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Hyperspectral and Laser Speckle Contrast Imaging for Monitoring the Effect of Epinephrine in Local Anesthetics in Oculoplastic Surgery

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Purpose: Epinephrine is used in local anesthetics to induce vasoconstriction and thus reduce bleeding and prolong the anesthetic effect. Finding the optimal delay between the administration of the anesthetic and skin incision to ensure vasoconstriction and minimize bleeding is important and has recently become the subject of debate. This is the first study to assess blood perfusion and oxygen saturation (sO_2) simultaneously in response to a local anesthetic containing epinephrine in human oculoplastic surgery.

Methods: A local anesthetic consisting of lidocaine and epinephrine (20 mg/ml + 12.5 μg/ml) was injected in the eyelids of 9 subjects undergoing blepharoplasty. The perfusion and sO_2 of the eyelids were monitored using laser speckle contrast imaging and hyperspectral imaging, respectively.

Results: Laser speckle contrast imaging monitoring showed a decrease in perfusion over time centrally at the site of injection. Half-maximum effect was reached after 34 seconds, and full effect after 115 seconds, determined by exponential fitting. The drop in perfusion decreased gradually further away from the injection site and hypoperfusion was less prominent 4 mm from the injection site, with a spatially dependent half-maximum effect of 231 seconds. Hyperspectral imaging showed only a slight decrease in sO_2 of 11 % at the injection site.

Conclusions: The optimal time delay for skin incision in oculoplastic surgery is approximately 2 minutes after the injection of lidocaine with epinephrine. Longer delay does not lead to a further decrease in perfusion. As sO_2 was only slightly reduced after injection, the results indicate that the use of epinephrine is safe in the periocular region.

(*Ophthalmic Plast Reconstr Surg* 2022;38:462–468)

Accepted for publication January 15, 2022.

Supported by the Swedish Government Grant for Clinical Research (ALF), Skåne University Hospital (SUS) Research Grants, Skåne County Council Research Grants, the Lund University Grant for Research Infrastructure, the Department of Research and Development Region Kronoberg, Crown Princess Margaret's Foundation (KMA), the Foundation for the Visually Impaired in the County of Malmöhus and the Swedish Eye Foundation.

The authors have no conflicts of interest to disclose.

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DOI: 10.1097/IOP.0000000000002163

Epinephrine has been used in local anesthetics to reduce perioperative bleeding since 1903.[1] Nevertheless, the optimal waiting time between the injection of local anesthetics containing epinephrine and skin incision, to minimize bleeding, has recently become the subject of debate. Textbooks often recommend a delay of 7 to 10 minutes after administration of local anesthetics.[2] However, McKee et al.[3] reported in 2013 that the lowest hemoglobin level was seen 26 minutes after injecting lidocaine and epinephrine (1 mg/ml + 10 μg/ml) in forearm skin. In another study, they found that the blood loss from the skin of patients undergoing carpal tunnel release surgery showed a significant reduction in bleeding when skin incision was delayed by 30 minutes, compared with 7 minutes.[4] These findings are in strong contrast to clinical experience in the periocular region, and if the findings of McKee et al. are truly representative, this would indicate a need to significantly extend the delay before skin incision. The response to epinephrine may indeed vary in different parts of the body, and as the periocular area is richly vascularized the effect should warrant special considerations. In a study on porcine eyelid flaps, the time from the injection of local anesthetic containing epinephrine to the stabilization of hypoperfusion was found to be 75 seconds.[5] These findings were confirmed in a study on porcine flank being 120 seconds[6] and a study on human forearm being 156 seconds.[7] However, the time to minimum perfusion in response to epinephrine in local anesthetics has not been studied in oculoplastic surgery in humans.

Laser speckle contrast imaging (LSCI) is a commercially available technique for noninvasive monitoring of blood perfusion in skin with high spatial and temporal resolution.[8] LSCI has been used in measurements of perfusion in human periocular flaps and reconstructive surgery with convincing results[9,10] and has also been used to study hypoperfusion in porcine eyelid flaps resulting from vasoconstrictive agents.[5]

Optical spectroscopy is the most common technique used to monitor sO_2 noninvasively. Tissue is exposed by light in the 690 to 1,000 nm spectral range where different molecular components exhibit unique absorption features. Hemoglobin has different absorption coefficients depending on the oxygenation state. The light that is not absorbed, but scattered back from the tissue, is measured, allowing the analysis of tissue oxygenation.[11] Spectroscopic techniques such as near-infrared spectroscopy and pulse oximetry typically have limited spatial resolution needed to identify heterogeneous tissue oxygenation. However, the noninvasive and contact-free technique of hyperspectral imaging (HSI) has recently been introduced in medicine. HSI uses a broad light spectrum with detection between 600 and 1,000 nm, forming a spectral map, from which for

example the blood oxygen saturation (sO_2) can be calculated. HSI has yet not been implemented clinically, but a few studies have demonstrated its applicability in monitoring oxygenation in in humans.[12–16] However, the technique has never been tested in monitoring sO_2 in oculoplastic surgery.

The present study was performed to simultaneously assess the hypoperfusion and sO_2 in response to a local anesthetic containing epinephrine in oculoplastic surgery. LSCI and HSI provided noninvasive mapping of the change in perfusion and sO_2 over time in the eyelid following the injection of lidocaine + epinephrine in preparation for blepharoplasty.

MATERIALS AND METHODS

Ethics. The study was approved by the Swedish Ethical Review Authority. The research adhered to the tenets of the Declaration of Helsinki as amended in 2008. All the subjects were thoroughly informed about the study, and the voluntary nature of participation, and gave their informed written consent.

Subjects. Patients scheduled for blepharoplasty of the upper eyelid with a skin type I to II on the Fitzpatrick scale were eligible for inclusion.[17] Those with any advanced medical condition that could contraindicate the injection of a local anesthetic containing epinephrine, such as ischemic heart disease, heart arrhythmia, lung disease, asthma, or previous adverse reaction to local anesthetics, were excluded. Subjects not able to provide their informed consent, or who did not have the physical or mental ability to cooperate during the local anesthetic procedure were also excluded. Nine subjects, 2 men and 7 women, were included in the study. The median age of the subjects was 58 years (range 46–74 years). All subjects had a skin type II on the Fitzpatrick scale. One subject had type 2 diabetes and hypertension, one was being medically treated for depression, one was taking a blood thinner (Apixaban) due to earlier deep vein thrombosis and 1 had mild cerebral palsy with full mental ability. Two of the subjects were smokers.

Laser Speckle Contrast Imaging. Blood perfusion was monitored using LSCI with a moorFLP-2 blood flow imager (Moor Instruments Ltd, Devon, UK). The tissue is illuminated by an infrared laser at 785 nm, and the interference of the backscattered light creates a speckled pattern. The system then calculates tissue perfusion by analyzing the variations in the speckle pattern. The recording rate is up to 100 fps at full field, and the highest achievable spatial resolution at maximum zoom is 3.9 μm per pixel. The perfusion is integrated over 1 second to optimize signal-to-noise ratio.

Hyperspectral imaging. HSI was used to monitor sO_2. The technique employs a customized hyperspectral camera in the HySpex model series (Norsk Elektro Optikk AS, Oslo, Norway), capable of providing high-resolution maps of sO_2. The camera acquires hyperspectral images by dispersive methods, unlike the commonly used spectral filtering methods. This means that instead of capturing consecutive full 2D images, where each image is filtered to extract a single wavelength, only a narrow line of the 2D image, where each point along that line contains the full spectrum, is captured in each instance. To capture the entire image, this procedure is repeated line-by-line by scanning over the stationary sample until the object under investigation has been covered.

A halogen lamp was used to illuminate the sample with broad-spectrum white light. The camera was aligned so that the line from which the signal was collected was centered on the illumination spot. Using this type of alignment ensures that the sample is evenly illuminated as the illumination profile is comparatively flat at the center. The region of interest was then scanned, and spectra were collected from each point along a single line ~10 cm in length, line-by-line, until the entire periocular region had been covered. Each line generated 640 individual spectra and the spatial resolution was 150 μm.

Experimental Procedure. The patients were prepared for blepharoplasty in the surgical room and rested in the supine position for 10 minutes to allow vital parameters to stabilize. Pulse and oxygen saturation were monitored using a pulse oximeter before and during the study, and were in all cases stable, from the start of the injection and throughout perfusion monitoring. The periocular area was cleaned and disinfected with chlorhexidine (cutaneous solution, chlorhexidine digluconate, 5 mg/ml, Fresenius Kabi AB, Uppsala, Sweden).

The lights were switched off and the windows were covered, and only dim room light was used while performing the LSCI and HSI measurements. The temperature in the room was maintained at about 22°C. A white reference material (Tube Holder, 708131, Mölnlycke, Gothenburg, Sweden) was placed on the forehead of the subject, which was imaged at the same time as the periocular region. After measuring the spectral response of the white reference against a spectrally calibrated surface, the spectral shape of the incoming light could be normalized and further analyzed.

The hyperspectral camera was placed 20 cm directly above the subject, and the LSCI device was placed next to this camera. The subjects were specifically asked not to move during the procedure, and the examiner paid special attention to this, as well as observing whether there were excessive involuntary eye movements.

The local anesthetic solution was preheated to body temperature (37°C) to avoid effects on perfusion due to cooling of the tissue. 1 ml local anesthetic (20 mg/ml lidocaine + 12.5 μg/ml epinephrine, Xylocaine Dental Adrenaline, Dentsply Ltd., York, PA, USA) was injected subcutaneously in the lateral part of the eyelid at a depth of approximately 1 mm. The injection was performed as uniformly as possible by 1 experienced oculoplastic surgeon. A 27-gauge needle (0.4 × 19 mm) was used with 1 entry point of injection, and 1 direction and position of the needle. The OD or OS was randomly infiltrated first. The effect on perfusion and sO_2 was monitored by LSCI and HSI, respectively. The blepharoplasty procedure was then performed outside the study according to standard clinical practice, with supplementary local anesthetics when needed. No sedation or hyaluronidase was used during the procedure.

LSCI and HSI measurements were conducted simultaneously. Baseline measurements were made before injection of the local anesthetic with epinephrine. During the 20 seconds period when the HSI camera was not acquiring data, its light source was shut off to not interfere with the acquisition of LSCI data. During the 10 seconds that the HSI light source was on, the perfusion signal artificially became saturated. Thus, these time frames needed removal during postprocessing. This was performed by setting a threshold value equal to the median perfusion from the entire acquisition, above which data were filtered out. This approach worked well in most cases, but sometimes failed to remove transition frames immediately before or after a period of artificially high perfusion. This resulted in periodic spikes in the data, that ultimately had little effect on the conclusions drawn from the results.

Calculations and Statistical Analysis. The LSCI speckle patterns were analyzed automatically, and the blood perfusion calculated by the software in the system. The results are presented in arbitrary perfusion units.

Blood perfusion was calculated as the percentage of the perfusion in the eyelid just before injection of the anesthetic. The data from each patient were extracted as a spatial average over a region covering the injected tissue with sufficient margin in each time frame. The perfusion curve was then normalized to the initial value to allow comparisons to other measurements.

LSCI and HSI were performed simultaneously. The eyelid with the best focus for HSI was chosen for analysis. The dataset from 1 patient was excluded due to motion artifacts, leaving a total of 8 datasets to be analyzed. Calculations and statistical analysis were performed using MATLAB (The MathWorks Inc. South Natick, MA, USA). As the study population was relatively small, the results are expressed as median values and 95% confidence intervals (CIs).

To determine the oxygen saturation (sO_2) from the HSI data, linear spectral unmixing was applied, as described by Merdasa[18] to each pixel in the measured spectra. In this method, the best linear combination of absorption spectra representing oxygenated (HbO_2) and

deoxygenated (HbR) hemoglobin[11] is determined through non-negative matrix factorization. The linear coefficients (a_{HbO2}, a_{HbR}) are then used to calculate the oxygen saturation using the relation $sO_2 = a_{HbO2}/(a_{HbO2} + a_{HbR})$.

To characterize the rate at which both perfusion and sO_2 decreased, a time-dependent exponential function was fitted to the data:

$$f(t) = A \times \exp\left(-\frac{\tau}{t}\right) + B$$

where A is the initial value at t = 0, τ is the time constant from which the half-life ($t_{1/2}$) is determined ($t_{1/2} = \ln^2 \times \tau$), and B is the *plateau* value. The decrease in perfusion and sO_2 was calculated on group level in a region of interest covering approximately 1 cm^2 centered on the site of injection.

RESULTS

Perfusion, presented as median values with 95% CIs, in the upper eyelids decreased with time (*t*) after injection. It can be seen from Figure 1A that the perfusion reached a plateau at 35 % of its initial value, which was considered to be maximum vasoconstriction. The time to half-maximum (*t*1/2) effect was 34 seconds, and the time to maximum effect was 115 seconds, after which no further decrease in perfusion was observed.

To demonstrate the ability to spatially resolve the perfusion and its evolution over time, the perfusion was analyzed along a line extending 5 mm on either side of the injection site, seen in Figure 2A. The change in perfusion after injection was monitored, and the results presented in Figure 2B. The rate at which the perfusion was reduced by epinephrine was analyzed at 3 distances from the injection site (0, 2, and 4 mm, denoted locations 1–3). An exponential function was fitted to the data collected at each of the locations to obtain the time to reach half the maximum effect of epinephrine (*t*1/2). At the site of the injection (location 1), this was 98 seconds. The time to maximum hypoperfusion increased with increasing distance from the site of injection and was also less pronounced.

Administration of the anesthetic containing epinephrine resulted in only a slight gradual median (95 % CI) reduction in sO_2 over the 7 minutes period during which HSI measurements were made (Fig. 3). The exponential fit yielded a time to half-maximum effect of 205 seconds, indicating that hypoperfusion does not lead to a momentary reduction in sO_2, but a delayed reduction. Furthermore, sO_2 was only reduced by 11%, suggesting that sO_2 is not significantly affected by the decrease in perfusion.

DISCUSSION

To the best of the authors' knowledge, the time to maximum hypoperfusion by epinephrine in local anesthetics has not been studied in oculoplastic surgery in humans. In the present study, LSCI was used to monitor blood perfusion in eyelids, showing that the injection of a local anesthetic containing epinephrine resulted in the stabilization of hypoperfusion after approximately 2 minutes. Our measurements showed a local decrease in perfusion at the site of injection, while the

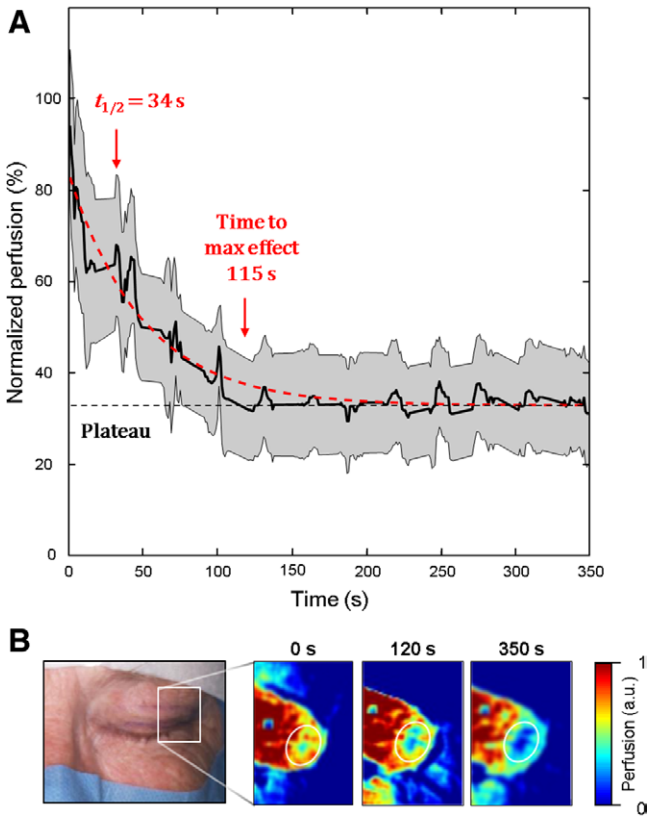

FIG. 1. **A**, Graph showing the decrease in perfusion in 8 eyelids after injection of a local anesthetic containing epinephrine prior to blepharoplasty. Data were analyzed in a ROI surrounding the site of injection. Data are expressed as the percentage (median values and 95% CIs) of the perfusion just before injection. The dashed red curve shows the exponential function fitted to the median, from which the time to half-maximum response of 34 seconds was determined. The time to maximum response was determined to be 115 seconds. The periodic spikes are artifacts arising from the HSI measurements. **B**, A representative example of an eyelid prepared for surgery. The white rectangle indicates the region in which the injection was given. The "heat maps" on the right show the perfusion with time following injection, with the ROI indicated. ROI indicates region of interest.

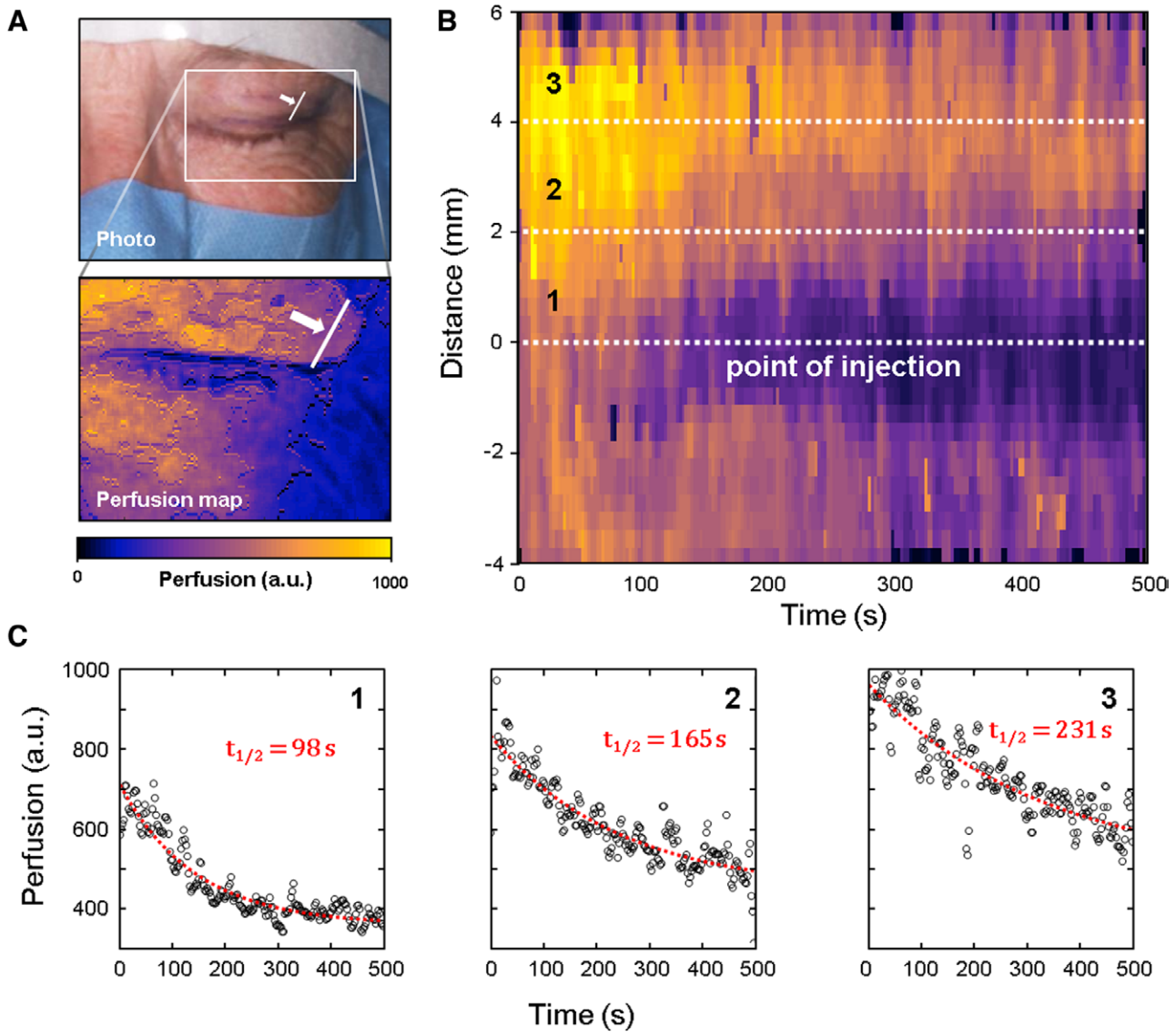

FIG. 2. **A**, Photograph and perfusion map of an eyelid showing the site of injection (arrow). Perfusion was analyzed along a 10-mm line passing through the injection site. **B**, "Heat map" showing the decrease in perfusion over time at increasing distance from the injection site. **C**, Perfusion as a function of time at the 3 locations in a representative example. The decrease in perfusion was fitted with an exponential function from which the time to half-maximum effect was extracted (*t*1/2). Note the longer time required to reach hypoperfusion with increasing distance from the injection site.

surrounding tissue was not affected to the same degree, and we therefore concluded that hypoperfusion at the injection site was mainly due to the effect of epinephrine.

Only one other study has been performed on the effect of epinephrine in human eyelids, in which perioperative bleeding was measured during blepharoplasty. In that study, blood-collecting swabs were weighed after incision, and it was concluded that bleeding was reduced by 75% when the surgical procedure was started 7 minutes after infiltration of lidocaine with epinephrine (20 mg/ml + 12.5 μg/ml).[19] A study on porcine eyelid flaps showed that maximum hypoperfusion was achieved 75 seconds after the administration of epinephrine (10 μg/ml) using laser Doppler flowmetry, LSCI and diffuse reflectance spectroscopy.[5]

The effect of epinephrine has been studied in animals[6,20] and in humans in other parts of the body.[4,21–23] In a study on the forearm of human volunteers, it was found that after injection with lidocaine + epinephrine (20 mg/ml + 12.5 μg/ml) perfusion reached a stable minimal value after 2.6 minutes, when measured with diffuse reflectance spectroscopy.[7] In a similar study using photoacoustic imaging, a technique able to measure sO_2 with high spatial resolution, a local decrease in sO_2 with maximum effect after 2 minutes was concluded.[24] Ghali et al.[21] studied the effects of injections of lidocaine + epinephrine (10 mg/ml + 10 μg/ml) on the arm and face of healthy volunteers with laser Doppler imaging, and found a maximal decrease in cutaneous blood flow after 10 minutes in the arm and 8 minutes in the face. O'Malley studied patients undergoing head and neck surgery, and found a maximum vasoconstrictive effect, measured with laser Doppler flowmetry, between 3 and 4 minutes after injection of lidocaine with epinephrine with varying concentrations (10 mg/ml + 2.5–20 μg/ml).[22] The majority of these studies show that stable vasoconstriction was obtained within 10 minutes of the administration of local anesthetics containing epinephrine,

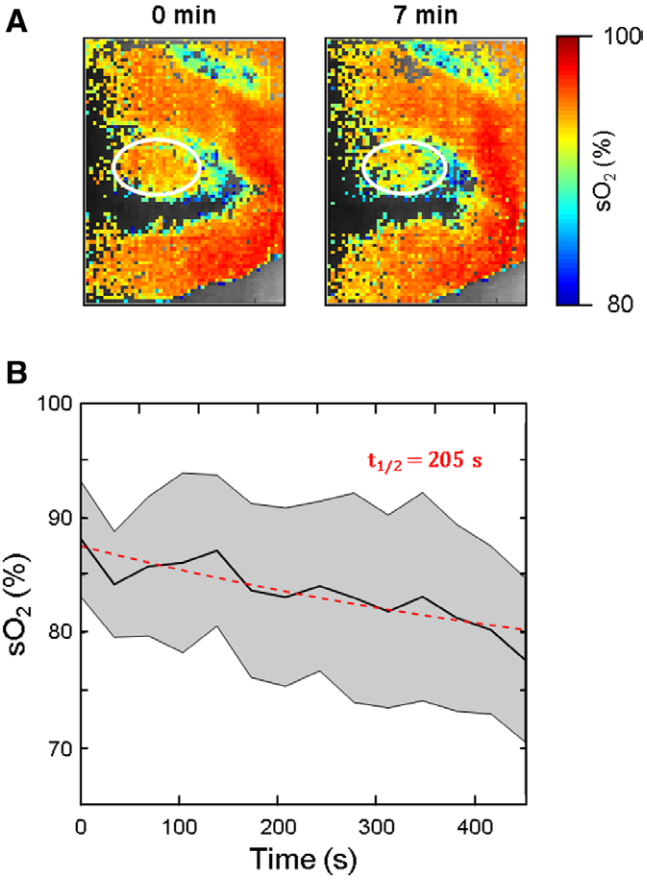

FIG. 3. **A**, A representative example of HSI measurements of sO_2 before (0 minutes) and after injection (7 minutes). The local anesthetic was administered in the region indicated by the white ring. **B**, Graph showing the decrease in sO_2 over time (median values and 95 % CIs). The dashed red curve shows the exponential function fitted to the median, from which the half-maximum response of 205 seconds was extracted. Note the delay in the maximal decrease in sO_2, indicating that epinephrine does not affect sO_2 in the same way as the perfusion.

and in some cases within only 2–3 minutes of administration, which is in line with the results of the present study.

We found a decrease in perfusion to about 35% of the initial value after the administration of epinephrine in the human eyelid. This is in line with similar studies on the effects of epinephrine in humans. Vag et al.[25] studied gingival blood flow with LSCI in human healthy volunteers and found a 50 % decrease after the administration of 2.24 µg epinephrine. Ghali et al.[21] and O'Malley et al. studied the effect of epinephrine in the head and neck, where perfusion was found to decrease to approximately 56% (10 µg/ml) and 25–50% (5–10 µg/ml)[22] of the initial value. This indicates that perfusion decreases to a level at which the perioperative bleeding is reduced, but presumably not to a level that could result in ischemia. This was supported by our measurements with HSI in the present study. Indeed, sO_2 was found to decrease only slightly during the 7 minutes period monitored. This indicates that the remaining perfusion is enough to maintain sufficient sO_2 of the tissue.

The use of local anesthetics containing epinephrine in organs with end arteries such as the ear, nose, penis and digits has long been the subject of debate. Early in the twentieth century, high levels of epinephrine were used, resulting in cases of ischemic necrosis.[26] Today, many studies have concluded a safe use, longer duration of anesthesia, decreased amount of anesthetic, and less bleeding as advantages of local anesthetics with epinephrine in digital blocks.[26–28] In reconstructive surgery, flap survival depends on sufficient perfusion and oxygenation of the tissue. In a recent porcine study, 20% perfusion was found in eyelid flaps after the injection of epinephrine in the flap base, suggesting that the use of epinephrine in eyelid flaps is safe.[5] Indeed, the present study confirms that the eyelid tissue remains well oxygenated despite the local hypoperfusion, indicating the safe use of epinephrine in the periocular region.

HSI is a novel technique for monitoring oxygenation clinically. The advantage is that the technique can be employed with white light and can therefore be used without risk in the periocular region. The preset study, for the first time, shows the capability of using HSI for monitoring sO_2 in oculoplastic surgery. Hitherto, HSI has been developed in animal studies, showing promising effects in monitoring sO_2 in for example irradiated skin in nude mice,[29] dorsal skin flaps in rats,[30] and during shock and resuscitation of pigs.[31] Only a few clinical studies have been performed on humans proving that HSI may be used to map sO_2 in diabetic foot ulcers,[32,33] peripheral arterial disease,[34] skin grafts[13] and during forearm occlusion.[35,36] Zusak used HSI to measure the spatial distribution of skin sO_2 and demonstrated a relationship between skin tissue sO_2 and forearm blood flow in patients with sickle cell disease.[37] The present study supports continued development of HSI for noninvasive monitoring in reconstructive surgery, but further studies are required in the clinical setting.

Measuring blood perfusion in the skin is difficult. Laser-based techniques are sensitive to motion, and it is well known that LSCI is highly sensitive to motion artifacts.[38] Although the patients in the present study were asked to lie as still as possible, it is difficult to avoid small involuntary eye movements that may

influence the perfusion signal. Since the signal is processed by the acquisition software to yield perfusion values from the measured speckle patterns, it is not possible to correct for such motion artifacts during postprocessing.

The simultaneous use of LSCI and HSI in this study was an advantage, allowing direct comparison of perfusion and oxygenation in the same patients, although it was difficult to achieve good focus on both eyelids at once due to the sidelong setup.[39] Therefore, only the data from the best focused eyelid were analyzed. Another problem associated with simultaneous measurements was the disturbances in the LSCI signal caused by the HSI scans, although these regions were easily identified and excluded from the analysis.

Davis et al.[40] performed Monte Carlo simulations on the measurement depth of LSCI which showed that 95 % of the signal originated from the upper 700 μm of the tissue.

The thickness of the eyelid skin in an elderly Caucasian population has been found to vary between 759 μm (medial upper eyelid) and 1,089 μm (lateral upper eyelid).[41] The depth of a blepharoplasty depends on the surgical technique employed, that is, on whether only skin is excised, or the orbicularis oculi muscle is included in the excision, which would result in a deeper incision below the measurement depth of LSCI and HSI.

The measurements in the present study were made on intact skin only. The measurement depth of LSCI is approximately 1 mm, and the time for hemostasis therefore applies only to the effect of epinephrine in the skin. The hemostatic effect on the orbicularis oculi muscle could thus not be deduced in the present study. It would, however, be interesting to perform a study to examine the hemostatic effect of epinephrine deeper in the tissue. This could preferably be performed using a novel imaging technique such as photoacoustic imaging, which has the capability to measure the change in oxygenation down to a depth of 20 mm but has hitherto not been clinically implemented.[24]

A further challenge in measuring perfusion and sO_2 during changes in blood volume in the skin is the so-called "window effect."[23] Vasoconstriction leads to blanching of the skin, which in turn causes a change in the optical properties of the skin and thus the measurement depth.[24,42,43] Blanching of the skin was not clinically assessed in this study. The exact measurement depth was not determined, but the window effect did not appear to have a significant impact on the results, possibly due to the high degree of vascularity in the eyelid[44] and the small measurement depth of the techniques used.[40,42,45] However, the window effect cannot be completely ruled out, and may explain why the perfusion did not decrease to even lower levels in this study.

CONCLUSIONS

In conclusion, this is the first clinical study on the effect of local anesthetic containing epinephrine on perfusion in the periocular region. The results suggest that a waiting time of 2 minutes after injection is sufficient to ensure maximum hypoperfusion before starting surgery, which is in line with common clinical practice, and in contrast to the longer time of 26 minutes suggested by McKee et al.[3] This is also the first time HSI has been applied in clinical practice for noninvasive measurements of oxygenation in oculoplastic surgery. We were thus able to show that sO_2 was only slightly reduced after the injection of epinephrine, despite the more distinct decrease in perfusion compared with baseline. This can probably be explained by the well vascularized eyelid, delivering sufficient blood to oxygenize the tissue, indicating that the use of epinephrine is safe with regard to oxygenation of the periocular region.

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