-healed. This wound did not heal, therefore in some outcome-measures this would be regarded as a failed treatment.

We believe that MDT should not only be instituted in order to close wounds, but it could also be applied in patients in whom the wounds will never heal, no matter what therapy is instituted. It could be used to rapidly clean a wound, to remove infection, after which for example smell is reduced, enabling the patient to socialize more. Unfortunately more than 20% of our patients died within the study-period. We believe this reflects our idea and our policy to use MDT for different indications; not only in the curation of wounds but also in the palliative setting in the last years of a patients life, like was the case in the patient presented in detail.

Table 1

	Deceased (n=24)	Alive (n=77)	P
ASA III/IV	91.7%	64.5%	0.007 *
Diabetes	70.8%	36.8%	0.004 *
Age at presentation	75.4 year	69.8 year	0.098
Vascular patient	66.7%	48.1%	0.086
Number of MDT-applications	3.2	2.2	0.008 *
Outcome beneficial	61% (14/23)	71.4% (55/77)	P=0.337

* significant ($p<0.05$)

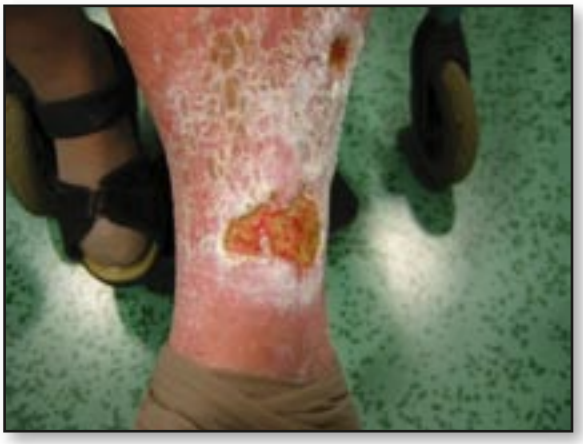

Figure 1: A 93-year old female with an active psoriasis presented with a mixed arterial-venous ulcer that had been present for 6 months.

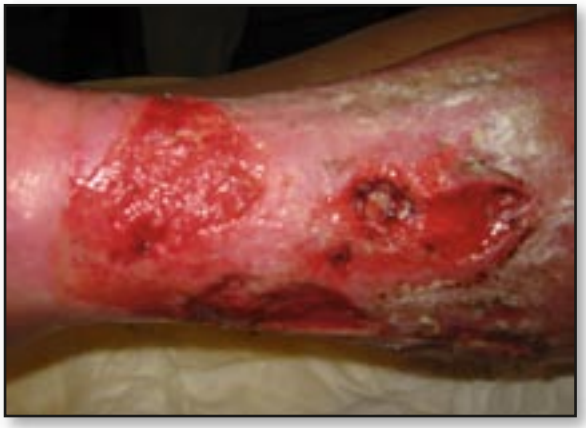

Figure 2: After 3 applications of maggots the wound is clearly fully debrided.

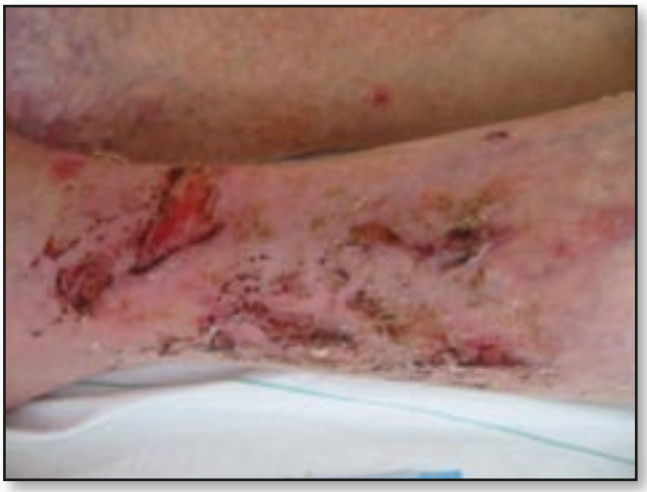

Figure 3: The wounds have a good healing tendency and are reducing in size; more importantly odour and pain are reduced.

Chapter

6

Adverse Effects and safety issues

6A The YUK-factor

Based on the following article:

Wound repair and Regeneration
P. Steenvoorde, T.J. Buddingh, A. van Engeland, J. Oskam
Department of Surgery Rijnland Hospital, Leiderdorp, The Netherlands.
Maggot therapy and the 'YUK factor'; an issue for the patient? Wound Repair Regen 2005; 13(3); 350-352.

Introduction

Maggots. the very word evokes images of rotting and decay. It's very easy to understand why the mere thought of using these creatures on infected wounds would not be a pleasant thought for many people. It's suggested that many patients are deterred by this therapy, mainly because of the "yuk factor"[163-164],but perhaps health care professionals have a bigger "yuk factor" as compared to patients.[165] Placement of maggots in socalled "biobags" makes them invisible, easier to apply, and may reduce the "yuk factor" in health care professionals and patients.[103;164] Others state that the acceptance of the therapy is high among patients.[166] In a phenomenological study on six patients receiving maggot therapy, the experience was not as scary as imagined.[167] We performed a survey among our patients to inquire whether the "yuk factor" is important for patients undergoing MDT.

Methods

To establish whether or not the "yuk factor" played a role for the patient in agreeing to maggot therapy, we performed a survey among all our maggot-treated patients, treated between september 2002 and december 2003. The maximum time between the questionnaire and maggot therapy was 11 months. The following questions were asked

1. What were your expectations prior to commencing maggot therapy?
2. Did maggots escape during the therapy?
3. Was there any adverse reaction from your surroundings?
4. Would you again agree to maggot therapy?
5. Would you recommend maggot therapy to other patients?

In addition, we asked about the smell of the wound and the itch over the body using a Visual Analog Scale (VAS), in which the patient had to record the smell prior to, during, and after maggot therapy and the itch over the body during maggot therapy.

Results

In the study period, 41 patients were treated with maggot therapy for nonhealing wounds in our hospital. There were 22 men and 19 women with an average age of 67 years (range: 25–93). Thirty-one patients were treated ambulatory, eight patients were treated while admitted, and two were both ambulatory and admitted. There was a variety of underlying comorbidities. Smoking (61%), diabetes mellitus (48%), and arterial insufficiency (34%) played a role in the wound pathology. The average time the wound

existed before maggot therapy was 14 months (range: 0.5–132). All maggot applications were performed in our outpatient department, including those on our admitted patients. There are three nurses and three physicians who performed the treatment. We used two application techniques: the first six patients were treated with biobags, which contained an average of 20 maggots in a fine polyvinylalcohol bag, which was placed on the wound. All other patients where treated with the free-range technique. In the latter technique the maggots are placed freely on the wound, covered only by a net. This net is taped to a skin adhesive, which is applied to the periwound skin. This adhesive together with the covering net acts like a barrier to reduce maggot migration. Over the net, wet gauze and a light bandage is wrapped. Patients were well instructed on how to treat the wound at home. Every 3–4 days new maggots were placed on the wound until thorough debridement was reached. The patients did not have to change their gauze at home. In our patients the average treatment time was 11 days.

All patients who were proposed for maggot therapy, agreed. None of the patients refused. In 19% of the patients an amputation was necessary, despite maggot therapy. We believe, however, that the amputation level was influenced by maggot therapy, leading to lower level amputations. Because three patients had died before the questionnaire was taken, 38 questionnaires were sent. There was a response rate of 37/38 (97%). In their expectations about maggot therapy none of the patients reported adverse feelings regarding maggots. High expectations were reported by 35%, 54% had no expectations, and 11% reported it to be their final hope for cure. Of all patients, 89% would agree again on maggot therapy, 11% would not. Of the four patients (11%) who would not agree again, three did not benefit from the therapy. When asked whether to recommend maggot therapy to others, 94% would and only 6% would not. For the smell of the wound before, during, and after maggot therapy a visual analog scale was used. The average score before maggot therapy was 3.1 (no smell is reported 0.0 and the most offensive smell is 10.0). During maggot therapy, the score was 5.2, and after therapy it returned to 3.0. Twenty-two patients (58%) reported a more offensive smell during maggot therapy. A VAS was also used for the itch over the body. During maggot debridement therapy the average score for itch over the body was 1.0 (0.0–7.3).

In 43% of the patients, at one time or another some maggots escaped. Because patients received several maggot applications, the escape rate was 12% for all free-range technique applications, and 11% for all applications. For all patients in whom maggots escaped, they all agreed on maggot therapy again if necessary. Adverse reactions from social interactions of the patients were reported by 22% of the patients. They consisted mostly of people finding the idea of maggots eerie. However, all of these patients agreed on maggot therapy again, and all recommended the therapy to others.

Discussion

From these results it seems our patients were not deterred by maggot therapy, in contrast to what has been suggested by others.[163-164] Rather, our results support the study by Thomas et al.[166] All patients agreed to maggot therapy when it was suggested by their physician. There were even some patients who presented the idea themselves after hearing it from others. None of the patients reported any adverse feelings toward maggots, and a high percentage was very positive about this therapy. All patients returned the questionnaire, which suggests a close involvement with the therapy or therapist. Most of our patients are positive about maggot therapy and would undergo it again if necessary. An even higher percentage of patients recommend this therapy to others. No difference is seen between patients who were treated with the biobag or with

the free-range technique. There was a high percentage of maggot escapes. Biobags instead of the free-range technique could reduce that percentage to a minimum, although we don't see a difference in acceptance between patients treated with biobags or with the free-range technique. We think this is also due to good instructions before therapy and preparing the patient for the possibility of maggot escapes. A high number of patients reported adverse reactions from their social environment, consisting mostly of people finding the idea of maggots eerie. Although it doesn't seem to influence the maggot therapy, we think acceptance is important to reduce adverse reactions to a minimum. To achieve this goal, one could think about good informative material (for example, in the form of a brochure) in which there is information for relatives— taking relatives to the hospital to attend the application of new maggots and thereby reducing the prejudice for maggot therapy—and about getting media attention, both national and local. When patients appear on radio, television, or in the newspaper telling their story, people might reconsider their ideas about maggots. As for the smell of the wound, we noted the VAS score increased from 3.1 before maggot therapy to 5.2 during therapy. This increase cannot be ignored, but until now we have found no answer for this problem. Pilot studies with active carbon did not show any positive results. Fortunately, the VAS score goes back to its original score after maggot therapy. As for the itch over the body, we conclude that this is not an important side-effect of maggot therapy for our patients. On the basis of this questionnaire, we think it's safe to state that the ''yuk factor'' does not seem to be an important factor for our maggot-treated patients. When patients are well informed and instructed, no one is deterred by the idea of maggots. When a patient with chronic or infected wounds who is not responding to conventional wound debridement therapy is suitable for maggot therapy,[102;163;165-166;168-169] the physician should offer this. Prejudicial thoughts about maggots should be eliminated by the physician in both patient and relative through good information and instructions. Also, health care workers should be well instructed, because they could also be deterred by maggots. In this way, maggot therapy can become a widely accepted, reasonable alternative for patients with chronic or infected wounds.

6B Bleeding complications

Based on the following article:

International Journal of Lower Extremity Wounds
P. Steenvoorde, J. Oskam
From the department of Surgery Rijnland Hospital, Leiderdorp, The Netherlands
Bleeding complications in patients treated with Maggot Debridement Therapy (MDT).
Int J LowExtrem Wounds 2005; 4(1):57-58.

Introduction

In literature maggot debridement therapy (MDT) or biosurgery is advocated as a safe, non-surgical debriding agent.[170] Theoretical contra- indications for MDT are patients with known allergies to eggs, soyabeans, fly larvae or any of the components of the dressing.[169;171] If complications of MDT are mentioned in literature, bleeding is not always reported.[166;170-173] Even in a handbook of maggot-assisted wound healing, bleeding is only mentioned in case maggots are placed near exposed bloodvessels.[174] Information sheets for physicians regarding MDT, report bleeding as a complication. According to reports, bleeding occurs in les than 1% of wounds dressed with maggots, especially if maggots are used in close proximity to major veins of vessels.[175] Maggot therapy in individuals with a natural of pharmocologically induced coaggulopathy should, if done at all, only at close supervision.[176] Church and Courtenay even reporteded mild bleeding to occur in 24 out of 70 patients (34%) treated with MDT. Treatment consisted of maggot removal and simple local measures.[169]

Study

In the period of august 2002 untill 1 january 2004 we treated 41 patients with MDT in our hospital. There was a variety of underlying co-morbidity that maintained the non-healing wounds, as diabetes mellitus, smoking, arterial pathology and corticosteroid use. On the average most treated wounds were leg ulcers. Prior to MDT, the average time the wound existed was 14 months. Average patient age was 67 years. We treated 22 men and 19 women. Most patients were treated ambulatory (31/41). The patients were treated either with biobags (8/41) or with the free-range technique. In total 4/41 patients (10%) experienced mild bleeding, one of the patients needed to be admitted to the hospital for this. In total 11 patients used oral anticoagulation therapy and 7 were on antiplateted therapy during MDT. Of the 4 patients experiencing mild bleeding, 2 were on oral anticoagulation therapy and 1 on antiplateted therapy. None of our patients had significant bloodloss, necessitating bloodtransfusion. In our serie, relative riskfactors for experiencing mild bleeding with MDT are 2.8 and 1.3, for patients on oral anticoagulation therapy and antiplatelet therapy respectively. There was no bleeding in any of the patients treated with the contained form of maggot therapy, in which the maggots are placed in a so-called biobag.[174] In our hospital biobags are used in wounds which are difficult to dress, or in which the chance of escaping maggots has to be almost nil (for example in a patient treated with a wound of her breast). The containment of maggots however reduces its effectiveness[177], therefore in principle, we use the free-range technique. There was no bleeding observed in an earlier study of 16 patients treated with the biobag-technique.[138] In the free-range therapy maggots can crawl around the wound

more freely, compared to the contained technique. Perhaps this freedom leads to a more easily damaged wound, leading to a mild bleeding.

Cathastrophic bleeding in patients treated with MDT has not been reported in literature. Minor bleeding in patients treated with the free-range technique seems to occur in 10% of treated patients. These minor bleedings can be treated simply by local measures and removal of the larvae. In patients on oral anticoagulation therapy mild bleeding seems to occur more frequently (relative risk 2.8). Therefore MDT in patients on oral anticoagulation therapy should be done under close supervision only or the contained form of MDT should be used.

6C Pain

Based on the following article:

Journal of Woundcare
P. Steenvoorde, T. Budding, J. Oskam
Department of Surgery Rijnland Hospital, Leiderdorp, the Netherlands
Determining pain levels in patients treated with maggot debridement therapy.
J Wound Care 2005; 14(10): 485-488.

Introduction

Pain as a complication of maggot debridement therapy (MDT) is controversial.[167] Whether or not there is pain appears to depend on the type of wound treated; for example, pain is not reported in spinal cord injury patients with pressure ulcers due to a lack of or altered sensation.[178] However, some authors suggest that MDT is not painful: mild ischaemia may be experienced in patients with ischaemic wounds;[171] most patients do not feel maggots, or the pain decreases or disappears after the maggots are applied.[174] By contrast, pain has been reported after the maggots have grown (one to three days after application)[179] and in patients who had significant wound pain before maggot therapy despite the use of analgesia.[4] In a study of 74 patients treated with MDT, Wolff et al. found 34% of maggot-treated patients felt increased pain during treatment, 25% less pain and 41% no difference in pain.[180] Courtenay reported severe pain in six of 23 patients, moderate pain in 11 out of 23 and mild pain in six of 23.[179] Approximately 20–25% of patients with painful wounds might complain of increased pain during MDT and should therefore be treated with analgesics.[55] In our experience there can be a significant difference in pain between diabetic patients and non-diabetic patients treated with MDT. This appears to be primarily due to neuropathy. Diabetic polyneuropathy is primarily a symmetrical sensory neuropathy, initially affecting the distal lower extremities.[181] To find out how pain was experienced by patients treated with MDT, a retrospective study was undertaken in which all those treated between September 2002 and 1 January 2004 were interviewed. In accordance with a standard protocol, patients are generally treated in the outpatient department at the Rijnland Hospital, although treatment is undertaken on an inpatient basis if necessary. Indications for MDT included: gangrenous or necrotic tissue, infected diabetic foot ulceration, arterial leg ulceration, traumatic infected ulcers and chronic wounds that would not heal despite treatment by the primary physician. Underlying comorbidities included chronic limb ischaemia,[182] diabetes mellitus, smoking and corticosteroid use.[59] Most of the wounds were worst case scenarios, for which the only other option was amputation or surgical debridement in theatre. Patients were excluded from receiving MDT if the treating surgeon believed an urgent amputation could not be postponed, for example in cases of severe sepsis, or if life expectancy was shorter than a few weeks. All patients gave informed consent. Most black dry necrotic tissue was removed prior to therapy. A diagnosis of infection was made if there was purulent discharge and/or two local signs present, such as warmth, erythema, lymphangitis, lymphadenopathy, oedema or pain.

Method

This was a retrospective study, in which a questionnaire was sent by post to those patients who had been treated with MDT during the study period. The maximum time

interval between the questionnaire and treatment was 11 months. A total of 41 patients were treated in the study period. Unfortunately, three diabetic patients died before the study got under way (one died of pneumonia, one of congestive heart failure and one of bowel ischaemia), so only 38 questionnaires were sent out. The patients were asked to rate and record their pain level before, during and after therapy using a visual analogue scale (VAS). An example of how to fill in the VAS score was attached to the questionnaire. A VAS score below 30mm was interpreted as low pain, 30–54mm as moderate pain and above 54mm as severe pain.[183] The VAS was chosen as it is commonly used for the evaluation of pain severity and relief. It is practical, reproducible, sensitive and easy to analyse.[184] However, it may be unreliable as pain experience is probably blurred by memory and by the end result of the therapy.

Pain management in MDT

Pain management during MDT is standardised in our hospital. Initially, all patients, including those with diabetes, were treated with paracetamol (1g three times daily) and Tramal (licensed as Tramadol in the UK) (50mg three times daily), the latter being changed to Durogesic plaster (25µg every three days and 50µg the day before the maggot change) to avoid the complications of Tramal intake. As previously mentioned, pain is generally experienced after one to three days when the maggots have grown. Therefore, in our protocol the patients received a higher dose of analgesic therapy on the day before the maggot change. If the analgesia was not sufficient or complications due to therapy occurred — for example, the maggots escaped — these were addressed accordingly (the maggots were removed; in one case an epidural anaesthestic was given). Where necessary, the maggots were removed; none of the patients wanted the maggots removed because of the pain; on four occasions, removal was due to mild bleeding.

Results

In the period under discussion, 41 patients (22 men, 19 women) with 46 wounds were treated. The average age was 67 years (range: 25–93 years). Of these, 31 were outpatients, eight were treated while admitted, and two were both ambulatory and admitted. Co-morbidities included: smoking (61%), diabetes mellitus (48%) and chronic limb ischaemia (34%). The average wound duration before starting the maggot therapy was 14 months (range: two weeks to 132 months). Previous treatment modalities included vascular interventions, topical negative pressure, surgical and enzymatic debridement, and other such as wet gauze. Seven wounds were treated using the contained technique, while the remainder were treated using the contained technique, including one patient who received both techniques (maggots escaped the first time with the free-range technique and we wanted to reduce the risk of any other maggots escaping). The mean time after MDT until wound closure was 2.8 months. The follow-up period after MDT ranged from three months to two years and three months, during which time wound improvement was noted in 77% of patients (the wounds were fully debrided and were one-third smaller than their initial wound size, or they were still the same size but had no necrosis or slough and were free of infection; necrosis and slough were measured subjectively). MDT was discontinued if there was a healthy granulating tissue and treatment continued with an alginate dressing (Kaltostat, ConvaTec) some in combination with plaster; some wounds were closed in theatre using a split-skin graft after MDT (in some cases, we removed the maggots in theatre just before applying the split-skin graft). In 65% of the patients the wound closed completely. In 19% of the

patients amputation was necessary despite MDT due to underlying disease, mostly chronic limb ischaemia. There was no effect in the remaining 4%.

In total, 38 questionnaires were sent (17 to patients with diabetes and 21 to non-diabetic patients). The response rate was 37 out of 38 (97%). One non-diabetic patient did not respond. This patient's chart contained no reports of excessive pain during MDT; however, as the patient did not return the questionnaire, there was no VAS score. The pain medication prescribed was recorded in her chart, but there are no available details of when and how often it was taken. Patients with diabetes experienced the same amount of pain before MDT as during it **(Table 1)**. However, eight out of 20 non-diabetic patients experienced more pain during MDT **(Table 2)**. These differences are statistically significant ($p<0.05$). Despite receiving morphine, seven of the 20 nondiabetic patients felt their analgesia was still inadequate (the eighth patient did not receive morphine). This increase in pain cannot be attributed to a negative wound outcome as all eight patients healed. However, it should be noted that the freerange technique was used.

Conclusion

Pain during MDT is a problem in non-diabetic patients. However, this was a retrospective study in which pain measurements were undertaken post and not during treatment. Factors such as memory, time and outcome might have affected how the patients described their pain. Pain is an issue for patients treated with MDT, although this depends on the underlying pathology. A standardised pain management protocol for patients receiving this therapy, which can be individually tailored, is recommended. Based on our experience, use of paracetamol and Durogesic plaster appears to be suitable for the outpatient clinic. Pain can be adequately treated with analgesic therapy in patients with diabetes who are receiving MDT. In non-diabetic patients, however, pain management is more problematic. If pain cannot be adequately treated, the options are admission to hospital, use of the contained technique or, in the worst case scenario, discontinuation of MDT.

Table 1. Pain measured using the VAS in diabetic patients treated with MDT.

No.	Sex	Age	Region	Technique	Pain medication used	Pain before MDT	Pain during MDT
1	M	67	Foot	contained	4	3	3
2	M	82	Foot	free-range	2	1	1
3	F	63	Heel	free-range	2	1	1
4	F	64	Too	free-range	5	3	3
5	M	80	Heel	free-range	4	3	3
6	M	63	Heel	contained	1	1	1
7	F	39	Heel	free-range	5	3	3
8	F	86	Lower leg	free-range	5	3	1
9	F	84	Heel	free-range	5	1	1
10	M	71	Foot	free-range	5	3	3
11	M	53	Foot	free-range	5	1	1
12	F	48	Lower leg	free-range	3	1	1
13	F	79	Lower leg	free-range	4	3	3

14	F	74	BKA	free-range	5	3	3
15	M	86	Heel	free-range	5	3	2
16	M	67	Foot	free-range	2	1	1
17	M	71	BKA	free-range	5	2	1

BKA = below knee amputation
Pain: 1 = mild; 2 = moderate; 3 = severe[11]
Pain medication used: 1 = none; 2 = paracetamol only;
3 = non-steroidal anti-inflammatory
drugs; 4 = morphine (Tramal); 5 = morphine (Durogesic plaster); 6 = morphine (Dipidolor); 7 = epidural

Table 2: Pain measured using the VAS in non-diabetic patients treated with MDT

No.	Sex	Age	Region	Technique	Pain medication used	Pain before MDT	Pain during MDT
1	M	59	BKA	contained	6	1	1
2	M	40	Upper leg	free-range	7	3	1
3	F	76	Lower leg	free-range	4	1	3
4	F	77	Lower leg	contained	2	3	3
5	F	93	Lower leg	free-range	4	3	3
6	F	69	Lower leg	free-range	5	1	3
7	F	80	Lower leg	free-range	5	3	3
8	M	44	Lower leg	free-range	5	1	1
9	M	37	Lower leg	free-range	4	3	3
10	F	77	Lower leg	free-range	5	2	3
11	M	75	Lower leg	free-range	5	1	1
12	M	58	Lower leg	free-range	5	2	3
13	M	84	Heel	free-range	1	1	1
14	F	60	Breast	Both	5	3	3
15	M	48	Lower leg	free-range	5	1	3
16	M	62	Foot	free-range	5	2	3
17	F	88	Heel	free-range	5	3	3
18	M	42	Lower leg	free-range	5	1	1
19	F	64	Lower leg	free-range	3	1	3
20	M	64	Foot	free-range	5	1	2

BKA = below knee amputation
Pain: 1 = mild; 2 = moderate; 3 = severe[11]
Pain medication used: 1 = none; 2 = paracetamol only;
3 = non-steroidal anti-inflammatory
drugs; 4 = morphine (Tramal); 5 = morphine (Durogesic plaster); 6 = morphine (Dipidolor); 7 = epidural

Chapter

7

Determinants of MDT Outcomes

7A Smoking and MDT

Based on the following article:

European Wound Management Association
Pascal Steenvoorde MD MSc[1,2], Catharina E. Jacobi Phd[3], Louk P. van Doorn MA[2], Jacques Oskam MD Phd[1,2]
From the department of Surgery[1] Rijnland Hospital Leiderdorp, the Rijnland Wound Clinic Leiderdorp[2] and the department of Medical Decision Making[3], Leiden University Medical Center, all in the Netherlands
Smoking is not contra-indicated in maggot debridement therapy in the chronic wound.
EWMA 2007; 7(1): 15-18.

Introduction

The negative effects of smoking on acute wound healing were first reported in 1977, in a smoker with impaired healing of a hand-wound.[185] Cigarette smoke contains over 4000 different components with different effects on a variety of tissues in the body.[186-187] There is a vast amout of literature describing the negative effects of smoking on acute wound healing.[188] There is also evidence that[189-193] smoking cessation programs improve healing rates, compared to patients that continue to smoke.[194] These effects are however less clear in the chronic wound.[187] Maggot debridement therapy (MDT) is effective in the debridement of the chronic sloughy or necrotic wound, with success percentages around 80%.[89] Patients with cutaneous ulcers should be instructed to refrain from smoking[109], but this is not always feasible in a chronic wound population.

Besides smoking many factors influence the healing of chronic wounds.[43] We questioned ourselves whether MDT-healing rates were influenced by smoking, because smoking is considered as a (relative) contra-indication for MDT in another hospital in the Netherlands. We believe this could be important in traumatic acute wounds, but believe this should be reconsidered in the chronic wound care group in whom amputation sometimes seems to be the only alternative. We believed MDT in smokers would be a better alternative than our standard surgical debridement that was performed before the introduction of MDT in our clinic. We report MDT-results on 125 wounds in 109 patients, with special emphasis on possible detrimental effects of smoking.

Methods

In the period August 2002 to March 2006, patients who presented with chronic wounds with signs of gangrenous or necrotic tissue at our surgical department and seemed suited for MDT were treated with MDT. This is a descriptive consecutive case-series. Chronic wounds were arbitrarily defined as wounds existing for more than four weeks. The accepted definition of a chronic wound relates to any wound that fails to heal within a reasonable period. There is no clear-cut definition that points to the chronicity of a wound.[109] Three physicians and three nurses and one nurse practitioner were involved in the actual maggot therapy. Patients were not eligible for the study if the treating surgeon believed an urgent amputation could not be postponed (for example in case of severe sepsis) or if life expectancy was shorter than a few weeks. All patients gave informed consent for MDT. Patient characteristics like age and sex were reported. The patient was recorded as a non-smoker if never smoker or non-smoking for more than three months.

Outcome

Maggots are debriding agents; if the wound is clean from bacteria, necrosis and slough maggots are no longer useful in the wound, and other wound-treatment must be followed in order to close the wound. In this study we defined 8 different outcomes of MDT, based on outcome definition in the literature.[55;88-90;93] and our own experience[107;162;195;196]

Effect of MDT observed (beneficial outcome)

1. Wound fully closed by second intervention (for example split skin graft);
2. Wound spontaneous fully closed;
3. Wound free from infection and <1/3 of original wound size;
4. Clean wound (free from infection/necrosis/slough), but same as initial size or up to 1/3 smaller;

Effect of MDT observed (unsuccesful outcome)

5. No difference observed between the pre- and post-MDT-treated wound;
6. The wound is worse;
7. Minor amputation (for example partial toe amputation);
8. Major amputation (for example below knee amputation).
9. Unknown outcome.

In this study outcomes 1-4 are arbitrarily determined beneficial outcomes and outcomes 5-9 are determined unsuccessful outcomes. It's arbirtrarily for in some patients a fully debrided wound does not offer any advantages for the patient (for example he/she still needs wound care) and for another patient only a partial toe amputation (which is defined as non-successful) could mean the difference between a wheelchair and fully ambulating.

Statistical analyses

To study the impact of smoking on the outcome of MDT, a univariate analysis using Chi-square statistics was performed.

Results

From august 2002 until March 2006, 109 patients with 125 wounds were treated with MDT in our hospital. In total 110 patients were asked for MDT, one alcoholic patient, with a psychiatric history refused. From one patient the outcome was not known, due to death of the patient during maggot treatment. The patient died in another hospital, due to a myocardial infarction, which was unrelated to the MDT. There were 59 male (54.1%) and 50 females treated. Average age is 71 years (range: 25-93 years). The wounds existed on average 7 months before starting with MDT (range 1 week-11 years). Of the 125 wounds treated with MDT, 76 (69.7%) had beneficial outcomes **(Table 1)**. MDT resulted in complete debridement and epithelialization, leading to a stable and pain-free scar with no subsequent breakdown in 64 of the 125 wounds (51.2%), 14 wounds (11.3%) were free from necrosis, slough and infection and the wound dimensions were less than one third of original wound size. A major amputation was needed in 28 patients (22.4%). In the current study there were 37 smokers and 72 non-smokers. Of the smokers 25 (67.7%) had a good result, compared to 51 (70.8%) in the non-smokers group. This difference was non-significant **(Table 1)**. The same result was true if success was defined only as a closed wound (outcome 1 or 2). Nor did smokers have a higher chance of amputation (outcome 7 and 8).

Discussion

Smoking is a risk factor for complicated wound healing; it is a systemic risk factor in line with diabetes and malnutrition. It seems to be one of the most important (preventable) risk factors for impaired healing, for more than 25 percent of the adult population smokes.[187] Smoking causes damage to blood vessels, there is decreased collagen production[197], increased aging of collagen[198] and keratinocytes show impaired migration.[199] Nicotine has been shown to impair wound contraction from the sixth to the tenth day in a rabbit-ear model.[200] Tobacco smoke contains over 4000 different compounds of particles or gases. There are many toxic components like nicotine, carbon monoxide, cyanide, heavy metals, additives and numerous different chemical compounds known as condenate.[187] The effect of the cigarette smoke is a thrombogenic state through an effect on the blood contituents, vasoconstricting prostaglandins and an effect on the dermal microvasculature.[201] Eventually all these factors lead to tissue hypoxia.

There is a vast amout of literature describing the negative effects of smoking on acute wound healing. Sternal woundhealing[188], hip and knee arthroplasty[189], ankle arthrodesis[202], spinal fusion[190], intra-oral implant placement[191], skin flaps[192], incisional hernia[203], leg amputation[204] and breast reduction[193] are all examples of acute wounds that have delayed healing in smokers. For example, delayed healing after breast reduction was significantly associated with smoking. In a study on 179 patients undergoing breast reduction surgery; 22 percent had delayed healing in the smoking group versus 7.7% in the non-smoking group ($p=0.03$)[193]; thus with a relative strong effect. Not only in (skin-)wound healing there is evidence of the negative effect of smoking, also in the field of (for example) fracture healing[205] and bowel anastomosis[206] it's shown that smoking negatively affects healing. There is a dose-response association in heavy smokers with all cause higher morbidity, however it is not clear if this is also the case for wound healing.[207] One study found that high-level smokers (› 1 pack per day) had developed tissue necrosis three time more frequently compared to low-level smokers (‹1 pack per day).[208]

In literature we could find no reports describing the differences between cigarette and cigar smokers, nor on passive smoke. Almost all smokers in the current study were cigarette smokers, there was one cigar smoker.

In patients undergoing elective hip or knee replacement a smoking intervention study (with smoking cessation or at least a 50% reduction in smoking) led, in a randomised controlled trial ($n=120$), to a reduction in the wound-related complications from 31% to 5% ($p=0.001$).[194] This effect was found if the patients had been subject to a 6-8 week program. In experimental rat studies, Kaufman and others found that exposure to tobacco smoke 7 days prior to the flap procedure affected flap survival more adversely than did smoking postoperatively. They, however, did not find cessation of smoking to greatly improve flap survival.[209] Others found a critical time period of 7 to 14 days of preoperative cessation of smoking before this increase in flap survival occurred.[210] It seems therefore that pre-operative smoking is more important than post-operative smoking. However, all these reports relate to acute wound healing, and we are dealing with patients with chronic wounds. In our study many patients claimed they would stop smoking during the MDT, but we classified them as smokers, because the duration of MDT is shorter than the time needed before healing rates would be comparable to non-smokers.

In this type of studies, with relative small sample sizes, one should always be carefull interpretting the results. In this study we found no indications that smoking should be considered a contra-indication in MDT of chronic wounds. It is always possible that there is an effect, but not shown by the statistics. Regarding our study, however, it is not very likely to have missed a negative effect of smoking in chronic wound therapy as even a

samewhat larger percentage of smokers had beneficial outcomes as compared to non-smokers.

In this study on maggot debridement therapy on chronic wounds , we could not observe any statistically significant difference between smokers and non-smokers in outcome. Tissue hypoxia is the end-result of the detrimental effects of smoking, which occurs through different pathways.[201] It has been shown in the acute wound that smoking has negative effects, and we hypothesize that this is due to tissue hypoxia in the smokers group. The patients in our study were a selection of many worst-case scenario's. We could postulate that all these wound had tissue hypoxia at presentation, caused by different mechanisms, such as like arterial insufficiency, diabetes mellitus or smoking. It could be that because all wounds were in some sort of tissue hypoxia at the start of MDT, that that is the reason why we didn't observe any difference between the smokers and the non-smokers in outcome.

Conclusion

Smoking has an adverse effect on acute wound healing, but in chronic wound care this effect has been less proven. In this study, smoking was not found to effect the results of maggot debridement therapy in chronic wounds, and smoking should, therefore, not be a contra-indication for maggot debridement therapy in these wounds.

Table 1: Results of MDT in 109 patients with 125 wounds, divided by smokers and non-smokers.

		All wounds†			All patients‡		
			Smokers	Non-smokers		Smokers	Non-smokers
		N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Total		125 (100)	41 (32.8)	84 (67.2)	109 (100)	37 (33.9)	72 (66.1)
Beneficial outcome		85 (68.0)	29 (70.7)	56 (66.7)	76 (69.7)	25 (32.9)	51 (67.1)
1.	Wound fully closed by second intervention (for example split skin graft)	23 (18.4)	9 (22.0)	14 (16.7)	23 (21.1)	9 (24.3)	14 (19.4)
2.	Wound spontaneous fully closed	41 (32.8)	16 (39.0)	25 (29.8)	34 (31.2)	13 (35.1)	21 (29.2)
3.	Wound free from infection and <1/3 of original wound size	14 (11.2)	2 (4.9)	12 (14.3)	13 (11.9)	2 (5.4)	11 (15.3)
4.	Clean wound (free from infection/ necrosis/slough), but same as initial size or up to 1/3 smaller	7 (5.6)	2 (4.9)	5 (6.0)	6 (5.5)	1 (2.7)	5 (6.9)
Unsuccessful outcome		40 (32.0)	12 (29.3)	28 (33.3)	33 (30.3)	12 (36.4)	21 (63.6)
5.	There is no difference between before and after MDT	5 (4.0)	2 (4.9)	3 (3.6)	3 (2.8)	2 (5.4)	1 (1.4)
6.	The wound is worse	1 (0.8)	0 (0.0)	1 (1.2)	1 (0.9)	0 (0.0)	1 (1.4)
7.	Minor amputation (for example toe)	5 (4.0)	2 (4.9)	3 (3.6)	5 (4.6)	2 (5.4)	3 (4.2)
8.	Major amputation (below knee amputation or above knee amputation)	28 (22.4)	8 (19.5)	20 (23.8)	23 (21.1)	8 (21.6)	15 (20.8)
9.	Unknown result	1 (0.8)	0 (0.0)	1 (1.2)	1 (0.9)	0 (0.0)	1 (1.4)

† Chi-square: smoker's/non-smoker's wounds vs. 2-group outcome: $X2=0.209$ (df=1), P-value=0.647 (via Fishers Exact correction: P-value=0.688)

‡ Chi-square: smoking/non-smoking patients vs. 2-group outcome: $X2=0.123$ (df=1), P-value=0.725 (via Fishers Exact correction: P-value=0.826)

7B MDT and chronic limb ischemia

Based on the following article:

Internet journal of Surgery
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The results of Maggot Debridement Therapy in the ischemic leg. A study on 89 patients with 89 wounds on the lower leg treated with maggots. Internet Journal of Surgery 2007; 9(1).

Introduction

Maggot Debridement Therapy (MDT) is a debridement method with great advantages over sharp debridement. It's highly selective, without inflicting to much damage to the healty tissue, moreover it has other beneficial effects wich promote woundhealing. Another advantage is that for MDT, no aneshtesia is needed and the patient does not need to be admitted, wich is of great importance in a time of an ever growing elderly population with co-morbidity. Some authors do not treat patients with inadequate vascular supply with MDT, unless healing is not the goal.[211] Hofman points out that in deep ischemic wounds maggots will die for they need oxygen to survive.[212] Sherman states that arterial insufficiency is a relative contra-indication for MDT.[152] Wound healing seems almost impossible if the absolute systolic ankle pressure is below 50 mmHg, it is difficult between 50-80 mm Hg, and good if above 80 mm Hg.[213-214] If a patient has an ischemic ulcer, and there are no possibilities to improve revasculazation, prognosis for the patient is poor. The patient is likely to end up with a major amputation. In our clinic MDT was started in august 2002. Contra-indications for the therapy were patient-preference, septicaemie and patients from whom informed consent could not be obtained. Vascular insufficiency was not a contra-indication, although we believed results in these patients would be worse. In this analysis we studied the results of MDT in patients with and without vascular problems in order to answer the question if MDT could be worthwile in the ischemic leg.

Methods

Patients with chronic wounds on the leg were found eligible for MDT treatment. Of each patient it was recorded whether arterial insufficiency was present. The diagnosis of arterial insufficiency was made if both pedal pulses of the involved foot were absent and/or the ankle-brachial pressure index was less than 0.6 and/or the absolute ankle pressure was below 50 mm Hg. Conservative wound healing usually takes place above the threshold of chronic critical limb ischemia. If the absolute systolic ankle pressure and/or the ankle-brachial index are below this threshold, foot pulses tend to be absent, the extremities are cold and wound pain is common. The Second European Consensus[74] has outlined the following criteria for a diagnosis of chronic limb ischemia: recalcitrant rest pain or distal necrosis of more than 2 weeks' duration in the presence of (1) a systolic ankle pressure of 50 mm Hg or less, or (2) systolic toe pressure of 30 mm Hg or less, or (3) a transcutaneous oxygen pressure of 10 mm Hg or less.

In this study a patient was recorded as a vascular patient if the patient met the criteria for chronic critical limb ischemia, or if the patient had a history of a peripheral bypass or

radiological intervention of the ipsilateral leg. The patient was recorded as a successfully re-vascularized patient if the patient had an ankle-brachial index of more or equal to 0.6 and/or the absolute ankle pressure was above 50 mm Hg and had a previous history of interventional vascular procedures of the involved leg, including both surgical and radiological procedures.

In the period August 2002 and the first of January 2006, all patients who presented at the surgical department of the Rijnland Hospital, Leiderdorp, The Netherlands, with infected wounds with signs of gangrenous or necrotic tissue who seemed suitable for maggot debridement therapy (MDT), were asked whether they would enrol in a prospective case series study regarding MDT. All types of patients were included: patients from the dermatology department sent directly for this therapy, patients with infected diabetic feet, with arterial leg ulcers, with traumatic infected ulcers and with chronic wounds that would not heal despite treatment by the primary physician. Patients were excluded from the study if the treating surgeon believed an urgent amputation could not be postponed (for example in case of severe sepsis) or if life expectancy was shorter than a few weeks (ASA V). For this current study, patients were also excluded if the wound was not located below the knee or if they died before the MDT results could be registered.

Of all wounds of patients, only the first wound with which they presented at the clinic was included. For analysis we grouped the patients according to their vascular status. As 3 groups of patients could be distinguished, 4 comparisons could be made. These encompassed: 1) Non-vascular patients vs. vascular patients; 2) Non-vascular patients vs. successful revascularized patients; 3) Non-vascular patients vs. vascular (non-revascularized) patients; and 4) Successful revascularized patients vs. vascular (non-revascularized) patients.

Results

In the study-period 101 patients with 117 wounds were treated with MDT. Excluded from this current study were patients with wounds localized above the knee (11 patients with 16 wounds) and if patients had more than one wound, all second wounds were excluded (11 wounds). One patient was excluded, for unfortunately the patient died before the result of the MDT could be obtained. The number of patients included in the present study 89 patients, with 89 wounds. There were 50 male patients (56%) and 39 females patients treated (see **Table 1** for patient-characteristics). The average age was 70.9 years (range: 25-93 years, SD: 14.7). The wounds existed on average 7 months before starting with MDT (range 1 week-11 years). Based on our definitions (see methods), 43 patients (48.3%) had no vascular problems, 19 patients (21.3%) had had vascular problems but underwent sucessful revascularization treatment, and 27 patients (30.3%) were (untreated) vascular patients.

In the vascular group of patients (n=46) significantly more often diabetes occcured (63% versus 35%; p=0.015), the wounds existed for a longer period, the wounds were more often deep and more often had a worse result, compared to the non-vascular-group. If we look at the succesfully revascularized patients, we found that there were no statistically significant differences between patient- and wound characteristics compared to the vascular patients. Good outcome was reached in 52% of all vascular patients, with 68% good outcome in the succesfully revascularized patients and 41% in the non-revascularized patients. This difference in outcome, however, was not statistically significant (p=0.12).

The univariate logistic regression analyses showed that sex and wound size had no impact at all on MDT results, i.e. good vs. bad outcome. These two characteristics were, therefore, not selected for the multivariate analysis. All other characteristics, i.e. age (split by age of 60 years), the presence of diabetes, wound duration, and wound depth had a statistically significant impact or showed a trend on the outcome of MDT. So, these characteristics were selected for the multvariate analysis. Regarding vascular problems, four univariate analyses were performed, as described in the methods. These analysis, looking at the impact of vascular problems on MDT results, showed that vascular patients had statistically significant more often a bad outcome after MDT compared to non-vascular patients (Odds ratio (OR): 12.2; 95% Confidence Interval: 3.3-45.2; P-value<0.001). Successful revascularized patients had statistically significant more often a bad outcome after MDT compared to non-vascular patients (OR: 6.2; 95% CI: 1.3-28.2; P=0.019). Similarly, vascular patients (non-recvascularized) had statistically significant more often a bad outcome after MDT compared to non-vascular patients (OR: 19.4; 95% CI: 4.8-78.8; P<0.001). With these univariate analysis, we could not show a statistically significant difference in MDT outcome between successful revascualrized patients and vascular (non-revascularized) patients (OR: 3.2; 95% CI: 0.9-10.8; P=0.068). Although this difference in MDT outcome was not statistically significant, but on a trend level, it might be clinically relevant, as we are dealing with small groups of patients. We also have to keep in mind that these results are unadjusted for differences in patient and clinical characteristics.

If we adjust for differences between groups, i.e. age, presence of diabetes, wound duration, and wound depth, the statistic significant difference in outcome between non-vascular patients and vascular patients (revascularized and non-revascularized) is no longer present **(Table 2)**. Only the trend regarding the difference in MDT outcome between successful revascularized patients and vascular patients holds (P=0.051).

In conclusion, these results indicate that, although it seems that vascular problems have a negative influence on wound healing, it might be the case that other patient characteristics have larger impact on MDT outcome than the vascular problems itself. The results, however, give the impression that a revascularization intervention does have some benificial effect, as we found even in the multivariate analysis that (on a trend level) revascularized patients have more often good results after MDT compared to vascular, non-revascularized, patients. Good outcome was reached in 52% of all vascular patients, with 68% good outcome in the succesfully revascularized patients and 41% in the non-revascularized patients. We therefore believe MDT could be used in the ischemic leg, escpecially for the lack of other treatment modalities besides amputation.

Table 1: Patient- wound- and Interventioncharacteristics of the studies group.

		All patients	Vascular patient			Revascularized patient		
			No	Yes	P*	Yes	No	P
		N=89 (100%)	N=43 (48.3%)	N=46 (51.7%)		N=19 (41.3%)	N=27 (58.7%)	
Sex	Male	50 (56.2)	20 (46.5)	30 (65.2)		11 (57.9)	19 (70.4)	
Age	Mean (SD)	70.9 (14.7)	69.9 (15.9)	71.8 (13.5)		67.9 (14.7)	74.5 (12.1)	
Diabetes		44 (49.4)	15 (34.9)	29 (63.0)	0.015	13 (68.4)	16 (59.3)	
Wound Duration	≥ 3 months	53 (59.6)	17 (39.5)	36 (78.3)	0.000	15 (78.9)	21 (77.8)	
Wound Depth	Deep*	49 (55.1)	12 (27.9)	37 (80.4)	0.000	15 (78.9)	22 (81.5)	
Wound Size	≥ 2 cm	66 (74.2)	33 (76.7)	33 (71.7)		14 (73.7)	19 (70.40	
Outcome**	1 2 3 4 5 6 7 8	21 (23.6) 27 (30.3) 11 (12.4) 5 (5.6) 1 (1.1) 1 (1.1) 5 (5.6) 18 (20.2)	16 (37.2) 17 (39.5) 5 (11.6) 2 (4.7) 0 (0.0) 1 (2.3) 0 (0.0) 2 (4.7)	5 (10.9) 10 (21.7) 6 (13.0) 3 (6.5) 1 (2.2) 0 (0.0) 5 (10.9) 16 (34.8)		2 (10.5) 6 (31.6) 4 (21.1) 1 (5.3) 0 (0.0) 0 (0.0) 3 (15.8) 3 (15.8)	3 (11.1) 4 (14.8) 2 (7.4) 2 (7.4) 1 (3.7) 0 (0.0) 2 (7.4) 13 (48.1)	
Result	Good (1-4) Bad (5-8)	64 (71.9) 25 (28.1)	40 (93.0) 3 (7.0)	24 (52.2) 22 (47.8)	0.000	13 (68.4) 6 (31.6)	11 (40.7) 16 (59.3)	0.121
Intervention	Radiologic Surgery None					8 (42.1) 11(57.9) 0 (0.0)	3 (11.1) 4 (14.8) 20 (74.1)	

* Deep visible bone, joint or tendons.

** Effect of MDT observed (beneficial outcome)

- Wound fully closed by second intervention (for example split skin graft);
- Wound spontaneous fully closed;
- Wound free from infection and ‹1/3 of original wound size;
- Clean wound (free from infection/necrosis/slough), but same as initial size;

No effect of MDT observed (unsuccessful outcome)

- No difference observed between the pre- and post-MDT-treated wound;
- The wound is worse;
- Minor amputation (for example partial too amputation);
- Major amputation (for example below knee amputation).

Table 2: Results of multivariate results of logistic regression analyses: impact on MDT outcome.

	Group 1	Group 2	Group 3	Group 4
	N=89 (43 vs 46)	N=62 (43 vs 19)	N=69 (43 vs 27)	N=46 (19 vs 27)
	OR (95%CI) P-value	OR (95%CI) P-value	OR (95%CI) P-value	OR (95%CI) P-value
Multivariate results				
Vascular problems*	2.27 (0.44-11.82) 0.329	1.08 (0.10-11.27) 0.952	3.59 (0.59-21.69) 0.164	6.05 (0.99-36.95) 0.051

* Adjusted for age, diabetes, wound duration, and wound depth.
Group 1: non-vascular patients vs. vascular patients; Group 2: Non-vascular patients vs. successful revascularized patients; Group 3: Non-vascular patients vs. vascular (non-revascularized) patients; and Group 4: Successful revascularized patients vs. vascular (non-revascularized) patients.

Chapter

8

General Discussion

Despite modern wound treatment and antibiotic treatment not all patients with chronic wounds heal. The appearance of antibiotic resistant bacteria, gave rise to a strong come-back of Maggot Debridement Therapy (MDT). Treatment with sterile maggots is becoming more and more common, also in the Netherlands. Popularity can be seen in several areas. Up to date, more than a 100 articles were published on the subject. The U.S. Food and Drug Administration allowed production and marketing of maggots as a medical device in 2004. The International Biotherapy Society, which held his first congress in 1996, has held it's 7th congres in 2007 in Korea. Large randomized studies are lacking, although one containing 600 venous ulcer patients was initiated in 2004. We were asked to participate in that study, but lack of funding unfortunately prevented this. Untill now, there have been three randomized studies performed. Only one of them has been reported in a peer-reviewed journal. In the specific article Wayman et al. have shown the cost effectiveness of larval therapy in venous ulcers compared to hydrogel dressing. In his study on 12 patients, it was shown that costs were reduced ($p<0.05$).[120] On the 36th annual meeting of the EASD, Markevich et al. reported on a randomized, mulitcenter, double blind controlled clinical trial (n=140) for neuropathic diabetic foot lesions compared to conventional treatment. They found a significant higher percentage of granulation tissue after 10 days, compared to the Hydrogel group.[215] However this study was never published and the authors, unfortunately could not be reached for further comments. The third presented randomized study was a poster-presentation, which was presented by Contreras et al. in 2004 on the 2nd World Union of Wound Healing Societies Meeting in Paris. The authors were unable to find a difference between MDT and curettage and topical silver sulfadiazine in 19 patients with venous leg ulcers.[216] In short, there has only been one peer-reviewed published randomized trial on MDT, including only 12 patients.

After having treated our second or third patient in 2002, we wanted to perform a randomised trial, for in literature proof of effectivity seemed lacking. One of the problems with starting a trial in an early stage is that of confouding factors. There were many questions that we could not find an answer for in literature. Some questions were very fundamental like what are indications and contra-indications for MDT. There is debate in literature regarding adverse reactions and safety isssues, even which application technique to use was not clear from literature. However, after internal discussions we believed it would be ethically unjust and unfair to put our patients in a trial of a therapy in which there is not a clear idea on indications, contra-indications, possible adverse effects, safety issues and discussions about application techniques. In the years that followed 2002, we have answered a lot of questions, but as was to be expected these answers let to new questions, necessitating further research.

What has this thesis brought us and what has the herein reported studies shown us? What are the questions raised, what are future perspectives and what sort of randomized trial should be performed in our opinion? After treating more than 150 patients, we now know that wounds treated with maggot therapy follow the same wound healing phases compared to non-maggot treated wounds. It was shown that clinical judgement could be guided by laboratory investigations. Wounds infected with Gram-positive bacteria can be treated more efficiently compared to wounds infected with Gram-negative bacteria. Therefore if a randomized trial is planned, this should be taken into account. In another publication it was discussed that all infected traumatic wounds healed with MDT and all patients with an arthritis could not be healed. In a separate publiciation it was discussed

that application technique matters, and that the free-range technique seems to be the most efficient technique. There were several publications on the feasibility of MDT in patients' wounds of different origin. MDT seemed usefull in traumatic wounds, amputation wounds, in breastcancer, in necrotizing fasciitis and the use of MDT in palliative medicine has it's value. The YUK-factor didn't seem to be an important factor for our patients. Pain can be managed with a good pain-protocol and as complications, besides maggot migration, bleeding occurs (mainly in patients on oral anticoagulation) most often, but never life threatening. We have shown that pain does occur, but that this can be managed effectively. Ischemia has a negative influence on MDT-outcome, but vascular problems are not an absolute contra-indication.

Based on our publications and experience, we would suggest a randomized trial on patients with neuropathic (non-ischemic) diabetic ulcers, in which infection is caused by gram-postive bacteria. Factors that should be stratified for are age (above or below 60 years) and depth of the wound (superficial or deep; in which deep is recorded as a wound in which there is visible bone, joint or tendons). Wounds in which there is a (septic) arthritis and all patients with appearent signs of septicemia should be treated surgically, and therefore be excluded from the trial. The application technique should be the free-range technique. Smoking, sex, quetelet index, location, woundsize and woundduration should be recorded but not adjusted for. Patients can be managed in the outpatient clinic, but if on oral anticoagulation patients should be admitted for bleeding might occur. All patients should be treated according to international standards, meaning adequate offloading. Antibiotic therapy should be given according to international standards, and is not a contra-indication for MDT. As a control debridement enzymatic-, mechanical- or surgical debridement could be chosen.

Future perspectives for MDT in our clinic are the incorporation of MDT in a holistic treatment modality in which it's clear from the start which patients are and which are not suited for MDT. In our opinion further international research should focus on mechanism of action of MDT, proof of effectivity in the form of randomized studies. Research in our clinic should focus more on implementing MDT at home and studying social and psychological factors that might improve MDT-results.

A large problem of MDT is the difficulty of this type of therapy to gain acceptance in the medical community. Maggots are associated with rotting and decay. The image is of filthy, low-life creatures that are ugly and disgusting. In an oral presentation we held recently at a Dutch scientific surgical meeting, a surgical professor in the audience said, "I will never allow those creatures in my ward, on which the whole crowd laughed."[217] This remark shows that widespread use and acceptance of MDT has not yet been reached. It seems there is still much work to do before MDT is generally accepted as a therapeutic method. Fortunately, the negative image that seems to exist among nurses and physicians does not seem to bother patients.[211] We have treated more than 150 patients in our clinic with MDT. All, but one, patients to whom we proposed MDT agreed to the therapy. All were allowed to discontinue the therapy whenever they wanted; none did. In a survey of the first 38 MDT-treated patients, 89% agreed to another session of MDT if the surgeon believed it would be beneficial, and 94% of the patients said that they would recommend it to others. This is despite the fact that the therapy was not successful in all patients (there was a below-knee of above the knee amputation-rate of 19% among patients who underwent MDT).[107]

It's important to remember that MDT is not holy water, in which all wounds that are touched with maggots heal. MDT has it's failures, and we have lost some extremities despite the use of MDT. We would like to prevent patients in whom amputation can't be avoided to be treated with maggots, so 'they know we have tried everything'. If amputation is inevetible then the amputation must not be postponed. We do not want our patients to get any false hopes. Maggots need to be used, knowing it's indications and limitations. In our opinion this does not necessarrily have to be in a specialized wound clinic, although results will be influenced by a dedicated team.

In the near future we hope that MDT is also possible in the home care setting (not in the clinic and not in outpatient department), so that specialised nurses or nurse-practioners do the actual treatment at home. This should be made possible financialy first. Norman Bethune a famous canadian thoracic surgeon (who published (70 years ago) on the use of maggots in the thoracic cavity) once said, while operating in the Japanse-Chinese war under terrible conditions: 'Doctors! Go to the Wounded! Do not wait for them to come to you'. I would like to finish this thesis with: 'Doctors! Go to your patients! Do not wait for them to come to you!'

Summary

In the first chapter, the history of Maggot Debridement Therapy (MDT) is presented. Starting from the oldest known written record to the introduction of MDT in the Netherlands. The presumed methods of action are discussed. It's stressed that MDT is a biological debridement. Other forms of debridement are briefly discussed.

Chapter 2 adresses soms fundamental questions. The first article describes histopathological examination of wounds prior, during an after MDT. In this study we have shown that maggot debrided wounds follow the same wound healing phases compared to non-maggot treated wounds. The other two articles in this chapter describe laboratory and microbiological effects of MDT. Furthermore tt was shown that Leucocyte count was significantly reduced after maggot therapy (8.4 x 10e9/L) compared to a baseline value of 10.5 x (10e9/L) prior to maggot therapy. It's stated that Leucocytes could be used as an indicator for succes of MDT. From in-vitro studies it's know that maggots are more capable of destroying gram-postive infections (like *S. Aureus*) compared to gram-negative infections (like *Pseudomonas*). In our study we could confirm this in an in-vivo study. The chance of culturing a gram-negative bacteria after MDT is significantly higher compared to before MDT (p=0.001), the (non-significant) reverse is true for gram-postive bacteria (p=0.07).

In the third chapter we have discussed patient-, wound- and therapy-characteristics predicting MDT-outcome. It was found that traumatic wounds are a good indication for MDT, artritis apperantly not. Based on a mulitvariate analysis outcome was negatively influenced by chronic limb ischemia (OR 7.5), depth of the wound (OR 14.0) and age over 60 years (OR 7.3). Outcome was not influenced (in this mulitvariate analysis) by sex, quetelet index, diabetes mellitus, smoking, ASA-classification, location of the wound, size of the wound or woundduration.

In chapter 4 two different application techniques; the free-range and the contained technique were compared. In a study on 64 patients presenting with 69 wounds treated with maggot therapy, it was found that the free-range technique significantly improved outcome compared to the contained technique (p=0.28).

In Chapter 5, several case-reports and case-series in which MDT was performed is presented. First, 11 patients are described of the Leiden University Medical Center, in whom treatment was initiated to avoid amputation. Treatment-time varied from 11-34 days, with 100-2900 maggots needed. Second, a patient with a breast wound is described. It's a chronic wound after breastconserving therapy (including radiotherapy) for a carcinoma. Despite repeated surgical debridements, alginate dressing, mechanical debridement and antibiotic therapy the wound didn't heal. Only After MDT was initiated the wound healed. Specific problems in this type of wound are discussed. Necrotizing fasciitis is the basis for article three and four of this chapter. In a study on 15 patients, it's argued that MDT could be beneficial if applied early (besides emergent radical surgical debridement and antibiotic therapy). If applied early the number of needed surgical debridements (=debridements in theatre) are significantly reduced from 4.1 to 1.8 (p=0.001). The next article describes 5 patients with infected amputation stumps. It's argued that with modern wound care (MDT and Vacuum assisted closure therapy) the conversion rate to an above knee amputation will be diminished. Finally a case is presented of a 94-year old female in whom MDT prevented amputation of her lower limb. The primary goal of MDT in this patients was prevention of amputation, without the intention of curation: curation was not the goal, palliation was.

In chapter 6 of this thesis adverse effects and safety issues are discussed. The first study on 41 patients assesed the YUK-factor, it was found not to be an important factor for

patients treated with maggot therapy. Bleeding complications occurred with MDT, but seemed to occur mainly in patients on oral anticoagluation therapy (relative risk 2.8). It was advised to only perform MDT on patient with oral anticoagluation therapy under close supervision. In the final article of this chapter, pain issues (41 patients) were adressed, for in literature there is much debate about this issue. We found that especially non-diabetic patients experienced pain during MDT, compared to diabetic patients ($p<0.05$). A standardised pain management protocol for patients receiving this therapy was advised.

In chapter 7 two articles are described. In the first, 125 wounds in 109 patients were studied. Smoking has proven negative effects on woundhealing, the question however was if smoking was of negative influence on maggot-treated wounds. In this study a positive outcome in smokers (67.7%) was equal to patients that were classified as non-smokers (70.8%). It was stated smoking is not considered a contra-indication for MDT. Ischemia is another factor, which is frequently stated to be a contra-indication for MDT. In the second article, this was subject of study. In a study on 89 patients treated with maggots for wounds on the lower extremity it was found that patients classified as vascular patients had significantly worse outcome ($p<0.001$), however still 52% had a beneficial outcome. If these patients were succesfully revascularised the woundhealing rates raised to 68% (non-significant). It's argued that ischemia plays a major role and should be corrected, but it's not an absolute contra-indication for MDT, for still 52% of vascular patients heal with MDT.

Samenvatting

Ondanks moderne wondbehandeling en antibiotica, bestaan er nog steeds patiënten met chronische wonden. Het ontstaan van antibioticaresistente bacteriën, zoals MRSA (Metilcilline-resistente Staphylococcus Aureus), heeft in de laatste 10 tot 20 jaar tot een ware opkomst van behandelingen met maden geleid. Ronald Sherman heeft in 1989 madentherapie als het ware opnieuw ontdekt. Behandeling met steriele maden (Engels: Maggot Debridement Therapy) krijgt een steeds grotere vlucht, ook in Nederland. Het feit dat er, in de laatste tien jaar alleen al, meer dan 100 artikelen over maden zijn verschenen, laat zien dat madentherapie een sterke comeback heeft gemaakt in de geneeskunde. In januari 2004 heeft de Amerikaanse FDA (U.S. Food and Drug Administration), regel 510(k)#33391 uitgegeven, waardoor de productie en verkoop van maden als een medisch apparaat is toegestaan. De International Biotherapy Society heeft haar zevende internationale congres in 2007. Er zijn nu ongeveer 300 centra in Amerika en meer dan 1000 in Europa die madentherapie toepassen.

Bewijs in de vorm van multi-center gerandomiseerd onderzoek van behandeling met maden is niet voorhanden. Er zijn drie gerandomiseerde studies naar madentherapie in de literatuur gepubliceerd, de patiëntenaantallen van de studies waren klein, en de kwaliteit van de studies kon in twee gevallen niet gecontroleerd worden. De studie waarvan de kwaliteit wel gecontroleerd kon worden, ging maar over een totaal van twaalf patiënten. Madentherapie heeft, na de introductie in het LUMC in 1999, ook een grote vlucht in Nederland genomen. In 2002 werd in het Rijnland Ziekenhuis een speciale wondenpolikliniek opgericht. Naast vele andere behandelmethoden is daar in 2007 de 150ste patiënt met maden behandeld. Bij de start van de madentherapie bleken er nog vele basale klinische vragen onbeantwoord. Die vragen vormden de basis voor dit proefschrift.

In HOOFDSTUK 2 worden histopathologische, laboratorium- en microbiologische bevindingen bij madentherapie beschreven. In het eerste artikel worden coupes bestudeerd van wonden die behandeld werden met maden. In deze studie vonden we dat met maden behandelde wonden in het pathologische beeld van de inflammatiefase naar de proliferatie fase gingen, zoals ook gebruikelijk is wanneer de wond geneest door andere behandelingen. In de tweede studie worden 16 patiënten beschreven bij wie we hebben aangetoond dat er na de behandeling met maden, een opmerkelijke daling optrad van het aantal Leukocyten in het bloed (van 10.5 x 10e9/L naar 8.4 x 10e9/L). Hiermee werd aangetoond dat de werkzaamheid van madentherapie niet alleen zichtbaar is in de wond, maar zich ook vertaalt in vermindering van de infectieparameters bij de patiënt. Een dergelijk effect werd voor bezinking en C-reactieve proteïne niet gezien. In de derde studie (zelfde 16 patiënten) is in-vivo gekeken naar de antimicrobiële effecten van madentherapie. Uit laboratoriumstudies (in vitro-studies) bleek dat madentherapie effectiever is tegen infecties die veroorzaakt worden door gram-positieve bacteriën, in vergelijking met infecties veroorzaakt door gram-negatieve bacteriën. In deze studie vonden we dat de kans op het vinden van een gram-negatieve bacterie na madentherapie opmerkelijk groter was dan voor de behandeling met madentherapie ($p=0.001$), voor gram-positieve infecties ($p=0.07$) werd het tegenovergestelde gevonden. In deze studies hebben we laten zien dat de effectiviteit van madentherapie het grootst is bij gram-positieve infecties. Daarom, op basis van een sub-analyse, adviseerden we het gebruik van meer maden bij gram-negatieve infecties. Dit advies is later ook door de FDA overgenomen.

In HOOFDSTUK 3 wordt ingegaan op patiënt-, wond- en therapiefactoren, die van invloed zijn op madentherapie (101 patiënten, 117 wonden). Hierdoor kan een betere patiëntselectie plaatsvinden. In 67% van de gevallen was er sprake van een positieve uitkomst na de inzet van madentherapie. Alle wonden met een traumatische origine (n=24) hadden een goed resultaat. Alle patiënten die zich presenteerden met een septische artritis en die werden behandeld met maden (n=13), hadden een slechte uitkomst. Daarnaast bleek bij een mulitvariate analyse dat vaatlijden (Odds Ratio (OR): 7.5), diepte van de wond (OR: 14.0) en oudere leeftijd (≥60 yrs) (OR: 7.3) de uitkomst negatief beïnvloedden. De uitkomst bleek niet beïnvloed te worden door geslacht, overgewicht, diabetes mellitus, roken, ASA-classificatie, locatie van de wond, wondgrootte of hoelang de wond al bestond.

In HOOFDSTUK 4 wordt een studie beschreven bij 64 patiënten met 69 wonden bij wie twee applicatietechnieken met elkaar worden vergeleken. Er zijn twee applicatiemethodes voor het aanbrengen van de maden. Traditioneel wordt de zogenaamde losse techniek gebruikt, hierbij worden maden los op de wond aangebracht, waarna een 'kooi' van hydrocolloïd, nylongaas en bruine pleister ontsnapping moet voorkomen. De losse techniek is niet goed te gebruiken als de wondranden slecht zijn af te plakken, zoals het geval kan zijn bij wonden rondom de anus. Het alternatief is de tweede applicatietechniek, die van de zogenaamde 'biobag': een techniek waarbij maden in een soort theezakje worden aangebracht. De applicatietechniek is vereenvoudigd, hoewel nog steeds een zelfde 'kooi' als bij de losse techniek noodzakelijk is, als een tweede barrière voor ontsnapte maden. In deze studie werd gevonden dat het de losse techniek tot opmerkelijk betere uitkomsten leidde dan de techniek waarbij de maden in een biobagtechniek worden geappliceerd (P=0.028).

In HOOFDSTUK 5 worden meerdere gevalsbeschrijvingen en kleine series gepresenteerd aan de hand van studies die verricht zijn naar madentherapie. Het eerste artikel is het eerste artikel dat we als onderzoeksgroep hebben gepubliceerd, het gaat over de eerste elf patiënten die in het LUMC zijn behandeld, waarbij 100 tot 2900 maden werden geappliceerd in 3 tot 10 behandelingen (11-34 dagen behandelduur), waarbij de maden een positieve invloed hadden op de uitkomst. Het tweede artikel beschrijft een patiënt die met een ernstige infectie te maken had na een mammasparende behandeling in verband met een mammacarcinoom. De wond genas niet ondanks langdurige behandeling met antibiotica, chirurgische necrotectomieën en andere behandelingen. De wond werd uiteindelijk succesvol behandeld met madentherapie. In dit artikel worden specifieke moeilijkheden (en mogelijke oplossingen) beschreven die de behandeling met zich meebracht. Necrotiserende fasciitis is een ernstig ziektebeeld, dat nog steeds een hoge mortaliteit heeft (in de literatuur tot 70%). De behandeling bestaat uit snelle chirurgische necrotectomie en het geven van antibiotica. Er worden twee artikelen gepresenteerd, het eerste is een gevalsbeschrijving en het tweede gaat over een serie van 15 patiënten die behandeld zijn met madentherapie. In deze serie konden we aantonen dat het vroegtijdig inzetten van madentherapie (naast chirurgisch debridement en antibiotische therapie) het totale aantal benodigde chirurgische necrotectomieën (op de operatiekamer) van 4.1 daalde naar 1.8 (p=0.001). Het volgende artikel beschrijft een serie van 5 patiënten. In dit artikel worden de mogelijkheden beschreven (waaronder behandeling met maden) om geïnfecteerde amputatiestompen succesvol te behandelen, waardoor er minder noodzaak is om een bovenbeenamputatie uit te voeren naar aanleiding van de indicatie "geïnfecteerde onderbeenstomp". Het laatste artikel beschrijft

een patiënt, bij wie blijkt dat madentherapie niet alleen ingezet kan worden ter genezing, maar ook goed ter palliatie. Het betreft een 94-jarige patiënt die gezien pijnlijke geïnfecteerde wonden aan het onderbeen een onderbeenamputatie moest ondergaan. De patiënt en familie wilden dit liever niet en met behulp van madentherapie waren we in staat om de wonden schoon te krijgen, zodat er geen infectie meer was en geen pijn.

In HOOFDSTUK 6 van dit proefschrift worden complicaties en veiligheidsaspecten besproken. In het eerste artikel wordt de zogenaamde 'jakkiebakkie'-factor (Engels: YUK factor) beschreven. Er werd een enquête verstuurd onder onze eerst behandelde patiënten (38 patiënten). Het bleek dat 89% van de patiënten weer een behandeling zou ondergaan indien dat nodig was, 94% van de patiënten raadde de therapie aan andere patiënten aan. Op basis van deze enquête blijkt de jakkiebakkiefactor niet zo'n grote rol te spelen bij onze patiënten. Van de nu meer dan 150 patiënten die in het Rijnland Ziekenhuis Leiderdorp zijn behandeld, was er maar 1 patiënt die de behandeling weigerde. In het tweede artikel wordt ingegaan op bloedingcomplicaties bij madentherapie. Uit een studie bij 41 patiënten bleek dat het relatieve risicofactor voor een niet-levensbedreigende bloeding 1.3 was voor patiënten die aspirines gebruiken (zoals Ascal) en 2.8 voor patiënten met orale antistollingmedicatie. Op basis van het feit dat er geen bloeding optrad bij het gebruik van de biobag, werd geadviseerd madentherapie bij patiënten met orale antistollingmedicatie alleen toe te passen gedurende een klinische opname en, indien mogelijk, tijdelijk te stoppen met de antistollingmedicijnen of gebruik te maken van de biobag. Pijn bij de behandeling met madentherapie is een belangrijk probleem, dat in de literatuur wisselend wordt beschreven. In een studie bij 38 patiënten bleek dat pijn bij 78% van de patiënten goed behandeld kon worden met pijnstillers. Vooral niet-diabeten kregen meer pijn tijdens de behandeling met maden, dit verschilt opmerkelijk met de resultaten bij diabeten ($p<0.05$). Geadviseerd werd om pijnstilling op maat voor te schrijven.

In HOOFDSTUK 7 worden nog twee studies beschreven, waarin wordt ingegaan op roken en vaatlijden als mogelijke contra-indicaties voor madentherapie. In de eerste studie wordt beschreven dat bij 109 patiënten (125 geïnfecteerde, chronische wonden) het succespercentage van madentherapie voor rokers (67.7%) gelijk is aan dat van niet-rokers (70.8%). In dit artikel wordt dan ook gesteld dat roken, met zijn bekende negatieve effecten op wondgenezing, voor madentherapie geen specifieke absolute contra-indicatie is. In het tweede stuk wordt een studie beschreven bij 89 patiënten die worden behandeld voor wonden aan de onderste extremiteiten. Vaatlijden wordt in veel studies als contra-indicatie gezien voor madentherapie. In deze studie vonden we dat vaat-patiënten een opmerkelijk slechtere uitkomst ($p<0.001$) hadden dan niet-vaatpatiënten; echter, 52% van de vaat-patienten had nog steeds een goede uitkomst. Als er succesvol gerevasculariseerd kon worden (chirurgisch of radiologisch), steeg dit percentage naar 68% (niet-opmerkelijk). In deze studie werd geconcludeerd dat vaatlijders met madentherapie weliswaar minder kans op genezing hebben, maar dat dit niet zwart-wit moet worden gezien. Madentherapie is niet meer weg te denken uit het arsenaal aan wondbehandelingstechnieken dat we in het ziekenhuis tot onze beschikking hebben.

Madentherapie is een methode waarbij necrose effectief kan worden verwijderd, zonder dat hiervoor opname en anesthesie nodig zijn. Dit zijn belangrijke factoren in de huidige tijdsgeest van efficiëntie en kostenbesparing in de zorg. Bovendien is het voor de patiënt een goed gegeven dat hij de behandeling in zijn eigen omgeving kan ondergaan.

Veel patiënten worden met madentherapie poliklinisch behandeld (in ons centrum ongeveer 60%). Door middel van de studies die we verricht hebben, kunnen we een betere inschatting maken welke patiënt / wond behandeld kan worden met maden.

Er is echter wel een kanttekening, madentherapie is geen heilig water; het is niet zo, dat alles wat wordt aangeraakt door maden, geneest. Er zijn ook wonden die niet door middel van madentherapie genezen kunnen worden en die uiteindelijk leiden tot een amputatie. Voorkomen moet worden dat iedere patiënt, hoe ernstig het ook is, eerst 'een rondje maden' moet krijgen, voordat hij een amputatie kan ondergaan, opdat hij weet dat 'alles geprobeerd is'. We willen geen valse hoop wekken bij onze patiënten. Madentherapie moet worden toegepast als de indicaties en limitaties bekend zijn. In onze ogen hoeft dat niet per se in een gespecialiseerde wondkliniek te zijn. Mogelijk dat door nieuwe ontwikkelingen, bijvoorbeeld door gebruik te maken van madensecreten in plaats van de maden zelf, de behandeling vereenvoudigd zou kunnen worden, waardoor meer hulpverleners en ziekenhuizen er gebruik van zullen maken.

In de nabije toekomst hopen we dat toepassing van madentherapie ook mogelijk is in de thuissituatie (dus niet in de kliniek en niet op de polikliniek), zodat gespecialiseerde verpleegkundigen of nurse-practitioners de behandeling thuis kunnen uitvoeren. Dit moet dan wel eerst financieel mogelijk gemaakt worden. Norman Bethune, een beroemde Canadese thorax chirurg (die maden toepaste in de thoraxholte) zei ooit terwijl hij in de Japans-Chinese oorlog in China, onder erbarmelijke omstandigheden aan het opereren (1937) was: 'Doctors! Go to the wounded! Do not wait for them to come to you.' Ik zou dit proefschrift willen afsluiten met: Dokters, ga naar je patiënten, wacht niet tot ze naar jou komen!

Publications on maggot therapy by Steenvoorde

Jukema GN, Menon AG, Bernards AT, **Steenvoorde P**, Dissel JT van 'Amputation-sparing surgery by nature: maggots revisited.' *Clin Infect Dis 2002 15; 35(12): 1566-71.*

Jukema GN, **Steenvoorde P**, Lindeman JHN, Hanemaaijer R, Bockel JH van, Terpstra OT. 'Severe infection in traumapatients: extended amputation or biosurgery by Maggots?' *European J. Trauma S 1/2002/Abstracts 188-189.*

Jukema GN, **Steenvoorde P**, Lindeman JHN, Haanemaaijer R, Bockel JH van. Osteomyelitis in Diabetes: Vacuum Sealing and Maggot Therapy. *Diabetic Foot Study Group of the European Association for the Study of Diabetes (EASD) 3rd Scientific Meeting Balontfured, Hungary 29th august /1 September 2002,*
http://dialex.co.uk/conferences/diabetic_foot_study_group_2002/diabetic_foot_study_group_2002.htm: page 13

Steenvoorde P, Jukema GN, Lindeman JHN, Hanemaaijer R, Taheri R, Bockel JH van, Terpstra OT. 'Steriele maden: Eéndagsvlieg of toekomstperspectief?' *Voorjaarsvergadering Chirurgendagen 31 mei 2002.*

Kortekaas RTJ, **Steenvoorde P**, Dissel JT van, Bernards AT, Jukema GN. 'Made in Holland'. Necrotomie van geinfecteerde wonden met behulp van 'chirurgische' maden. *Voorjaarsvergadering Chirurgendagen,2003; P11:161.*

Steenvoorde P, Jukema G N. Can laboratory investigations help us to decide when to discontinue larval therapy? *J Wound Care 2004 Jan; 13(1):38-40.*

Steenvoorde P. So28 – Maggot debridement therapy in chronic wounds: the antimicrobial activity of maggots: in vivo results.: *2nd World Union of Wound Healing Societies'Meeting, 8th/13th july 2004, Abstract book: 221.*

Steenvoorde P, Jukema GN. The anti-microbial activity of maggots, in vivo-results. *J Tissue Viability 2004; 14(3): 97-101.*

Steenvoorde P. So28 – Maggot debridement therapy in chronic wounds: the antimicrobial activity of maggots: in vivo results.: *2nd World Union of Wound Healing Societies'Meeting, 8th/13th july 2004.*

Steenvoorde P, Oskam J. Bleeding complications in patients treated with Maggot Debridement Therapy (MDT). *Int J LowExtrem Wounds 2005; 4(1):57-58.*

Steenvoorde P, Oskam J. Use of larval therapy to combat infection after breast- conserving surgery. *J Wound Care 2005; 14(5): 212-213.*

Steenvoorde P, Budding TJ, Engeland A. van, Oskam J. Maggot therapy and the 'YUK factor'; an issue for the patient? *Wound repair and Regeneration 2005; 13(3); 350-352.*

Steenvoorde P, Jacobi CE, Oskam J. Maggot Debridement Therapy: Free-range or contained? An In-vivo study. *Adv Skin Wound Care 2005 18(8):430-435.*

Steenvoorde P, Budding TJ, Oskam J. Pain levels in patients treated with maggot debridement therapy. *J Wound Care 2005; 14(10): 485-488.*

Jukema GN, Wong CY, **Steenvoorde P**, Plas MJA van der, Bernards AT, Dissel JT van. Der Weichteilschaden ein Problem? Die Verwendung von 'chirurgische' Maden in der Traumatologie. 122 Kongress der Deutsche Gesellschaft fur Chirurgie. Munchen, 5-8 april 2005. http://www.egms.de/en/meetings/dgch2005/05dgch761.shtml

G.N. Jukema, C.Y. Wong, **P Steenvoorde**, and J.T. v Dissel
Euroepan Bone and Joint Infection Society (Milan 2004) : Experiemtnal use of 'surgical' maggots for preventiong amutations in trauma surgery. J Bone Joint Surg Br Proceedings, Sep 2005; **87-B**: 251. http://proceedings.jbjs.org.uk/cgi/search?fulltext=steenvoorde&volume=87-B&issue=SUPP+III&journalcode=jbjsbrproc

Jukema GN, Wong CY, **Steenvoorde P**, Dissel JT van. Surgical maggots for treatment of periarticular infections and osteomyelitis, when future meets history. *European Bone and Joint Infection Society annual meeting 19-21 May Ljubljana, Slovenia 2005.*

Jukema GN, Wong CY, **Steenvoorde P**, Plas MJA van der, Bernards AT, Nibbering PH, Dissel JT van. New approach in traumasurgery: can maggots reduce invalidating amputations in case of serious injury wiht severe infection? P36 ZfW Sonderhelft 2/2005 Wound Rep Reg 2006 14 A17-51: A13. (Abstract 411)
http://www.stuttgart2005.0rg/document/poster_abstracts/Poster%2021-40.pdf

Steenvoorde P, Jacobi CE, Oskam J. Re: Maggot Therapy in "Lower-Extremity Hospice" Wound Care. *J Am Podiatr Med Assoc 2006 96(1):82-83.*

Steenvoorde P, Oskam J. 'Modern Wound treatment of infected transtibial amputation: Case reports' *J Prosthet Orthot 2006; 18:17-20.*

Rozeboom A, **Steenvoorde P**, Hartgrink H, Jukema GN. Necrotizing fascitits following a simple pelvic fracture: case report and literature review. *J Wound Care 2006;* 15(3):117-120.

Steenvoorde P, Oskam J. Maggot therapy in the Netherlands; Rijnland Hospital Leiderdorp. *BioTherapeutics Education and Research Foundation. The BeTer Letter 2006; 3(1); 1-5.*
www.bterfoundation.org/tbl/tbl_0301.pdf

Steenvoorde P, Doorn L van, Jacobi CE, Oskam J The Yuk Factor: Maggot debridement therapy: the ancient treatment for chronic wounds makes comeback.
The Hospitalist 2006 10(8): 16-21.

Steenvoorde P, Doorn LP van, Jacobi CE, Kaptein AA, Oskam J. Re: Patient acceptability of larval therapy for leg ulcer treatment: a randomised survey to inform the sampe size calculation of a randomised trial. BMC Medical Research Methodology 2006; 6:43 (www.biomedcentral.com/1471_2288/6/43/comments)

Steenvoorde P, Calame J, Oskam J Maggot-treated wounds follow normal wound healing phases. *Int J Dermatol 2006; 45(12):1477-9.*

Steenvoorde P, Doorn LP van, Jacobi CE, Oskam J. Behandeling met steriele maden kan nu poliklinisch plaatsvinden. *Nederlands Tijdschrift voor Wondverzorging 2006; nr 6/7, art 3.*

Jukema GN, **Steenvoorde P**, Wong CY, Bernards SAT, Dissel JT van. [Maggot Therapy for Treatment of Severe Infections in Trauma Surgery: "Back to the Future!" *Zentralbl Chir 2006; 131(S 1): 75-78.*

Jukema G, **Steenvoorde P**, Wong CY, Bernards SAT, Dissel JT van. Ein neues konzept in der Behandlung von Infectionen in der Traumatologie – 'Back to the Future'. *Drei-Lander-Kongres Nurnberg 28-29 April 2006. /www.drei-laender-kongress.de/programm.pdf*

Steenvoorde P, Doorn L van, Jacobi CE, Oskam J: Maggot Debridement Therapy: Free-range or contained? *European Tissue Repair Society 16th annual meetig. Pisa 13-16 september 2006, Abstractbook page 161.*

Steenvoorde P, Doorn L van, Jacobi CE, Oskam J. V03.08 Madentherapie: retrospectieve vergelijking tussen twee verschillende applicatie-technieken. *Nederlands Tijdschrift voor Heelkkunde 2006; 15(4): 86.*

Steenvoorde P, Jacobi CE. Oskam J. The results of Maggot Debridement Therapy in the ischemic leg. A study on 89 patients with 89 wounds on the lower leg treated with maggots. *The Internet Journal of Surgery 2007 9(1).*

Steenvoorde P, Jacobi CE, Wong C, Jukema GN. Maggot debridement therapy in necrotizing fasciitis reduces the numer of surgical debridements. Report on 15 treated patients. *Wounds 2007; 19(3): 73-78.*

Steenvoorde P, Jacobi CE, Doorn L van, Oskam J. Smoking is not contra-indicated in maggot debridement therapy. Based on a study on 125 wounds in 109 patients. *European Wound Management Association 2007; 7(1):17-20.*

Steenvoorde P, Jacobi CE, Doorn, L. van, Oskam J. Maggot Debridement Therapy of chronic infected ulcers: factors influencing outcome. A Study on 101 patients with 117 chronic wounds treated with maggots. *Ann of the Royal Coll of Surg England 2007; 89(6):596-602.*

Steenvoorde P, Doorn L van, Jacobi CE, Oskam J. Maggot Debridement Therapy in the palliative setting. *Am J Hosp Palliat Care 2007; 24(4): 308-10.*

Naves C, **Steenvoorde P,** Oskam J, Costa A da, Jacobi CE. Maggot debridement therapy: which patients are suitable for treatment at home. Poster presentation ETRS Southampton 26-28 september 2007. *Lower Extremity Wounds 2007; 6(3): 223.*

Curriculum vitae

Pascal Steenvoorde, geboren op 7 november 1974 te Bilthoven (gemeente de Bilt).

Mijn jeugd heb ik doorgebracht in Lelystad en later in Lisse. Mijn VWO diploma behaalde ik in 1993 op het Rijnlands Lyceum in Sassenheim. In datzelfde jaar startte ik met de studie Geneeskunde in Leiden. Tevens startte ik in 1994 met de studie psychologie. Mijn doctoraal examen geneeskunde behaalde ik in 1997. Het artsexamen behaalde ik in 1999, met een onderzoek onder leiding van Prof. E.K.J. Pauwels, naar schadelijke effecten van diagnostisch nucleair onderzoek voor de ongeboren vrucht. In het jaar 2000 sloot ik mijn studie Psychologie succesvol af. Het onderzoek waarmee ik deze opleiding beindigde, onder leiding van M. van de Reijden, had als titel: *Optimalisering Ouderschap, in Een tot Drie Gesprekken*.

Na een korte periode als arts werkzaam te zijn geweest in Tanzania, bracht ik twee jaar door als AGNIO (assistent geneeskunde niet in opleiding). Allereerst bij B. Lether in het Spaarne Ziekenhuis Haarlem en later in het Leids Universitair Medisch Centrum. In dat jaar, in 2001, werd mijn interesse voor wondbehandeling gewekt door G.N. Jukema. In 2002 heb ik samen met J. Oskam, chirurg in het Rijnland Ziekenhuis, het Rijnland Wond Centrum opgericht, een polikliniek waar patiënten met chronische en complexe wonden worden behandeld.

De interesse voor wondproblemen heeft geleid tot presentaties op nationale en internationale congressen, naast het feit dat er meerdere publicaties op wondgebied volgden. In 2002 startte ik de opleiding Heelkunde, allereerst in het Rijnland Ziekenhuis Leiderdorp (opleider F. Rijksen en later S.A. da Costa). Na 2 jaar in het LUMC (opleider Prof. O.T.T. Terpstra en later Prof. J. Hamming) heb ik het laaste gedeelte van de opleiding weer in het Rijnland Ziekenhuis verricht. Het laatste jaar van deze opleiding differentieer ik in de gastroenterologie richting en op 1 januari 2008 zal ik de opleiding tot chirurg hebben afgerond.

REFERENCES

1. http://en.wikipedia.org/wiki/Image:Norman_Bethune_graduation_1922.jpg . 2007.
2. Osteomyelitis- the use of maggots. *Med Ann*. 1933;51:321.
3. Wainwright M. Maggot therapy - a backwater in the fight against bacterial infection. *Pharm Hist*. 1988;30:19-26.
4. Church JC. The traditional use of maggots in wound healing, and the development of larva therapy (biosurgery) in modern medicine. *J Altern Complement Med*. 1996;2:525-527.
5. Grassberger M. Ein historischer Ruckblick auf den therapeutischen einsatz von fliegenlarven. *NTM*. 2002;10:13-24.
6. Dunbar GK. Notes on the Ngemba tribe of the Central Darlin River of Western New South Wales. *Mankind*. 1944;3 S:177-180.
7. Greenberg B. *Flies and Diseases. Biology and disease transmission*. 1973.
8. Weil JC, Simon RJ, Sweadner WR. A biological, bacteriological and clinical study of larval or maggot therapy in the treatment of acute and chronic pyogenic infections. *American Journal of Surgery*. 1933;19 S:36-48.
9. Goldstein HI. Maggots in the treatment of wound and bone infections. *J Bone Joint Surgery*. 1931;13:477-478.
10. Coppi C. I dressed your wounds, God healed you - a wounded person's psychology according to Ambroise Pare. *Ostomy Wound Manage*. 2005;51:62-64.
11. http://ca.wikipedia.org/wiki/Ambroise_Par%C3%A9
12. Adams GW. Wartime Surgery. In: New York HS, ed. *Doctors in blue: The medical history of the Union Army in the Civil War*. 1952.
13. Baer WS. The treatment of chronic osteomyelitis with the maggot (larva of the blow fly). *J Bone Joint Surgery*. 1931;13:438-475.
14. Puckner WA. New and nonofficial remedies, surgical maggots-Lederle. *J Am Med Assoc*. 1932;98:401.
15. Sherman RA, Wyle F, Vulpe M. Maggot therapy for treating pressure ulcers in spinal cord injury patients. *J Spinal Cord Med*. 1995;18:71-74.
16. Dissemond J, Koppermann M, Esser S, Schultewolter T, Goos M, Wagner SN. [Treatment of methicillin-resistant Staphylococcus aureus (MRSA) as part of biosurgical management of a chronic leg ulcer]. *Hautarzt*. 2002;53:608-612.
17. Church JC. The traditional use of maggots in wound healing, and the development of larva therapy (biosurgery) in modern medicine. *J Altern Complement Med*. 1996;2:525-527.
18. Church J. Biosurgery in wound healing. *J Wound Care*. 1996;5:51.
19. Thomas S, Jones M, Shutler S, Andrews A. Wound care. All you need to know about ... maggots. *Nurs Times*. 1996;92:63-6, 68, 70.
20. Thomas S, Jones M, Shutler S, Jones S. Using larvae in modern wound management. *J Wound Care*. 1996;5:60-69.
21. Jukema, G. N. Made in Holland, Het gebruik van maden in de wondbehandeling. Abstract book 29 november 2002, 1. 2002.
22. Steenvoorde, P, Jukema, G. N., Lindeman, J. H. N., and Hanemeijer, R. Steriele maden in de heelkunde: Eendagsvlieg of toekomstperspectief. Lustrumcongres 2002 samenvattingen, 116. 2002.
23. Personal communication of a patient in our wound clinic february 2007.
24. Nederlands Instituut voor Militaire Historie. 22-3-2007.
25. Steenvoorde P. Moderne wondbehandeling. Huisartsen symposium diabetische voet, Alphen a/d Rijn 21 november. 2002.
26. Steenvoorde P., Engeland, A. van, and Oskam, J. Nieuwe ontwikkelingen in de wondgenezing. Wetenschapsdag Rijnland ziekenhuis; Leiderdorp 25 november. 2002.
27. Reinders, R. Rijnland Ziekenhuis onderzoekt effect maden op genezing wonden. Leidsch Daglad 2 januari 2003.
28. Steenvoorde P, Doorn L, Jacobi CE, Oskam J. Behandeling met steriele maden kan nu poliklinisch plaatsvinden. *Nederlands Tijdschrift voor Wondverzorging*. 2006;artikel 3.

29. Steenvoorde P, Oskam J. Modern Wound treatment of infected transtibial amputation: Case reports. *J Prosthet Orthot*. 2006;18:17-20.
30. Steenvoorde P, Doorn L, Jacobi CE., and Oskam J. The use of OASIS wound Martrix in 20 chronic wounds. European Tissue Repair Society 16th annual meetig. Pisa 13-16 september, Abstractbook page 42. 2006.
31. http://www.icb2007.org/main/index.php?page=home . 2007.
32. http://biotherapy.md.huji.ac.il/newsletter09.htm . 2007.
33. Raynor P, Dumville J, Cullum N. A new clinical trial of the effect of larval therapy. *J Tissue Viability*. 2004;14:104-105.
34. Petherick ES, O'Meara S, Spilsbury K, Iglesias CP, Nelson EA, Torgerson DJ. Patient acceptability of larval therapy for leg ulcer treatment: a randomised survey to inform the sample size calculation of a randomised trial. *BMC Med Res Methodol*. 2006;1:43.
35. Debridement. http://en.wikipedia.org/wiki/debridement . 2007.
36. Leaper, D. Sharp technique for wound debridement. World wide wounds www. worldwidewounds.com/2002/december/Leaper/sharp-debridement.html. 2002.
37. Kirshen C, Woo K, Ayello AE, Sibbald RG. Debridement: A Vital Component of Wound Bed Preparation. *Adv Skin Wound Care*. 2006;19:506-517.
38. Baharestani M. The clinical relevance of debridement. In: Baharestani M, ed. *The clinical relevance of debridement.* Berlin: Springer-Verlag; 1999.
39. Mulder GD, Vande Berg JS. Cellular senescence and matrix metalloproteinase activity in chronic wounds. *JAPMA*. 2002;92:34-37.
40. Kirsner R. Wound bed preparation. *Ostomy Wound Manage*. 2003;49:2-3.
41. Robson MC, Stenberg BD, Heggers JP. Wound healing alterations caused by infection. *Clin Plast Surg*. 1990;17:485-492.
42. Zacur H, Kirsner RS. Debridement: Rationale and Therapeutic optons. *Wounds*. 2007;14:2s-6s.
43. Hunt TK, Hopf H, Hussain Z. Physiology of wound healing. *Adv Skin Wound Care*. 2000;13:6-11.
44. Bradley M, Cullum N, Sheldon T. The debridement of chronic wounds: a systematic review. *Health Technology Assessment*. 1999;3.
45. National Institute for clinical Excellence. Guidance for the use of debriding agents for difficult to heal surgical wounds. *London: Nice*. 2000.
46. Smith J. Debridement of diabetic foot ulcers. *Cochrane Databases of Systematic Reviews*. 2002.
47. Lewis R, Whiting P, Riet Gt, O'Meara S, Glanville J. A rapid and systematic review of the clinical effectiveness and cost-effectiveness of debriding agens in treating surgical wounds healing by secondary intention. *Health Technol Assess*. 2001;5.
48. Bonn D. Maggot therapy: an alternative for wound infection. *Lancet*. 2000;356:1174.
49. Prete PE. Growth effects of Phaenicia sericata larval extracts on fibroblasts: mechanism for wound healing by maggot therapy. *Life Sci*. 1997;60:505-510.
50. Stoddard SR, Sherman RA, Mason BE, Pelsang DJ, Sherman RM [corrected to Sherman RA]. Maggot debridement therapy. An alternative treatment for nonhealing ulcers. *J Am Podiatr Med Assoc*. 1995;85:218-221.
51. Ziffren SE, Heist HE, May SC, Womack NA. The secretions of collagenase by maggots and its implication. *Ann Surg*. 1953;138:932-934.
52. Jarvis A. Maggot therapy. *Lancet*. 2000;356:2016.
53. Pavillard ER, Wright E.A. An antiobiotic from Maggots. *Nature*. 1957;180:916-917.
54. Thomas S, Jones M, Shutler S, Andrews A. Wound care. All you need to know about ... maggots. *Nurs Times*. 1996;92:63-6, 68, 70.
55. Mumcuoglu KY, Ingber A, Gilead L et al. Maggot therapy for the treatment of intractable wounds. *Int J Dermatol*. 1999;38:623-627.
56. Nigam Y, Bexfield A, Thomas S, Ratcliffe NA. Maggot Therapy: The Science and Implication for CAM Part II- Maggots combat infection. *Advance Access Publication*. 2006;3:303-308.
57. Sibbald R, Williamson D, Orsted H. Preparing the wound bed- debridement, bacterial balance, and moisture balance. *Ostomy Wound Manage*. 2007;46:14-22, 24-8, 30-5.
58. Sieggreen MY, Maklebust J. Debridement: Choices and challenges. *Adv Wound Care*. 1997;10:32-71.
59. Vowden, K. and Vowden, P. Wound bed preparation. http://www.worldwidewounds.com/2002/april/Vowden/Wound-Bed-Preparation.html . 2002. 1-8-2005.

60. Mosti G, Iabichella ML, Picerni P, Maqliaro A, Mattaliano V. The debridement of hard to heal leg ulcers by means of a new device based on Fluidjet technology. *International Wound Journal. 2005;2:307-314*.
61. Kiernan M. Nurse prescriber. Wet, sloughy and necrotic wound management. *Community Nurs*. 1999;5:51-52.
62. Varghese MC, Balin AK, Carter DM, Caldwell D. Local environment of chronic wounds under synthetic dressings. *Arch Dermatol*. 1986;122:52-57.
63. Mekkes JR, Zeegelaar JE, Westerhof W. Quantitative and objective evaluation of wound debriding properties of collagenase and fibrinolysin/desoxyribonuclease in a necrotic ulcer animal model. *Archives of Dermatological Research*. 1998;290:152-157.
64. Muller E, Leen MWv, Bergemann R. Economic evalutation of collagenase-containing ointment and hydrocolloid dressing in the treatment of pressure ulcers. *Pharmacoeconomics*. 2001;19:1209-1216.
65. Marazzi M, Stefani A, Chiaratti A, Ordanini MN, Falcone L, Rapisarda V. Effect of enzymatic debridement with collagenase on acute and chronic hard-to-heal wounds. *J Wound Care*. 2006;15:222-227.
66. Bott R, Crissman J, Kollar C et al. A silicone-based controlled-release device for accelerated proteolytic debridement of wounds. *Wound Repair Regen*. 2007;15:227-235.
67. Ziegler UE. Enzymatic Debridement. In: Cherry GW, Harding KG, Ryan TJ, eds. *Wound Bed Preparation*. London: Royal Society of Medicine Press Ltd; 2001:99-104.
68. Abdulwadud, O. What is the effectiveness of a dressing moistend with sodium hypochlorite (Dakin's) solution compared to other dressings in improving wound healing? Centre for clinical effectiveness evidence report . 2000.
69. Singhal A, Reis ED, Kerstein MD. Options for nonsurgical debridement of necrotic wounds. *Adv Skin Wound Care*. 2001;14:96-100.
70. Ayello EA, Cuddigan JE. Debridement: controlling the necrotic/cellular burden. *Adv Skin Wound Care*. 2004;17:66-75.
71. Jukema GN, Menon AG, Bernards AT, Steenvoorde P, Dissel JTv. Amputation-sparing surgery by nature: maggots revisited. Clin Infect Dis. 2002;35:1566-1571.
72. Fleischmann W, Russ MK, Moch D. Chirurgische Wundbehandlung. Chirurg. 1998;69:222-232.
73. Sherman RA, Sherman J, Gilead L, Lipo M, Mumcuoglu KY. Maggot debridement therapy in outpatients. Arch Phys Med Rehabil. 2001;82:1226-1229.
74. Second European Consensus. Second European Consensus document on chronic critical leg ischemia. Circulation. 1991;84:1-26.
75. Mauro T. Natural course of wound repair versus impaired healing in chronic skin ulcers. In: Shai A, Maibach HI, eds. Wound healing and ulcers of the skin. Heidelberg: Springer; 2005:7-18.
76. Hersh RE, Jack JM, Dahman MI, Morgan RF, Drake DB. The vacuum-assisted closure device as a bridge to sternal wound closure. Ann Plast Surg. 2001;46:250-254.
77. Gustafsson R, Johnsson P, Algotsson L, Blomquist S, Ingemansson R. Vacuum-assisted closure therapy guided by C-reactive protein level in patients with deep sternal wound infection. J Thorac Cardiovasc Surg. 2002;123:895-900.
78. Simmons S. The bactericidal properties of excrfetions of the maggot of Lucilia sericata. Bull Entomol Res. 1935;26:559-563.
79. Thomas S, Andrews AM, Hay NP, Bourgoise S. The anti-microbial activity of maggot secretions: results of a preliminary study. J Tissue Viability. 1999;9:127-132.
80. Jukema GN, Bohm HJ, Hierholzer G. Vacuum occlusion: a new concept in treatment of soft tissue and bone infections. Langenbecks Arch Chir Suppl Kongressbd. 1997;114:581-585.
81. Argenta LC, Morykwas MJ. Vacuum-assisted closure: a new method for wound control and treatment: clinical experience. Ann Plast Surg. 1997;38:563-576.
82. Pellizzer C, Strazzabosco M, Presi S et al. Deep tissue biopsy vs. superficial swab culture monitoring in the microbiological assessment of limb-threatening diabetic foot infection. *Diabetic Medicine*. 2001;18:822-827.
83. Robinson W, Norwood VH. The role of surgical maggots in the disinfection of osteomyelitis and other infected wounds. *J Bone Joint Surgery*. 1933;15:409-412.

84. Robinson W, Norwood VH. Destruction of pyogenic bacteria in the alimentary tract of surgical maggots implanted in infected wounds. *The Journal of Laboratory and clinical medicine*. 1933;19:581-585.
85. Mumcuoglu KY, Miller J, Mumcuoglu M, Friger M, Tarshis M. Destruction of bacteria in the digestive tract of the maggot of Lucilia sericata (Diptera: Calliphoridae). *J Med Entomol*. 2001;38:161-166.
86. Fitzpatrick M. Tiny „surgeons“ prove surprisingly effective. *JAMA*. 2000;284:2306-2307.
87. Thomas S, Jones M, Wynn K, Fowler T. The current status of maggot therapy in wound healing. *Br J Nurs*. 2001;10:S5-8, S10, S12.
88. Church JCT, Courtenay M. Maggot debridement therapy for chronic wounds. *Lower extremity Wounds*. 2002;1:129-134.
89. Wolff.H., Hansson C. Larval therapy - an effective method for ulcer debridement. *ClinExp Dermat*. 2003;28:137.
90. Courtenay M, Church JC, Ryan TJ. Larva therapy in wound management. *J R Soc Med*. 2000;93:72-74.
91. International consensus working group on antimicrobial therapy of diabetic foot infections, chaired by Professor Beanjamin A. Lipsky. International Consensus on the Management and the prevention of the diabetic foot. 2003.
92. Sherman RA, Wyle FA, Thrupp L. Effects of seven antibiotics on the growth and development of Phaenicia sericata (diptera: calliphoridae) Larvae. *J Med Entomol*. 1995;32:646-648.
93. Wollina U, Liebold K, Schmidt W-D, Hartmann M, Fassler D. Biosurgery supports granulation and debridement in chronic wounds - clinical data and remittance spectroscopy measurement. *Int J Dermatol*. 2002;41:635-639.
94. Wilson JA, Clark JJ. Obesity: Impediment to postsurgical wound healing. *Adv Skin Wound Care*. 2004;17:426-432.
95. Schomig M, Ritz E, Standl E, Allensberg J. The diabetic foot in the dialyzed patient. *J Am Soc Nephrol*. 2000;11:1153-1159.
96. Wollina U, Karte K, Herold C, Looks A. Biosurgery in wound healing--the renaissance of maggot therapy. *J Eur Acad Dermatol Venereol*. 2000;14:285-289.
97. Oyibo SO, Jude EB, Tarawneh I et al. The effects of ulcer size and site, patient‘s age, sex and type and duration of diabetes on the outcoume of diabetic foot ulcers. *Diabetic Medicine*. 2001;18:133-138.
98. Rijswijk Lv. Full-thickness leg ulcers: patient demographics and predictors of healing. Multi-center leg ulcer study group. *J Fam Pract*. 1993;36:625-632.
99. Sherman RA. A new dressing design for use with maggot therapy. *Plast Reconstr Surg*. 1997;100:451-456.
100. Sherman RA, Tran JM, Sullivan R. Maggot therapy for venous stasis ulcers. *Arch Dermatol*. 1996;132:254-256.
101. Sherman R.A. Maggot Therapy Information Sheet for Physicians (FAQs). http://www.ucihs.uci.edu/com/pathology/sherman/mdt_info.pdf . 2005. 22-6-0005.
102. Jukema GN, Menon AG, Bernards AT, Steenvoorde P, Dissel JTv. Amputation-sparing surgery by nature: maggots revisited. *Clin Infect Dis*. 2002;35:1566-1571.
103. Grassberger M, Fleischmann W. The biobag - A new device for the application of Medicinal Maggots. Dermatology. 2002;204:306.
104. Thomas S, Wynn K, Fowler T, Jones M. The effect of containment on the properties of sterile maggots. Br J Nurs, Tissue Viability Supplement. 2002;11:S21-S28.
105. Ganz ohne skalpell. Maden putzen wunden. Hospital Tribune. 2003;26.
106. Kitching M. Patient‘s perceptions and experiences of larval therapy. J Wound Care. 2004;13:25-29.
107. Steenvoorde P, Budding TJ, Engeland Av, Oskam J. Maggot therapy and the ‚YUK factor‘; an issue for the patient? Wound Repair Regen. 2005;13:350-352.
108. Chronic decubitus, ulcus cruris. Maggots feed for wound healing. MMW Fortschr Med. 2002;144:69.
109. Shai A, Maibach HI. *Wound Healing and Ulcers of the Skin. Diagnosis and Therapy - The practical approach*. Heidelberg: Springer-Verlag; 2005:1-268.

110. Simmons S. A bactericidal principle in excretions of surgical maggots which destroys important etiological agents of pyogenic infetions. *J Bacteriol*. 1935;30:253-267.
111. Wollina U, Karte K, Herold C, Looks A. Biosurgery in wound healing--the renaissance of maggot therapy. *J Eur Acad Dermatol Venereol*. 2000;14:285-289.
112. Sherman RA, Hall MJ, Thomas S. Medicinal maggots: an ancient remedy for some contemporary afflictions. *Annu Rev Entomol*. 2000;45:55-81.
113. Robinson W. Stimulation of healing in non-healing wounds. *J Bone Joint Surgery*. 1935;17:267-271.
114. Steenvoorde P, Oskam J. Use of larval therapy to combat infection after breast-conserving surgery. *J Wound Care*. 2005;14:212-213.
115. Steenvoorde P, Oskam J. Bleeding complications in patients treated with Maggot Debridement Therapy (MDT). Letter to the editor. *IJLEW*. 2005;4:57-58.
116. Chernin E. Surgical maggots. *South Med J*. 1986;79:1143-1145.
117. Larrey DJ. Observations on wounds, and their complications by erysipeals, gangrene and tetanus etc. [in French]. *Riviunus EF, trans Philadelphia: Key, Mielke, & Biddle*. 1832;34.
118. Fleischmann W, Russ MK, Moch D. Chirurgische Wundbehandlung. *Chirurg*. 1998;69:222-232.
119. Galeano M, Ioli V, Colonna M, Risitano G. Maggot therapy for treatment of osteomyelitis and deep wounds: an old remedy for an actual problem. *Plast Reconstr Surg*. 2001;108:2178-2179.
120. Wayman J, Nirojogi V, Walker A, Sowinski A, Walker MA. The cost effectiveness of larval therapy in venous ulcers. *J Tissue Viability*. 2000;10:91-94.
121. Gupta R, Sinnett D., Carpenter R, Preece PE, Royle GT. Antibiotic prophylaxis for post-operative wound infection in clean elective breast surgery. *Eur J Surg Oncol*. 2000;26:363-366.
122. Claxton MJ, Armstrong DG, Short B, Vazquez JR, Boulton AJ. 5 Questions - and answers - about maggot debridement therapy. *Adv Skin Wound Care*. 2003;16:99-102.
123. Horn KL, Cobb AH, Jr., Gates GA. Maggot therapy for subacute mastoiditis. *Arch Otolaryngol*. 1976;102:377-379.
124. Dunn C, Raghavan U, Pfleiderer AG. The use of maggots in head and neck necrotizing fasciitis. *J Laryngol Otol*. 2002;116:70-72.
125. Sealby N. The use of maggot therapy in the treatment of a malignant foot wound. *Br J Community Nurs*. 2004;9:S16-S19.
126. Kahn DC. Myiasis secondary to dermatobia hominis (humon botfly) presenting as a long-standing breast mass. *Arch Pathol Lab Med*. 1999;123:829-831.
127. Furey PC, Macgillivray DC, Castiglione CL, Allen L. Wound complications in patients receiving adjuvant chemotherapy after mastectomy and immediate breast reconstruction for breast cancer. *J Surg Oncol*. 1994;55:194-197.
128. Fleischmann W, Grassberger M, Sherman R. *Maggot Therapy: A Handbook of Maggot-Assisted Wound Healing*. New York: Thieme; 2004:81-85.
129. Singh G, Sinha SK, Adhikary S, Babu KS, Ray P, Khanna SK. Necrotising infections of soft tissues - a clinical profile. *Eur J Surg*. 2002;168:366-371.
130. Elliot DC, Kufera JA, Meyers RAM. Necrotizing soft tissue infections: risk factors for mortality and strategies for management. *Ann Surg*. 1996;224:672-683.
131. Wong CH, Wang YS. The diagnosis of necrotizing fasciitis. *Curr Opin Infect Dis*. 2005;18:101-106.
132. Hasham S, Matteucci P, Stanly PRW, Hart NB. Necrotising fasciitis. *BMJ*. 2005;330:830-833.
133. Ledingham IMCA, Tehrani MA. Diagnosis, clinical course and treatment of acute dermal gangrene. *Br J Surg*. 1975;62:364-372.
134. Cunningham JD, Silver L, Rudikoff D. Necrotizing fasciitis: a plea for early diagnosis and treatment. *Mt Sinai J Med*. 2001;68:253-261.
135. Childers BJ, Potyondy LD, Nachreiner R. Necrotizing fasciitis: a fourteen-year retrospective study of 163 consecutive patients. *Am Surg*. 2002;68:109-116.
136. Beck W, Weckbach A. [Necrotizing fasciitis after closed pelvic ring fracture. Case report and review of the literature]. *Unfallchirurgie*. 1993;19:234-239.
137. Thomas S, Andrews AM, Hay NP, Bourgoise S. The anti-microbial activity of maggot secretions: results of a preliminary study. *J Tissue Viability*. 1999;9:127-132.
138. Steenvoorde P, Jukema GN. The anti-microbial activity of maggots, in vivo-results. *J Tissue Viability*. 2004;14:97-101.

139. Jukema GN, Steenvoorde P, Timmers M. Installationvacuumsealing Technique with PVA-foam and Lavasept for treatment of osteomyelitis and soft tissue infection. *3th Conference European wound management association Pisa (Italy), 22-24 may.* 2003;http://www.ewma.org/pisa2003/pdf/044.pdf.
140. Fleischmann W, Russ M, Westhauser A, Strampehl M. Vacuum sealing as carrier system for controlled local drug administration in wound infection. *Unfallchirurg.* 1998;101:649-654.
141. Steenvoorde P, Jukema GN. Can laboratory investigations help us to decide when to discontinue larval therapy? *J Wound Care*. 2004;13:38-40.
142. Jones J. Investigation on the nature, causes and treatment of hospital gangrene as it prevails in the confederate armies 1861-1865. *In: Hasting Hamilton F, editor Surgical memoirs of the war of rebellion New York: Sanitary Commission*. 1871.
143. Descamps V, Atiken J, Lee MG. Hippocrates on necrotizing fasciitis. *Lancet*. 1994;344:556.
144. Meleney FL. Haemolytic strepotococcus gangrene. *Arch Surg*. 1924;9:317-364.
145. Wilson B. Necrotizing fasciiitis. *Am Surg*. 1952;18:416-431.
146. Endorf FW, Supple KG, Gamelli RL. The evolving characteristics and care of necrotizing soft-tissue infections. *Burns*. 2005;31:269-273.
147. Efem SE. The features and aetiology of Fournier's gangrene. *Postgrad Med*. 1994;70:568-571.
148. Eke N. Fournier's gangrene: a review of 1726 cases. *Br J Surg*. 2000;87:718-728.
149. Schnall SB. Necrotizing fasciitis: clinical presentation, microbiology and determinants of mortality. *J Bone Joint Surgery*. 2003;85A:869-870.
150. Teich S, Myers RA. Maggot therapy for severe skin infections. *South Med J*. 1986;79:1153-1155.
151. Contreras RJ. Contraindications to Maggot Debridement Therapy. *CAWC*. 2003;www.cawc.net/open/wcc/3-1/contreras.html.
152. Sherman RA. Maggot therapy for foot and leg wounds. *Lower Extremity Wounds*. 2002;1:135-142.
153. Steenvoorde P, Doorn Lv, Brehm V, Verdegaal S. The use of cadaveric donor fascia lata in open knee-joint due to necrotizing fasciitis. *European Tissue Repair Society, Pisa 13-16 september*. 2006;Abstractbook page 160.
154. Rozeboom A, Steenvoorde P, Hartgrink H, Jukema GN. Necrotizing fascitits following a simple pelvic fracture: case report and literature review. *J Wound Care*. 2006;15:117-120.
155. Geus de HR, Klooster van der JM. Vacuum-assisted closure in the treatment of large skin defects due to necrotizing fasciitis. *Intensive Care Med*. 2005;31:601.
156. Aulivola B, Hile CN, Hamdan AD et al. Major lower extremity amputation. *Arch Surg*. 2004;139:395-399.
157. Toursarkissian B, Shireman PK, Harrison A, Dayala M, Schoolfield J, Sykes MT. Major lower-extremity amputation: contemporary experience in a single Veterans Affairs instituation. *Am Surg*. 2002;68:606-610.
158. Keagy BA, Schwartz JA, Kotb M, Burnham SJ, Johnson G, Jr. Lower extremity amputation: the control series. *J Vasc Surg*. 1986;4:321-326.
159. Sibbald RG, Mahoney J. A consensus report on the use of vacuum-assisted closure in chronic, difficult-to-heal wounds. *Ostomy Wound Manage*. 2003;49:52-66.
160. Hess CL, Howard MA, Attinger CE. A review of mechanical adjuncts in wound healing: hydrotherapy, ultrasound, negative pressure therapy, hyperbaric oxygen, and electrostimulation. *Ann Plast Surg*. 2003;51:210-218.
161. Moues CM, Vos MC, van den Bemd GJ, Stijnen T, Hovius SE. Bacterial load in relation to vacuum-assisted closure wound therapy: a prospective randomized trial. *Wound Repair Regen*. 2004;12:11-17.
162. Steenvoorde P, Jacobi CE, Doorn Lv, Oskam J. Maggot Debridement Therapy of infected ulcers: patient and wound factors influencing outcome. *Ann of the Royal Coll of Surg England 2007; 89(6):596-602*.
163. Sherman RA. Maggot therapy for treating diabetic foot ulcers unresponsive to conventional therapy. *Diabetes Care*. 2003;26:446-451.
164. Evans P. Larvae therapy and venous leg ulcers: reducing the yuk factor. *J Wound Care*. 2002;11:407-408.
165. Bonn D. Maggot therapy: an alternative for wound infection. *Lancet*. 2000;356:1174.

166. Thomas S, Jones M, Wynn K, Fowler T. The current status of maggot therapy in wound healing. *Br J Nurs*. 2001;10:S5-8, S10, S12.
167. Kitching M. Patient's perceptions and experiences of larval therapy. *J Wound Care*. 2004;13:25-29.
168. Sherman RA, Pechter EA. Maggot therapy: a review of the therapeutic applications of fly larvae in human medicine, especially for treating osteomyelitis. *Med Vet Entomol*. 1988;2:225-230.
169. Courtenay M, Church JC, Ryan TJ. Larva therapy in wound management. *J R Soc Med*. 2000;93:72-74.
170. Ballard K, Baxter H. Developments in wound care for difficult to manage wounds. *Br J Nurs*. 2000;9:405-8, 410, 412.
171. Drisdelle R. Maggot Debridement therapy: A Living cure. *Nursing*. 2003;17.
172. Beasley WD, Hirst G. Making a meal of MRSA- the role of biosurgery in hospital-acquired infection. *J Hosp Infect* . 2004;56:6-9.
173. Mumcuoglu KY. Clinical applications for maggots in wound care. *Am J Clin Dermatol*. 2001;2:219-227.
174. Fleischmann W, Grassberger M, Sherman R. *Maggot Therapy: A Handbook of Maggot-Assisted Wound Healing*. New York: Thieme; 2004:81-85.
175. ww.larve.com/maggot_manual/docs/contraindications.html . 12-10-2004.
176. *www ucihs uci edu/com/pathology/sherman/mdt_info pdf 12-10-2004*.
177. Thomas S, Wynn K, Fowler T, Jones M. The effect of containment on the properties of sterile maggots. *Br J Nurs, Tissue Viability Supplement*. 2002;11:S21-S28.
178. Sherman RA, Wyle F, Vulpe M. Maggot therapy for treating pressure ulcers in spinal cord injury patients. *J Spinal Cord Med*. 1995;18:71-74.
179. Courtenay M. The use of larval therapy in wound management in the UK. *J Wound Care*. 1999;8:177-179.
180. Wolff.H., Hansson C. Larval therapy - an effective method for ulcer debridement. *Clin Exp Dermat*. 2003;28:137.
181. Dyck PJ, Kratz KM, Karnes JL et al. The prevalence by staged severity of various types of diabetic neuropathy, retinopathy, and nephropathy in a population-based cohort: the Rochester Diabetic Neuropathy Study. *Neurology*. 1993;43:817-824.
182. *Second European Consensus document on chronic critical leg ischemia Circulation 1991 84:1-26*. 2006.
183. Collins SL, Moore R.A., McQueay HJ. The visual analoque pain intensity scale: what is moderate pain in millimeters? *Pain*. 1997;72:95-97.
184. Wallerstein SL. Scaling clinical pain and pain relief. Bromm B, ed. Pain measurement in man: neurophysiological correlates of pain. New York: Elsevier; 1984.
185. Mosley LH, Finseth F. Cigarette smoking: Impairment of digital blood flow and wound healing in the hand. *Hand*. 1977;9:97-101.
186. Peto R, Lopez AD, Borehain J. Mortality from tobacco in developed countries: indirect estimation from national statistics. *Lancet*. 1992;339:1268-1278.
187. Sorensen LT. Smoking and wound healing. *EWMA Journal*. 2003;3:13-15.
188. Golosow LM, Wagner JD, Feeley M et al. Risk factors for predicting surgical salvage of sternal wound-healing complications. *Ann Plast Surg*. 1999;43:30-35.
189. Moller AM, Pedersen T, Villebro N, Munksgaard A. Effect of smoking on early complications after elective orthopaedic surgery. *J Bone Joint Surg Br*. 2003;85:178-181.
190. Glassman SD, Anagnost SC, Parker A, Burke D, Johnson JR, Dimar JR. The effect of cigarette smoking and smoking cessation on spinal fusion. *Spine*. 2000;25:2608-2615.
191. Jones JK, Triplett RG. The relationship of cigarette smoking to impaired intraoral wound healing: a review of evidence and implications for patient care. *J Oral Maxillofac Surg*. 1992;50:237-239.
192. Nolan J, Jenkins RA, Kurihara K, Schultz RC. The acute effects of cigarette smoke exposure on experimental skin flaps. *Plast Reconstr Surg*. 1985;75:544-551.
193. Cunningham BL, Gear AJL, Kerrigan CL, Collins ED. Analysis of breast reduction complications derived from the Bravo study. *Plast Reconstr Surg*. 2005;115:1597-1604.
194. Moller AM, Villebro N, Pedersen A, Tonnesen H. Effect of preoperative smoking intervention on postoperative complications: a randomised clinical trial. *Lancet*. 2002;359:114-117.

195. Steenvoorde P, Budding TJ, Oskam J. Pain levels in patients treated with maggot debridement therapy. *J Wound Care*. 2005;14:485-488.
196. Steenvoorde P, Jacobi CE, Oskam J. Maggot Debridement Therapy: Free-range or contained? An In-vivo study. *Adv Skin Wound Care*. 2005;18:430-435.
197. Jorgensen LN, Kallehave F, Christensen E, Siana JE, Gottrup F. Less collagen production in smokers. *Surgery*. 1998;123:450-455.
198. Rickert WS, Forbes WF. Changes in collagen with age- II Modification of collagen structure by exposure to gaseous phase of of tobacco smoke. *Exp Geront*. 1972;7:99.
199. Zia S, Ndoye A, Lee TX, Webber RJ, Grando SA. Receptor-mediated inhibition of keratinocyte migration by nicotine involves modulations of calcium influx and intracellular concentration. *J Pharmacol Exp Ther*. 2000;293:973-981.
200. Mosely LH, Finseth F, Goody M. Nicotine and its effect on wound healing. *Plast Reconstr Surg*. 1978;61:570-575.
201. Chang LD, Buncke G, Slezak S, Buncke HJ. Cigarette smoking, plastic surgery, and microsurgery. *J Reconstr Microsurg*. 1996;12:467-474.
202. Cobb TK, Gabrielsen TA, Campbell DC, Wallrichs SL, Ilstrup DM. Cigarette smoking and nonunion after ankle arthrodesis. *Foot Ankle Int*. 1994;15:64-67.
203. Sorensen LT, Hemmingsen UB, Kirkeby LT, Kallehave F, Jorgensen LN. Smoking is a risk factor for incisional hernia. *Arch Surg*. 2005;140:119-123.
204. Lind J, Kramhoft M, Bodtker S. The influence of smoking on complications after primary amputations of the lower extremity. *Clin Orthop Relat Res*. 1991;211-217.
205. Schmitz MA, Finnegan M, Natarajan R, Champine J. Effect of smoking on tibial shaft fracture healing. *Clin Orthop Relat Res*. 1999;184-200.
206. Sorensen LT, Jorgensen T, Kirkeby LT, Skovdal J, Vennits B, Wille JP. Smoking and alcohol abuse are major risk factors for anastomotic leakage in colorectal surgery. *Br J Surg*. 1999;86:927-931.
207. Sorensen LT, Horby J, Friis E, Pilsgaard B, Jorgensen T. Smoking as a risk factor for wound healing and infection in breast cancer surgery. *Eur J Surg Oncol*. 2002;28:815-820.
208. Goldminz D, Bennet RG. Cigarette smoking and flap and full-thickness graft necrosis. *Arch Dermatol*. 1991;127:1012.
209. Kaufman T, Eicheulaub EH, Levin M. Tobacco smoking: impairment of experimental flap survival. *Ann Plast Surg*. 1984;13:468.
210. Hardesty R.A., West SS, Schmidt S. Preoperative cessation of cigarette smoking and its relationship to flap survival. *Presented at the 69th Annual Meeting, American Association of Plastic Surgeons, Hot Springs, VA.* 1990.
211. Contreras RJ, Fuentes SA, Karam-Orantes M, Lourdes D-CJ. Larval Debridement Therapy in Mexico. *Wound Care Canada*. 2005;3:42-46.
212. Hofman D. Concepts in clinical Wound Healing: Debridement - A Nursing issue? *European Itssue Repair Society Buleletin*. 2002;9.
213. Hafner J, Schaad I, Schneider E, Seifert B, Burg G, Cassina PC. Leg ulcers in peripheral arterial disease (arterial leg ulcers): Impaired wound healing above the threshold of chronic critical limb ischemia. *J Am Acad Dermatol*. 2000;43:1001-1008.
214. Wutschert R, Bounameaux H. Predicting healing of arterial leg ulcers by means of segmental systolic pressure measurements. *Vasa*. 1998;27:224-228.
215. Markevich, Y. O., McLeod-Roberts, J., Mousley, M., and Melloy, E. Maggot Therapy for Diabetic Neuropathic Foot Wounds: a Randomized study. 36th Annual Meeting of the EASD 17-21 september 2000.
216. Contreras RJ, Fuentes SA, Arroyo ES, Sosa MCd, Maravilla FE, Dominques CJ. Larval debridment therapy and infection control in venous ulcers: a comparative study. *2nd World Union of Wound Healing Societies'Meeting, 8th/13th july*. 2004.
217. Steenvoorde P, Doorn Lv, Jacobi CE, Oskam J. [Maggot therapy: retrospective study comparing two different application-techniques. (Dutch)]. *Nederlands Tijdschrift voor Heelkunde*. 2006;15:86.