

Journal Pre-proof



Buffered lidocaine 1%, epinephrine 1:100'000 with sodium bicarbonate (hydrogen carbonate) in a 3:1 ratio is less painful than a 9:1 ratio: A double-blind, randomized, placebo-controlled, crossover trial

Alexandra Vent, MD, med.pract., Christian Surber, Pharmacist, Prof. Dr. phil. nat., Nicole Tracy Graf Johansen, Biostatistician, Dr. phil. nat., Verena Figueiredo, Pharmacist, MSc, Georg Schönbacher, Pharmacist, Dr. sc. nat., Laurence Imhof, MD, Dr. med., Caroline Buset, MD, med.pract., Jürg Hafner, MD, Prof. Dr. med.

PII: S0190-9622(20)30066-9

DOI: <https://doi.org/10.1016/j.jaad.2019.09.088>

Reference: YMJD 14145

To appear in: *Journal of the American Academy of Dermatology*

Received Date: 12 December 2018

Revised Date: 1 September 2019

Accepted Date: 12 September 2019

Please cite this article as: Vent A, Surber C, Graf Johansen NT, Figueiredo V, Schönbacher G, Imhof L, Buset C, Hafner J, Buffered lidocaine 1%, epinephrine 1:100'000 with sodium bicarbonate (hydrogen carbonate) in a 3:1 ratio is less painful than a 9:1 ratio: A double-blind, randomized, placebo-controlled, crossover trial, *Journal of the American Academy of Dermatology* (2020), doi: <https://doi.org/10.1016/j.jaad.2019.09.088>.

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2020 Published by Elsevier on behalf of the American Academy of Dermatology, Inc.

1 **Buffered lidocaine 1%, epinephrine 1:100'000 with sodium**
2 **bicarbonate (hydrogen carbonate) in a 3:1 ratio is less painful than**
3 **a 9:1 ratio: A double-blind, randomized, placebo-controlled,**
4 **crossover trial**

5
6 Alexandra Vent, MD, med. pract. ¹

7 Christian Surber, Pharmacist, Prof. Dr. phil. nat. ¹

8 Nicole Tracy Graf Johansen, Biostatistician, Dr. phil. nat. ²

9 Verena Figueiredo, Pharmacist, MSc ³

10 Georg Schönbächler, Pharmacist, Dr. sc. nat. ⁴

11 Laurence Imhof, MD, Dr. med. ¹

12 Caroline Buset, MD, med. pract. ¹

13 Jürg Hafner, MD, Prof. Dr. med. ¹

14

15 ¹ Department of Dermatology, CH-8091 University Hospital of Zurich

16 ² Graf Biostatistics, CH-8400 Winterthur

17 ³ Hospital Pharmacy of the University Hospital Zurich, CH-8052 Schlieren

18 ⁴ Institute of Psychology, 8091 University of Zurich

19 Switzerland

20 Corresponding author

21 Prof. Dr. med. Jürg Hafner, MD

22 Department of Dermatology

23 Gloriosastrasse 31

24 CH-8091 University Hospital of Zurich, Switzerland

25 E-mail: juerg.hafner@usz.ch

26 +41 44 255 25 33

27

28 e-Mail addresses of co-authors

29 alexandra.vent@usz.ch

30 christian.surber@unibas.ch

31 graf@biostatistics.ch

32 verena.figueiredo@kaz.zh.ch

33 georg.schoenbaechler@access.uzh.ch

34 laurence.imhof@usz.ch

35 caroline.buset@usz.ch

36 Funding sources:37 JH Rahn Foundation, Zurich (www.jhrah-foundation.ch)38 Bruno Bloch Foundation, Zurich (<https://www.brunoblochstiftung.ch>)

39

40 Conflict of interest:

41 The authors have no conflict of interest to declare.

42

43 Prior presentation:

44 This study has not yet been presented.

45

46 Reprint request:47 The undersigned author transfer all copyright ownership of this manuscript to the
48 American Academy of Dermatology, in the event the work is published.

49

50 Word count (main text): 2473

51 Word count (abstract): 195

52 Word count (capsule summary): 45

53 References: 20

54 Figures: 5

55 Table: 0

56

57 Consort statement: Included

58 Trial protocol statistical analysis: Included

59 Ethical approval of local ethical committee (KEK): Included

60 Ethical approval of Swissmedic: Included

61 Trial Registration: ClinicalTrials.gov NCT03238625

62 **Abstract**

63 **Background**

64 Neutralizing (buffering) lidocaine 1%, epinephrine 1:100,000 solutions (Lido/Epi) with sodium
65 hydrogen carbonate (NaHCO_3) (bicarbonate) is widely used to reduce burning sensations
66 during infiltration of Lido/Epi. Optimal mixing ratios have not been systematically
67 investigated.

68 **Objectives**

69 To determine whether the Lido/Epi- NaHCO_3 mixing ratio 3:1 (IMP1) causes less pain during
70 infiltration than the mixing ratio 9:1 (IMP2) or unbuffered Lido/Epi (IMP3).

71 **Methods**

72 Double-blind, randomized, placebo-controlled, crossover trial ($n=2 \times 24$) with 4 investigational
73 medicinal products (IMP1-4).

74 **Results**

75 The 3:1 mixing ratio was significantly less painful than the 9:1 ratio ($p = 0.044$). Unbuffered
76 Lido/Epi was more painful than the buffered Lido/Epi ($p=0.001$ vs IMP1; $p=0.033$ vs IMP2).
77 IMP4 (NaCl 0.9%=placebo) was more painful than any of the anesthetic solutions ($p=0.001$
78 vs IMP1; $p=0.001$ vs IMP2; $p=0.016$ vs IMP3;). In all cases the anesthesia was effective for
79 at least 3 hours.

80 **Limitations**

81 Results of this trial cannot be transferred to other local anesthetics such as prilocaine,
82 bupivacaine, or ropivacaine which precipitate with NaHCO_3 admixtures.

83 **Conclusions**

84 Lido/Epi- NaHCO_3 mixtures effectively reduce burning pain during infiltration. The 3:1 mixing
85 ratio is significantly less painful than the 9:1 ratio. Reported findings are of high practical
86 relevance given the extensive use of local anesthesia today.

87 **Introduction**

88 Lidocaine, an anesthetic of the amide class, is one of the most commonly used local
89 anesthetics. It is available in a variety of concentrations (0.5% to 2.5%). A concentration of
90 1%, with or without epinephrine, is the most commonly used. Epinephrine is added at a
91 concentration of 1:100,000. It causes vasoconstriction resulting in less bleeding, longer
92 action, and less systemic toxicity.¹

93 To aid manufacture and stability, commercial lidocaine products with or without epinephrine
94 have a pH of 2.5-4.0.²⁻⁴ Acidity is assumed to be responsible for the burning sensation
95 during infiltration.⁵ Seventeen peer-reviewed studies have confirmed significant pain
96 reduction during infiltration of lidocaine when sodium hydrogen carbonate (NaHCO_3) 8.4%
97 (synonymous: bicarbonate 8.4%) was added in various mixing ratios (10:1-5:1) to buffer the
98 solution at a neutral, more physiologic pH.^{1,6} All studies consistently reported that buffering
99 did not reduce or shorten the anesthetic effect.

100 Based on this information it has become common to mix Lidocaine 1%, Epinephrine
101 1:100'000 (Lido/Epi) with NaHCO_3 at a 9:1 ratio (9ml Lido/Epi plus 1ml NaHCO_3 8.4%). In
102 daily practice, however, many patients still report distressing pain during infiltration. We
103 therefore empirically extended mixing ratios and found that a 3:1 ratio led to virtually painless
104 infiltrations.

105 In order to scientifically substantiate our observation, we conducted a phase II, monocentric,
106 double-blind, randomized, placebo-controlled, crossover trial to assess pain during infiltration
107 of two solutions with different ratios of Lido/Epi- NaHCO_3 (3:1 and 9:1), unbuffered Lido/Epi,
108 and sodium chloride (NaCl) 0.9% (placebo).

109 **Materials and Methods**

110 **Approvals**

111 The study was approved by the local Ethical Committee (KEK-ZH, Nr.2015-0531), and by
112 *Swissmedic* (national authorization and supervisory authority of Switzerland for drugs and
113 medical products). Written informed consent was obtained from all volunteers. The trial was
114 registered at www.clinicaltrials.gov (NCT03110393).

115 **Volunteers**

116 48 healthy volunteers were included and distributed to two groups. In each group every
117 volunteer was randomly allocated a different order of injections (figure 1).

118 *Inclusion criteria* were age (18-75 years), proficiency in German, and sufficient intellectual
119 and linguistic abilities to fully understand and follow all trial procedures and instructions.

120 *Exclusion criteria* were hypersensitivity or allergies to local anesthetics of the amide type or
121 to auxiliary substances such as sulfites, pregnancy (secured with testing), damaged skin on
122 the arms, or inability to give informed consent.

123 **Investigational Medicinal Products (IMP)**

124 Four IMP (IMP1,2,3,4) were prepared and labelled according to a packaging- and
125 randomization-plan by the hospital pharmacy of the University Hospital Zurich according to
126 current Good Manufacturing Guidelines. To ensure a blind test and to guarantee product
127 conformity/stability on the day of injection, IMP1,2,3,4 were prepared as sets of two identical
128 vials (5ml) – one containing NaHCO₃ and one containing Lido/Epi in appropriate
129 concentrations. For further details see figure 2. The mixing took place within 1 minute prior
130 to infiltration. This procedure was explicitly chosen to guarantee product conformity/stability.

131 **Injection Sites and Injection Procedure**

132 *Group 1* received two infiltrations. The injection sites were on the right (A) and left (B)
133 palmar forearm.

134 *Group 2* received four infiltrations into the palmar forearms. The injection sites were
135 alphabetically assigned: (A) right radial, (B) right ulnar, and (C) left ulnar and (D) left radial
136 palmar forearm, respectively.

137 All sites were 5 cm distal from the cubital fossa. Five seconds after skin puncture 2ml of the
138 IMP was slowly infiltrated into the superficial subcutis over a period of 15s with a 30G needle.
139 The residual 8ml in the syringe was discarded. The injections were performed by the same
140 study physician throughout the investigation. The mixed study medication was at room
141 temperature.

142 **pH and Osmolality of Investigational Medicinal Products (IMPs)**

143 The pH of the IMPs was determined with the potentiometer Titrand 906 (Metrohm AG,
144 Herisau, Switzerland), according to European Pharmacopoeia (PhEur) 2.2.3. Osmolality of
145 the IMPs was determined with the Advanced-3320 micro-osmometer (Advanced Instruments,
146 Norwood MA, USA) according to PhEur 2.2.35.

147 **Measuring Parameters and Hypotheses**

148 The following three measurements were recorded 1) pain during infiltration, 2) patient
149 comfort during infiltration and 3) duration of local anesthesia (numbness).

150 *Quantitative rating of pain during infiltration* was recorded on a 10-point numerical rating
151 scale (NRS) immediately after infiltration of the study solution (a few seconds after removing
152 the needle). For each volunteer data was recorded on a sheet of paper with a printed
153 numerical rating scale (from 0 to 10; 0=no pain; 10=unacceptable pain).⁷

154 *Qualitative rating of patient comfort during infiltration* was recorded using a sheet of paper
155 with a choice of four categorical terms – *desirable* (wünschenswert), *acceptable*
156 (akzeptabel), *less acceptable* (weniger akzeptabel), and *almost or totally unacceptable*
157 (kaum oder gar nicht akzeptabel) which could be ticked by the volunteers. This was done
158 directly after recording the *pain during infiltration (NSR)*-measurement.

159 *Duration of local anesthesia* (numbness) was recorded after infiltration (after recording the
160 pain during infiltration and patient comfort during infiltration) within 3-5 minutes of removing
161 of the needle, and at 30-minute intervals up to 3 hours, each time using a standardized laser
162 stimulus which left the skin barrier intact (Erbium: Glass non-ablative fractional laser [NAFL],
163 1540nm, 10mm tip, fluence 30mJ, pulse width 15ms, Cynosure Inc.).⁸ Numbness was
164 recorded as present (YES) or *absent* (NO).

165 The following hypotheses were tested for *pain during infiltration*:

166 **Group 1 (primary end point):**

- 167 • IMP1 (Lido/Epi-NaHCO₃=3:1) causes less *pain during infiltration* than IMP2 (Lido/Epi-
168 NaHCO₃=9:1)

169 **Group 2 (secondary end points):**

- 170 • IMP1 (Lido/Epi-NaHCO₃=3:1) causes less *pain during infiltration* than IMP3
171 (unbuffered Lido/Epi) and IMP4 (placebo)
172 • IMP2 (Lido/Epi-NaHCO₃=9:1) causes less *pain during infiltration* than IMP3
173 (unbuffered Lido/Epi) and IMP 4 (placebo).

174 **Statistics**

175 *Group 1, primary end point* (n=24): We planned for a two-sided Mann-Whitney test of the
176 CROS-estimator, measured as the score for *pain on infiltration* of the first injection minus
177 the score for *pain on infiltration* of the second injection. We estimated that 24 cross-overs
178 would be needed to have at least 80% power with significance testing at the $\alpha=0.05$ level to
179 detect a difference between the two randomization groups (two different order of injection
180 sequence) of 3 NRS values, assuming a standard deviation of 2.5. Variability was
181 estimated based on a 2010 Cochrane review.⁶

182 *Group 2, secondary end points* (n=24): We decided to test all four IMPs on a second group
183 of volunteers to avoid possible interferences with the investigation of the primary end point
184 (IMP1 vs IMP2). Every volunteer of group 2 was randomly allocated a different order of
185 injections.

186 The randomization list was computer generated by the hospital pharmacy, using block
187 sizes of 6 for Group 1 (4 blocks of 6 participants, 3 start with IMP1, and 3 with IMP 2) and
188 of one block of 24 participants for Group 2 (this block contains 24 possible combinations).
189 The tests for differences in *pain during infiltration* are based on a two-sided exact Mann-
190 Whitney test of the CROS-estimator.

191 *Patient comfort during infiltration and local anesthesia* (numbness) information were
192 analyzed descriptively. All analyses were performed in the R-programming language
193 (version 3.3.3).⁹

Journal Pre-proof

194 **Results**

195 48 volunteers were included (21 males, 27 females), with mean age 31.4 (21–62) years. All
196 completed the study.

197 **Pain measurements**

198 ***Quantitative rating of pain during infiltration***

199 *Group 1:* IMP1 (Lido/Epi-NaHCO₃=3:1) was significantly less painful than IMP2 (Lido/Epi-
200 NaHCO₃=9:1) ($p = 0.044$). When IMP1 was followed by IMP2, IMP1 had a median pain
201 score 1.5 points lower (less painful) than IMP2 (1st to 3rd quartile range -3.0 – -1.0). When
202 IMP 2 was followed by IMP 1, the IMP 1 median pain score was 0.5 points lower than for
203 IMP2 (1st to 3rd quartile range -2.0 – 1.25) (figure 3).

204 *Group 2:* IMP3 (unbuffered Lido/Epi) was more painful than both IMP1 (Lido/Epi-
205 NaHCO₃=3:1) ($p=0.001$) and IMP2 (Lido/Epi-NaHCO₃=9:1) ($p=0.033$). IMP4 (placebo) was
206 more painful than IMP1 ($p=0.001$), IMP2 ($p=0.001$), and IMP 3 ($p=0.016$) (figure 4).

207 [IMP1: median NRS 2.0 (1st to 3rd quartile range 1.0 – 4.0); IMP2: median NRS 3.0 (1st to 3rd
208 quartile range 2.0 – 4.25); IMP3: median NRS 4.5 (1st to 3rd quartile range 3.0 – 7.0); IMP4:
209 median NRS 6.0 (1st to 3rd quartile range 3.75 – 8.0)]

210 ***Qualitative rating of patient comfort during infiltration***

211 Qualitative rating score data is presented in figure 5.

212 ***Duration of local anesthesia (numbness)***

213 In all volunteers, laser-induced pain was absent in the injection areas of IMP1,2,3 and
214 present in the injection areas of IMP4 between 5 minutes and 3 hours after infiltration.

215 **pH and Osmolality of IMPs**

216 pH information is presented in figure 2. Osmolality (mosm/kg) of IMP1,2,3,4 was 674.6,
217 467.1, 315.0, 285.3, respectively.

218 **Adverse events**

219 No serious adverse events occurred during the study. Five test persons experienced local
220 tenderness for 1-2 days. One test person had a deep subcutaneous nodule of approximately

221 5mm diameter which completely disappeared after 2 months. We assume it must have been
222 a small hematoma in the deeper subcutis.

Journal Pre-proof

223 **Discussion**

224 In a phase II, monocentric, double-blind, randomized, placebo-controlled, crossover trial we
225 scientifically substantiated that the mixing of Lido/Epi-NaHCO₃ at a 3:1 ratio causes less *pain*
226 *during infiltration* than at a 9:1 ratio (fig 3). Furthermore, we showed that unbuffered Lido/Epi
227 is more painful than buffered Lido/Epi at ratios 3:1 and 9:1, and that the placebo was more
228 painful than any of the anesthetic solutions (fig 4). *Patient comfort during infiltration*
229 assessed with qualitative pain scores showed a clear preference for the buffered Lido/Epi at
230 a ratio of 3:1. Placebo received the most negative scores (fig 5). All anesthetic solutions led
231 to numbness in the injection areas between 5 minutes and 3 hours after infiltration. The
232 lower lidocaine concentration in the 3:1 Lido/Epi-NaHCO₃ did not affect local anesthesia,
233 compared to the 9:1 ratio, within the observation time.

234 ***Acidity causes the burning sensation during infiltration***

235 Acidity has been assumed to be responsible for the burning sensation during infiltration.
236 Meanwhile the detection of acid-sensing ion channel receptors or nociceptors fully supports
237 this explanation.⁵ The causal link between pH and burning pain during infiltration is also
238 supported by our trial with the unbuffered Lido/Epi solution (IMP3) at pH 3.4 and with the
239 pharmacologically inactive placebo (IMP4) at pH 6.2. The pain during infiltration with the
240 neutralized solutions (IMP1: pH 7.5, IMP2: pH 7.3) was significantly reduced.

241 ***At neutral pH, lidocaine is predominantly present in its active form***

242 Injection solutions contain lidocaine in an uncharged, non-ionized and in a charged, ionized
243 form, respectively. The uncharged form - also known as the active form - is lipophilic and in
244 contrast to the charged (hydrophilic) form readily permeates the nerve membrane to bind
245 from the cytosol to the acid-sensing ion channel receptors.¹⁰⁻¹¹ According to the Henderson-
246 Hasselbalch equation, in any sample of a lidocaine solution the ratio of the non-ionized
247 (active form) to ionized species of the anesthetic depends on the pH. At a more acidic pH,
248 the ionized, cationic form predominates. For instance, at a pH of 3.8, a typical cartridge of
249 Lido/Epi contains only 1 molecule of non-ionized (active form) anesthetic for every 10,000
250 molecules of ionized anesthetic. On the other hand, closer to physiologic pH, more non-
251 ionized (active form) anesthetic is present. For instance, at the physiologic pH 7.4 there is 1
252 molecule of non-ionized (active form) lidocaine in solution for every 4 molecules of ionized
253 lidocaine. At the physiologic pH, there are 2'500 times more of the active form available than
254 at a pH of 3.8.

255 ***NaCl 0.9% is more painful than unbuffered Lido/Epi***

256 During injection of unbuffered Lido/Epi (pH 3.8), the buffering system of the body will bring –
257 with a very short time lag - the unphysiologic pH of the solution to a more neutral level.¹²
258 During neutralization the continuously formed non-ionized lidocaine (active form) can
259 penetrate nerve cells and block the acid-sensing ion channel receptors from inside the
260 synapse.^{13,14} With NaCl 0.9% (pH 6.2) the buffering system of the body will also neutralize
261 the solution. However, due to the absence of lidocaine, acidity will cause a noticeably longer
262 duration of burning sensation until neutralization of the solution.

263 ***CO₂ has analgesic effects***

264 When NaHCO₃ is mixed with acidic lidocaine hydrochloride solution, water (H₂O) and carbon
265 dioxide (CO₂) are formed. Condouris et al. demonstrated that CO₂ develops an independent,
266 direct local anesthetic effect.¹⁵ Based on these observations, Catchlove et al. were able to
267 demonstrate that CO₂ enhances the action of lidocaine. CO₂ directly deactivates the nerve
268 axon, and indirectly increases the anesthetic effect of lidocaine by changing its electrical

269 charge.¹⁶ This may explain why an excess of CO₂, as formed in 3:1 mixture, more effectively
270 reduces the burning sensation of lidocaine relative to the 9:1 mixture. Both mixtures
271 effectively neutralize the Lido/Epi solution, but the 3:1 mixture provides more local CO₂
272 anesthesia.

273 ***Osmolality and pain during infiltration***

274 Osmolality may impair tolerability (including pain on infiltration) of injection solutions.¹⁷ The
275 general osmolality recommendations for injection and infusion solutions is to not greatly
276 exceed the value of 600 and 1,000 mOsm/kg respectively.¹⁷ As yet, no mOsm/kg-to-pain
277 relationship could be found between 300 and 1100 mOsm/kg in a study after intra-muscular
278 injection of vaccine suspensions in healthy adults.¹⁸ Osmolality of Lido/Epi-NaHCO₃ at a
279 ratio of 3:1 (IMP1) was 674.6 mosm/kg and higher than in the other solutions (467.1, 315.0,
280 285.3 mosm/kg). In a similar investigation, Parham et al. found that pain during infiltration of
281 physiological NaCl 0.9% (285.3 mosm/kg) was significantly more painful than their more than
282 twice as osmolar Lido/Epi-NaHCO₃ mixtures.¹⁹ Our data confirms this observation.

283 ***Stability of bicarbonate neutralized Lido/Epi solution***

284 The stability of neutralized Lido/Epi solutions with NaHCO₃ is limited²⁰ which is the major
285 reason why there are no commercial products on the market. Neutralized Lido/Epi solutions
286 are compounded in advance – either preoperatively by nurses or physicians, or by a
287 specialized pharmacy.²⁰ The manner in which the solutions are mixed often depends on the
288 available bulk containers (drug/buffer), syringes, and local situations and customs. This
289 makes the storage and subsequent stability of the compounded product an important issue.
290 Despite numerous examinations on the stability of neutralized anesthetics/epinephrine
291 solutions, there is still a considerable degree of uncertainty on the topic. Due to the lack of
292 proper method validation and study design, and the large number of influences when
293 compounding (such as temperature, time, packaging material, light, and oxygen level), no
294 general rules have emerged to simplify compounding and ensure product quality. In the
295 context of our trial we therefore limited the time between mixing and infiltrating the NaHCO₃ -
296 Lido/Epi solution to one minute so that degradation would not be a factor. In our everyday

297 practice we limit the shelf-life of the ad hoc prepared Lido/Epi-NaHCO₃ solutions to the
298 duration of the surgical intervention (less than 2 hours).

299 **Conclusions**

300 The Lido/Epi-NaHCO₃ mixtures effectively reduce burning pain during infiltration. The 3:1
301 mixing ratio was significantly less painful than the 9:1 ratio. The reported findings are of high
302 practical relevance given the extensive use of local anesthesia today.

303 **Limitations**

304 The results of this clinical trial cannot be transferred to other local anesthetics, such as
305 prilocaine, bupivacaine, or ropivacaine, which precipitate with bicarbonate admixture.
306 NaHCO₃ admixtures with other concentrations of lidocaine, e.g., 0.5% or 2.0%, and with
307 lidocaine without epinephrine are expected to function the same way, but this has not been
308 formally tested.

309 **Acknowledgements**

310 This study was supported by the JH Rahn Foundation, Zurich (www.jhrah-foundation.ch)
311 and the Bruno Bloch Foundation, Zurich (www.brunoblochstiftung.ch). We thank all
312 participants.

313 **Conflict of interest**

314 None.

315 **References**

- 316 1. Burns CA, Ferris G, Feng C, et al. Decreasing the pain of local anesthesia: a
317 prospective, double-blind comparison of buffered, premixed 1% lidocaine with
318 epinephrine versus 1% lidocaine freshly mixed with epinephrine. *J Am Acad*
319 *Dermatol.* 2006;54:128-131.
- 320 2. Christoph RA, Buchanan L, Begalla K, Schwartz S. Pain reduction in local anesthetic
321 administration through pH buffering. *Ann Emerg Med.* 1988;17:117-120.
- 322 3. Masters JE. Randomised control trial of pH buffered lignocaine with adrenaline in
323 outpatient operations. *Br J Plast Surg.* 1998;51:385-387.
- 324 4. Crews JC, Clark RB. Effect of alkalization on the pH of local anesthetic solutions.
325 *Anesth Analg.* 1987;66:1203.
- 326 5. Yoder N, Yoshika C, Gouaux E. Gating mechanisms of acid-sensing ion channels.
327 *Nature* 2018;555(7696):397-401.
- 328 6. Cepeda MS, Tzortzopoulou A, Thackrey M, et al. Adjusting the pH of lidocaine for
329 reducing pain on injection. *Cochrane Database Syst Rev.* 2010(12):CD006581.
- 330 7. Miro J, Castarlenas E, de la Vega R, et al. Validity of three rating scales for measuring
331 pain intensity in youths with physical disabilities. *Eur J Pain.* 2016;20:130-137.
- 332 8. Plaghki L, Mouraux A. How do we selectively activate skin nociceptors with a high
333 power infrared laser? Physiology and biophysics of laser stimulation. *Neurophysiol*
334 *Clin.* 2003;33:269-277.
- 335 9. *A Language and Environment for Statistical Computing* [computer program]. Vienna,
336 Austria: R Foundation for Statistical Computing; 2017.
- 337 10. Hille B. The pH-dependent rate of action of local anesthetics on the node of Ranvier.
338 *J Gen Physiol.* 1977;69:475-496.
- 339 11. Hille B. Local anesthetics: hydrophilic and hydrophobic pathways for the drug-
340 receptor reaction. *J Gen Physiol.* 1977;69:497-515.
- 341 12. Gardner JH, Semb J. The relation of pH and surface tension to the activity of local
342 anesthetics. *J Pharmacol Exp Ther.* 1935;54:309-319.
-

- 343 13. Talu H, Elibol O, Yanyali A, et al. Effect of warming and buffering lidocaine on pain
344 during facial anesthesia. *Ann Ophthalmol.* 2001;33:43-47.
- 345 14. Richtsmeier AJ, Hatcher JW. Buffered lidocaine for skin infiltration prior to
346 hemodialysis. *J Pain Symptom Manage.* 1995;10:198-203.
- 347 15. Condouris GA, Shakalis A. Potentiation of the nerve-depressant effect of local
348 anesthetics by carbon dioxide. *Nature.* 1964;204(Oct 3):57-58.
- 349 16. Catchlove RFH. The influence of CO₂ and pH on local anesthetic action. *J Pharmacol*
350 *Exp Ther.* 1972;181:298-309.
- 351 17. Wang W. Tolerability of hypertonic injectables. *Int J Pharm.* 2015;490:308-315.
- 352 18. Nony P, Girard P, Chabaud S, et al. Impact of osmolality on burning sensations during
353 and immediately after intramuscular injection of 0.5 ml of vaccine suspensions in
354 healthy adults. *Vaccine.* 2001;19:3645-3651.
- 355 19. Parham SM, Pasioka JL. Effect of pH modification by bicarbonate on pain after
356 subcutaneous lidocaine injection. *Can J Surg.* 1996;39:31-35.
- 357 20. Pascuet E, Donnelly RF, Garceau D, et al. Buffered lidocaine hydrochloride solution
358 with and without epinephrine: stability in polypropylene syringes. *Can J Hosp Pharm.*
359 2009;62:375-380.
-

360 **Figure legends**

361

362 **Figure 1: CONSORT study flow diagram.**

363 IMP 1 (Lido/Epi-NaHCO₃ = 3:1)

364 IMP 2 (Lido/Epi-NaHCO₃ = 9:1)

365 IMP 3 (Lido/Epi without NaHCO₃)

366 IMP 4 (NaCl 0.9%)

367

368 **Figure 2: Preparation, labelling and packaging of IMP 1-4**

369 Investigational medicinal products 1-4 were prepared, labelled and packed according
370 to a randomization list (double-blinded to study participant and physician)

371

372 **Figure 3: Quantitative rating of pain during infiltration, IMP 1 vs IMP 2**

373 Group 1 (n=24), primary end point. The volunteers of group 1 compared IMP 1 vs

374 IMP 2 in a randomized sequence (block randomization, 4 blocks of 6 volunteers):

375 IMP 1 was significantly less painful during infiltration than IMP 2 (p=0.044).

376 When IMP1 was followed by IMP2, IMP1 had a median pain score 1.5 points lower

377 (less painful) than IMP2. When IMP 2 was followed by IMP 1, the IMP 1 median pain

378 score was 0.5 points lower than for IMP2.

379 IMP 1: Lido/Epi : NaHCO₃ = 3:1. IMP 2: Lido/Epi : NaHCO₃ = 9:1.

380 Box plots: The line in the box corresponds to the median, the lower and upper hinges

381 correspond to the first and third quartiles, the upper/lower whisker extends from the hinge to

382 the largest/smallest value no further than 1.5 * IQR from the hinge. Dots show each of the 24

383 NRS score points.

384

385

386

387 **Figure 4: Quantitative rating of pain during infiltration, IMP 1 vs IMP3 and IMP4,**
388 **IMP2 vs IMP3 and IMP 4**

389 Group 2 (n=24), secondary end point. The volunteers of group 2 compared IMP 1 vs
390 IMP3 and IMP4, and IMP 2 vs IMP3 and IMP4 in a randomized sequence (block
391 randomization, 1 block of 24 different combinations):

392 Statistical significance:

393 IMP 1 vs IMP 3 (p = 0.001)

394 IMP 2 vs IMP 3 (p = 0.033)

395 IMP 1 vs IMP 4 (p = 0.001)

396 IMP 2 vs IMP 4 (p = 0.001)

397 IMP 3 vs IMP 4 (p = 0.016)

398

399 Group 2: IMP 1: Lido/Epi : NaHCO₃ = 3:1; IMP 2: Lido/Epi : NaHCO₃ = 9:1; IMP 3: Lido/Epi without
400 NaHCO₃; IMP 4: NaCl 0.9% (placebo)

401 Box plots: The line in the box corresponds to the median, the lower and upper hinges correspond to
402 the first and third quartiles, the upper/lower whisker extends from the hinge to the largest/smallest
403 value no further than 1.5 * interquartile ranges from the hinge. Dots show each of the 24 NRS data.

404

405 **Figure 5:** Qualitative rating of patient comfort during infiltration of local anesthetics,
406 captured with categorical terms. The first two bars represent results from group 1,
407 while the next four bars represent results from group 2.

408 IMP 1 (Lido/Epi : NaHCO₃ = 3:1)

409 IMP 2 (Lido/Epi : NaHCO₃ = 9:1)

410 IMP 3 (Lido/Epi without NaHCO₃)

411 IMP 4 (NaCl 0.9%)

412 **Abbreviation and acronym list**

413	IMP:	Investigational Medicinal Product
414	GMP:	Good Manufacturing Production
415	NRS:	Numerical Rating Scale
416	PhEur:	European Pharmacopoeia
417	NaCl:	Sodium chloride
418	NaHCO ₃	Sodium hydrogen carbonate
419		(synonymous: bicarbonate)
420	Lido/Epi	Lidocaine 1%, Epinephrine 1:100'000

421 **Key Words**

422 Local anesthesia, lidocaine, epinephrine, sodium hydrogen carbonate, bicarbonate,
423 admixture, ratio, 3:1, unbuffered, placebo, burning sensation, double-blind, randomized,
424 controlled, trial

Journal Pre-proof

48 volunteers assessed for elegibility

- No exclusions

48 participants consecutively enrolled

Group 1 (n = 24) (block randomization, 4 blocks with n = 6)

Double-blind RCT

to compare IMP1 vs IMP2

12 participants start with IMP 1 and 12 start with IMP 2

Group 2 (n = 24) (block randomization, 1 block with n = 24)

Double-blind RCT

to compare IMP 1 vs IMP 3 and IMP 2 vs IMP 3

to compare IMP 1 vs IMP 4 and IMP 2 vs IMP 4

to compare IMP 3 vs IMP 4

IMPs 1-4 in random order (24 possible combinations)

Group 1

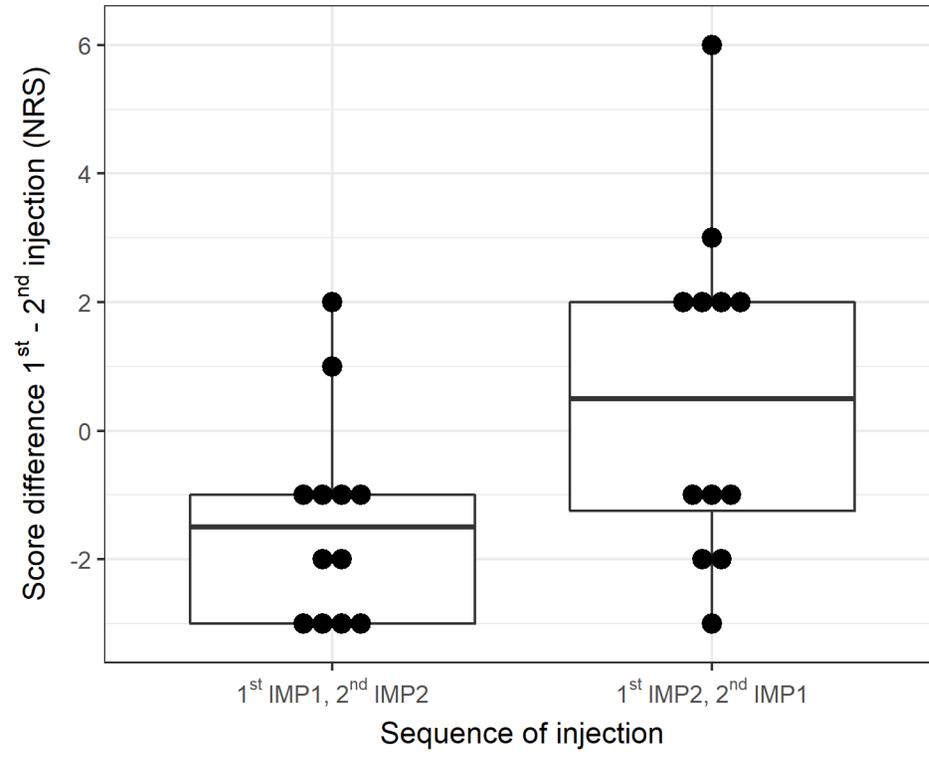
<p>IMP 1 1 vial (5ml) with Lido/Epi 15mg/ml, 15µg/ml 1 vial (5ml) with NaHCO₃ 42mg/ml > mixed, resulting in 10ml Lido/Epi-NaHCO₃ = 3:1 (pH 7.5) containing Lido 7.5mg/ml, NaHCO₃ 21mg/ml</p>	set
<p>IMP 2 1 vial (5ml) with Lido/Epi 18mg/ml, 18µg/ml 1 vial (5ml) with NaHCO₃ 16.8mg/ml > mixed, resulting in 10ml Lido/Epi-NaHCO₃ = 9:1 (pH 7.3) containing Lido 9mg/ml, NaHCO₃ 8.4mg/ml</p>	set
kit	

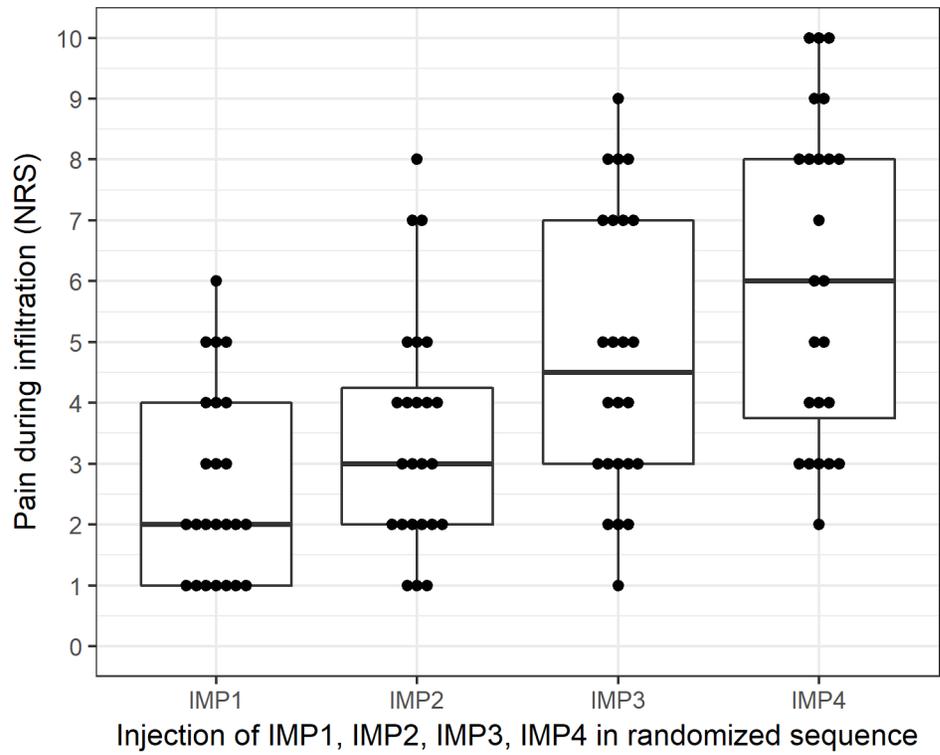
<p>IMP 1 vs IMP 2</p> <ul style="list-style-type: none"> • n = 24, block randomization in 4 blocks (n = 6) • Each participant receives 1 encoded kit containing 2 encoded sets, each set containing 2 blinded vials • Infiltration of 2ml IMP per site (8ml discarded) • 12 participants start with IMP 1 and 12 with IMP 2

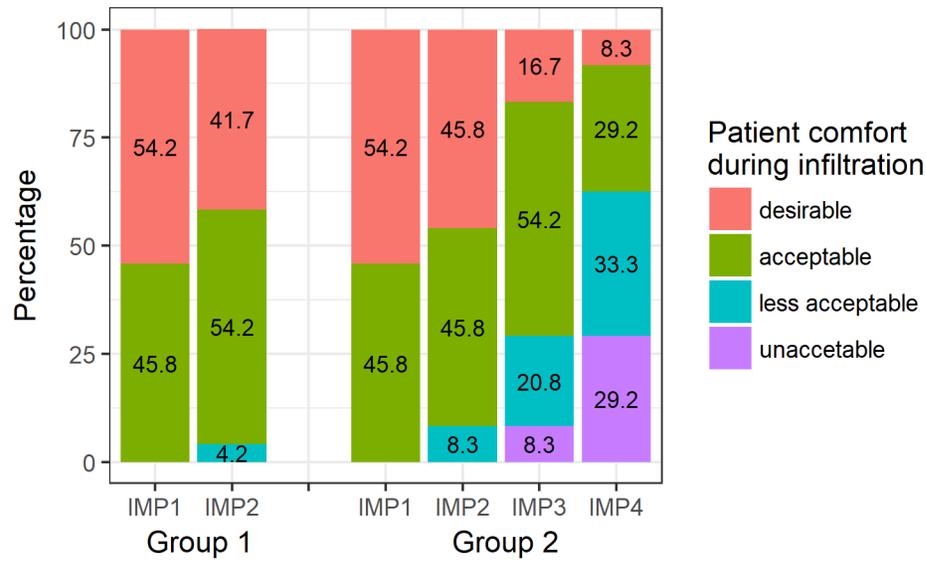
Group 2

<p>IMP 1 1 vial (5ml) with Lido/Epi 15mg/ml, 15µg/ml 1 vial (5ml) with NaHCO₃ 42mg/ml > mixed, resulting in 10ml Lido/Epi-NaHCO₃ = 3:1 (pH 7.5) containing Lido 7.5mg/ml, NaHCO₃ 21mg/ml</p>	set
<p>IMP 2 1 vial (5ml) with Lido/Epi 18mg/ml, 18µg/ml 1 vial (5ml) with NaHCO₃ 16.8mg/ml > mixed, resulting in 10ml Lido/Epi-NaHCO₃ = 9:1 (pH 7.3) containing Lido 9mg/ml, NaHCO₃ 8.4mg/ml</p>	set
<p>IMP 3 1 vial (5ml) with Lido/Epi 10mg/ml, 10µg/ml 1 vial (5ml) with Lido/Epi 10mg/ml, 10µg/ml > mixed, resulting in 10ml Lido (pH 3.4) containing Lido 10mg/ml</p>	set
<p>IMP 4 (placebo) 1 vial (5ml) with NaCl 0.9% 1 vial (5ml) with NaCl 0.9% > mixed, resulting in 10ml NaCl 0.9% (pH 6.2) containing NaCl 0.9mg/ml</p>	set
kit	

<p>IMP 1 vs IMP 2 vs IMP 3 vs IMP 4 (placebo)</p> <ul style="list-style-type: none"> • n = 24, block randomization in 1 block (n = 24) • Each participant receives 1 encoded kit containing 4 encoded sets, each set contains 2 blinded vials • Each participant receives the 4 IMPs in a different order (24 possibilities). • Infiltration of 2ml IMP per site (3cm wheal) (8ml discarded)







Capsule Summary

- Admixture of sodium hydrogen carbonate significantly reduces the strong burning sensation during infiltration of lidocaine 1% with epinephrine (1:100000). The recommended ratios vary from 5:1 to 10:1.
- This double-blind, randomized, placebo-controlled, crossover trial demonstrates superiority of a 3:1 mixing ratio over 9:1 or unbuffered lidocaine.

Journal Pre-proof