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A Cohort Study to Evaluate the Predictions of the Tardivo Algorithm and the Efficacy of Antibacterial Photodynamic Therapy in the Management of the Diabetic Foot

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Abstract

Objective: A large percentage of diabetic patients receives neither proper foot screening nor treatment. We aim to test whether the efficacy of an algorithm that predicts amputation risk coupled to an effective antimicrobial therapy will reduce the percentage of amputations in patients with diabetic foot.

Research Design and Methods: A cohort of two hundred eighteen patients with complex diabetic foot ulcers (DFU) were organized in six sub-groups, which were: i) infected DFU without surgery (39.91%); ii) infected DFU post-surgical treatment (29.82%); iii) ischemic DFU (9.17%); iv) DFU without infection (11.01%), v) Charcot foot (6.42%); vi) Diabetic leg ulcers (3.67%); and were treated with antimicrobial Photodynamic Therapy (a-PDT). The outcome of the different groups was compared among themselves and with literature results. Descriptive statistics was performed by absolute and relative frequencies for qualitative variables at 95% confidence intervals.

Results: Patients with ischemic parts had low recovery rates (20%) compared with the other groups (94% of recovery). Peripheral arterial disease proved to be an important complication in DFU and a-PDT was discontinued in these patients. The rate of previous amputations was much higher in the debrided group, 69% against 29% in the non-debrided group. By using the Tardivo´s algorithm, amputation frequency of those classified with scores greater than 12 was 63% (n=17) compared to 9% (n=17) in the group with scores up to 12 ($p<0.001$).

Conclusions: The score obtained with Tardivo´s algorithm showed a good level of prediction for the extended set of patients. a-PDT is highly effective in controlling and treating severe and deep infections in ulcerated feet, allowing resolution of osteomyelitis, with no need for surgery and hospitalization. This low-cost therapy is a good solution to avoid amputations in patients with diabetic foot.

Keywords: Diabetic Foot Ulcer (DFU), Antimicrobial Photodynamic Therapy (a-PDT), Diabetes, Foot, LED, Methylene Blue, Photosensitizers, Osteomyelitis, Amputation, Limb Salvage

Introduction

Diabetes patients are at increased risk of lower-limb amputations, and the main cause is the peripheral arterial disease (PAD). High levels of glucose damage the blood vessels and the peripheral nerves. Neuropathy favors ulcerations and wound healing is also impaired by a deficiency in collagen synthesis. Consequently, foot ulcers affect 10 to 25% of diabetic patients during their lives [1-4].

Diabetic foot ulcers (DFU) represent significant complications, as they require long and intensive treatment and affect patients' quality of life, contributing with high costs to the health care systems. The population of diabetic patients who present foot ulceration is heterogeneous. Although most patients have peripheral polyneuropathy, other characteristics may vary between patients, such as PAD, infection, or other comorbidities [4,5].

PAD is considered an important predictor of outcome [4,5] Osteomyelitis is present in approximately 10–15% of individuals with moderate infection and in 50% of patients with severe infectious processes [5]. To avoid amputation, antibiotics have to be used for a long time with adequate good blood perfusion [4-7] The rapid development of microorganism resistance to antibiotics, their improper perfusion in the bones' contamination sites, and the side effects of antibiotic therapy at high doses and for long periods, increase the need for debridement and amputation [8], suggesting that alternative antimicrobial procedures are highly necessary [9,10].

Lower limb amputations have a huge impact on the quality of life of the individual, their relatives, and the community. Amputations are related to an increased risk of mortality (greater than 50%) in the first five years after the event. Besides the age, PAD, diabetes, and kidney failure are factors that increase the mortality risk [11,12]. Štotl and coworkers succeeded in developing a model for predicting complications in the diabetic foot, by analyzing the history of symptoms, by performing careful foot inspection (monofilament testing, palpation of peripheral pulses), by evaluating the presence of foot symptoms (pain, tingling, numbness or restless legs) and of foot deformities (hallux valgus, muscle atrophy, nail deformity or claw toe) [13].

Antibacterial photodynamic therapy (a-PDT) is a viable option to avoid amputation of the diabetic foot [14], being effective even against multidrug-resistant pathogens, including vancomycin-resistant Enterococcus ssp and methicillin-resistant strains Staphylococcus aureus [15-18]. Preliminary results indicated that a-PDT is very effective in treating and preventing amputation of DFU. DFU patients treated with a-PDT had only a 2.9% chance of amputation, compared with 100% in the control group (classical antibiotic therapy, without a-PDT). a-DPT also seem to reduce the need for surgical debridement of DFU. Additionally, diabetic patients with osteomyelitis have excellent chances of cure with a-PDT [19-21].

The Tardivo Algorithm is a prognostic score developed in a cohort study of 62 patients submitted to a-PDT. It has been used to determine the risk of amputation and to predict the best therapeutic options for the treatment of DFU. [21] The score is based on three main factors: Wagner's classification, signs of PAD, and location of foot ulcers. Other medical research groups have been successfully using the Tardivo Algorithm [22-24]. The present study's main objective is to evaluate the algorithm with a larger cohort of 218 patients. The extended cohort also allowed the evaluation of a-PDT efficacy in osteomyelitis DFU associated with osteomyelitis and surgical debridement, with pressure DFU without infection, with Charcot foot and with severe PAD.

Methods

Population

Patients with DFU were referred from emergency care services in São Bernardo do Campo to hospitals linked to the Vascular Surgery Service of the Vascular Surgery Discipline of the ABC Medical School. After being evaluated by the team's vascular surgeons, these patients were either hospitalized and evaluated for surgical treatment of debridement and/or amputation or were referred directly to the diabetic foot clinic. The physician always adopted the clinical or surgical approach on duty at the hospital, and the medical group that attended the diabetic foot outpatient clinic had no interference in the initial conduct and the choice between the two treatment alternatives. Furthermore, patients who had been hospitalized at the time of hospital discharge were all referred to the diabetic foot clinic to continue treatment until the outcome.

Recruitment period was from March 2011 to July 2019. The selection process started with a total pool of 395 patients. We opted to remove from the study 44 patients that had two sequential ulcers, resulting in 351 participants. 83 of them were excluded for presenting arterial, renal, cardiac and orthopedic complications. From the remaining 268 patients, 50 discontinued the treatment resulting in the group of 218 patients (Figure 1).

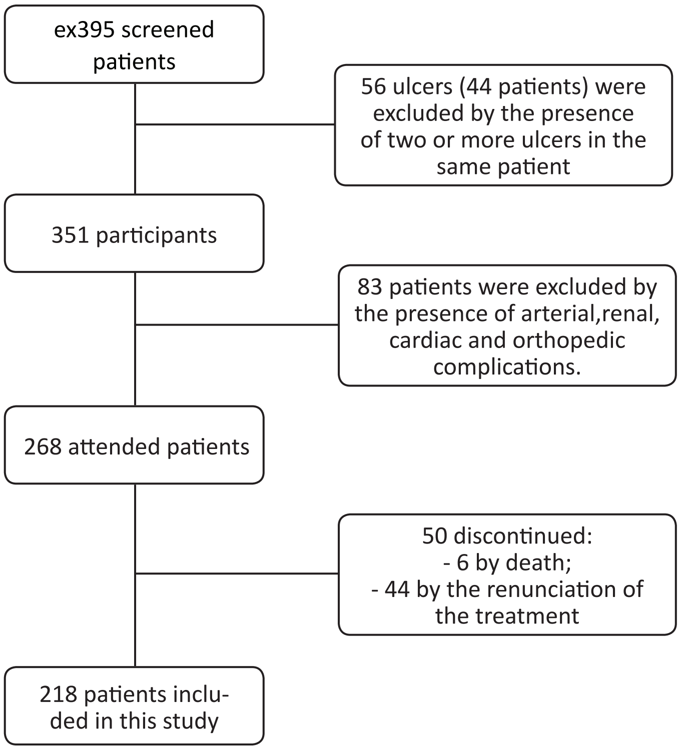

Figure 1: The Recruitment and selection process. Initially 395 patients were transferred to our service. 44 patients were removed for having two sequential ulcers, resulting in 351 participants. 83 of them were excluded for presenting arterial, renal, cardiac, and orthopedic complications. From the remaining 268 patients 50 discontinued the treatment resulting in the group of 218 patients

Patients with DFU (diabetic foot ulcer) were submitted to antibiotic therapy, usually with Ciprofloxacin 500 mg every 12 hours and Clindamycin 300 mg every 6 hours, for 10 to 14 days. Laboratory evaluations of blood count, glycated HB, c-reactive protein, fasting blood glucose, foot radiography and Doppler ultrasound of lower limb arteries, were performed.

All patients participating in this study had diabetes and had DFU associated or not with infections and/or peripheral arterial disease. All patients with Wagner Classification grades 1 to 4 were admitted. Patients without ulcers or with total gangrene of the foot (Wagner 0 and 5, respectively) were not treated with a-PDT. The inclusion criteria were age over 18 years, with diabetes and with any injury in the foot or lower limbs and who did not have critical ischemia of the extremities, always respecting the Wagner classification with grades 1 to 4. The only exclusion factor was the presence of severe ischemia, grade Wagner 5. Patients who met all inclusion criteria were invited to participate in the study and to sign the informed consent form. The two main patient outcomes were: i) classify as foot saved or amputated, ii) quantify the average time that the outcome took to occur.

The study was approved by the Research Ethics Committee ABC Medical School, process nº 257 / 2010. The clinical trial was registered at Rebec with UTN U1111 1273 1796. After the treatment, patients were followed by the hospital staff. None cured patient relapsed in the same ulcer.

Patient characteristics

A group of 218 patients of both genders was selected and received a-PDT treatment in the diabetic foot. (Table 1). There was a prevalence of male patients, 68.81% (n=150), against 31.19% (n=68) female. The age of these patients ranged from 34 to 82 years, with the highest frequency in the range of 56 to 66 years, with a mean age of 59 years. Considering Wagner's classification, grade 1 is superficial ulcers, grade 2 is deep ulcers, grade 3 is infection with or osteomyelitis, and grade 4 is forefoot gangrene. Most patients, 74.31% (n=162) had Wagner grade 3, followed by Wagner 2, 12.30% (n=27), Wagner 4 10.55%(n=23) and finally, only 3.72%(n=6) Wagner 1. The location of the lesions most frequently in the forefoot, reaching the phalanges and/or metatarsals in its distal portion in 70.64% (n=154) of the cases, followed by 18.35% (n=40) in the middle foot and only 11.01%(n=24) in the rear foot. PAD with important clinical repercussion was present in 57 patients. Two hundred patients with peripheral neuropathy had a sensation loss of feet, 94 had been subjected to previous amputations and 58 % of the patients were using insulin.

Outcomes	**Groups n[%]**							
	Total	**Infection No Surgery**	**Infection Pos Surgery**	**Ischemic DFU**	**DFU No Infection**	**Charcot foot**	**Leg ulcer**	**p**
Gender								
Female	68 [31,19]	26 [28,89]	16[24,62]	8[40,00]	8[33,33]	5[35,71]	5[62,50]	0,312*
Male	150 [68,87]	61 [70,11]	49[75,38]	12[60,00]	16[66,67]	9[64,29]	3[37,50]	
Wagner grade								
1	6 [2,75]	0 [0,00]	0[0,00]	0[0,00]	5[20,83]	0[0,00]	1[12,50]	<0,001*
2	28 [12,84]	1 [1,15]	0[0,00]	0[0,00]	19[79,17]	6[42,86]	2[25,00]	
3	161 [73,85]	84 [96,55]	65[100,00]	0[0,00]	0[0,00]	8[57,14]	4[50,00]	
4	23 [10,55]	2 [2,30]	0[0,00]	20[100,00]	0[0,00]	0[0,00]	1[12,50]	
Peripheral Arterial Disease Classification								
PAD 1	161 [73.85]	73 [83.91]	53[81.54]	0[0.00]	17[70.83]	13[92.86]	5[62.50]	<0,001*
PAD 2	57 [26.15]	14 [16.09]	12[18.46]	20[100.00]	7[29.17]	1[7.14]	3[37.50]	
Neuropathy								
No	17 [7,83]	1 [1,15]	7[10,77]	3[15,00]	2[8,70]	0[0,00]	4[50,00]	<0,001*
Yes	200 [92,17]	86 [98,85]	58[89,23]	17[85,00]	21[91,30]	14[100,00]	4[50,00]	
PA****								
No	124 [56.88]	62 [71.26]	20[30.77]	14[70.00]	12[50.00]	10[71.43]	6[75.0]	<0,001*
Yes	94 [43.12]	25 [28.74]	45[69.23]	6[30.00]	12[50.00]	4[28.57]	2[25.0]	
Insulin use								
No	91 [41,74]	32 [36,78]	39[60,00]	9[45,00]	5[20,83]	5[35,71]	1[12,50]	0,004*
Yes	127[58,26]	55 [63,22]	26[40,00]	11[55,00]	19[79,17]	9[64,29]	7[87,50]	
Quantitative measures								
RS Score median	6	3	9	16	4	6	12	<0,001**
Time (weeks) median	16	13	21,9	8	17,1	40,7	23,2	<0,001**
Average age	59	59,4	57,4	60,3	59,3	62,1	59,9	0,540***

*Chi-square, **Kruskal-Wallis, ***ANOVA, ****PA is previous amputation

Considering the clinical presentation of peripheral arterial disease (PAD), ankle-arm index less than 0.7 or other signs such as the absence of pedis or posterior tibial pulse, intense pallor, fixed cyanosis, or dry gangrene of the extremities, 25.22% (n= 55) of the patients were thus classified as having clinical PAD, without the need for additional tests. The remaining 74.77% (n=163) had satisfactory peripheral perfusion.

All patients were classified according to the degree of risk of amputation, using the Tardivo Algorithm [21], where higher scores represent greater risk, with 12 being the cutoff score, with 152 times higher chance of amputation, which corresponded to 10.09% (n=22). Lower risk scores were present in most patients, 77.52% (n=169), while scores above 12, with worse prognosis, were observed in 12.38% (n=27) of cases.

Insulin-dependent patients accounted for 58.71% (n=128). Peripheral neuropathy was found in 70.18% (n=153) of the cases and patients with some previous lower limb amputation corresponded to 43.57% (n=95).

Due to the complexity of the clinical presentations of the diabetic foot, we separated these patients into six groups to compare outcomes: Group 1, infected DFU without surgical debridement: Infected ulcers and/or osteomyelitis 39.90% (n=87); Group 2 with surgical wounds infected after debridement and/or residual osteomyelitis: 29.81% (n=65); Group 3 with dry forefoot gangrene, Ischemic DFU group, Wagner grade 4, 9.17%(n=20) patients; Group 4 without infection with plantar foot ulcer 6.42%(n=14), and surgical wounds without infection 4.58%(n=10); Group 5 acute or chronic Charcot's neuro-arthropathy group, representing 6.42% (n=14) patients and Group 6 diabetic leg ulcers, 3.66% (n=8).

We did not consider DFU, 8 diabetic patients who had complex leg ulcers and received photodynamic treatment and whose results will be presented below.

Antimicrobial Photodynamic Therapy (a-PDT)

In this work, the photosensitizers used were phenothiazine dyes 2% Methylene Blue and 2% Toluidine Blue, in aqueous solution, manipulated for topical use. Before applying the dye, a 0.2% solution of Chlorhexidine Antiseptic in an aqueous solution was used to clean the wound area and, when necessary, remove slough, dead tissue, and crumbs with tweezers. Hydrogen peroxide was mixed with the dye to irrigate deeper cavities, where the expansion of the hydrogen peroxide allowed to carry the dye to all internal contaminated spaces.

Then red-light sources were positioned to irradiate the diseased areas already soaked with the photosensitizers. Three prototypes of light sources were used: -1. RL50 prototype with a 50 W halogen dichroic source, filtered with a 49 mm S&K R2 red photographic filter, with 100mW output power. - 2. Fasa FR-100 prototype with an optical fiber 1.5 mm in diameter with 25 mW output power. – 3. GD prototype with 16 red LED board with 50 mW output power in each LED.

The mean fluency was 45 J/cm² (30-60 J/cm²), and the sessions were weekly. In some cases, those with an exuberant purulent secretion, the PDT sessions were performed every 3 days.

All patients received antibiotics in the first 14 days and were only medicated again when there was the presence of purulence in the lesions. The standard antibiotic therapy was Ciprofloxacin 500 mg every 12 hours and Clindamycin 300 mg every 6 hours.

Before starting the irrigation with phenothiazine, the diabetic foot ulcers were photographed in all sessions with the cameras: Sony camera model DSC-W310 and Samsung Camera model SM-A520F.

All patients received outpatient treatment. Hospitalization occurred in cases that the patient did not respond to a-PDT, with persistent severe infection or sepsis in those with critical ischemia or required amputation.

The Tardivo Algorithm

Score calculation by the Tardivo Algorithm is obtained by multiplying the values of its three individual factors, which are: Wagner classification (1 to 4), PAD (1 or 2), and the location of the DFU (1 to 4). Concerning PAD, patients with good peripheral vascularization received a value of 1, while those with clinical signs of ischemia received a value of 2. Ulcer location was defined as toes (1), forefoot (2), midfoot (3) and hind foot (4). [21]

Statistical analysis

Descriptive statistics were performed by absolute and relative frequencies for qualitative variables and by the medians or means and respective 95% confidence intervals, according to the adherence of quantitative variables to the normal distribution (assessed by Shapiro-Wilk test). To assess the relationship between qualitative variables and groups, the Fisher's test or Chi-square's test was used according to their assumptions. To assess the relationship between quantitative variables and groups, the Kruskal-Wallis test was used for non-normal variables (Shapiro-Wilk, $p<0.05$) and ANOVA for normal variables (Shapiro-Wilk, $p\geq0.05$). For all analyses, the confidence level was 5%. Stata (StataCorp, LC) version 11.0 was used.

Results

Two hundred eighteen patients with complex DFU were treated with a-PDT. As described in Table 1, DFU were classified in six groups: 1. Infected DFU without surgery; 2. Infected DFU Post-surgical treatment; 3. Ischemic DFU; 4. DFU without infection; 5. Charcot foot, and 6. Diabetic leg ulcers. Note that Table 1 shows an overview of the sample groups, demonstrating the homogeneity within the groups and statistically significant differences among the groups: "classification of peripheral arterial disease" (p<0.001), prevalence of neuropathy (p<0.001), previous amputation (p<0.001), and isulin usage rate (p=0.004). Differences were also found for the median RS score (p<0.001) and for the median time in weeks until healing (p<0.001).

A large percentage of feet were rescued by a-PDT in almost all DFU groups. 65 patients that had previously received surgical treatment but that still had active foot infections, responded positively to a-PDT (**Table 2**). This is particularly interesting considering that the treatment is performed in outpatient facilities. Ischemic group responded poorly to a-PDT (20% of salvage) compared with 92.1% of salvage in no ischemic patients (**Table 2**), (**Figure 2**). Undoubtedly, PAD is an important complication in DFU, because different degrees of blockage of blood flow can reduce the chances of saving limbs.

Among the amputees with osteomyelitis, 68,75% had severe PAD (Table 3). This group was divided into two subgroups, as differences were observed in the results in cases in patients submitted to previous surgical debridement procedures. The rate of previous amputations was much higher in the debrided group, 69.23% against 28.73% in the non-debrided group, strongly indicating that many amputations occurred during debridement surgery. (**Table 2**) The Infected DFU without surgery group presented 86,20% of feet salvage. There was not any bone remotion, and a-PDT seems to stimulate bone reconstruction (**Figure 3**).

Table 2: Rescue of non-ischemic DFU limbs

Groups	Total	Infection No Surgery	Infection Pos Surgery	DFU No Infection	Charcot foot	Leg ulcer*	p
n	198	87	65	24	14	8	
Rescue %	90.91	86.20	93.85	100	92.86	87.50	0.192**
Amputations %	9.10	13.79	6.15	0.00	7.14	12.5	
Previous amputations %	44.44	28.74	69.23	50.00	28.57	25.00	<0.001**
PAD %	18.69	16.09	18.46	29.17	7.14	37.50	0.259**
Average Risk Score	8.6	12	9	5	8.5	*	
Week average Healing	23.77	16.90	28.03	19.10	51.73	28.82	<0.001***

*Tardivo Algorithm does not apply to leg ulcers, **Fisher's test, ***ANOVA

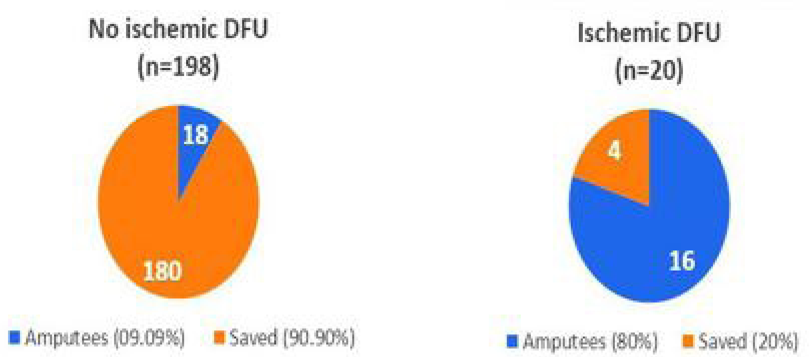

Figure 2: Main outcome comparing the level of amputation in no ischemic and ischemic DFU

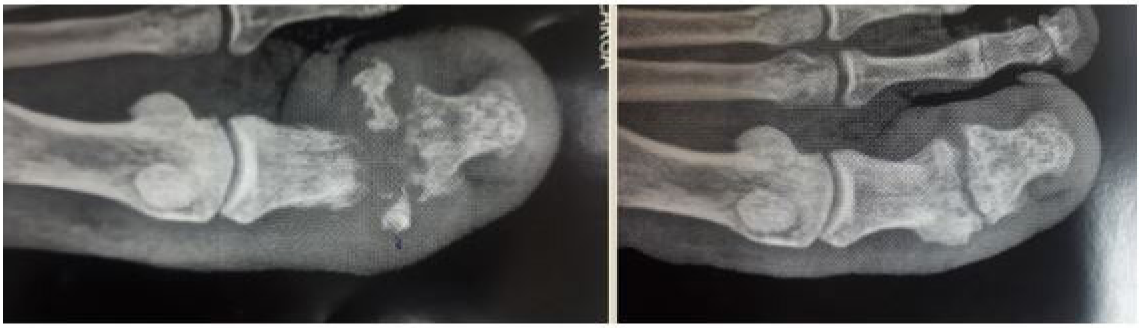

Figure 3: Radiography of a patient of this study, before (left) and after (right) a-PDT

The frequency of ulcers in the forefoot was greater than in the midfoot and hindfoot together, corresponding to 70.64% of the 218 cases. The salvage rate was equal between forefoot 85.06% in 154 patients versus 84.37% salvage in 64 cases in the midfoot and hindfoot. Mean healing time on the forefoot was 19 weeks versus 38 weeks on the midfoot and hindfoot. Although the healing time was very different, there was the same rate of rescue.

The greater number of sessions is related to the longer treatment time until the outcome (Inf without Surg: 0.73; p<0.001; Inf with Surg: 0.79; p<0.001; Ischemia PAD: 0, 92; p<0.001; DFU without inf: 0.87; p<0.001; Charcot foot: 0.79; p<0.001), with the exception of the group with leg ulcers that did not present this correlation (rho =0.71; p=0.073) (Table 3).

Following the predictions by the Tardivo algorithm, we observed that the amputation rate of patients classified with scores greater than 12 (62.96%, n=17) was much larger than the amputation rate in the group of patients with scores up to 12 (8.9%, n=17). This analysis presented p<0.001.

Table 3: Main outcome of sub-groups

Outcomes	**Groups**							
	Total	**Infection No Surgery**	**Infection Pos Surgery**	**Ischemic DFU**	**DFU No Infection**	**Charcot foot**	**Leg ulcer**	**p**
Time of disease diabetes (median of years)	15	16	13.44	19	17.86	15.25	9.85	0.086*
insulin use %	58.26	63.21	40	55	79.16	64.28	87.5	0.004**
PAD %	26.15	16.09	16.92	100	29.16	7.14	37.5	<0.001**
Previous amputations %	15.6	28.73	69.23	30	50	28.57	25	<0.001**
Risk score (Median of Tardivo Algor)	6	5	9	16	5	8	***	<0.001*
Salvage %	84.4	86.2	93.84	20	100	92.85	87.58	<0.001**
Sessions until outcome (n)	10	12	18	6	9	19.42	13	<0.001*
Treatment time (median of weeks)	6	16.9	28.03	_	19.1	51.73	28.82	<0.001*

*Kruskal-Wallis test, **Chi-square test, **** Tardivo Algorithm does not apply to leg ulcers

Discussion

Osteomyelitis is a common complication of a diabetic foot ulcer that occurs following a soft tissue infection of the ulcerated area and spreads to the bone.

Ulcers and osteomyelitis have been established as important risk factors for amputations. On the other hand, PAD often coexists in patients with diabetes. The association of PAD and infection influences the evolution of the diabetic foot, increasing the risk of not healing and is associated with a poor prognosis. It is generally recommended that patients having PAD undergo revascularization to restore adequate blood flow in the infected limb [1-5,13]. Our experience with a-PDT, which is a highly effective local treatment of the foot ulcer, is no different. PAD patients responded poorly to a-PDT and had to be vascularized to had a better chance to have the foot rescued.

In osteomyelitis cases, a bone sample should be collected and sent for culture and antibiotic sensitivity analysis. However, these interventions require hospitalization of patients, increasing the risk of cross-infection and the surgical procedure often ends in finger amputations, which could be avoided if other approaches were used, for example, photodynamic therapy. The key for chronic osteomyelitis treatment of the diabetic foot is excision of the bone involved. However, inappropriate removal can lead to reoperation [4-7]. Frequently, successive amputations of part of the foot can occur, due to the inefficiency in fighting the infection surgically. Recent studies have shown that antibiotics can be used for a long time to treat osteomyelitis, avoiding amputation. [6,7] However, the prolonged use of antibiotics can further compromise the renal function of diabetics, since due to diabetic microangiopathy, there is already a certain degree of impairment of the renal filtration function.

A substantial proportion of limb amputations, particularly in diabetic patients, are preventable through adequate health care. Much effort has been made to reduce the risk of amputation in the population with diabetes, such as introducing multidisciplinary centers for the care of these patients' feet. A study in Belgium observed a reduction of 8% per calendar year in major amputations among people with diabetes [8].

Chronic bacterial infection of the bone blocks the cortical blood supply and leads to the formation of sequestrations, necrotic cortical bone bags, which are avascular and difficult to treat. The presence of sequestration in chronic osteomyelitis usually requires surgical intervention [12]. The local use of antibiotics to prevent skeletal infections was incorporated into general practice with joint arthroplasty in Europe in the 1970s [13]. Placing plasters with dual mechanical function and chemical / biochemical with a long release of antibiotics proves to be advantageous for treating of chronic osteomyelitis and has evolved from permanent resins to biodegradable materials. [25-27] Nevertheless, this technique does not apply to the diabetic foot, so there is a high rate of amputations people with diabetes. Infection in diabetic ulcers often persists, even after surgical debridement, and as a consequence, new surgeries are needed, and the infection spreads over the limb, transforming a minor into a major one. Several other forms of local treatment have been developed, such as ozone, hyperbaric chamber [28].

The economic burden associated with hospitalizations related to amputation of extremities is considerable. Diabetes, old age, and socio-demographic factors can influence amputation, generating a high economic cost to the health system. [8, 12] The a-PDT allows the entire treatment of the infected diabetic foot in an outpatient setting, avoiding hospitalization, and reducing costs in the healthcare system. [19-21] Our data, which was obtained with an expanded data set of patients, indicates a salvage rate of 94% for patients without PAD, proving that patients with diabetic foot ulcers and with amputation indication will respond properly to a-PDT, avoiding amputation. a-PDT benefits the patients and offers enormous cost savings in surgical procedures, rehabilitation, and aftercare [12].

Light-activated methylene blue is currently being used against methicillin-resistant Staphylococcus aureus (MRSA), with subsequent decreases in postoperative infection rates [29]. Photons in the red band that can penetrate well into biological tissue are the main antimicrobial agents. a-PDT treats the focus of the bone lesion effectively because the photoactive molecules are directed to the foci of bone sequestration, staining the microorganisms present in these lesions, and once activated by light sources, start to oxidize multiple structures of the microorganisms, and thus eliminating them, including multi-resistant strains, without running the risk of developing resistant strains. [14-16,19-21,29]

We also aimed to answer whether the surgical approach should be the first choice in managing patients with diabetic foot. There is no doubt that the surgical approach often leads to amputations of important parts of the foot and is not always effective in eradicating the regional loci of infection. In our study, patients with infected DFU had a much higher chance of having had a history of previous amputation (69.23%), while only 29.73% of the patients that had suffered no previous surgical procedure, had DFU (Table 3). Indeed, diabetic patients have a history of frequent repetitive surgeries, especially when the infection ascends via tendons and anatomical compartments of the limb. This condition tends to become more severe when there is poor peripheral arterial perfusion associated [8,20].

Only one in five patients with PAD had the foot rescue by a-PDT (Table 3). The association of infection with PAD greatly increased the risk of limb loss or part of it. The number of patients treated with a-PDT in cases of severe ischemia was small (n=20), because when we noticed the high rate of amputations in this group, we started to refer these cases to the vascular team for revascularization treatment, since without good blood irrigation a-PDT is not as effective. Therefore, our data attest that a conservative treatment (a-PDT, for example) should be considered before amputation. However, our data also indicate that revascularization is key to allow the salvage of the ischemic diabetic foot. The length of diabetes disease correlates with the onset of complications and their severity. The mean time of diabetes in the severe ischemia group was 19 years, and among the amputees, the meantime of diabetes was 20 years and of the rescued, 10 years. In the group of ulcerated diabetic feet without infection, we noticed an important difference regarding the use of insulin, because 79% of these patients were users of this hormone therapy and did not have infection in their ulcers. This data seems to correspond to the publications on the importance of insulin in immunity [30,31].

In the Charcot Foot Group, the meantime to healing was very high, ~52 weeks. This data shows a great disparity compared to the action of the other groups, meaning that a-PDT alone is not ideal for these patients since removing the overload from these feet is fundamental, and corrective orthopedic surgery is the most indicated treatment [32].

Tardivo's algorithm proved to be highly effective; since in 33 amputated patients the average risk score was 15, confirming previous studies. The amputation rate of those classified with scores greater than 12 was 63% (n=17), compared to 9% (n=17) in the group with scores up to 12 (this analysis presented $p<0.001$).

The main limitation of this study was not having compared the results of a-PDT with that of the conventional treatment (antibiotic therapy and surgery). We preferred to accept this limitation than to cope with the consequences of having high rate of unnecessary amputations in the control group. Another limitation is that all patients were treated by the same medical team. We aim to expand the evaluation of the efficacy of a-PDT and of the Tardivo algorithm, by gathering data from other medical teams in a multicentric study that has just started.

Conclusion

In a population of 218 patients with DFU the overall salvage rate was 84.4% and it was 89.47% in the population of 152 patients with osteomyelitis. We concluded that a-PDT was effective in limb salvage of diabetic patients with DFU and it was highly effective in controlling and treating severe and deep infections in ulcerated feet, with the resolution of osteomyelitis, requiring neither surgery nor hospitalization. Patients with severe ischemia and with significant PAD, did not benefit from a-PDT, requiring referral for revascularization treatment. Tardivo's algorithm proved reliable as a predictive index of amputation/salvation. More studies are needed to understand whether early use of insulin would improve the immunity of diabetics and thus decrease their risk of infections and peripheral arterial disease.

Authors contributions

JPT designed the experiment, treated the patients, analyzed the data, wrote the manuscript; JAC, designed the experiment, analyzed the data; MASP, designed the experiment, analyzed the data; FWSF, statistical analysis; FA, statistical analysis; LMZ, designed the experiment, treated the patients; STC, designed the experiment, treated the patients; MSB, designed the experiment, analyzed the data, wrote the manuscript.

Competing Interests

The authors declare no competing interests.

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