Communication

Sonication of Kirschner wire as a tool for the microbiological diagnosis of diabetic foot infection – preliminary results

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Abstract:

Background: Diabetic foot infection (DFI) is the commonest diabetic problem requiring hospital admission. Culture yield can be challenging, particularly in the presence of biofilms. Literature confirms biofilms are ubiquitous in diabetic foot ulcer, although, there is not a microbiologic diagnostic approach regarding biofilm disruption on DFI. We postulated sonicating a stainless-steel wire along with tissue samples into the thioglycollate broth media (TBM) may improve the diagnosis of DFI. Method: Prospective unicentric study that assessed patients with DFI who underwent surgical debridement. The vascular surgery team collected tissue fragments and inoculated the specimens into three TBM to execute the conventional culture method (CCM), and additional fragments to place into other TBM along with a Kirschner wire (K-wire – Kw method). The microbiologist processed the samples and the resultant sonication fluid in aerobic sheep-blood agar after 24 hours, 5 and 10 days of incubation. Both methods were compared (Wilcoxon test; $p < 0.05$). Results: The number of pathogens isolated in each method was not statistically significant ($p = 0.414$): CM = 1.67 (± 0.92); KwM = 1.75 (± 0.94). The KwM was not inferior to CCM. In addition, despite the absence of statistical significance, the KwM detected more pathogens than CCM.

Keywords: Diabetic foot infection; biofilm disruption; Kirschner wire; sonication

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1. Introduction

DFI is the most familiar diabetic problem requiring hospital admission and a major part of the amount of work of clinical specialists. Besides, DFIs are associated with substantial morbidity, mortality, and reduced quality of life. Early suspicion diagnosis is essential to improve outcomes [1]. The microbiology of DFI modifies by characteristics of the patient (e.g., previous antibiotic course, recent hospitalisation) as well as the severity of disease. Minor DFI tend to be caused by Gram-positive cocci, and moderate DFI by Gram-positive and Gram-negative pathogens. In severe DFI, the infection can be polymicrobial, concerning Gram-positive and Gram-negative bacteria along with *Candida* spp. [1]. Obtaining a specimen for culture provides valuable information on the causative pathogen(s) along with their antibiotic susceptibility, allowing appropriate selection of antibiotic therapy. Though, culture yield can be challenging, particularly in the presence of biofilms [2]. Rising evidence surrounded by the literature confirms biofilms are ubiquitous in diabetic foot ulcer (DFU) and suggested that they participate to delayed wound healing [3]. Johani *et al*. investigated the presence of biofilm in DFU applying microscopy combined with molecular approaches. All 65 DFU specimens evaluated by microscopy contained biofilm, ($P < 0.001$). The researchers detected the existence of mono and multi-species biofilms in the same tissue segments, and when DNA sequencing analysis showed varied polymicrobial communities [4]. The consequences of harboring biofilms are negative, since it increases the chances of therapeutic failure while its complex structure hinder immune action, antimicrobial penetration, and wound healing. Therefore, the biofilm disruption may be crucial to obtain better outcomes, since it could allow the identification sessile pathogenic microorganisms. Until now, there is not a microbiologic diagnostic approach regarding biofilm disruption on DFI. Here, we postulated that sonication of a stainless steel wire along with tissue samples into the TBM could disrupt the biofilm, allowing an improvement on microbiological diagnosis of DFI. In the background, we aimed to evaluate the risk factors for amputation.

2. Materials and Methods

We performed a prospective unicentric study that assessed patients with DFI who underwent surgical debridement between April 2018 and April 2021 at a 250-bed tertiary hospital centre. The vascular surgery team collected three fragments of soft tissue or bone then inoculated the specimens into three TBM to posterior execution of the conventional culture method (CCM), and extra three fragments along with a sterile Kirschner wire (K-wire) gaging 5 cm were inoculated together into other three TBM (Kw method). Each TBM received only one specimen. The TBM harboring a K-wire was vortexed for 30 seconds then sonicated for 1 minute, and vortexed (30 seconds) again as soon as they arrived at the laboratory, on the fifth and tenth days of incubation at 35 to 37 °C in 5% to 7% CO2. We sonicated earlier since distinct bacteria could be attached to surfaces for seconds to minutes. The microbiologist processed the tissue fragments and the resultant fluid (sonicated TBM containing

a K-wire) in aerobic sheep-blood agar plates at 24h, 5 and 10 days. We do not proceed with anaerobic culture. The microorganisms were identified by means of the VITEK 2 system. Both methods were compared (Wilcoxon test). In addition, we applied the Mann-Whitney test to assess whether two independent samples were taken from populations with equal means. All results were considered significant for a probability of significance of less than 5% ($p < 0.05$), thus having at least 95% confidence in the conclusions presented.

3. Results

Overall, twenty-four patients were enrolled, 29.2% female and 70.8% male. The patient's age ranged from 33 to 86 years (57.8 ± 12.2); 58.3% and 41.7% were PEDIS 3 and 4, respectively. The number of pathogens isolated in each method was not statistically significant ($p = 0.414$): CMC = 1.67 (± 0.92); KwM = 1.75 (± 0.94) (Table 1). There was a microbiological agreement in 75% of the situations (18/24 cases) concerning the two methods. The CCM detected an additional pathogen in two different cases, (*Pseudomonas aeruginosa* and *Enterococcus faecalis*). The KwM identified an extra pathogen in four patients: *Klebsiella pneumoniae* and *Proteus mirabilis* (in the same case); *P. aeruginosa*, *Morganella morganii* and *Streptococcus agalactiae* in three different cases. Table 2 shows the association between the need for amputation and the factors of interest. There was a significant association between amputation and hospitalisation in the last six months ($p = 0,037$). All patients in which amputation was performed were not hospitalized in the last six months, against 38.9% in the group where amputation was not performed.

Table 1. Characterization of patients regarding the number of microorganisms considering the method.

Method	Descriptive measures		*P*
	Minimum-Maximum	Average + SD [3]	
CCM [1]	0,0 – 4,0	1,67 ± 0,92	0,414
KwM [2]	0,0 – 4,0	1,75 ± 0,94	

[1] Conventional conventional method
[2] Kirschner wire method
[3] Standard deviation

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Table 2. The association between amputation and the variables of interest.

Variables	Amputation		*p*
	No	Yes	
Sex			
Female	12 (66,7%)	4 (80%)	1,000 [1]
Male	6 (33,3%)	1 (20%)	
Age	57,4 ± 12,9	58,8 ± 11,9	0,801
P_{50} (P_{25} – P_{75})	57,5 (46,8 – 64,3)	55,0 (49,0 – 70,5)	
PEDIS severity			
PEDIS 3	11 (61,1%)	2 (40%)	0,618 [1]
PEDIS 4	7 (38,9%)	3 (60%)	
Hemoglobin (g/dL)			
Average ± SD	9,6 ± 2,1	10,2 ± 1,5	0,612 [2]
P_{50} (P_{25} – P_{75})	9,0 (8,0 – 11,0)	10,0 (9,0 – 11,5)	
Antibiotic use us last 3 months			
No	4 (22,2%)	1 (20%)	1,000 [1]
Yes	14 (77,8%)	4 (80%)	
Hospitalisation in the last 6 months			
No	7 (38,9%)	5 (100%)	**0,037** [1]
Yes	11 (61,1%)	0 (0%)	
Use of CIP [3] or LEV [4] in the last 3 months			
No	8 (44,4%)	3 (60%)	0,529 [1]
Yes	4 (22,2%)	2 (40%)	
Unknown	6 (33,3%)	0 (0%)	
Gram-negative			
No	12 (66,7%)	4 (80%)	1,000 [1]
Yes	6 (33,3%)	1 (20%)	

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Osteomyelitis			
No	11 (61,1%)	1 (20%)	0,155 [1]
Yes	7 (38,9%)	4 (80%)	

[1] The probability of significance refers to Fisher's exact test.

[2] The probability of significance refers to the Mann-Whitney test.

[3] Ciprofloxacin

[4] Levofloxacin

4. Discussion

Despite the absence of statistical significance, it is important to consider the low sampling and the absence of a sub-analysis of patients with chronic diabetic foot infection (so far, 17 patients), often harboring extensive, polymicrobial and mature biofilms [4–11]. In addition, we do not perform anaerobic culture due to the insufficiency of the essential supplies. It is possible to obtain expressive results from the statistical point of view with a larger sampling, which allows the sub analysis of chronic infections and anaerobic cultivation. There are many questions still unanswered, such as (1) the ideal media to insert the tissue samples, (2) which material composition is most appropriate to incubate along with the tissues - stainless steel, silicone, or polyurethane, for example, (3) the number of samples to take in, and (4) the impact of early sonication as well as the instances chose to sonicate. It must not be forgotten that we intend an early diagnosis, which positively impacts treatment, for cure or disease-free survival reasons.

We do not know certainly why the association between amputation and the absence of previous hospitalisation. It is possible that non-adherent diabetes mellitus patients avoid an early medical consultation for any cause, including DFI, or do not recognize the solemn threat providing by DFI. A meta-analyses from Shen et al. does not find this association, as other studies regarding amputation as an outcome [12–15].

The KwM may be a useful microbiologic diagnostic tool to complement the conventional culture method since it identifies pathogens that have not been previously diagnosed minimizing the chance of missing significant pathogenic agents. Despite the absence of statistical significance, the KwM detected more pathogens than CCM, which deserves additional investigations in prospective trials containing an appropriate number of

participants, and anaerobic culture application. Not less crucial are the questions regarding the ideal media, type of material composition, the optimal number of tissue samples, and the ideal moments to sonicate.

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1. because it is an observational and prospective study, which will employ mostly information from medical records, institutional information systems and other sources of data and clinical information available in the institution,
2. because all data was carried out and analyzed anonymously,
3. because the results resulting from the study will be presented in aggregate form, not allowing the individual identification of the participants, and
4. because it is a non-interventional study and without alterations or influences in the routine and treatment of the research participant, and consequently without adding risks or damage to their well-being.

Informed Consent Statement: Ethical review and approval were waived for this study. The reasons are explained on "**Institutional Review Board Statement".**

Data Availability Statement: Data available on https://figshare.com/

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References

1. Saeed, K.; Esposito, S.; Akram, A.; Ascione, T.; Bal, A.M.; Bassetti, M.; Carnelutti, A.; Chan, M.; Davis, J.; Dryden, M.; et al. Hot topics in diabetic foot infection. *Int. J. Antimicrob. Agents* **2020**, *55*, 105942, doi:10.1016/j.ijantimicag.2020.105942.
2. Lipsky, B.A. Stopping Antibiotic Therapy for a Diabetic Foot Infection. *Int. J. Low. Extrem. Wounds* **2015**, *14*, 307–308, doi:10.1177/1534734615607145.
3. Lipsky, B.A.; Senneville, É.; Abbas, Z.G.; Aragón-Sánchez, J.; Diggle, M.; Embil, J.M.; Kono, S.; Lavery, L.A.; Malone, M.; Asten, S.A.; et al. Guidelines on the diagnosis and treatment of foot infection in persons with diabetes (IWGDF 2019 update). *Diabetes. Metab. Res. Rev.* **2020**, *36*, doi:10.1002/dmrr.3280.
4. Johani, K.; Malone, M.; Jensen, S.; Gosbell, I.; Dickson, H.; Hu, H.; Vickery, K. Microscopy visualisation confirms multi-species biofilms are ubiquitous in diabetic foot ulcers. *Int. Wound J.* **2017**, *14*, 1160–1169, doi:10.1111/iwj.12777.
5. Pouget, C.; Dunyach-Remy, C.; Pantel, A.; Schuldiner, S.; Sotto, A.; Lavigne, J.P. Biofilms in diabetic foot ulcers: Significance and clinical relevance. *Microorganisms* 2020, *8*, 1–15.
6. Armstrong, D.G.; Boulton, A.J.M.; Bus, S.A. Diabetic Foot Ulcers and Their Recurrence. *N. Engl. J. Med.* **2017**, *376*, 2367–2375, doi:10.1056/nejmra1615439.
7. Dowd, S.E.; Wolcott, R.D.; Sun, Y.; McKeehan, T.; Smith, E.; Rhoads, D. Polymicrobial nature of chronic diabetic foot ulcer biofilm infections determined using bacterial tag encoded FLX amplicon pyrosequencing (bTEFAP). *PLoS One* **2008**, *3*, doi:10.1371/journal.pone.0003326.
8. Redel, H.; Gao, Z.; Li, H.; Alekseyenko, A. V.; Zhou, Y.; Perez-Perez, G.I.; Weinstock, G.; Sodergren, E.; Blaser, M.J. Quantitation and composition of cutaneous microbiota in diabetic and nondiabetic men. *J. Infect. Dis.* **2013**, *207*, 1105–1114, doi:10.1093/infdis/jit005.
9. Gontcharova, V. A Comparison of Bacterial Composition in Diabetic Ulcers and Contralateral Intact Skin. *Open Microbiol. J.* **2010**, *4*, 8–19, doi:10.2174/1874285801004010008.
10. Jneid, J.; Cassir, N.; Schuldiner, S.; Jourdan, N.; Sotto, A.; Lavigne, J.-P.; La Scola, B. Exploring the Microbiota of Diabetic Foot Infections With Culturomics. *Front. Cell. Infect. Microbiol.* **2018**, *8*, doi:10.3389/fcimb.2018.00282.
11. Pereira, S.G.; Moura, J.; Carvalho, E.; Empadinhas, N. Microbiota of chronic diabetic wounds: Ecology, impact, and potential for innovative treatment strategies. *Front. Microbiol.* 2017, *8*.
12. Sen, P.; Demirdal, T.; Emir, B. Meta-analysis of risk factors for amputation in diabetic foot infections. *Diabetes. Metab. Res. Rev.* **2019**, *35*, doi:10.1002/dmrr.3165.
13. Pickwell, K.; Siersma, V.; Kars, M.; Apelqvist, J.; Bakker, K.; Edmonds, M.; Holstein, P.; Jirkovská, A.; Jude, E.; Mauricio, D.; et al. Predictors of lower-extremity amputation in patients with an infected diabetic foot ulcer. *Diabetes Care* **2015**, *38*, 852–857, doi:10.2337/dc14-1598.
14. Uysal, S.; Arda, B.; Taşbakan, M.I.; Çetinkalp, Ş.; Şimşir, I.Y.; Öztürk, A.M.; Uysal, A.; Ertam, İ. Risk factors for amputation in patients with diabetic foot infection: a prospective study. *Int. Wound J.* **2017**, *14*, 1219–1224, doi:10.1111/iwj.12788.
15. Lu, Q.; Wang, J.; Wei, X.; Wang, G.; Xu, Y. Risk Factors for Major Amputation in Diabetic Foot Ulcer Patients. *Diabetes. Metab. Syndr. Obes.* **2021**, *14*, 2019–2027, doi:10.2147/DMSO.S307815.