Frontal infrared thermography in healthy individuals and chronic migraine patients: reliability of the method

Journal:	*Cephalalgia*
Manuscript ID	CHA-00474-OA-2017.R2
Manuscript Type:	Original Article
Date Submitted by the Author:	n/a
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Key Words:	Frontal thermography, chronic migraine, cold patch, standard procedure, reliability

Frontal infrared thermography in healthy individuals and chronic migraine patients: reliability of the method

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Keywords

Frontal Thermography (FIT); Chronic Migraine; Cold Patch; Standard Procedure; Reliability

Abbreviations

AI – Asymmetry Index

ANOVA – analysis of variance

BMS - between-subjects mean square

CV - Coefficient of Variation

EMS - residual mean square

FIT – Frontal Infrared Thermography

ICC - intra-class correlation coefficient

JMS - within-subjects mean square

tDCS - transcranial direct current stimulation

VAS - Visual Analogue Scale

Abstract

Background: The use of Frontal infrared thermography in the diagnosis of primary headaches provided scattering results due to measurement fluctuations and different types of headaches or research protocols.

Objective: This study aims to assess the reliability of Frontal infrared thermography in healthy individuals and provide a preliminary evaluation in chronic migraine patients using a commercial infrared thermal camera.

Methods: Thermographic images were acquired in 20 controls and 15 patients at 3 consecutive time-points in two daily sessions. The Side Difference and Asymmetry Index parameters were defined. The reproducibility of the measurements, the correlation of Asymmetry Index and Side Difference with clinical evaluations and patient perceptions, and the ability of the parameters to discriminate between patients and controls were investigated.

Results: We reported a good reproducibility of the measurements (Inter-class Correlation Coefficient>0.75 and Coefficient of Variation<13.4%), independent from external factors. The Side Difference was significantly different between patients and controls ($p<0.001$). The Asymmetry Index showed good correlation with the side of unilateral pain ($p=0.0056$).

Conclusions: Frontal infrared thermography can be used to quantify the difference between the right and the left side of frontal vascular changes in chronic migraine patients, provided that standardized conditions are satisfied.

Introduction

Infrared Thermography detects infrared light emitted by the human body to visualize changes in heat due to abnormalities in the skin surface blood flow of diseased areas. This non-invasive and non-radiative imaging technique has different clinical applications including the detection of circulatory and/or inflammatory disorders such as rheumatoid arthritis, Raynaud's disease or osteoarthritis of the knee[1]. It was also demonstrated that thermography is able to capture thermal gradient in facial areas characterizing healthy individuals[2].

However, the literature investigating the use of external carotid region (forehead) thermal imaging for the characterization of vascular headaches did not reach consistent results due to different types of headache evaluated, different timing of patients' evaluation (during headache attack or in headache free interval), different technologies used for image acquisition, and different methods for imaging or statistical analysis.

In 1986, Swerdlow and Dieter[3] comparing electronic thermography between 275 headache patients and 45 controls, defined the "Cold Patch" as a region of the face where the temperature is cooler (less than 0.5°C) than the surrounding areas[3,4]. The presence of the cold patch is more frequent in vascular headaches than in healthy subjects or patients with tension type headache, psychogenic headaches or post-traumatic headaches.

There is an open question on whether the cold patch is a "fixed" entity[5] or whether it decreases with therapy[6,7]. In fact, whereas according to Swerdlow and Dieter, "the vascular cold patch is independent of prognosis and is most likely a permanent element of a vascular headache sufferer's facial thermal pattern"[5], in 1991, Dalla Volta et al. [7] suggested that the "cold patch" in vascular headache patients is ipsilateral to the prevailing side of pain and that the cooler area decreased after 6 months of prophylactic therapy. The differences observed by the two groups may be explained in terms of experimental protocol and patient's selection that introduced a higher variability in the cold patch response [7].

Anyway, there is large consensus on the fact that the cold patch represents an asymmetry in the forehead of migraine patients. Unfortunately, the location of the cold patch is seldom related to the side of the pain[5] probably due to the variation of temperature during headache attack or due to the lateralization of the headache (unilateral or bilateral) as measured by Drummond and Lance[8,9].

Finally, FIT, in conjunction with nitroglycerine administration, was suggested as a novel non-invasive approach to study vascular processes underlying headaches[10].

Taken together, all these studies demonstrate that FIT can be used as diagnostic tool in migraine with and without aura, in cluster headache and in other headache types.

In the light of previous observations and of new possible therapeutic applications, the present study has two primary end points: (1) to evaluate the reliability of FIT measurements in controls and chronic migraine patients using modern commercial infrared thermal camera; (2) to verify whether FIT-based parameters correlate both with the visual evaluation of FIT by an expert clinician and with patient's perception of pain side (at least in case of unilateral pain).

Materials and methods

Subjects

Thirty five right handed volunteers (26 females and 9 males) with a mean age of 35±11.6 years (range: 20-55) were enrolled at the Headache centre of the Fondazione IRCCS Istituto Neurologico Nazionale Casimiro Mondino. All the subjects were not medicated at the time of testing.

15 of the 35 volunteers (3 males) suffered from chronic migraine with medication overuse, as assessed according to the IHS Classification[11]. They were examined while hospitalized and during a washout period from analgesics or other symptomatic treatment, including non-headache medication. Patients were not on dietary/smoking restrictions ahead of

measurements. Patients did not use preventive treatment and underwent daily parenteral detoxification (saline, cyanocobalamin, folic acid, nicotinammide, glutathione, delorazepam and metoclopramide on demand). Patients were assessed on the second or third day after symptomatic medication withdrawal that started on the day of hospital admission. No symptomatic medication was allowed during the evaluation period but local ice bag (at least one day apart from the examination). Hence, patients were generally with headache during the examination. Pain severity was evaluated before each examination on a 0-3-score Visual Analogue Scale (0: no pain,1: mild pain, 2: moderate pain 3: severe pain) with a mean value of [mean±SE] 1.88 ± 0.078.

The remaining 20 of the 35 volunteers were used as controls since they suffered from migraine or tension type headache no more than 1-2 times a year.

The study was approved by the institutional review board (Ethical Committee of the Fondazione IRCCS Istituto Neurologico Nazionale Casimiro Mondino, date of approval: July 29th 2013) and conformed with the Declaration of Helsinki. All patients signed written informed consent before participating to the study.

Frontal Infrared Thermography

FIT was assessed with a modern infrared thermal camera (model LT3, produced by Zhejiang Dali Technology Co. Ldt) characterized by a thermal sensitivity below 0.08°C at 30°C, according to suggested guidelines [12,13]. FIT measured the spatial distribution of the heat over the human face (object emissivity 0.98): the camera was placed at distance of 1 meter from the subject in a room with stable temperature (23.6 ± 1.57°C). To ensure comparability between patients and controls, since controls do not have a "cold area", the temperature was evaluated in two target points (left and right side) in the frontal polar sites. In patients, we identified the coolest point in the side showing the cold patch, and then we identified its symmetric on the other side of the head (equidistant from the nasion, Fig.1). In controls there

is no cold patch, so we used two symmetric points equidistant from the nasion on a radius of 2cm (Fig.2). This protocol allowed repeatability and comparability among subjects.

Study design

All measurements were taken in two test sessions (T1 and T2) for all subjects. The second session (T2) was run at least one day apart in order to evaluate the intra-subject variations in FIT. During each session (T1 and T2), the measurements were repeated three times (m1, m2, m3) by the same experimenter after 10 minutes of rest between each measurement. Room temperature was recorded during each session. Images where taken approximately at the same time of the day in each patient (10-12 a.m. or 2.00-4.00 p.m.).

Data analysis

Reliability of Frontal Thermography (FIT)

In order to verify whether measurements were influenced by external factors, correlation analysis was computed with respect to room temperature, sex and age of subjects, both at T1 and at T2, using m1, m2, and m3 as dependent variables.

Student's paired t-Test was used to determine whether three measurements belonging to the same session (m1 vs m2, m2 vs m3, m1 vs m3) had the same mean. The Bonferroni correction for repeated measures was applied ($p<0.016$).

Reliability of measurements was investigated using the intra-class correlation coefficient (ICC), defined as the fraction of variance that is caused by the variation between subjects. Thus, if the variance between tests is smaller than the variance between subjects then ICC is close to 1. According to Fleiss, ICC values above 0.75 generally mean “excellent” reliability[14]. The intra-class correlation coefficient (ICC) was calculated for FIT at each point: data during a single session (T1 and T2) were analysed using a Two Factor ANOVA without

Replication (factors: subjects and FIT readings m_i) and the value from the analysis of variance table were substituted into equation (1):

$$ICC\ 2,1 = (BMS - EMS)/(BMS + EMS + 2(JMS-EMS)/n) \quad (1)$$

where BMS is the between-subjects mean square, EMS is the residual mean square, JMS is the within-subjects mean square, n is number of subjects[15].

The reproducibility of the method was tested by calculating the Coefficient of Variation (CV) during the first test session[16,17]. For each comparison between measurements (m1 vs m2, m2 vs m3, m1 vs m3) the coefficient of variation (equation 2) was calculated as the absolute value of:

$$CV = 100 * | (m_i - m_{i+1}) / ((m_i + m_{i+1}) /2) | \quad (2)$$

where m_i is the first reading (test) and m_{i+1} is the second one (retest). The numerator is the difference between two consecutive measurements and the denominator is the average of the two measurements. In this way, the Coefficient of Variation is the percentage difference between two readings. For each subject the coefficient of variation was computed for the three intra-session comparisons and then averaged across subjects.

In order to evaluate the intra-subject variations in FIT, a two-way analysis of variance with replication (2-way ANOVA) was performed for a statistical comparison between two different test sessions (T1 and T2).

Characterization of images comparing controls and patients

In order to verify the difference between migraine FIT measurement and healthy subject FIT measurements, we defined two parameters:

- the Asymmetry Index [14] (equation 3)

$$AI=2 \times ((Tleft-Tright)/(Tleft+Tright)) \quad (3)$$

Where Tleft is the temperature on the left forehead and Tright is the temperature of the right forehead. AI was calculated in order to assess the lateralization of FIT. If the temperature

measurement on the two sides is the same, then the asymmetry index is equal to 0. Positive AI value means that the cold patch is located on the right side of the forehead. Conversely, a negative asymmetry index means a lateralization on the left side.

- the absolute value of percentage difference between left and right side

Side Difference (%)= 100 * | 2 * (Tleft - Tright / Tleft + Tright) | (4)

SideDifference (equation 4) is the difference between left and right side as a percentage of the average temperature in forehead.

Both parameters were calculated from all the measurements of each patients (15*6=90 observations) and controls (20*6=120 observations). A comparison between the two groups was conducted in order to test whether there was a significant difference of AI or Side Difference. In headache patients, the Asymmetry Index was compared with FIT visual inspection by the doctor and with declarations of patient before each session (the side of pain and intensity of pain using VAS scores).

Results

Reliability of frontal infrared thermography

FIT readings did not correlate with external factors during both during T1 and T2 sessions as far as sex (Tright r = 0.20, p = 0.24; Tleft r = 0.23, p = 0.17), room temperature (Tright r = 0.22, p = 0.20; Tleft r = 0.19, p = 0.27) and age (Tright r = -0.017, p= 0.92; Tleft r = 0.035, p = 0.83) is concerned.

During each session, paired t-Test analysis revealed no significant difference between consecutive FIT measurements during the same session (p>0.016) for all the three comparisons (m1 vs m2, m2 vs m3, m1 vs m3) in both the right and left measurement side (Figure 3).

The reliability analysis demonstrated "excellent"[14] results during T1 (ICC values: right side, 0.73; left side, 0.81). The same result was confirmed during T2 (ICC values: right side, 0.80; left side, 0.74).

Regarding the reproducibility, mean CV between consecutive FIT measurements in the right and left side were similar in controls (T1: left side: 1.63% ± 1.79; right side: 1.69% ± 2.09; T2: left side: 2.71% ± 3.26; right side: 2.67% ± 3.22) and in patients (T1: left side: 3.77% ± 3.57; right side: 3.87% ± 3.62; T2: left side: 2.71% ± 3.26; right side: 2.67% ± 3.22). However, in patients, CV range was slightly greater than in controls both in T1 and T2 session (patients: 0.00%-13.40%; controls: 0.00% - 10.04%). In addition, the average smallest CV was observed among m2m3 measurements (m2m3: 1.56% ± 0.62 vs m1m2: 3.13% ± 1.29 and m1m3: 3.54% ± 1.25). Despite this, the overall reproducibility of the measurements was very good because the maximum CV was less than 13.40%.

When examining intra-subject variations between two different days (inter-test), CV represents the percentage difference between two daily sessions (T1 and T2). The maximum CVs between test sessions (T1 and T2) were very low (patients range: 0.19 - 8.24%, controls range: 0.00 - 7.56%) thus suggesting a good reproducibility of the measurements over different days.

The range of temperature was not different between days or between the two groups of subjects (controls: T1: 33.10-36.73°C; T2: 34.13 – 36.70°C; patients: T1: 32.30 – 37.60; T2: 32.30 – 36.17°C). Patients were characterized by a larger standard deviation of temperature than controls (Figure 3). The two-way ANOVA with replication over 35 subjects demonstrated that there were no significant differences between FIT measurements in different days.

Characterization of frontal infrared thermography comparing patients and controls

In controls, the temperature measurements during T1 varied from a minimum of 32.60°C to a maximum value of 38.90°C (mean on the two sides: 35.05°C, standard deviation: 1°C, corresponding to 2.8% of the mean value). In patients with chronic migraine the temperature measurements during T1 varied from a minimum of 31.90°C to a maximum value of 38.30°C with a larger standard deviation from the mean than controls (mean on the two sides 35.47°C, standard deviation 1.59°C, corresponding 4.5% of the mean value).

Whereas average AI was not different ($p>0.05$) between patients (-0.00002 ± 0.0164) and controls (-0.00118 ± 0.0091), the Side Difference, representing the absolute value of the difference between temperature measured in the left and right side, significantly discriminated controls (mean ± std: 0.73% ± 0.55) from patients ([mean ± SD]: 1.37% ± 0.87, P-value one tail<0.0001) (Fig. 4).

Conversely, AI can be used to locate the cold patch in patients. In fact, AI polarity (positive or negative, where positive AI value means that the cold patch is located on the right side of the forehead) was in consort with the visual inspection of the FIT by the examiner (correlation, $r^2=0.7$, $p=0.00072$). In addition, when the patient referred a bilateral pain before the test session, both the visual inspection and the AI index revealed an asymmetry in the forehead, thus suggesting that the AI can be a reliable index for the localization of the coldpatch also when the patient's perception is not reliable.

Finally, in patients with unilateral pain, the correlation between AI and the patient-referred pain side before FIT was good ($r^2=0.6$, $p=0.0056$). Conversely, even though patients were examined mainly during attacks with different pain severities, as measured by VAS, the correlation between AI and pain severity was very low ($r^2=0.2$, $p=0.37$).

Discussion

In this study, we examined the usability and reproducibility of FIT measurement in patients with headache and in healthy subjects, in a research scenario in which previous experience using FIT were not conclusive [1,3,4,6,7,18].

In order to obtain comparable results between patients and controls, we used a thermal punctual evaluation instead of an evaluation by area. In fact, whereas patients showed a cooler area (the cold patch), controls did not, thus making the evaluation by area unreliable and not reproducible. We therefore chose to evaluate the temperature in two symmetric points equidistant from the nasion. In patients, one point was the coolest point in the cold patch, and the second was its symmetric, in controls we took two symmetric points located on a circle of fixed radius. With this setup, our data showed that FIT is a reproducible tool provided that standard location and standard measurement procedure is carried out. In fact, we found good reproducibility of the measurements within the same session and between the two sessions, as measured by CV. However, we also found the smallest CV in the comparison between the second reading and the third reading during each test sessions, while the first reading, even though statistically not different from the others, cannot be always reliable during thermography. Since the first reading was taken immediately when the patient entered the room, it is likely that, after some minutes, there is some stabilization of the subject at the room temperature that affects the absolute measurements. This observation suggests that a stabilization period of the subject at the room temperature is recommended before the thermograph exam in order to guarantee an effective measurement. This is in line with available guidelines suggesting that thermography measures should be taken after a stabilization period of 15-20 minutes to allow equilibrating with the environment.[12,13]

Our data also suggest that FIT is a reliable procedure in detecting the cold patch location in headache patients and that the AI parameter is able to localize the cold patch even when the patient referred a bilateral pain before the image is recorded. In previous studies, no

correlation was found between FIT measurements and pain side or VAS score in patients [5,18]. Instead, our results demonstrate that the Asymmetry Index defined by Kurth et al.[19] well describe the lateralization of cold patch in patients. Moreover, AI index correlates with doctor's visual inspection of FIT and in case of unilateral pain there is a good correlation between AI and pain side. Even if the correlation between AI and VAS score is low, AI index could be a useful tool in order to localize the pathological side (where there is the coldpatch) in each patient. Our results further confirm that regardless to patient's perception, the cold patch can be viewed as a "unilateral" entity, representing the neurochemical imbalance between the two sides in terms of microcirculation in the facial district. This hypothesis is in line with neurophysiological studies on Visual Evoked Potentials showing a prominent laterality of neurophysiological signatures in headache [20–24] . Therefore, the AI estimated by FIT can have a relevant prognostic role going beyond patient's perception.

Finally, the SideDifference parameter was able to discriminate headache patients from controls, even if the FIT image was taken with patients experiencing different pain intensities. These observations suggest that FIT can be a useful neurophysiological aid in the diagnosis for pain medicine. An add-on marker in diagnosis may be useful in difficult case or when there is a risk for diagnostic mistakes concerning unilateral or bilateral headaches form[26]. We foresee the time when FIT could be used in evaluating pharmacological and non-pharmacological treatment in headache and other facial neuralgia: FIT with a commercially available camera is a relatively inexpensive procedure that can be used in headache and pain centre setting.

However, even though promising, our results were obtained in a relatively small number of subjects, and acquisitions followed a shared protocol that guaranteed consistency across subjects. Therefore, for future applications, it will be important to create a consensus procedure for FIT acquisition, able to support diagnosis.

From a practical viewpoint, in the current therapeutic scenario, where non-invasive neuromodulation techniques are increasingly used for the treatment of migraine [21–23][25–27] , these results can reach an even more interesting meaning. In fact, transcranial direct current stimulation (tDCS) has been proposed for the treatment of migraine [27,28,30,31]. tDCS is a non-invasive technique that acts sub-threshold (<1 V/m vs. 100 V/m produced by other supra-threshold techniques[32], producing an excitability change in the area below the electrode. Even though the electric field induced by tDCS spread over neighbouring areas, the electrode montage is a crucial element in the optimization of tDCS therapy[29]. In migraine, electrodes are usually placed in the frontal area, according to patient's perception and subjective analysis. Conversely, the cold patch, representing a vasoconstriction area characterizing the migraine may be an effective target for neuromodulation intervention. In migraine patients, the cold patch corresponds to a vasoconstriction within the external carotid territory, which represents the end result of the haemodynamic changes due to the activity of the autonomic and the trigeminovascular systems. In this case, cathodal tDCS over this area may reduce the pathological frontal asymmetry. For this reason, the automatic localization of cold patch using FIT based parameters, as proposed in this study, can be applied for the optimization of tDCS treatment in migraine patients.

Taken together, our results suggest that FIT could be useful as a diagnostic tool, to localize the cold patch and to study of unilateral headache without side shift like Cluster Headache, Paroxysmal Hemicrania, SUNCT or cranial neuralgias (trigeminal neuralgia). However, the case series is relatively small in order to evaluate the sensitivity and specificity of FIT in discriminating between different headache entities. In addition, even though at present there is no strong evidence supporting the change of FIT after a pharmacological or non-pharmacological treatment, the use tDCS or prophylactic agents (beta blockers, Calcium

antagonist) show a tendency to reducing or disappearing of FIT asymmetry, thus suggesting the use of FIT also as a prognostic tool.

Conclusions

In conclusion, in respect to previous studies, this work introduces the possibility to use modern commercial infrared thermal camera for the analysis of frontal thermography in migraine patients. Moreover, two new parameters (AI and Side Difference) provided meaningful results, being one able to localize the cold patch and the other one able to distinguish patients from controls.

Article Highlights

- The use of Frontal Infrared Thermography (FIT) in the diagnosis of primary headaches is still debated.
- In this study, we showed that FIT measurements have good reproducibility even when using commercial cameras in both patients and controls.
- We defined a new parameter, the Side Difference, that is able to discriminate patients with headache from controls.
- We defined another parameter, the Asimmetry Index, that correlates with the side of pain.
- FIT can be used to characterize vascular changes in chronic migraine patients but standardized recording conditions are needed to guarantee reliability.

Funding resources

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Institutional Review Board Approval:

The study was approved by the institutional review board (Ethical Committee of the Fondazione IRCCS Istituto Neurologico Nazionale Casimiro Mondino, date of approval: July 29th 2013) and conformed with the Declaration of Helsinki. All patients signed written informed consent before participating to the study.

Conflict of interest statement

Elena Rossi, at the time of the work, was employed by Newronika Srl, a spin-off company of the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico and of the University of Milan. Sara Marceglia is founder and shareholder of Newronika Srl.

The other authors declare no conflict.

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Figures legends

Figure 1- Measurement point identification in patients. The radius of the circle was calculated as the distance between the Nasion and the coolest point in the cold patch. Once defined the radius, the second point was the point equidistant from the Nasion and with the same vertical coordinate of the first point of measurement (symmetric point).

Figure 2- Measurement point identification in controls. The radius of the circle was constant.

Figure 3 – Mean FIT measured over 20 controls (left side) and 15 patients (right side) in three consecutive readings (m1, m2, m3 - x axis) with 10 minutes of rest between readings during T1 and T2. Error bars represent standard errors.

Figure 4 - Difference between left and right side as a percentage of the average temperature in forehead (SideDifference) in patients (N=20) and controls (N=15).

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Keywords

Frontal Thermography (FIT); Chronic Migraine; Cold Patch; Standard Procedure; Reliability

Abbreviations

AI – Asymmetry Index

ANOVA – analysis of variance

BMS - between-subjects mean square

CV - Coefficient of Variation

EMS - residual mean square

FIT – Frontal Infrared Thermography

ICC - intra-class correlation coefficient

JMS - within-subjects mean square

tDCS - transcranial direct current stimulation

VAS - Visual Analogue Scale

Abstract

Background: The use of Frontal infrared thermography in the diagnosis of primary headaches provided scattering results due to measurement fluctuations and different types of headaches or research protocols.

Objective: This study aims to assess the reliability of Frontal infrared thermography in healthy individuals and provide a preliminary evaluation in chronic migraine patients using a commercial infrared thermal camera.

Methods: Thermographic images were acquired in 20 controls and 15 patients at 3 consecutive time-points in two daily sessions. The Side Difference and Asymmetry Index parameters were defined. The reproducibility of the measurements, the correlation of Asymmetry Index and Side Difference with clinical evaluations and patient perceptions, and the ability of the parameters to discriminate between patients and controls were investigated.

Results: We reported a good reproducibility of the measurements (Inter-class Correlation Coefficient>0.75 and Coefficient of Variation<13.4%), independent from external factors. The Side Difference was significantly different between patients and controls ($p<0.001$). The Asymmetry Index showed good correlation with the side of unilateral pain ($p=0.0056$).

Conclusions: Frontal infrared thermography can be used to quantify the difference between the right and the left side of frontal vascular changes in chronic migraine patients, provided that standardized conditions are satisfied.

Introduction

Infrared Thermography detects infrared light emitted by the human body to visualize changes in heat due to abnormalities in the skin surface blood flow of diseased areas. This non-invasive and non-radiative imaging technique has different clinical applications including the detection of circulatory and/or inflammatory disorders such as rheumatoid arthritis, Raynaud's disease or osteoarthritis of the knee[1]. It was also demonstrated that thermography is able to capture thermal gradient in facial areas characterizing healthy individuals[2].

However, the literature investigating the use of external carotid region (forehead) thermal imaging for the characterization of vascular headaches did not reach consistent results due to different types of headache evaluated, different timing of patients' evaluation (during headache attack or in headache free interval), different technologies used for image acquisition, and different methods for imaging or statistical analysis.

In 1986, Swerdlow and Dieter[3] comparing electronic thermography between 275 headache patients and 45 controls, defined the "Cold Patch" as a region of the face where the temperature is cooler (less than 0.5°C) than the surrounding areas[3,4]. The presence of the cold patch is more frequent in vascular headaches than in healthy subjects or patients with tension type headache, psychogenic headaches or post-traumatic headaches.

There is an open question on whether the cold patch is a "fixed" entity[5] or whether it decreases with therapy[6,7]. In fact, whereas according to Swerdlow and Dieter, "the vascular cold patch is independent of prognosis and is most likely a permanent element of a vascular headache sufferer's facial thermal pattern"[5], in 1991, Dalla Volta et al. [7] suggested that the "cold patch" in vascular headache patients is ipsilateral to the prevailing side of pain and that the cooler area decreased after 6 months of prophylactic therapy. The differences observed by the two groups may be explained in terms of experimental protocol and patient's selection that introduced a higher variability in the cold patch response [7].

Anyway, there is large consensus on the fact that the cold patch represents an asymmetry in the forehead of migraine patients. Unfortunately, the location of the cold patch is seldom related to the side of the pain[5] probably due to the variation of temperature during headache attack or due to the lateralization of the headache (unilateral or bilateral) as measured by Drummond and Lance[8,9].

Finally, FIT, in conjunction with nitroglycerine administration, was suggested as a novel non-invasive approach to study vascular processes underlying headaches[10].

Taken together, all these studies demonstrate that FIT can be used as diagnostic tool in migraine with and without aura, in cluster headache and in other headache types.

In the light of previous observations and of new possible therapeutic applications, the present study has two primary end points: (1) to evaluate the reliability of FIT measurements in controls and chronic migraine patients using modern commercial infrared thermal camera; (2) to verify whether FIT-based parameters correlate both with the visual evaluation of FIT by an expert clinician and with patient's perception of pain side (at least in case of unilateral pain).

Materials and methods

Subjects

Thirty five right handed volunteers (26 females and 9 males) with a mean age of 35±11.6 years (range: 20-55) were enrolled at the Headache centre of the Fondazione IRCCS Istituto Neurologico Nazionale Casimiro Mondino. All the subjects were not medicated at the time of testing.

15 of the 35 volunteers (3 males) suffered from chronic migraine with medication overuse, as assessed according to the IHS Classification[11]. They were examined while hospitalized and during a washout period from analgesics or other symptomatic treatment, including non-headache medication. Patients were not on dietary/smoking restrictions ahead of

measurements. Patients did not use preventive treatment and underwent daily parenteral detoxification (saline, cyanocobalamin, folic acid, nicotinammide, glutathione, delorazepam and metoclopramide on demand). Patients were assessed on the second or third day after symptomatic medication withdrawal that started on the day of hospital admission. No symptomatic medication was allowed during the evaluation period but local ice bag (at least one day apart from the examination). Hence, patients were generally with headache during the examination. Pain severity was evaluated before each examination on a 0-3-score Visual Analogue Scale (0: no pain,1: mild pain, 2: moderate pain 3: severe pain) with a mean value of [mean±SE] 1.88 ± 0.078.

The remaining 20 of the 35 volunteers were used as controls since they suffered from migraine or tension type headache no more than 1-2 times a year.

The study was approved by the institutional review board (Ethical Committee of the Fondazione IRCCS Istituto Neurologico Nazionale Casimiro Mondino, date of approval: July 29th 2013) and conformed with the Declaration of Helsinki. All patients signed written informed consent before participating to the study.

Frontal Infrared Thermography

FIT was assessed with a modern infrared thermal camera (model LT3, produced by Zhejiang Dali Technology Co. Ldt) characterized by a thermal sensitivity below 0.08°C at 30°C, according to suggested guidelines [12,13]. FIT measured the spatial distribution of the heat over the human face (object emissivity 0.98): the camera was placed at distance of 1 meter from the subject in a room with stable temperature (23.6 ± 1.57°C). To ensure comparability between patients and controls, since controls do not have a "cold area", the temperature was evaluated in two target points (left and right side) in the frontal polar sites. In patients, we identified the coolest point in the side showing the cold patch, and then we identified its symmetric on the other side of the head (equidistant from the nasion, Fig.1). In controls there

is no cold patch, so we used two symmetric points equidistant from the nasion on a radius of 2cm (Fig.2). This protocol allowed repeatability and comparability among subjects.

Study design

All measurements were taken in two test sessions (T1 and T2) for all subjects. The second session (T2) was run at least one day apart in order to evaluate the intra-subject variations in FIT. During each session (T1 and T2), the measurements were repeated three times (m1, m2, m3) by the same experimenter after 10 minutes of rest between each measurement. Room temperature was recorded during each session. Images where taken approximately at the same time of the day in each patient (10-12 a.m. or 2.00-4.00 p.m.).

Data analysis

Reliability of Frontal Thermography (FIT)

In order to verify whether measurements were influenced by external factors, correlation analysis was computed with respect to room temperature, sex and age of subjects, both at T1 and at T2, using m1, m2, and m3 as dependent variables.

Student's paired t-Test was used to determine whether three measurements belonging to the same session (m1 vs m2, m2 vs m3, m1 vs m3) had the same mean. The Bonferroni correction for repeated measures was applied ($p<0.016$).

Reliability of measurements was investigated using the intra-class correlation coefficient (ICC), defined as the fraction of variance that is caused by the variation between subjects. Thus, if the variance between tests is smaller than the variance between subjects then ICC is close to 1. According to Fleiss, ICC values above 0.75 generally mean “excellent” reliability[14]. The intra-class correlation coefficient (ICC) was calculated for FIT at each point: data during a single session (T1 and T2) were analysed using a Two Factor ANOVA without

Replication (factors: subjects and FIT readings m_i) and the value from the analysis of variance table were substituted into equation (1):

$$ICC\ 2,1 = (BMS - EMS)/(BMS + EMS + 2(JMS-EMS)/n) \quad (1)$$

where BMS is the between-subjects mean square, EMS is the residual mean square, JMS is the within-subjects mean square, n is number of subjects[15].

The reproducibility of the method was tested by calculating the Coefficient of Variation (CV) during the first test session[16,17]. For each comparison between measurements (m1 vs m2, m2 vs m3, m1 vs m3) the coefficient of variation (equation 2) was calculated as the absolute value of:

$$CV = 100 * | (m_i - m_{i+1}) / ((m_i + m_{i+1}) /2) | \quad (2)$$

where m_i is the first reading (test) and m_{i+1} is the second one (retest). The numerator is the difference between two consecutive measurements and the denominator is the average of the two measurements. In this way, the Coefficient of Variation is the percentage difference between two readings. For each subject the coefficient of variation was computed for the three intra-session comparisons and then averaged across subjects.

In order to evaluate the intra-subject variations in FIT, a two-way analysis of variance with replication (2-way ANOVA) was performed for a statistical comparison between two different test sessions (T1 and T2).

Characterization of images comparing controls and patients

In order to verify the difference between migraine FIT measurement and healthy subject FIT measurements, we defined two parameters:

- the Asymmetry Index [14] (equation 3)

$$AI=2 \times ((Tleft-Tright)/(Tleft+Tright)) \quad (3)$$

Where Tleft is the temperature on the left forehead and Tright is the temperature of the right forehead. AI was calculated in order to assess the lateralization of FIT. If the temperature

measurement on the two sides is the same, then the asymmetry index is equal to 0. Positive AI value means that the cold patch is located on the right side of the forehead. Conversely, a negative asymmetry index means a lateralization on the left side.

- the absolute value of percentage difference between left and right side

Side Difference (%)= 100 * | 2 * (Tleft - Tright / Tleft + Tright) | (4)

SideDifference (equation 4) is the difference between left and right side as a percentage of the average temperature in forehead.

Both parameters were calculated from all the measurements of each patients (15*6=90 observations) and controls (20*6=120 observations). A comparison between the two groups was conducted in order to test whether there was a significant difference of AI or Side Difference. In headache patients, the Asymmetry Index was compared with FIT visual inspection by the doctor and with declarations of patient before each session (the side of pain and intensity of pain using VAS scores).

Results

Reliability of frontal infrared thermography

FIT readings did not correlate with external factors during both during T1 and T2 sessions as far as sex (Tright r = 0.20, p = 0.24; Tleft r = 0.23, p = 0.17), room temperature (Tright r = 0.22, p = 0.20; Tleft r = 0.19, p = 0.27) and age (Tright r = -0.017, p= 0.92; Tleft r = 0.035, p = 0.83) is concerned.

During each session, paired t-Test analysis revealed no significant difference between consecutive FIT measurements during the same session (p>0.016) for all the three comparisons (m1 vs m2, m2 vs m3, m1 vs m3) in both the right and left measurement side (Figure 3).

The reliability analysis demonstrated "excellent"[14] results during T1 (ICC values: right side, 0.73; left side, 0.81). The same result was confirmed during T2 (ICC values: right side, 0.80; left side, 0.74).

Regarding the reproducibility, mean CV between consecutive FIT measurements in the right and left side were similar in controls (T1: left side: 1.63% ± 1.79; right side: 1.69% ± 2.09; T2: left side: 2.71% ± 3.26; right side: 2.67% ± 3.22) and in patients (T1: left side: 3.77% ± 3.57; right side: 3.87% ± 3.62; T2: left side: 2.71% ± 3.26; right side: 2.67% ± 3.22). However, in patients, CV range was slightly greater than in controls both in T1 and T2 session (patients: 0.00%-13.40%; controls: 0.00% - 10.04%). In addition, the average smallest CV was observed among m2m3 measurements (m2m3: 1.56% ± 0.62 vs m1m2: 3.13% ± 1.29 and m1m3: 3.54% ± 1.25). Despite this, the overall reproducibility of the measurements was very good because the maximum CV was less than 13.40%.

When examining intra-subject variations between two different days (inter-test), CV represents the percentage difference between two daily sessions (T1 and T2). The maximum CVs between test sessions (T1 and T2) were very low (patients range: 0.19 - 8.24%, controls range: 0.00 - 7.56%) thus suggesting a good reproducibility of the measurements over different days.

The range of temperature was not different between days or between the two groups of subjects (controls: T1: 33.10-36.73°C; T2: 34.13 – 36.70°C; patients: T1: 32.30 – 37.60; T2: 32.30 – 36.17°C). Patients were characterized by a larger standard deviation of temperature than controls (Figure 3). The two-way ANOVA with replication over 35 subjects demonstrated that there were no significant differences between FIT measurements in different days.

Characterization of frontal infrared thermography comparing patients and controls

In controls, the temperature measurements during T1 varied from a minimum of 32.60°C to a maximum value of 38.90°C (mean on the two sides: 35.05°C, standard deviation: 1°C, corresponding to 2.8% of the mean value). In patients with chronic migraine the temperature measurements during T1 varied from a minimum of 31.90°C to a maximum value of 38.30°C with a larger standard deviation from the mean than controls (mean on the two sides 35.47°C, standard deviation 1.59°C, corresponding 4.5% of the mean value).

Whereas average AI was not different ($p>0.05$) between patients (-0.00002 ± 0.0164) and controls (-0.00118 ± 0.0091), the Side Difference, representing the absolute value of the difference between temperature measured in the left and right side, significantly discriminated controls (mean ± std: 0.73% ± 0.55) from patients ([mean ± SD]: 1.37% ± 0.87, P-value one tail<0.0001) (Fig. 4).

Conversely, AI can be used to locate the cold patch in patients. In fact, AI polarity (positive or negative, where positive AI value means that the cold patch is located on the right side of the forehead) was in consort with the visual inspection of the FIT by the examiner (correlation, $r^2=0.7$, $p=0.00072$). In addition, when the patient referred a bilateral pain before the test session, both the visual inspection and the AI index revealed an asymmetry in the forehead, thus suggesting that the AI can be a reliable index for the localization of the coldpatch also when the patient's perception is not reliable.

Finally, in patients with unilateral pain, the correlation between AI and the patient-referred pain side before FIT was good ($r^2=0.6$, $p=0.0056$). Conversely, even though patients were examined mainly during attacks with different pain severities, as measured by VAS, the correlation between AI and pain severity was very low ($r^2=0.2$, $p=0.37$).

Discussion

In this study, we examined the usability and reproducibility of FIT measurement in patients with headache and in healthy subjects, in a research scenario in which previous experience using FIT were not conclusive [1,3,4,6,7,18].

In order to obtain comparable results between patients and controls, we used a thermal punctual evaluation instead of an evaluation by area. In fact, whereas patients showed a cooler area (the cold patch), controls did not, thus making the evaluation by area unreliable and not reproducible. We therefore chose to evaluate the temperature in two symmetric points equidistant from the nasion. In patients, one point was the coolest point in the cold patch, and the second was its symmetric, in controls we took two symmetric points located on a circle of fixed radius. With this setup, our data showed that FIT is a reproducible tool provided that standard location and standard measurement procedure is carried out. In fact, we found good reproducibility of the measurements within the same session and between the two sessions, as measured by CV. However, we also found the smallest CV in the comparison between the second reading and the third reading during each test sessions, while the first reading, even though statistically not different from the others, cannot be always reliable during thermography. Since the first reading was taken immediately when the patient entered the room, it is likely that, after some minutes, there is some stabilization of the subject at the room temperature that affects the absolute measurements. This observation suggests that a stabilization period of the subject at the room temperature is recommended before the thermograph exam in order to guarantee an effective measurement. This is in line with available guidelines suggesting that thermography measures should be taken after a stabilization period of 15-20 minutes to allow equilibrating with the environment.[12,13]

Our data also suggest that FIT is a reliable procedure in detecting the cold patch location in headache patients and that the AI parameter is able to localize the cold patch even when the patient referred a bilateral pain before the image is recorded. In previous studies, no

correlation was found between FIT measurements and pain side or VAS score in patients [5,18]. Instead, our results demonstrate that the Asymmetry Index defined by Kurth et al.[19] well describe the lateralization of cold patch in patients. Moreover, AI index correlates with doctor's visual inspection of FIT and in case of unilateral pain there is a good correlation between AI and pain side. Even if the correlation between AI and VAS score is low, AI index could be a useful tool in order to localize the pathological side (where there is the coldpatch) in each patient. Our results further confirm that regardless to patient's perception, the cold patch can be viewed as a "unilateral" entity, representing the neurochemical imbalance between the two sides in terms of microcirculation in the facial district. This hypothesis is in line with neurophysiological studies on Visual Evoked Potentials showing a prominent laterality of neurophysiological signatures in headache [20–24] . Therefore, the AI estimated by FIT can have a relevant prognostic role going beyond patient's perception.

Finally, the SideDifference parameter was able to discriminate headache patients from controls, even if the FIT image was taken with patients experiencing different pain intensities. These observations suggest that FIT can be a useful neurophysiological aid in the diagnosis for pain medicine. An add-on marker in diagnosis may be useful in difficult case or when there is a risk for diagnostic mistakes concerning unilateral or bilateral headaches form[26]. We foresee the time when FIT could be used in evaluating pharmacological and non-pharmacological treatment in headache and other facial neuralgia: FIT with a commercially available camera is a relatively inexpensive procedure that can be used in headache and pain centre setting.

However, even though promising, our results were obtained in a relatively small number of subjects, and acquisitions followed a shared protocol that guaranteed consistency across subjects. Therefore, for future applications, it will be important to create a consensus procedure for FIT acquisition, able to support diagnosis.

From a practical viewpoint, in the current therapeutic scenario, where non-invasive neuromodulation techniques are increasingly used for the treatment of migraine [21–23][25–27] , these results can reach an even more interesting meaning. In fact, transcranial direct current stimulation (tDCS) has been proposed for the treatment of migraine [27,28,30,31]. tDCS is a non-invasive technique that acts sub-threshold (<1 V/m vs. 100 V/m produced by other supra-threshold techniques[32], producing an excitability change in the area below the electrode. Even though the electric field induced by tDCS spread over neighbouring areas, the electrode montage is a crucial element in the optimization of tDCS therapy[29]. In migraine, electrodes are usually placed in the frontal area, according to patient's perception and subjective analysis. Conversely, the cold patch, representing a vasoconstriction area characterizing the migraine may be an effective target for neuromodulation intervention. In migraine patients, the cold patch corresponds to a vasoconstriction within the external carotid territory, which represents the end result of the haemodynamic changes due to the activity of the autonomic and the trigeminovascular systems. In this case, cathodal tDCS over this area may reduce the pathological frontal asymmetry. For this reason, the automatic localization of cold patch using FIT based parameters, as proposed in this study, can be applied for the optimization of tDCS treatment in migraine patients.

Taken together, our results suggest that FIT could be useful as a diagnostic tool, to localize the cold patch and to study of unilateral headache without side shift like Cluster Headache, Paroxysmal Hemicrania, SUNCT or cranial neuralgias (trigeminal neuralgia). However, the case series is relatively small in order to evaluate the sensitivity and specificity of FIT in discriminating between different headache entities. In addition, even though at present there is no strong evidence supporting the change of FIT after a pharmacological or non-pharmacological treatment, the use tDCS or prophylactic agents (beta blockers, Calcium

antagonist) show a tendency to reducing or disappearing of FIT asymmetry, thus suggesting the use of FIT also as a prognostic tool.

Conclusions

In conclusion, in respect to previous studies, this work introduces the possibility to use modern commercial infrared thermal camera for the analysis of frontal thermography in migraine patients. Moreover, two new parameters (AI and Side Difference) provided meaningful results, being one able to localize the cold patch and the other one able to distinguish patients from controls.

Article Highlights

- The use of Frontal Infrared Thermography (FIT) in the diagnosis of primary headaches is still debated.
- In this study, we showed that FIT measurements have good reproducibility even when using commercial cameras in both patients and controls.
- We defined a new parameter, the Side Difference, that is able to discriminate patients with headache from controls.
- We defined another parameter, the Asimmetry Index, that correlates with the side of pain.
- FIT can be used to characterize vascular changes in chronic migraine patients but standardized recording conditions are needed to guarantee reliability.

Funding resources

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Institutional Review Board Approval:

The study was approved by the institutional review board (Ethical Committee of the Fondazione IRCCS Istituto Neurologico Nazionale Casimiro Mondino, date of approval: July 29th 2013) and conformed with the Declaration of Helsinki. All patients signed written informed consent before participating to the study.

Conflict of interest statement

Elena Rossi, at the time of the work, was employed by Newronika Srl, a spin-off company of the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico and of the University of Milan. Sara Marceglia is founder and shareholder of Newronika Srl.

The other authors declare no conflict.

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Figures legends

Figure 1- Measurement point identification in patients. The radius of the circle was calculated as the distance between the Nasion and the coolest point in the cold patch. Once defined the radius, the second point was the point equidistant from the Nasion and with the same vertical coordinate of the first point of measurement (symmetric point).

Figure 2- Measurement point identification in controls. The radius of the circle was constant.

Figure 3 – Mean FIT measured over 20 controls (left side) and 15 patients (right side) in three consecutive readings (m1, m2, m3 - x axis) with 10 minutes of rest between readings during T1 and T2. Error bars represent standard errors.

Figure 4 - Difference between left and right side as a percentage of the average temperature in forehead (SideDifference) in patients (N=20) and controls (N=15).

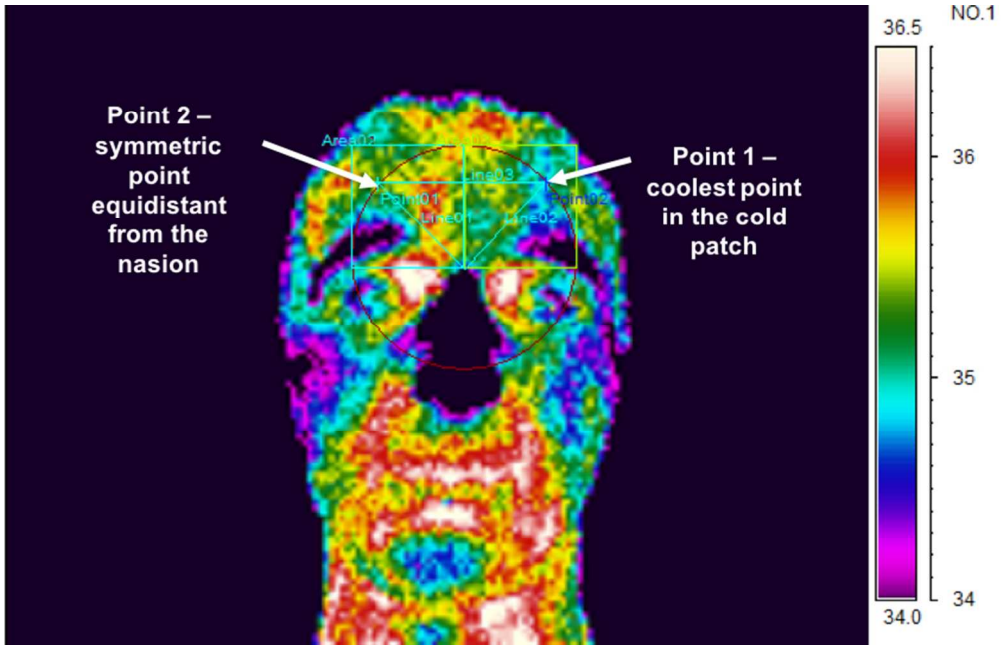

Figure 1

297x190mm (72 x 72 DPI)

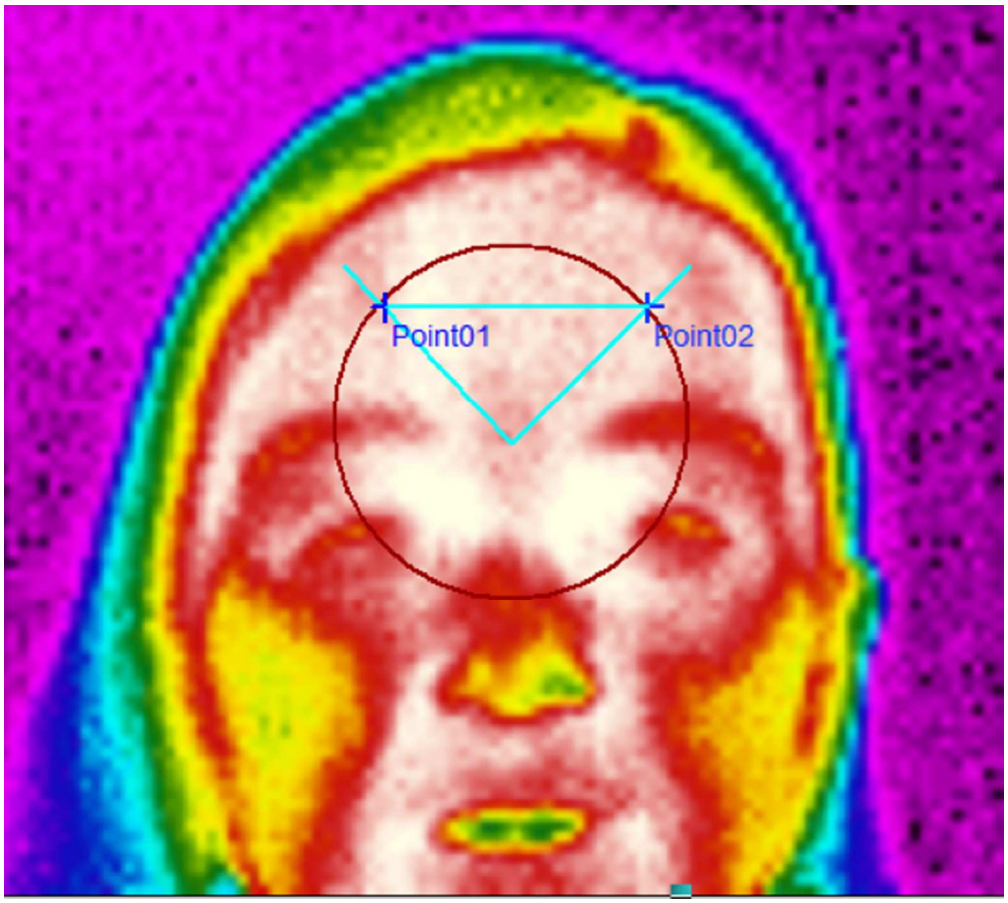

Figure 2

213x190mm (72 x 72 DPI)

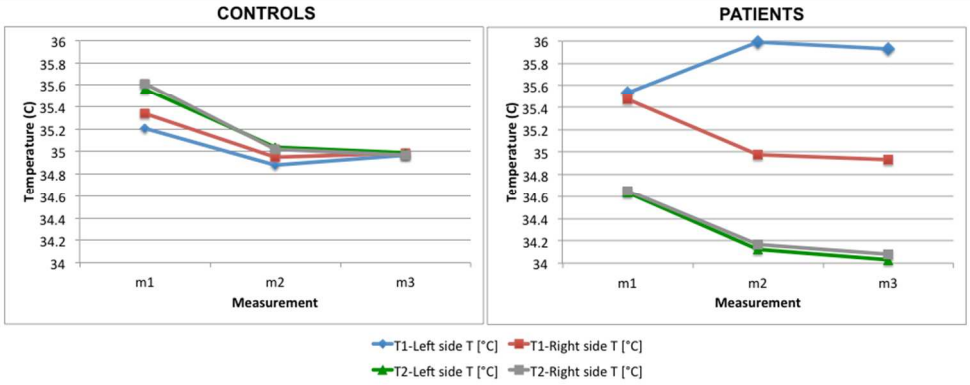

Figure 3

360x141mm (72 x 72 DPI)

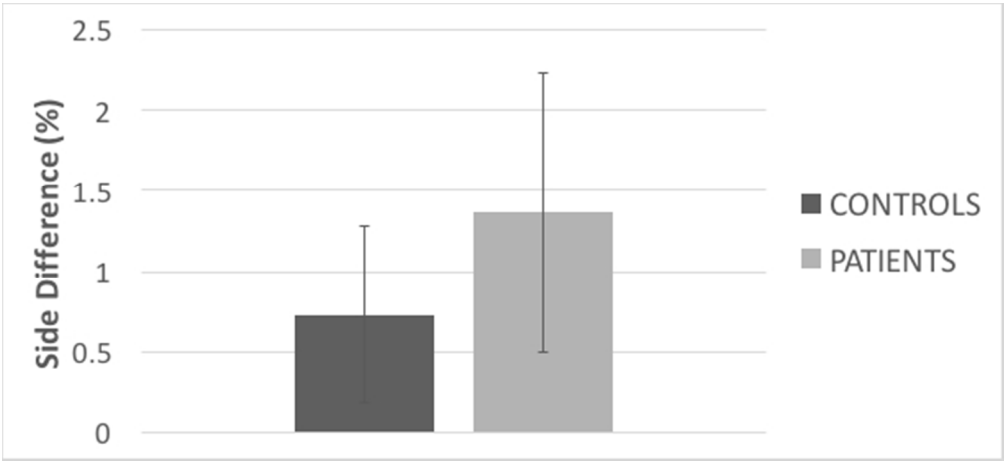

Figure 4

215x98mm (72 x 72 DPI)

STROBE Statement—checklist of items that should be included in reports of observational/case controlled studies, etc. **YOU MUST NOTE THE PAGE NUMBER WHERE EACH ITEM IS REPORTED INSIDE BRACKETS [ ] FOR EACH ITEM #. IF NOT APPLICABLE WRITE N/A**

	Item #	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
	[3]	(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2 **[4]**	Explain the scientific background and rationale for the investigation being reported
Objectives	3 **[5/6]**	State specific objectives, including any prespecified hypotheses
Methods		
Study design	4 **[7]**	Present key elements of study design early in the paper
Setting	5 **[6]**	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6 **[6]**	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7 **[7-9]**	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8* **[7-9]**	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9 **[n/a]**	Describe any efforts to address potential sources of bias
Study size	10 **[n/a]**	Explain how the study size was arrived at
Quantitative variables	11 **[7-9]**	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12 **[7-9]**	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was

	addressed
	Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy
	(*e*) Describe any sensitivity analyses

Continued on next page

Results		
Participants	13* **[n/a]**	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14* **[6]**	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data	15* **[n/a]**	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures
Main results	16 **[n/a]**	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17 **[10-12]**	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18 **[12]**	Summarise key results with reference to study objectives
Limitations	19 **[13]**	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20 **[12-13]**	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21 **[13-14]**	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22 **[15]**	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.