Maggot debridement therapy in surgery

Steenvoorde, P.

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.

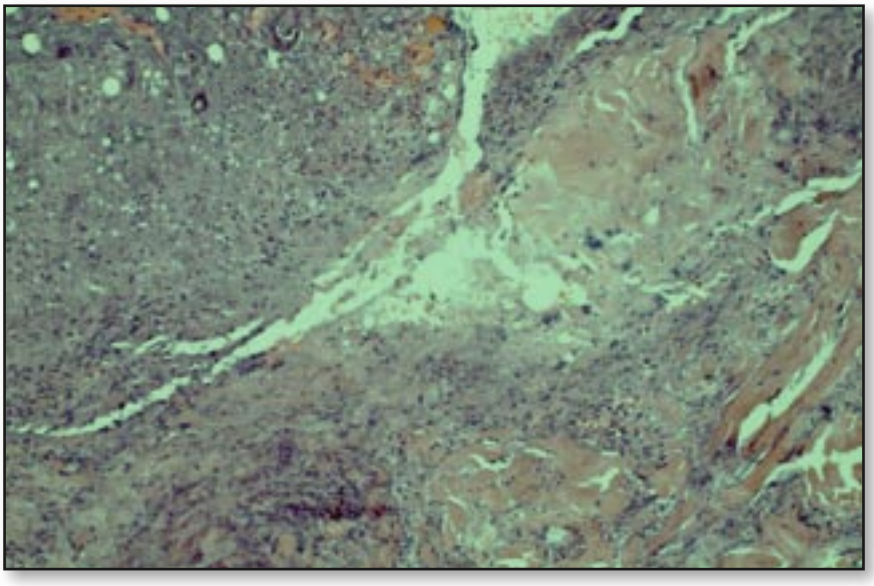

Figure 1: Showing pathological examination of the wound of patient no. 3 prior to MDT; bacteria and leucocytes are predominantly apparent; there is no angiogenesis, nor any sign of fibroblast proliferation.

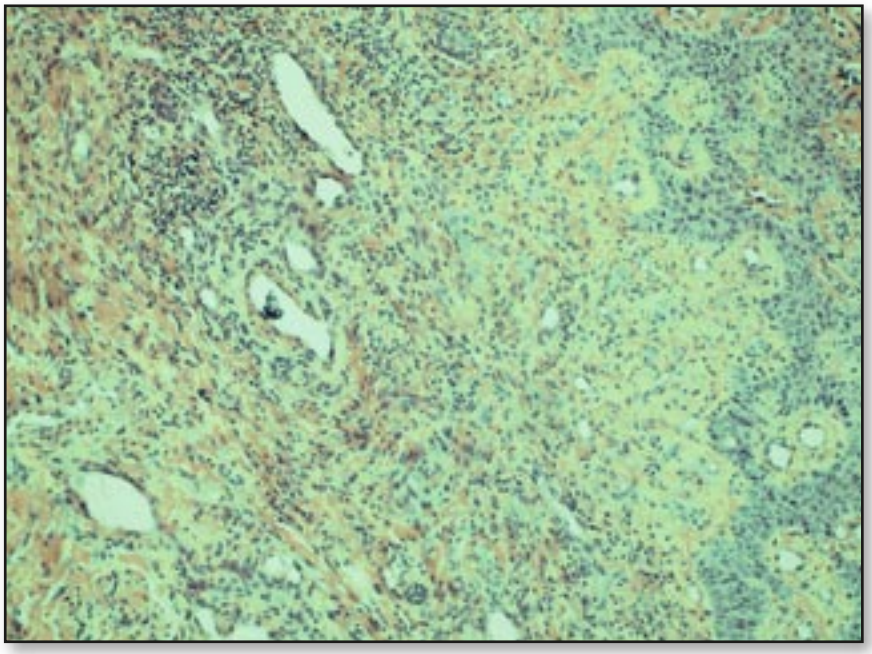

Figure 2: Showing pathological examination of the wound of patient no.3 after MDT; there are no bacteria; leucocytes are still present; but now angiogenesis and fibroblasts are also appearing.

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.