ORIGINAL RESEARCH

Impact of Temperature on Injection-Related Pain Caused by Subcutaneous Administration of Ustekinumab: A Three-Arm Open-Label Randomized Controlled Trial

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ABSTRACT

Background: Adherence to subcutaneous biologic agents for the treatment of psoriasis can be negatively influenced by injection pain.

Objective: To explore the differences in injection site pain when patients are pre-treated with heat or cold, versus no pre-treatment prior to administration of a subcutaneous biologic agent.

Methods*:* In an observational cohort study, patients receiving subcutaneous injections of ustekinumab were randomly assigned to receive pretreatment with ice, heat, or no intervention over three visits. Post-dose, patients rated pain on a 100 mm visual analogue scale (VAS).

Results*:* There was an overall increase in the VAS score for both heat (2.51, P=0.30) and ice (3.33, P=0.16), compared to no intervention. No differences were found between the two intervention groups (-0.83, P=0.73). On average, females had the same VAS scores with ice compared to that of no intervention (-0.12, P=0.97) and a non–significant decrease of 3.29 points (P=0.38) with heat. Males had increased pain scores by 5.65 points (P=0.07) with ice and by 6.39 points (P=0.04) with heat.

Limitations*:* Pain is a subjective measurement and objective quantification is difficult.

Conclusions*:* On average, neither heat nor cold application reliably reduced pain. Our results do not support the application of heat or cold prior to ustekinumab injection.

INTRODUCTION

Our improved understanding of the immune pathways involved in various skin diseases has allowed us to use more targeted therapeutics such as biologic agents. However, the long-term efficacy of these medications is directly dependent upon the degree of patient compliance and medication adherence.[1] While usually well-tolerated, most biologic agents are injectable drugs. The subcutaneous injections can be painful, and fear of the needle can be a real issue, and may even prevent some patients from undergoing treatment with a biologic agent.[2] Adherence thus is greatly influenced by fear of needles, as well as pain from injection administration.[2] In fact, previous studies have noted that injection-related pain and discomfort alone can lead to discontinuation of medications.[3] In a recent population-based multinational assessment of psoriasis and psoriatic arthritis, anxiety and fear related to injections and side effects were the most common reason for finding injectable biologics burdensome, with 10% of patients reporting pain and discomfort caused.[4] Therefore, reduction in injection-site pain could potentially result in improved adherence with better outcomes and patient satisfaction. Methods of reducing pain from injections are thus appealing to both patients and practitioners.

Various methods can be used prior to needle injection to ease the discomfort. The use of icing or cooling the skin is the most commonly reported intervention to reduce pain, and is being used prior to various interventions, such as physical therapy, laser treatment, and minor surgical procedures. The use of skin cooling has been reported to be successful in reducing pain from various injections, such as goserelin,[5] local anesthesia, botulinum toxin, and intralesional steroid injection,[6-10] local anesthesia injections during dental procedures,[11] and heparin injections.[12] Other reported methods include vibration anesthesia.[13-14] Warming the injected product has been reported, especially with local anesthetics.[15-17]

Pain is a subjective experience and can be difficult to measure. Various methods have been used in experimental studies, with the visual analogue scale (VAS) being the preferred approach.[18-19] The VAS is a commonly used outcome to assess the degree of pain a patient experiences.[18-20] A horizontal line measuring 100 millimeters is used to represent the degree of pain. The left end of the line represents “no pain at all”, while the right end represents “worst pain imaginable”. In clinical studies, the patient documents pain intensity by depicting a vertical line that bisects the scale. The VAS is a well-validated scale for characterizing the degree of pain associated with a specific intervention.[18+20]

Here we aimed to investigate whether the application of either heat or cold prior to an injection would be associated with lower pain scores, when compared to no pre-treatment. As the conditions requiring biologic use are chronic illnesses with negative impact on health-related quality of life, it becomes imperative to define ways to reduce administration discomfort and increase compliance. More specifically, we aimed to use the VAS to assess whether pre-treatment with heat or cold would reduce injection site pain, versus no pre-injection intervention at all prior to administration of a subcutaneous biologic agent.

METHODS

Study design

In this three-arm crossover open-label randomized controlled trial, we enrolled adults receiving treatment with the subcutaneous biologic agent ustekinumab at the Mount Sinai Medical Centre in New York, NY. Prior to beginning this study, approval for all study related documents was obtained from the Mount Sinai School of Medicine Program for the Protection of Human Subjects (Institutional Review Board). Patient demographics, medical history, and treatments were obtained through medical records. Patients provided written informed consent. To be eligible for the study, patients were required to be receiving treatment with a subcutaneous biologic agent. Our initial aim was to include patients receiving any of the approved subcutaneous biologics. However, contrary to the other agents, which are self-administered at home, ustekinumab is administered in the office, and it was therefore easier for us to recruit patients on ustekinumab. Exclusion criteria were cold-, heat-, or pressure-induced urticaria, and analgesic use within 12 hours of their injection. Our aim was to enroll a maximum of 200 subjects, to allow for a ten percent attrition rate, with a target of 100 patients completing the study.

Each patient required three study visits. Patients were randomly assigned the order of intervention received (1/3 received ice first, 1/3 received heat first, 1/3 received no intervention first; the same randomization ratio applied for the second and third injections). Randomization was performed using the second generator from www.randomization.com, where each subject must receive all the treatments in random order. The first patient was enrolled in the study in May 2014, while there was a delay in the trial registration on the clinicaltrials.gov website, which occurred in June 2014. Only the first visit of the first study subject occurred prior to study registration (Figure 1).

Patients then either received no pre-injection intervention, pre-injection intervention with ice, or pre-injection intervention with heating packs prior to administration of their subcutaneous biologic therapy. Immediately post-dose, patients were asked to rate pain associated with the injection using a 100 mm VAS pain score, which was described from 0 mm (no pain at all) to 100 mm (worst pain imaginable). This randomization aimed to reduce error in pain score measurement that may result if the order of pre-injection intervention was always the same (e.g. no intervention always first). Interval level data were obtained by measuring the distance from the low end of each scale to the subject's marked VAS. Pre-injection intervention included an ice pack covered by a disposable paper drape and placed against the skin for 2-3 minutes prior to injection, a reusable heating pack that was heated in the microwave for 45 seconds, covered by a disposable paper drape, and then applied to the skin for 2-3 minutes, or no pre-treatment prior to the injection. Two investigators administered the drug injections, while one patient requested on injecting himself.

Endpoints

The primary endpoint for this study was the difference in the pain Visual Analogue Scale (VAS) scores between heat and cold pre-treatment as compared to no pre-treatment prior to ustekinumab injection. We hypothesized that pre-treatment with either heat or cold would reduce the pain scores when compared to no pre-treatment prior to ustekinumab injection.

Statistical analysis

Sample size: As there were no preliminary data to base the sample size calculations on, the sample size was determined based on enrolment feasibility in our department. A sample size of 100 patients would have been adequate to detect moderated effect sizes (ES= 0.28), with >80% power on a 2-sided paired t-test.

Descriptive analysis was carried out to characterize the cohort in terms of demographic information. Mean and standard deviation (SD) were calculated for continuous variables, along with median and interquartile range (IQR). Count and percentages were calculated for categorical outcomes. One-way analysis of variance (ANOVA) was used for the mean difference of age and VAS scores across the three different intervention groups. Chi-square tests were conducted to test the association between the categorical variables (gender and injector) and the intervention group, respectively.

The primary analysis was to assess the efficacy of the pre-injection intervention (ice or heat) effect. A linear mixed-effect model was applied to take fixed effects (any potential covariates) and random effects (subject) into account, while also taking into account the correlation of different measurements for the same patient. The mixed model we applied is more advanced and adequate than other approaches (such as t test or ANOVA) in this study since it can take three measurements into account and use as much information as possible in the presence of missing values. Based on the Likelihood ratio test and the Akaike Information criteria, the final model was fitted with an unstructured correlation and assuming homoscedastic within-group errors. According to the protocol, we primarily assessed the model with only the intervention group as the fixed effect. In an unplanned analysis,, we included all potential covariates (age, gender, time, injector) and their interaction with intervention in a multivariate linear mixed model and performed a step-wise backward selection algorithm to define the final model. Interaction was also considered if appropriate. The final model identified by the backward selection algorithm included intervention, gender and the gender-intervention interaction. No missing values were imputed in this analysis and all available data was used.

RESULTS

Patient characteristics

A total of 118 patients currently on treatment were enrolled in the study, with 107 completing all three injections, ten patients receiving two injections, and one patient receiving only one injection (Figure 1). The majority of these patients were receiving treatment for psoriasis, with a small number being treated for other skin disease. All available data were included in the primary analysis. The average age was 51.16 years (range 22-86, SD=16.37), and 41% were female. For this cohort, pain VAS was 18.7 (SD=20.8) 107 patients received all three

injections, 10 patients received two, and one patient received only one injection. One patient moved away, another stopped the drug due to poor efficacy, and the remaining 9 dropped out of the study due to scheduling difficulties. Site of injection was the upper arm. The box plot indicated similarities of VAS scores in different intervention groups, in terms of their median and IQR (Figure 2A).

Safety Endpoints

In terms of safety and side effects related to ice or heating packs, none of the patients reported any clinically significant side effects.

Efficacy Endpoints

The primary efficacy endpoint for this study was the VAS score. To assess the effect of the intervention on the VAS score, a linear mixed-effect model was fitted considering treatment as fixed effect and a random intercept for each patient (Figure 2B). Results, summarized in Table 1 showed an overall increase in the VAS score for both heat (2.51, P=0.30) and ice (3.33, P=0.16) intervention. No differences were found between the two intervention groups (-0.83, P=0.73). To study the effect of the recorded variables in the analysis, we modelled the VAS score including all the potential covariates and their interactions with the interventions in the model as fixed effects. Model selection used a stepwise backward selection, with only intervention and gender (and its interaction) remaining in the model (Table 2 and Figure 2D). The distribution of the VAS scores by gender (Figure 2C) indicated that female patients had twice the pain scores than males even with no intervention (24.05 vs. 11.85, P=0.002).

Our model indicated that on average, female patients had the same VAS scores when treated with ice compared to that of those with no intervention (-0.12, P=0.97), and a small, non–significant decrease of 3.29 points in the pain scores was observed (P=0.38) while applying heat pre-injection. On the contrary, male patients increased their pain scores by 5.65 points (P=0.07) with ice and by 6.39 points (P=0.04) with heat. Of note, no significant differences between the effects of ice and heat were found in either gender group.

We also compared the proportion of patients that decrease, have no change or increase the pain score in the pre-treatment group from non-intervention (Table 3). Results largely agree with the analysis of continuous VAS scores with a larger percentage of females reporting a decrease in pain scores than males (42.86% in females vs. 36.92% in males for ice, and 45.24% in females vs. 33.85% in males for heat). These associations were not statistically significant (P=0.805 and P=0.342 for ice and heat, respectively).

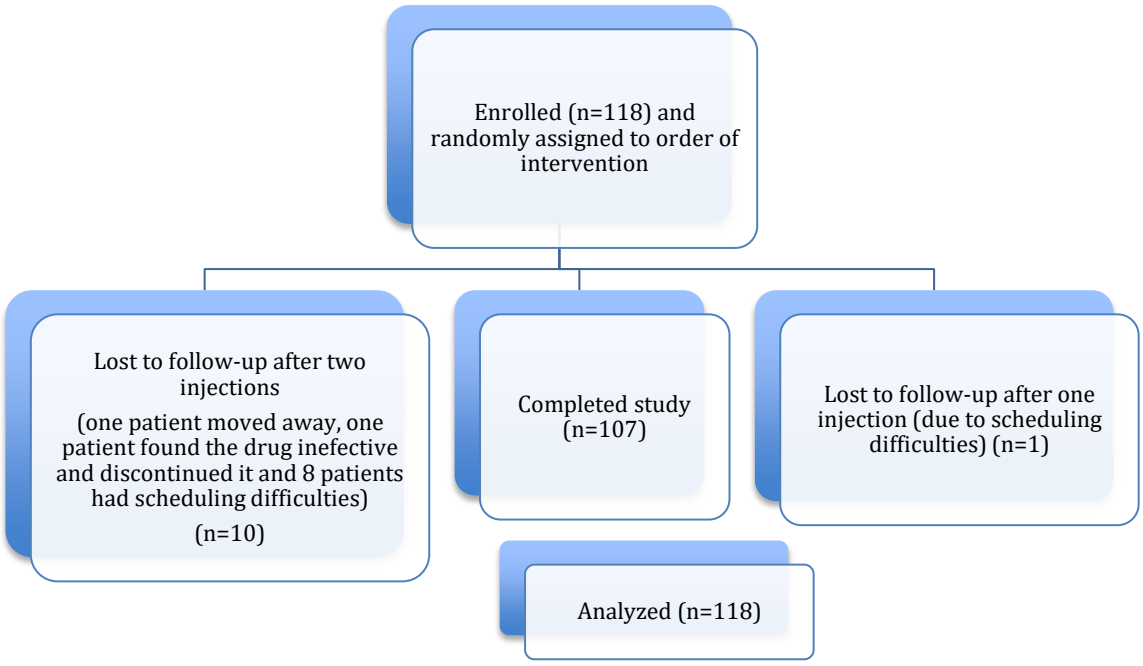

Figure 1: Subject flow diagram

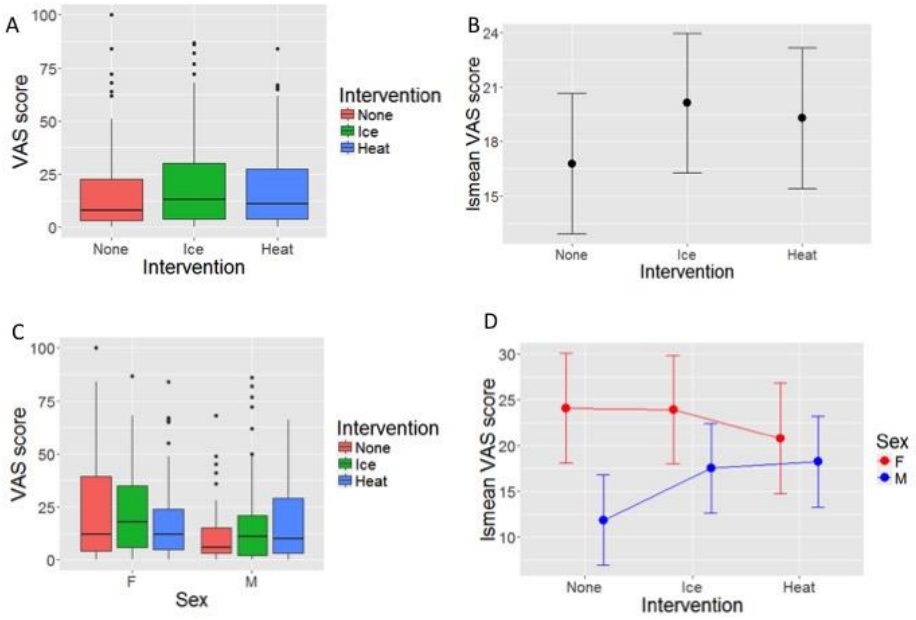

Figure 2: Pain perception with subcutaneous injection of Ustekinumab by intervention first randomized by (A,B), and stratified by gender (C,D)

Table 1. Primary Efficacy Analysis for the VAS score

Intervention	LS-mean (Estimated mean)	SEM	Lower CI	Upper CI	P-value*
None	16.78	1.95	12.9	20.6	-
Ice	20.12	1.93	16.3	23.9	0.16
Heat	19.29	1.96	15.4	23.2	0.30
Treatment Effect					
Intervention	LS-mean (Estimated mean)	SEM	Lower CI	Upper CI	P-value*
Heat-None	2.51	2.40	-2.2	7.2	0.30
Ice-None	3.33	2.38	-1.4	8.0	0.16
Heat-Ice	-0.83	2.39	-5.5	3.9	0.73

Notes:
* P-values comparing the mean for each group with the reference group (Female, None)
# P-values for gender effect, ice intervention effect, ice and gender interaction, heat intervention effect, heat and gender interaction.
Estimated Means are the least square means estimated for each contrast, SEM is the standard error of the mean, CI: lower and upper bound for the 95% Confidence Interval

Table 2. Model with covariates (selection was based on backward model selection)

Intervention	Sex	Estimated mean	SEM	Lower CI	Upper CI	P-value*	
None	F	24.05	3.02	18.07	30.03	-	
None	M	11.85	2.49	6.93	16.78	0.002	
Ice	F	23.93	2.99	18.01	29.86	0.974	
Ice	M	17.51	2.47	12.62	22.40	0.232	
Heat	F	20.77	3.05	14.73	26.81	0.385	
Heat	M	18.25	2.50	13.29	23.20	0.048	
Treatment Effect							
Treatment Effect	Sex	Estimated mean	SEM	Lower CI	Upper CI	P-value	P-value#
Ice - None	F	-0.12	3.72	-7.46	7.22	0.97	
Ice - None	M	5.65	3.07	-0.39	11.70	0.07	0.232
Heat - None	F	-3.29	3.77	-10.72	4.15	0.38	
Heat - None	M	6.39	3.09	0.31	12.48	0.04	0.048

Notes:
* P-values comparing the mean for each group with the reference group (Female, None)
§ P-values for comparing the difference between the sex in the change of VAS score for Ice and Heat intervention group.
# P-values for gender effect, ice intervention effect, ice and gender interaction, heat intervention effect, heat and gender interaction.
Estimated Means are the least square means estimated for each contrast, SEM is the standard error of the mean, CI: lower and upper bound for the 95% Confidence Interval

Table 3. Contingency tables for Change in VAS score by gender in Ice and Heat group

Table of VAS Score change by sex

Ice	Sex			Heat	Sex		
%column	F	M	Total	%column	F	M	Total
Increase	21 (50%)	35 (53.85%)	56	Increase	19 (45.24%)	39 60.00	58
No change	3 (7.14%)	6 (9.23%)	9	No change	4 (9.52%)	4 6.15	8
Decrease	18 (42.86%)	24 (36.92%)	42	Decrease	19 (45.24%)	22 33.85	41
Total	42	65	107	Total	42	65	107
Fisher's exact			P=0.805	Fisher's exact			P=0.342

Note: Fisher's exact test is used to assess the association of VAS score change and gender in ice and heat intervention.

DISCUSSION/CONCLUSIONS

A variety of studies in the literature report successful pain reduction by skin cooling. Our study demonstrated reduced pain scores in women after warming the skin prior to injection of biologic agents, although these findings were not statistically significant. We found that gender was an important factor in patients' perception of pain during the injection of subcutaneous biologic agents. Interestingly, there was an opposite effect in the pain perception amongst males (Figure 2D), who reported a pain increase after application of heat packs (95% CI: 0.403-12.281; P=0.036), as well after application of ice packs (95% CI: -0.66-11.136, P=0.081). This may be either due to a lower pain threshold in females, or an under-reporting of pain in male subjects.

While numerous studies have shown that pain perception, pain thresholds, the prevalence of chronic pain conditions, and response to analgesia differ amongst the genders,[21-27] these previous mentioned studies did not report such a marked difference between the sexes in pain perception after interventions to reduce injection-related pain. It is possible that the researchers did not take the subjects' gender into account when analyzing the results. Studies of cold-evoked pain found cold thresholds to be lower in hairy skin compared to glabrous skin,[25] which may be explained by anatomical differences between the skin sites such as increased thickness of the epidermis and decreased density of cold receptors in glabrous skin. This would, however, not explain the contrast in pain perception noted between men and women in our study. Furthermore, Kennard *et al*. failed to show an effect of skin thickness between men and women in pain thresholds.[26] A meta-analysis of gender differences in mechanically induced pain demonstrated lower pain thresholds and

lower tolerance to pain in women, with the largest differences seen in pressure-pain and electrical stimulation.[27] It has been suggested that the social norms may influence men into appearing more stoic and under-reporting,[28] especially in the presence of female researchers,[29] as was the case in our study. However, contradictory evidence has also been shown, such as a study that demonstrated lower pain thresholds in females following noxious heat stimuli, independent of the gender of the experimenter,[30] as well as a study that measured pupil diameter in response to pain, which is caused by sympathetic stimulation and is therefore not under conscious control by the patient. The results showed similar response in pupil dilation in both genders.[31] These conflicting reports highlight the complexity of pain sensitivity and make interpretation difficult. One possible explanation for the lack of pain reduction following heat and cold application in our study, as compared to previous reports, may be the different drug composition of biologics.

The decision whether or not to offer a patient a method of pain reduction prior to the injection of subcutaneous biologics therefore needs to be made by the respective clinician in every particular patient, while weighing the pros and cons of the different available methods. In particular, the gender of the patient needs to be taken into consideration, as this can greatly affect pain perception. While the application of both heat and ice packs is simple, inexpensive and safe, on average, neither heat nor cold application reliably reduced pain. We also could not identify which patients would be more likely to respond to heat or cold, except that female patients would more likely experience pain reduction with heat compared to males, while males experienced increased pain after heat application. Further research is required into pain caused by injectable therapeutics and pain reduction methods, while taking into account possible gender differences in pain perception, and the drug composition and properties.

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References:

1. Bluett J, Morgan C, Thurston L, *et al.* Impact of inadequate adherence on response to subcutaneously administered anti-tumour necrosis factor drugs: results from the Biologics in Rheumatoid Arthritis Genetics and Genomics Study Syndicate cohort. *Rheumatology (Oxford)*. 2015; 54:494-9. doi: 10.1093/rheumatology/keu358.

2. Betegnie AL, Gauchet A, Lehmann A, *et al.* Why do patients with chronic inflammatory rheumatic diseases discontinue their biologics? An assessment

of patients' adherence using a self-report questionnaire. *J Rheumatol*. 2016; 43:724-30. doi: 10.3899/jrheum.150414.

3. Bolge SC, Goren A, Tandon N. Reasons for discontinuation of subcutaneous biologic therapy in the treatment of rheumatoid arthritis: a patient perspective. *Patient Prefer and Adherence.* 2015; 9:121–31. doi: 10.2147/PPA.S70834.

4. Lebwohl MG, Kavanaugh A, Armstrong AW, Van Voorhees AS. US perspectives in the management of psoriasis and psoriatic arthritis: Patient and physician results from the population-based multinational assessment of psoriasis and psoriatic arthritis (MAPP) survey. *Am J Clin Dermatol.* 2016; 17:87-97. doi: 10.1007/s40257-015-0169-x.

5. Kinoshita H, Kawa G, Hiura Y, *et al*. Effectiveness of skin icing in reducing pain associated with goserelin acetate injection. *Int J Clin Oncol.* 2010; 15:472-5. doi: 10.1007/s10147-010-0095-0.

6. Al-Qarqaz F, Al-Aboosi M, Al-shiyab D, *et al*. Using cold air for reducing needle-injection pain. *Int J Dermatol.* 2012; 51:848-52. doi: 10.1111/j.1365-4632.2011.05383.x.

7. Skiveren J1, Kjaerby E, Nordahl Larsen H. Cooling by frozen gel pack as pain relief during treatment of axillary hyperhidrosis with botulinum toxin A injections. *Acta Derm Venereol.* 2008; 88:366-9. doi: 10.2340/00015555-0465.

8. Leff DR, Nortley M, Dang V, *et al*. The effect of local cooling on pain perception during infiltration of local anaesthetic agents, a prospective randomised controlled trial. *Anaesthesia.* 2007; 62:677-82.

9. Kuwahara RT, Skinner RB. Emla versus ice as a topical anaesthetic. *Dermatol Surg.* 2001; 27:495-6.

10. Bechara FG, Sand M, Altmeyer P, *et al*. Skin cooling for botulinum toxin A injection in patients with focal axillary hyperhidrosis: a prospective, randomised, controlled study. *Ann Plast Surg*. 2007;58(3):299-302.

11. Davoudi A, Rismanchian M, Akhavan A, *et al*. A brief review on the efficacy of different possible and nonpharmacological techniques in eliminating discomfort of local anaesthesia injection during dental procedures. *Anesth Essays Res*. 2016; 10:13-6. doi: 10.4103/0259-1162.167846.

12. Kuzu N, Ucar H. The effect of cold on the occurrence of bruising, haematoma and pain at the injection site in subcutaneous low molecular weight heparin. *Int J Nurs Stud.* 2001; 38:51-9.

13. Smith KC, Comite SL, Balasubramanian S, *et al*. Vibration anaesthesia: a noninvasive method of reducing discomfort prior to dermatologic procedures. *Dermatol Online J.* 2004; 10:1.

14. Mally P, Czyz CN, Chan NJ, *et al*. Vibration anaesthesia for the reduction of pain with facial dermal filler injections. *Aesthetic Plast Surg*. 2014; 38:413-8. doi: 10.1007/s00266-013-0264-4.

15. Hogan ME, vanderVaart S, Perampaladas K, *et al*. Systematic review and meta-analysis of the effect of warming local anaesthetics on injection pain. *Ann Emerg Med*. 2011; 58:86-98.e1. doi: 10.1016/j.annemergmed. 2010.12.001.

16. Mader TJ, Playe SJ, Garb JL. Reducing the pain of local anaesthetic infiltration: warming and buffering have a synergistic effect. *Ann Emerg Med.* 1994; 23:550-4.

17. Colaric KB, Overton DT, Moore K. Pain reduction in lidocaine administration through buffering and warming. *Am J Emerg Med*. 1998; 16:353-6.

18. Scott J, Huskisson EC. Graphic representation of pain. *Pain*. 1976; 2:175-84.

19. Ohnhaus EE, Adler R. Methodological problems in the measurement of pain: a comparison between the verbal rating scale and the visual analogue scale. *Pain.* 1975; 1:379-84.

20. Price DD, Bush FM, Long S, Harkins SW. A comparison of pain measurement characteristics of visual analogue and simple numerical rating scales. *Pain.* 1994; 56:217-26.

21. Maurer AJ, Lissounov A, Knezevic I, *et al*. Pain and sex hormones: a review of current understanding. *Pain Manag*. 2016; 6:285-96. doi: 10.2217/pmt-2015-0002.

22. Fillingim RB, King CD, Ribeiro-Dasilva MC, *et al*. Sex, gender, and pain: a review of recent clinical and experimental findings. *J Pain*. 2009; 10:447-85. doi: 10.1016/j.jpain.2008.12.001.

23. Manson JE. Pain: sex differences and implications for treatment. *Metabolism.* 2010; 59 Suppl 1:S16-20. doi: 10.1016/j.metabol.2010.07.013.

24. Craft RM, Mogil JS, Aloisi AM. Sex differences in pain and analgesia: the role of gonadal hormones. *Eur J Pain*. 2004; 8:397-411.

25. Harrison JL, Davis KD. Cold-evoked pain varies with skin type and cooling rate: a psychophysical study in humans. *Pain.* 1999; 83:123-35.

26. Kennard MA. The responses to painful stimuli of patients with severe chronic painful conditions. *J Clin Invest.* 1952; 31:245-52.

27. Riley JL 3rd, Robinson ME, Wise EA, *et al*. Sex differences in the perception of noxious experimental stimuli: a meta-analysis. *Pain.* 1998; 74:181-7.

28. Otto MW, Dougher MJ. Sex differences and personality factors in responsivity to pain. *Percept Mot Skills.* 1985; 61:383-90.

29. Levine FM, De Simone LL. The effects of experimenter gender on pain report in male and female subjects. *Pain.* 1991; 44:69-72.

30. Feine JS, Bushnell MC, Miron D, *et al*. Sex differences in the perception of noxious heat stimuli. *Pain.* 1991;44:255-62.

31. Bertrand AL, Garcia JB, Viera EB, *et al*. Pupillometry: the influence of gender and anxiety on the pain response. *Pain Physician.* 2013; 16:E257-66.