Noninvasive Blood Flow Measurement over Acupuncture Points (Gb21): A Pilot Study

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ABSTRACT

Objective: Noninvasive evaluation of the cutaneous (cBF) and subcutaneous (sBF) blood flow over the acupuncture point (AP) Gb21 to assess a characteristically increased microcirculation at AP.

Background: AP show several anatomic, histologic and physiologic peculiarities that indicate a specifically elevated microcirculation. Nevertheless cBF and sBF over AP have not yet been established by noninvasive measurements. The laser Doppler spectroscope O2C© allows a direct and real-time detection of the local cBF (depth: 1 mm) and sBF (depth: 8 mm) in vivo.

Methods: In 28 subjects (41.1 ± 9.8 years) the glass fiber probes of the O2C were placed over Gb21 and an adjacent asymptomatic reference point (RP) at the Trapezius Muscle. The relative blood flow was measured over a period of 3 minutes in each case. Microcirculation comparisons over AP and RP were made using multivariate analysis of variance.

Results: The results showed significantly higher sBF compared to cBF over Gb21 (F = 89.95, p < 0.01) and RP (F = 88.47, p < 0.01). A significantly higher microcirculation was evaluated for all subjects over Gb21 compared to RP (cBF: F = 7.35, p < 0.05; sBF: F = 8.56, p < 0.01).

Conclusion: The employment of the O2C permitted for the first time noninvasive evidence of a significantly increased cBF and sBF over AP (Gb21). This evaluation of an initial microcirculatory state of AP should be determined for several AP and larger sampling sizes. The results could be the basis for following controlled acupuncture trials. Regarding a close correlation of AP with myofascial trigger points (TP), further studies in patients with myoskeletal pain could quantify noninvasively a decreased perfusion in the area of TP that might indicate a pathologic condition.

The findings of this study encourage the implementation of the laser Doppler spectroscope O2C as a reliable diagnostic tool for various pain syndromes in prevention, therapy, and rehabilitation.

INTRODUCTION

The scientific basis for acupuncture points (AP) has not yet been satisfactorily clarified. Although few investigators found no sufficient evidence to conclude that AP have consistent anatomic features,1 there is a body of evidence relating several structural subjective and objective specificities at these loci.

Regarding histologic characteristics, there are indications that AP show several uniform receptor organs, consistent arrangements of nerve fibers, or correspondence with other neuromuscular phenomena.2 Thirty years ago, several investigators found different neural structures in both superficial and deep structures beneath acupuncture loci.3 In the later 1970s, Senelar and coworkers were one of the first to delineate the histologic structure of acupuncture points and reported neurovascular/lymphovascular bundles as unique anatomic and/or physiologic properties of AP.4,5 In good agreement with Plummer,6 Senelar5 described AP as vertical columns of loose connective tissue containing lymphatic...
trunks coupled to veins and arterioles and surrounded by denser connecting tissue of the skin and a rich plexus of un-myelinated cholinergic nerve fibers. Heine\(^7\) revealed arterial vessels (diameter between 0.6 and 0.7 mm) as leading structures of a nerve vessel bundle that perforates the superficial body fascia through openings of 2–7 mm in diameter. In histologic investigations, Draehmpaehl\(^8\) confirmed a typical accumulation of multiple sensory receptors and found congregations of neuromuscular spindles in deeper tissue layers in the region of AP.

In terms of physiologic specificities, AP are associated with local tenderness and pressure sensitivity.\(^2\) In thermographic measurements, AP appear to be warmer than adjacent points.\(^9\) Narongpunt et al.\(^10\) recently demonstrated thermal activity (heat emission) on Bladder meridian acupuncture points after acupressure stimulation due to unknown neurovascular effects measured by infrared thermographic visualization. Likewise, electrical skin resistance or impedance over AP\(^1\) and some meridians,\(^11\) is said to be lower than over nonacupuncture loci. Some investigators localized spontaneous electrical activity over AP,\(^12\) but these are still controversial subjects.\(^13\) Pulpation or acupuncture treatments of AP elicit the characteristic needling sensation (de qi).\(^2\) Furthermore, AP seem to be associated with measurable biomechanical differences (needle grasp) while needling.\(^14\)

These histologic and physiologic characteristics indicate a specifically elevated microcirculation over classically defined AP. Nevertheless, the cutaneous (cBF) and subcutaneous (sBF) blood flow over AP has not yet been established by noninvasive measurements.

Whereas perfusion of the large vessels currently is recorded by angiography and Doppler ultrasound, and blood gases by laboratory diagnosis and cuvette spectroscopy,\(^15\) the methods of studying microcirculation in vivo are relatively limited. Invasive measurements (e.g., within biopsy studies)\(^16,17\) are associated with a perceivable risk of “manipulated” results. The inflammatory reaction of the tissue released by the physical intervention could have crucial effects on the blood flow parameters.

The reliable \((r = 0.83, \ p < 0.01)\) and valid\(^18\) laser Doppler spectroscope O2C\(^\circledast\) (Lea Medizintechnik,\(^\circledast\) Geißen, Germany) (abbreviation for “oxygen to see”) applied in this study allows for the first time a direct, real-time, and noninvasive detection of the relative local cBF (depth: 1 mm) and sBF (depth: 8 mm).

The aim of this study was to determine resting cBF and sBF over Gallbladder 21 (Gb21) to prove a characteristically increased microcirculation over acupuncture loci by noninvasive laser Doppler spectroscopy. Gb21 is a frequently examined traditional AP located at the upper free border of the Trapezius Muscle and related to the Gallbladder meridian. Furthermore, Gb21 is a loci of response to diseases in the Shoulder region. Melzack et al.\(^19\) found that almost all AP correlate with myofascial trigger points (TP). This association is best illustrated by the TP often found precisely at Gb21.\(^20\)

**MATERIALS AND METHODS**

**Subjects**

Twenty-eight (28) healthy subjects ages 23–63 years (41.1 ± 9.8 years) participated in this study. To avoid influences of gender on microcirculatory parameters, only women were included in the study.

According to the results of previous studies,\(^21\) there were no restrictions regarding the age of the sample. The body–mass index of the cohort averaged 23.4 ± 2.44. Because of the direct vasoconstrictive effects of cigarette smoking, only nonsmokers were accepted. Persons with diseases concomitant with vascular disorders (e.g., venous diseases, decreased reactivity of the vessels, declined vasomotion, or reduced blood circulation within several skin diseases, (i.e., scleroderma, eczema), diabetes mellitus, ischemic heart diseases, occlusive peripheral arterial disease, and Raynaud’s disease were excluded. Furthermore, the intake of medication considerably influencing the cardiovascular system (e.g., β-blockers, corticosteroids, or acetylsalicylic acid resulted in exclusion from the trial.

Before examination, each subject received a booklet containing information about the experimental procedure, a form for declaration of informed consent, and an agreement for participation. The subjects were requested to refrain from coffee, tea, and alcohol as well as to avoid exhausting physical activity and applying lotions and ointments for 12 h preceding the test.

**Measuring points**

The measuring point Gb21 is defined as the highest point of the Shoulder. In Traditional Chinese Medicine (TCM), this alarm point (Jianjing) is equivalent to the “shoulder fountain” of the Gallbladder Meridian. Gb21 is located midway between Dazhui (Du14) and the Acromion. Gb21 is innervated by the posterior branch of the Supravacular Nerve, the Accessory Nerve. Perfusion is provided by the Transverse Cervical Artery and Vein.\(^22\)

An asymptomatic nonacupuncture point at the Trapezius Muscle was used as a reference point (RP). RP was located within a radius of 3–5 cm from Gb21.

**FIG. 1.** Glass fiber probe of the Laser Doppler Spectroscope O2C\(^\circledast\) (Lea Medizintechnik,\(^\circledast\) Geißen, Germany).
Experimental procedure and data collection

In the first examination step, the localization and marking of Gb21 and RP took place. The glass-fiber probe of the O2C (Fig. 1) was first placed parallel to the surface over Gb21 (Fig. 2). Subjects were requested to lie down quietly for a preparatory phase of 15 minutes to exclude relevant emotional, physical, or external influences on the perfusion. After the relative resting, blood flow was measured for a duration of 3 min.

Subsequently, the Doppler probe was applied parallel to the surface over the RP. After a 5-minute rest, the data acquisition was started. cBF and sBF were monitored for 3 minutes. The second period of equilibration time should guarantee identical examination conditions on both measuring points.

Standardization procedures were carried out according to Sprott et al. and Ghazanfari et al. The measurements were performed in a quiet, slightly illuminated room with a constant temperature between 22°C and 24°C. Speaking or physical activities not related to the testing procedure were not permitted during the recordings and acclimatization phases. The examinations were conducted constantly by the same investigator. During the entire procedure, the participants lay in the prone position. The tests were performed on one randomly selected body site.

Measurement instrument and analysis of the data

The principle of the O2C is based on the registration of moving erythrocytes in the tissue. The quantitative assessment of cBF and sBF is provided by the multiplication of the number and velocity of red blood cells in the observed tissue.

The measuring depths of 1 mm (cBF) and 8 mm (sBF) were provided by the application of a special glass fiber probe (Fig. 1) with a thickness of 2.4 mm and a width of 14 mm. The probe consists of an illuminating fiber of 400-μm diameter that emits infrared laser light at a wavelength of 820 nm into the tissue to be examined. The photon of the laser light penetrates the tissue and is scattered back by the motion of the blood particles, resulting in frequency shifts according to the Doppler principle. The frequency shift is measured in relation to the laser light that is emitted into the tissue. Two photodetectors in the scanner head with distances of 1 mm and 8 mm from the illuminating fiber detect the scattered and frequency-shifted light from the observed depths.

The plotting unit of the O2C comprises a four-channel digital-signal-processing card. To improve the signal-to-noise ratio, the back-scattered and frequency-shifted light from different depths was generated in a differential amplifier and then submitted to an analogue and digital processor. A spectrum analysis from the data of each location was performed using a Fast Fourier Transform algorithm. The generated laser Doppler signals are expressed in arbitrary units (AU) which represent the relative microcirculation in the observed tissue. By multichannel analysis of the blood flow signals from different depths, it is possible to measure depth selectively up to approximately 8 mm, with up to 80 measurements per second noninvasively.

On the basis of plausibility considerations, “steady-state” conditions of 120 seconds were extracted from the monitored relative cBF and sBF over Gb21 and RP using LabView® (National Instruments,™ LabView,™ Austin, TX) and Soleasy® ALEA-Solutions, Zurich, Switzerland). In terms of quantifying the mean local perfusion, a moving average was calculated for the generated data of the extracted timeframes. Thus, 60 AU-values per measuring point and depth could be computed by generating one mean value for every 2 seconds.

For further statistical processing, one mean AU-value per measuring point and measuring depth was calculated for each subject.

Statistical analysis

All statistical computations were made using the commercial statistics program SPSS (SPSS, Chicago, IL) for Windows. The Levene test for homogeneity of variances and the Kolmogorov–Smirnov test for verification of sampling distribution was carried out. Microcirculation comparisons (cBF, sBF) over Gb21 and RP were made using multivariate analysis of variance.

RESULTS

Significant main effects for the whole sample were found for resting cBF and sBF over Gb21 (F = 89.95, p < 0.01) and RP (F = 88.47, p < 0.01). This result is conterminous with significantly higher sBF values compared to blood flow values in a depth of 1 mm at both observed measuring points.
Mean relative cBF and sBF (AU) and standard deviations over Gb21 and RP are shown in Figure 3. Main effects were found for cBF over Gb21 and RP (F = 7.35, p < 0.05) as well as for sBF over Gb21 and RP (F = 8.56, p < 0.01). These findings are based on the significantly elevated microcirculation in both depths over Gb21 compared to RP.

High standard deviations were obvious, especially within the resting perfusion values over Gb21. The appropriate confidence intervals shown in Table 1 present the range of the blood flow values over both measuring points and for the observed depths.

**DISCUSSION**

The results demonstrate significant differences in relative local cutaneous and subcutaneous microcirculation over Gb21 compared to RP. The employment of the O2C permitted for the first time noninvasive evidence of a significantly increased cBF and sBF over the acupuncture point Gb21 in comparison to an adjacent nonacupuncture control point. This finding emphasizes the thesis of a specifically inclined microcirculation over Gb21 concomitant with several anatomic and physiologic characteristics at acupuncture loci.

As a second result, a significantly higher sBF compared to cBF was detected over both measuring points. This finding is in good agreement with the fact that blood circulation is much higher in deeper tissue because of an increased metabolism rate and a higher cutaneous density.27 Thus, this result emphasizes the validity of the laser Doppler spectroscope O2C.

The relatively high standard deviations are originated in numerous external and internal influencing variables on microcirculation (e.g., heart rate, vegetative tonus, and psychophysic conditions).28 Likewise, the microcirculatory heterogeneity is caused by the phenomenon of flowmotion.15 Because of the relatively wide measurement range of the O2C, the microcirculatory variations do not affect the blood flow values as much as in other laser Doppler methods.25 Nevertheless, the inhomogeneity of perfusion must be considered in standardization of the examination as well as in the evaluation of the readings.

The outcome measures of this study suggest a specific initial microcirculatory state at the AP. When interpreting the results, it must be considered that the size of the applied probe is related to the distance of the illuminating fiber, and the photodetectors partly overlap the AP area. Therefore, an underestimation of the actual blood flow rates can be suspected for both measuring depths over Gb21. Whether a significantly raised blood circulation is actually a specificity of all AP must be verified in future investigations and for larger sampling sizes. In addition, further studies should prove whether the implicated O2C warrants a noninvasive detection of acupuncture points independently from palpating or triggering a de qi sensation.

As mentioned above, Gb21 is prevalently affected by trigger points.29 Because there is clearly considerable overlap between trigger point pain and referral patterns and meridians,20 trigger points at Gb21 do not only provoke local pain but also induce microcirculatory indispositions and painful disorders in distant muscles.30 Based on the thesis that a specifically elevated microcirculation at acupuncture loci represents a physiologic condition, further studies in patients with myoskeletal pain could quantify noninvasively a decreased perfusion in the area of trigger points, which might indicate a pathologic condition. In this context, the effectiveness of different treatments (e.g., acupuncture or physical therapy) could be evaluated by using the laser Doppler

**Table 1. 95% Confidence Intervals (CI) of the Relative Cutaneous (cBF) and Subcutaneous (sBF) Blood Flow over the Acupuncture Point Gb21 and the Reference Point (RP)**

<table>
<thead>
<tr>
<th>Measuring point</th>
<th>Gb21</th>
<th>RP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Measuring depth</strong></td>
<td><strong>cBF</strong></td>
<td><strong>sBF</strong></td>
</tr>
<tr>
<td>95% CI AU</td>
<td>13.13–44.44</td>
<td>41.27–72.44</td>
</tr>
</tbody>
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AU, arbitrary units.
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spectroscopy O2C in future controlled trials. Increased blood flow values according to acupuncture or physical exercise may lead to released muscular tension and decreased pain in the affected muscles.

The findings of this study encourage the implementation of the proposed method as a reliable diagnostic tool for various pain syndromes in prevention, therapy, and rehabilitation.

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