


# Synergistic effect of two formulations of hypochlorous acid in the treatment of 346 chronic ulcers

Rafael Herruzo PhD, MD<sup>1</sup>  | Erika Fondo Alvarez MSc<sup>2</sup> | Irene Herruzo PhD<sup>3</sup> | Emma Santiso Casanova MSc<sup>2</sup> | Silvia Cerame Perez PhD<sup>2</sup>

<sup>1</sup>Facultad de Medicina, Universidad Autónoma Madrid, Madrid, Spain

<sup>2</sup>Centro Multidisciplinar Ulceras Crónicas, Madrid, Spain

<sup>3</sup>Facultad de Medicina, Universidad Francisco Vitoria, Madrid, Spain

## Correspondence

Rafael Herruzo, Facultad de Medicina, Universidad Autónoma, Madrid, Spain.  
Email: [rafael.herruzo@uam.es](mailto:rafael.herruzo@uam.es)

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## Abstract

Our objective was to assess the efficacy of two successive applications of hypochlorous acid, first as a liquid and then as a gel because liquid hypochlorous acid is effective but has little residual effect, while the gel form has more residual power, and compare it with that of other products. An experimental non-randomised study was carried out, treating 346 chronic ulcers in 220 patients. The antiseptic treatment has been divided into 'hypochlorous acid' (Clortech), 'hypochlorous acid liquid + gel' (Clortech + Microdacyn60<sup>R</sup>-hydrogel) and 'Others' (Prontosan or Chlorhexidine or Microdacyn60<sup>R</sup>-hydrogel). Bivariate and multivariate studies analysed the characteristics of the patients and their ulcers, including size, symptoms, signs, treatments received and their duration, and so on. The ulcers were complicated, of long evolution, and most had a vascular origin. On average, antiseptic treatment lasted 14 weeks. At the time of their discharge, or last treatment in the clinics, 59% of the ulcers had healed completely, 9.5% worsened, and 6.9% had become infected during this period. In the bivariate and multivariate studies, we took as reference the 'others' treatments that showed no significant differences in healing time or infection rates compared with *liquid* hypochlorous acid 100–500 mg/L alone. However, hypochlorous acid liquid + gel showed a synergistic effect, with a higher probability of achieving complete healing (four times) and a lower probability of infection (a fifth), compared to the 'other' antiseptics. In conclusion, a synergistic effect was found with the successive application of hypochlorous acid in liquid followed by gel, an effect that increased healing probability and decreased the risk of the ulcer becoming infected.

## KEYWORDS

HOCl-gel, HOCl-liquid, synergy, ulcers healing

## 1 | INTRODUCTION

Hypochlorous acid (HOCl) has been used to treat ulcers and wounds of various etiologies including those with arterial, venous, pressure, surgery,

**Abbreviations:** liq, liquid; log<sub>10</sub>, decimal logarithm; ppm, mg/L; OR, odds ratio.

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or diabetic foot origin, among others,<sup>1–7</sup> since it combines antiseptic properties with those of non-cytotoxicity to the underlying tissue, which is key to healing these skin lesions.<sup>8–17</sup> In addition, the treatment has anti-inflammatory and antipruritic effects,<sup>18,19</sup> which also help healing. Many of the CIHO formulations are liquid, but some are gels, which are more versatile since a gel can be applied to wounds, bedsores, ulcers, and so on, to create a hydrated environment and maintain an antimicrobial effect for one or more days, which is very important for healing.<sup>20</sup>

In 2020,<sup>21</sup> we published an *in vitro* study about a more concentrated and stable formula of HOCl comparing it with other antiseptics, either based on HOCl or on other components, for example, iodines, surfactants or biguanides, using a model of germ carriers, with or without biofilm. We concluded that HOCl at concentrations of 300–500 mg/L was a good antiseptic that could be used on wounds, as it had high antimicrobial efficacy: in 5–10 min, it reduced eight types of germs by 5 log<sub>10</sub>, even in the presence of biofilm. However, lower concentrations of HOCl had less antimicrobial efficacy (the reduction was only 1–2 log<sub>10</sub>) even if they were allowed to act for the maximum time used in the study, and they were even less effective if the substrate had a biofilm.

Based on these findings the following hypothesis was proposed: a faster, more effective and safer cure for ulcers can be achieved by treating them with two successive formulations of HOCl: First, wound antiseptics and cleaning, with a moderate concentration of liquid HOCl (300–500 mg/L), which is an effective microbicide but has little residual effect, and, after that, applying gel-HOCl (60 mg/L) to the wound, because, although it has little microbicidal power, it does maintain a residual antimicrobial effect (and is non-cytotoxic) in the ulcer. We call this mixture ‘HOCl liq + gel’.

Objective: To study whether the application of HOCl liq + gel is more effective than that of other antiseptic products in a large sample of ulcers with various aetiologies.

## 2 | MATERIALS AND METHODS

An experimental non-randomised study has been carried out at the ‘Centro Multidisciplinar de Úlceras Crónicas’ (CMUC), treating 346 chronic ulcers in 220 patients, who gave their informed consent.

Three time periods were studied, before 2018, 2018–2019 and 2020–2021 (with 50, 104 and 192 ulcers, respectively, included in each period). The patients are assigned to the year they enter CMUC, regardless of whether their treatment lasts until the following year or not.

The treatment consisted of cleaning the ulcers with one of the following antiseptics: HOCl (Clortech® 100–500 mg/L, pH 5.2, Clortech Lab, Spain), polymeric biguanide (Prontosan® B Braun Lab, Germ), 2% aqueous chlorhexidine (Lainco lab, Spain), HOCl-gel (hypochlorous acid 0.008% w/v, oxidised water 97.64% w/v and sodium hypochlorite 0.002% w/v, pH 6.2–7.8, Microdacyn60<sup>R</sup> Oculus Innovative Sciences Lab, USA). If the treatment failed, a second antiseptic was chosen. Alternatively, we treat the ulcers with HOCl liq + gel (Clortech and Microdacyn60).

### 2.1 | Ulcer treatment

The ulcers were treated following the CMUC protocols: a vascular examination is performed on admission for each patient and the ankle-brachial index (ABI) is calculated. Then, the ulcer is photographically recorded, its dimensions calculated, and the tissue at the base of the lesions assessed. All procedures maintain the three skin ‘H’s’ (hydration, hygiene and humidity) and follow the TIME-RS concept (T = assessment of non-viable tissue, I = infection control, M = exudate control, E = edge of the wound stimulation, R = use of advanced products and S = social, i.e., involving the patient in their own care).

Treatments begin by cleaning the ulcer with a soap made from ozonised oils (Ozoaqua Lab, Spain) and drying it thoroughly, emphasising difficult areas like the interdigital spaces. Then, the wound is ‘sanitised’ using the antiseptic of choice to remove the debris that is present on its surface. If there is devitalised tissue, an attempt will be made to eliminate said tissue using enzymatic and/or cutting debridement. Once debridement is complete, an antiseptic is applied to the wound for 15 min, in order to destroy its bacterial load. After that, in some cases in which cleaning has been carried out with HOCl 100–500 mg/L, a second application of HOCl will be made in gel format.

Finally, depending on the type of tissue present in the wound bed, it will be dressed to either promote, or not promote, a moist environment, and the type of bandage will be chosen depending on the aetiology of the wound. For example, in the case of patients whose wounds have a vascular venous aetiology, compression bandages would be chosen.

In some ulcers, especially those with a vascular origin, the above treatment is accompanied by the non-invasive application of ozone, similar to what has been done in other studies.<sup>22</sup>

If the wound progresses well, it should be treated, approximately every 2 days, until complete healing, or until voluntary discharge, either due to treatment failure, hospitalisation, or, in a few cases, due to death, generally related to other ongoing chronic diseases.

### 2.2 | Data analysis

Data on each ulcer were collected by CMUC nurses, following a specific and exhaustive protocol, and then revised and processed by their Nursing Director.

The variable of interest was the cleaning solution, divided into three categories: ‘HOCl’ (Clortech), ‘HOCl liq + gel’ (Clortech + Microdacyn60<sup>R</sup> hydrogel) and ‘Others’ (Prontosan, Chlorhexidine or Microdacyn60<sup>R</sup> hydrogel; in cases in which treatment failed, the cleaning solution was substituted by a different antiseptic from this group of ‘others’).

A descriptive analysis was carried out using cross tabulations of the different variables in each cleaning treatment category to determine their distribution. Statistical associations were calculated using the Chi-square test when variables were qualitative. Quantitative variables assessed differences between the cleaning treatments using an analysis of variance with Bonferroni correction, calculating the mean, standard deviation and internal comparison between the three categories of the target variable.

**TABLE 1** Variables of the 220 patients divided by cleaning solution

| N and % with respect to the total number of ulcers in the corresponding column |                |                           |                  |    |              |
|--|----------------|---------------------------|------------------|----|--------------|
| Variables  | HOCl<br>N = 45 | HOCl liq + gel<br>N = 103 | Others<br>N = 72 | p  | Total        |
| Sex  |                |                           |                  |    |              |
| Women  | 29 (64.4%)     | 52 (50.5%)                | 48 (58.6%)       |    | 139 (58.6%)  |
| Men  | 16 (35.6%)     | 51 (49.5%)                | 24 (33.7%)       |    | 91 (41.4%)   |
| Comorbidities  |                |                           |                  |    |              |
| Diabetes   | 15 (33.1%)     | 44 (42.7%)                | 25 (34.7%)       |    | 84 (38.2%)   |
| Heart disease  | 6 (13.3%)      | 49 (47.6%)                | 42 (68.4%)       | ** | 97 (44.1%)   |
| Peripheral venous<br>Disease   | 24 (53.3%)     | 48 (46.6%)                | 42 (58.3%)       |    | 114 (51.8%)  |
| Peripheral arterial<br>Disease   | 7 (15.6%)      | 36 (35%)                  | 12 (16.7%)       | ** | 55 (25%)     |
| Neurologic disease   | 9 (20%)        | 19 (18.4%)                | 15 (20.8%)       |    | 43 (19.5%)   |
| Cancer 2 (4.4%)  | 7 (6.8%)       | 3 (4.2%)                  |                  |    | 12 (5.5%)    |
| Others 16 (35.6%)  | 12 (11.7%)     | 19 (26.4%)                |                  |    | 47 (21.4%)   |
| BMI  |                |                           |                  |    |              |
| Underweight (BMI < 17)   | 0 (0%)         | 5 (4.9%)                  | 0 (0%)           |    | 5 (2.3%)     |
| Healthy weight<br>(BMI: 17–25)   | 20 (44.4%)     | 36 (35%)                  | 31 (43.1%)       |    | 87 (39.5%)   |
| Overweight (BMI: 25–30)  | 9 (20%)        | 30 (29.1%)                | 32 (44.4%)       |    | 71 (32.3%)   |
| Obese (BMI > 30)   | 16 (35.6%)     | 32 (31.1%)                | 9 (12.5%)        |    | 57 (25.9%)   |
| Mobility   |                |                           |                  |    |              |
| Normal 39  | (86.7%)        | 74 (71.8%)                | 53 (73.6%)       |    | 116 (75.5 %) |
| Seated patient   | 3 (6.7%)       | 16 (15.5%)                | 8 (11.1%)        |    | 27 (12.3%)   |
| Bed-ridden patient<br>(≥1 h walking/sitting)                                   | 3 (6.7%)       | 10 (9.7%)                 | 5 (6.9%)         |    | 18 (8.2%)    |
| (<1 h walking/sitting)   | 0 (0%)         | 3 (2.9%)                  | 6 (8.3%)         |    | 9 (4.1%)     |
| Age (years)  |                |                           |                  |    |              |
| Mean and SD  | 68.5 (16.1)    | 72.4 (13.8)               | 74.9 (15.6)      |    | 72.4 (15)    |

Note: Statistical significance: \* =  $p < 0.05$ ; \*\* =  $p < 0.01$ .

Finally, multivariate logistic regression was used to assess the efficacy of the cleaning products used after controlling for other variables that could also have influenced ulcer healing or infection and may have acted as confounders of these antimicrobials.

All analyses were performed using SPSS, version 25.

Last, a sub-analysis of the most serious ulcers was made, thus, if a patient had more than one ulcer, only the largest one (perimeter) was studied. In this way, 220 ulcers in 220 patients have been selected for this subanalysis of the more severe ulcers.

### 3 | RESULTS

The number of ulcers treated with each antiseptic was as follows: HOCl (Clortech solution):  $N = 60$  (17.3% of the total); HOCl liq + gel: 201 (58.1%) and 'Other' antiseptics:  $N = 85$  (24.6%). The latter can

be broken down into: Prontosan  $N = 20$  (5.8%); Microdacyn60<sup>R</sup>,  $N = 22$  (6.4%) and if one treatment failed, this antiseptic was replaced with one of the 'others',  $N = 24$  (6.9%). The results are detailed in Tables 1–4 (qualitative or quantitative variables and multivariable analysis by logistic regression). Figure 1 compares the effect of antiseptics on the healing or infection of the ulcers, taking as reference those grouped under the name 'others'. The variables in Tables 1 and 3 can be summarised into three types:

- 'Descriptive' variables about the clinical history of the 220 patients.
- 'Descriptive' variables about the 436 ulcers and their severity, for example, sex, some previous illnesses, nutritional status, mobility, aetiology of the ulcer, its evolution time, its dimensions at the start of treatment, as well as some signs of the ulcer, for example, heat, oedema, poor granulation, pain when changing the dressing. Half

**TABLE 2** Qualitative variables of the 346 ulcers studied by cleaning solution.

| (a) N and % with respect to the total number of ulcers in the corresponding column |                |                           |                  |    |             |
|--|----------------|---------------------------|------------------|----|-------------|
| Variables  | HOCl<br>N = 60 | HOCl liq + gel<br>N = 201 | Others<br>N = 85 | p  | Total       |
| Ulcer location   |                |                           |                  |    |             |
| Sacrum   | 3 (5%)         | 15 (7.5%)                 | 2 (2.3%)         |    | 20 (5.7%)   |
| Upper leg  | 0 (0%)         | 7 (3.5%)                  | 2 (2.3%)         |    | 9 (2.6%)    |
| Lower leg  | 38 (63.3%)     | 119 (59.2%)               | 58 (68.2%)       |    | 215 (62.1%) |
| Foot   | 16 (26.7%)     | 54 (26.9%)                | 18 (21.2%)       |    | 88 (25.4%)  |
| Other  | 3 (5%)         | 8 (4%)                    | 5 (5.9%)         |    | 14 (4%)     |
| Weeks of evolution of the ulcer (on admission)                                     |                |                           |                  | ** |             |
| 0–16 weeks   | 34 (56.6%)     | 144 (71.6%)               | 35 (41.2%)       |    | 213 (61.6%) |
| 17–52 weeks  | 13 (21.7%)     | 32 (15.9%)                | 27 (31.7%)       |    | 72 (20.8%)  |
| >1 year  | 13 (21.7%)     | 25 (12.4%)                | 23 (27.1%)       |    | 61 (17.6%)  |
| Ulcer aetiology  |                |                           |                  |    |             |
| Vascular: Arterial   | 13 (21.7%)     | 40 (19.9%)                | 14 (16.5%)       |    | 67 (19.4%)  |
| Vascular: Venous   | 29 (48.3%)     | 69 (34.3%)                | 48 (56.5%)       |    | 146 (42.2%) |
| Pressure   | 5 (8.3%)       | 37 (18.4%)                | 4 (4.7%)         |    | 46 (10.3%)  |
| Surgical   | 2 (3.3%)       | 8 (4%)                    | 2 (2.3%)         |    | 12 (3.5%)   |
| Diabetic foot  | 7 (11.7%)      | 27 (13.4%)                | 12 (14.1%)       |    | 46 (13.3%)  |
| Traumatic (other than burn)  | 4 (6.6%)       | 17 (3.5%)                 | 4 (4.7%)         |    | 25 (7.2%)   |
| Burn   | 0 (0%)         | 3 (1.5%)                  | 1 (1.2%)         |    | 4 (1.2%)    |
| Year of admission  |                |                           |                  | ** |             |
| Before 2018  | 5 (8.3%)       | 0 (0%)                    | 45 (52.9%)       |    | 50 (14.5%)  |
| 2018–9   | 55 (91.7%)     | 9 (4.5%)                  | 40 (47.1%)       |    | 104 (30.1%) |
| 2020–1   | 0 (0%)         | 192 (95.5%)               | 0 (0%)           |    | 192 (55.5%) |
| (b) N and % with respect to the total number of ulcers in the corresponding column |                |                           |                  |    |             |
| Variables  | HOCl<br>N = 60 | HOCl liq + gel<br>N = 201 | Others<br>N = 85 | p  | Total       |
| Exudate (quantity on admission)  |                |                           |                  | ** |             |
| Abundant   | 17 (28.3%)     | 38 (18.9%)                | 32 (37.6%)       |    | 87 (25.1 %) |
| Moderate   | 33 (55%)       | 85 (42.3%)                | 40 (47%)         |    | 158 (45.7%) |
| Scarce   | 10 (16.7%)     | 78 (38.8%) 13 (15.3%)     |                  |    | 101 (29.2%) |
| Exudate (characteristics on admission)   |                |                           |                  |    |             |
| Serous liquid  | 56 (93.3%)     | 177 (88%)                 | 82 (96.5%)       |    | 315 (91 %)  |
| Purulent   | 3 (5%)         | 16 (8%)                   | 3 (3.5%)         |    | 22 (6.4%)   |
| Bloody   | 1 (1.6%)       | 8 (4%) 0 (0%)             |                  |    | 9 (2.6%)    |
| Ulcer signs (on admission)   |                |                           |                  |    |             |
| Heat   | 35 (58.3%)     | 138 (68.7%)               | 26 (30.1%)       | ** | 199 (57.5%) |
| Swelling   | 28 (46.7%)     | 108 (53.7%)               | 23 (27%)         | ** | 159 (46%)   |
| Bad odour  | 18 (30%)       | 51 (25.4%)                | 20 (23.5%)       |    | 89 (25.7%)  |
| Altered granulation  | 10 (16.7%)     | 46 (22.9%)                | 7 (8.2%)         | *  | 63 (18.2%)  |
| Pain when changing dressing (0–10 points)  |                |                           |                  | *  |             |
| 0 point  | 9 (15%)        | 64 (31.8%)                | 10 (11.8%)       |    | 83 (23.9)   |
| 1–5 points   | 25 (41.7%)     | 66 (32.8%)                | 34 (40%)         |    | 115 (33.2%) |
| 6–10 points  | 26 (43.3%)     | 71 (35.3%)                | 41 (48.2%)       |    | 148 (42.8%) |
| Ozone  |                |                           |                  | *  |             |
| Yes  | 51 (85.6%)     | 106 (52.7%)               | 20 (23.5%)       |    | 177 (50.9%) |

**TABLE 2** (Continued)

| (b) N and % with respect to the total number of ulcers in the corresponding column |                |                           |                  |    |             |
|--|----------------|---------------------------|------------------|----|-------------|
| Variables  | HOCI<br>N = 60 | HOCI liq + gel<br>N = 201 | Others<br>N = 85 | p  | Total       |
| Enzymatic debridement  |                |                           |                  |    |             |
| Yes  | 58 (96.7%)     | 183 (91.4%)               | 64 (75.3%)       |    | 313 (90.5%) |
| Compressive bandage  |                |                           |                  | *  |             |
| Yes  | 36 (60%)       | 110 (54.7%)               | 63 (74.1%)       |    | 209 (60.4%) |
| (c) N and % with respect to the total number of ulcers in the corresponding column |                |                           |                  |    |             |
| Variables  | HOCI<br>N = 60 | HOCI liq + gel<br>N = 201 | Others<br>N = 85 | p  | Total       |
| General antibiotherapy   |                |                           |                  | *  |             |
| Yes  | 31 (50%)       | 113 (56.2%)               | 31 (37.6%)       |    | 175 (50.6%) |
| Type of antibiotic   |                |                           |                  |    |             |
| Quinolone  | 17 (28.3%)     | 55 (27.4%)                | 21 (24.7%)       |    | 93 (26.1%)  |
| Beta-lactam  | 5 (8.3%)       | 12 (6%)                   | 2 (2.3%)         |    | 19 (5.5%)   |
| Others   | 4 (6.7%)       | 10 (5%)                   | 5 (5.90%)        |    | 19 (5.5%)   |
| Combinations of the above products   | 5 (8.3%)       | 36 (17.9%)                | 3 (3.5%)         |    | 44 (12.7%)  |
| Ulcer healing speed  |                |                           |                  |    |             |
| >5% ulcer/week   | 32 (53.3%)     | 134 (66.6%)               | 32 (3.6%)        | ** | 198 (57.2%) |
| >12.5% ulcer/week  | 15 (25%)       | 64 (31.8%)                | 16 (18.8%)       |    | 95 (27.5%)  |
| Healing ulcer  |                |                           |                  | ** |             |
| Complete   | 24 (40%)       | 142 (70.6%)               | 37 (43.5%)       |    | 203 (58.7%) |
| Incomplete   | 30 (50%)       | 52 (25.9%)                | 28 (33%)         |    | 140 (31.3%) |
| Failure (increase of the perimeter)  | 6 (10%)        | 7 (3.5%)                  | 20 (23.5%)       |    | 33 (9.5%)   |
| Ulcer infection  |                |                           |                  |    |             |
| Yes  | 5 (8.3%)       | 8 (4%)                    | 11 (12.9%)       |    | 24 (6.9%)   |

\* $p < 0.05$ ; \*\* $p < 0.01$ .

of the patients received general antibiotic therapy (oral, intramuscular or intravenous). The most used antibiotic was quinolones for an average of 2 weeks while the antiseptic treatment averaged 19 weeks. Many variables are significantly associated with the three types of antiseptics used (this association is represented by asterisks on the right margin of the tables).

- c. Variables associated with the 'healing process' expressed the speed of healing, both in absolute values (7–12 mm/week) and relative ones (6%–10% of the ulcer healed per week); these two variables are strongly correlated. Other variables reflect that the ulcer healed in 14–24 weeks, regardless of its initial size on admission to the CMUC. All these variables showed an increase in healing speed when the HOCl liq + gel process was used. A failure of healing was defined as an increase in the diameter of the ulcer (about 10% of cases). This occurred more frequently when using 'other' antiseptics and was less frequent in those treated with HOCl liq + gel.
- d. Two 'final result' variables: complete healing or infection, which occurred in 58% and almost 7%, respectively, of the ulcers. The greater efficacy of HOCl liq + gel can be verified because the ulcers healed better (70%) and fewer became infected (4%) than

when the other products were used, including HOCl alone (Clorteck liquid without the addition of gel), since only about 40% achieved complete healing while 8%–13% of ulcers so treated became infected.

To quantify the advantage of the association between the two HOCl formulations, comparisons (bi- and multivariate) were made, taking the set of antiseptics grouped under the name 'others' as reference.

We studied the two final outcome variables (complete healing and infection) with respect to the variable that described the cleaning solution (antiseptics) controlled by other variables about previous diseases, year of treatment, location of the ulcer, weeks of evolution and perimeter of the ulcer at the beginning of the treatment, amount of exudate and other lesion signs, including pain, whether spontaneous or after changing dressings, antibiotic therapy received from the start of treatment and speed of healing by the ulcer. The results of liquid HOCl, either at 100, 300 or 500 ppm, did not differ significantly from those of 'others' in the probability of cure, both in bivariate studies (OR or RR measured as incidence density ratio, see Figure 1) or in

**TABLE 3** Quantitative variables of the 346 ulcers studied by cleaning solution. Mean (and standard deviation).

| Variables   | HOCl<br>N = 60 | HOCl liq + gel<br>N = 201 | Other<br>N = 85 | p  |
|---|----------------|---------------------------|-----------------|----|
| Baseline status                                   |                |                           |                 |    |
| Ulcer evolution (weeks)                           | 42.7 (85)      | 36.6 (83.3)               | 76 (120)        | ** |
| Ulcer perimeter (mm)                              | 133 (101)      | 134 (180)                 | 214 (247)       | ** |
| Ulcer necrosis area (%)                           | 28.3 (38.5)    | 27.7 (38.8)               | 17.9 (32.8)     |    |
| Ulcer granulation area (%)                        | 22 (28)        | 37.6 (37.5)               | 27.3 (30.4)     | ** |
| Ulcer pain scale (0–10 points)                    | 4.8 (3)        | 3.8 (3.6)                 | 5 (3.4)         | *  |
| Follow-up status                                  |                |                           |                 |    |
| Antibiotherapy (days)                             | 12 (5.5)       | 13.1 (7.1)                | 18.7 (14.2)     | ** |
| Cleaning solution (weeks)                         | 17.3 (14.5)    | 14.6 (12.1)               | 29.8 (46.2)     | ** |
| Healing speed (% ulcer perimeter/week)            | 6.5 (7.2)      | 10.2 (10.1)               | 6.4 (10.4)      | ** |
| Ulcer healing speed (mm/week)                     | 6.8 (7.5)      | 12.1 (19.8)               | 7.5 (10.7)      | *  |
| Uncured residual ulcer (%)                        | 3.5 (46)       | 17.4 (35.6)               | 51.1 (70)       | ** |
| ID of ulcer complete healing/1000 week—antiseptic | 23 (15.1–33.8) | 48 (41–57.2)              | 14 (10.4–19.9)  | *  |
| ID of ulcer infection/1000 week—antiseptic        | 4.8 (1.7–10.7) | 2.7 (1.9–5.4)             | 4.3 (2.2–7.7)   | *  |

Abbreviation: ID, incidence density.

\* $p < 0.05$ ; \*\* $p < 0.01$ .

multivariate studies (Table 4). In the latter, the OR is 0.81 with 95% confidence intervals (CI-95%) ranging between 0.38 and 1.67. The probability of ulcer infection, although lower than with “others”, does not differ significantly from them, either. However, the HOCl liq + gel treatment multiplied the probability of complete healing by more than 4 (OR: 4.37 CI 95%: 2.36–8.1) and also reduced the risk of infection of the ulcer to almost the fifth part (OR: 0.22 CI 95%: 0.07–0.64).

The 220 most severe cases were distributed as follows: 45 treated with HOCl-liquid, 103 with HOCl-liq + gel and 72 with ‘other antiseptics’. Of these, 40% (HOCl-liquid), 60.2% (HOCl liq + gel) and 41.7% (‘other antiseptics’) healed correctly, and 8.9%, 5.8%, and 13.9%, respectively, became infected.

In the analysis of the most severe cases, as was logical, the size of the ulcers ceased to have statistical significance, but the significance of the weeks of evolution remained, consequently this variable was part of all the logistic regression equations, to control for its effect as a confounding factor. Table 4 shows that the equations are very similar (and with a good mathematical fit) whether we analyse the 346 ulcers or only assess the 220 most severe lesions, highlighting the improvement in ulcer healing (OR 4.37 in the complete sample, which falls to 3.47 in the most serious ulcers) when using HOCl liq + gel therapy, while the reduction in the risk of infection was similar in both samples (OR = 0.22 vs. 0.24).

## 4 | DISCUSSION

The patients' characteristics, including diabetes (40%), vascular insufficiency (70%), neurological diseases (22%), immobility (seated or bedridden) for a long time (>30%), and so on, as well as the characteristics of the ulcers (39% showed a chronicity longer than 4 months), sometimes with a long history of treatments and failures, most with signs

like heat, oedema, bad smell, and so on, are predictive of difficult healing and long treatment, which notably complicated the permanence of the patients in the study and made it difficult to evaluate the efficacy of the antiseptics used.

The ulcers were treated following a standard protocol with debridement, enzymatic in most (90%), cleaning with antiseptics and wet dressing in two out of three cases. In almost 50% of the ulcers, ozone was also used when cleaning the wound, although in the 2015 Cochrane review<sup>22</sup> ulcers with this additive did not show better evolution.

Healing speed (the initial minus final size, divided by number of treatment weeks) is a good indicator of progression towards healing or, on the contrary, of failure if the speed is ‘negative’ (ulcer size increases). ‘Positive’ speed is closely associated with the type of antiseptic used, and doubled in the case of HOCl liq + gel. On the other hand, without having to wait for the end of treatment, some of these variables can be used as prognostic indicators. For example, if more than 5% of the ulcer heals each week, we can calculate that treatment can be expected to last about 5 months, which should help reduce the patient's anxiety by giving them a time horizon. Conversely, ulcer enlargement in the first few weeks of treatment may be a clear indicator of healing failure and would indicate treatment should be modified.

Multivariate methods (logistic regression) were used to determine the effect of other variables that can influence healing and would act as confounding factors in the analysis. The values and their confidence intervals showed that HOCl liq + gel multiplied the probability of complete healing by more than 4 (OR 4.37) and also reduced the risk of ulcer infection to almost a fifth (OR 0.22) of the ‘other’ group.

Table 4 shows how similar the equations calculated from the complete database are with respect to the one that only includes the most serious cases. This indicates that the logistic regression controlled the main confounding factors well and no more variables that

**TABLE 4** Multivariable study by logistic regression of the 346 ulcers (or 220 more grave ulcers) studied by cleaning solution.

| <b>(A) Equations with all ulcers, N = 346</b>  |        |             |         |                     |
|--|--------|-------------|---------|---------------------|
| <b>(a) Healing</b>   |        |             |         |                     |
| Variable   | Beta   | 95% CI-Beta | p value | OR (95% CI)         |
| Age (years)  | −0.03  | 0.009       | 0.001   | 0.97 (0.95–0.99)    |
| Ulcer evolution (weeks)  | −0.005 | 0.002       | 0.001   | 0.995 (0.992–0.998) |
| Unhealthy granulation (y/n)  | −0.95  | 0.33        | 0.004   | 0.38 (0.203–0.74)   |
| Antibiotic (y/n)   | −0.93  | 0.26        | <0.001  | 0.39 (0.23–0.66)    |
| HOCI liq + gel versus ‘others’   | 1.47   | 0.31        | <0.001  | 4.37 (2.36–8.1)     |
| HOCI liquid versus ‘others’  | −0.21  | 0.37        | 0.56    | 0.81 (0.38–1.67)    |
| Hosmer–Lemeshow goodness of fit = 0.4  |        |             |         |                     |
| <b>(b) Infection</b>   |        |             |         |                     |
| Variable   | Beta   | 95% CI-Beta | p value | OR (95% CI)         |
| Ulcer evolution (weeks) <sup>a</sup>   | 0.002  | 0.002       | 0.23    | 1.002 (0.999–1.005) |
| Bad odour (y/n)  | 2.71   | 0.53        | 0.005   | 15.1 (5.2–43.5)     |
| HOCI liq + gel versus ‘others’   | −1.49  | 0.53        | 0.005   | 0.22 (0.07–0.64)    |
| HOCI liquid versus ‘others’  | −0.77  | 0.63        | 0.22    | 0.46 (0.13–1.59)    |
| <b>(B) Equations with only one ulcer/patient and if there are more than one, the largest ulcer was included, N = 220</b> |        |             |         |                     |
| <b>(a) Healing</b>   |        |             |         |                     |
| variable   | Beta   | 95% CI Beta | p value | OR (95% CI)         |
| Age (years)  | −0.032 | 0.011       | 0.004   | 0.96 (0.95–0.99)    |
| Ulcer evolution (weeks)  | −0.009 | 0.003       | 0.001   | 0.99 (0.98–0.996)   |
| Unhealthy granulation (y/n)  | −0.788 | 0.42        | 0.06    | 0.45 (0.2–1.04)     |
| Antibiotic (y/n)   | −1.3   | 0.33        | 0.001   | 0.27 (0.14–0.52)    |
| HOCI liq + gel versus ‘others’   | 1.26   | 0.39        | 0.001   | 3.47 (1.62–7.4)     |
| HOCI liquid versus ‘others’  | −0.1   | 0.43        | 0.81    | 0.9 (0.38–2.1)      |
| Hosmer–Lemeshow goodness of fit = 0.959  |        |             |         |                     |
| <b>(b) Infection</b>   |        |             |         |                     |
| Variable   | Beta   | 95% CI-Beta | p value | OR (95% CI)         |
| Ulcer evolution (weeks) <sup>a</sup>   | 0.002  | 0.002       | 0.22    | 1.002 (0.998–1.006) |
| Bad odour (y/n)  | 2.79   | 0.61        | <0.001  | 16.3 (4.9–54.5)     |
| HOCI liq + gel versus ‘others’   | −1.42  | 0.6         | 0.01    | 0.24 (0.07–0.79)    |
| HOCI liquid versus ‘others’  | −0.92  | 0.7         | 0.19    | 0.39 (0.1–1.5)      |

Note: (A) Hosmer–Lemeshow goodness of fit = 0.23. (B) Hosmer–Lemeshow goodness of fit = 0.24.

<sup>a</sup>No significative but important in the good fit of this equation.

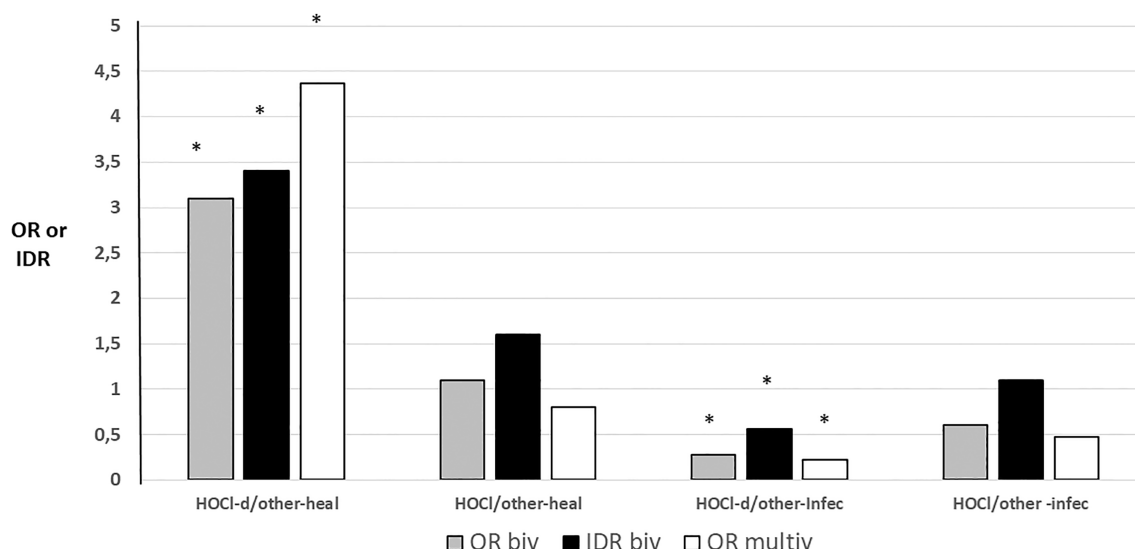
influenced ulcer healing or infection had to be included. For this reason, the effect obtained from HOCI liq + gel is very valuable, as it is associated with both a greater probability of healing in the ulcers (OR 3.4) and a smaller likelihood of becoming infected (OR 0.24).

The OR for the efficacy of the antiseptics used here, calculated by multivariate analysis, can be compared with the Relative Risks, obtained through Incidence Density, in which the time is integrated in the same variable. Here, time was registered as ‘treatment weeks’. Interestingly, that measurement of Relative Risk is much easier to calculate and gives similar results to those of a multivariate logistic regression analysis (Figure 1). This may be due to the fact that the

treatment time summarises many characteristics that influence the healing of ulcers, since lesions that occur in people with more serious diseases, or that have larger dimensions, or a more torpid evolution, and so on require longer treatments, and, by incorporating the time dimension in the Incidence Density, we are, inadvertently, controlling those variables associated with treatment time. That is something similar to what we did with the multivariate analysis, but in a much simpler way.

However, the most interesting, and practical finding, is that the two formulations of HOCI act synergistically, since, although, they did not produce substantial healing by themselves (around 40%), together they reach 70% (or 62% in the most severe lesions). Additionally, if





**FIGURE 1** Comparison of the effect of various cleaning solutions on chronic ulcers through bivariate and multivariate studies. \* $p < 0.05$ . biv, bivariate; HOCl-d, HOCl liq + gel; HOCl, HOCl-liquid; heal, ulcer healing; infec, ulcer infection; IDR, incidence density ratio; multiv, multivariate; other, other antiseptic as HOCl-gel or biguanides.

we assess the increased probability of healing when comparing HOCl liq + gel with the results of the rest of the antiseptic treatments used in this study, the probability of healing is multiplied 3.3–4.3 times. Something similar happens with infection, which is reduced to almost a fifth. This increase in curative efficacy and infection prevention in ulcers is explained by the fact that the liquid formula has good direct efficacy, but little residual effect, while the gel has the opposite effect, but when the latter is applied to an ulcer with a surface that has just been well disinfected by the liquid formula (which had a higher concentration of HOCl), it can maintain asepsis much better than either of the products on its own. In addition, the gel has a low concentration of HOCl, so it does not interfere with healing and can even stimulate it.

## 4.1 | Limitations and strengths of this study

### 4.1.1 | Limitations

The study was not randomised, but we did control, through multivariate analysis, the possible confounding factors for the treatment being used. Nevertheless, we cannot guarantee there is no inadvertent bias in the analysis. Therefore, a randomised experimental study would help to ratify these findings and provide definitive evidence.

### 4.1.2 | Strengths

- The follow-up is long-term, unlike other studies which conclude after only 3–4 weeks, when the only thing that can be assessed, in 1 month, is whether the healing process is correct, or not, but the

final result is not evaluated. In our case, the patients remained in the study for 17 weeks (median).

- The large number of ulcers studied with many different aetiologies, allows adequate assessment of the global efficacy of this mixture of HOCl formulations.
- The protective effect of the HOCl liq + gel combination is maintained both in the complete database and in the subanalysis that only includes the most serious ulcers, giving consistency to the study.

## 5 | CONCLUSION

The use of HOCl in two formulations applied successively within the same treatment-session of a chronic ulcer (Clorteck 300–500 ppm plus Mycrodacyn60-hydrogel) is a highly recommended technique since it has a synergistic effect that combines the immediate efficacy of the first with the residual effect of the second, obtaining:

- increased healing speed compared to other antiseptics,
- four-fold multiplication of the probability that the ulcer is cured when the patient is discharged, and
- decreases the probability that the ulcer will become infected by five.

## CONFLICT OF INTEREST STATEMENT

There is no potential conflict of interest regarding the publication of this manuscript.

## DATA AVAILABILITY STATEMENT

Data available on request due to privacy/ethical restrictions.



## ORCID

Rafael Herruzo  <https://orcid.org/0000-0001-7804-6680>

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