Mapping the architecture of the temporal artery with photoacoustic imaging for diagnosing giant cell arteritis

Magdalena Numovska, Aboma Merdesa, Björn Hammar, John Albinsson, Ulf Dahlstrand, Magnus Cinthio, Rabi Sheikh, Malin Malmsjo

ARTICLE INFO

Keywords:
Photoacoustic imaging
Giant cell arteritis
Unsupervised spectral analysis
Noninvasive, clinical diagnosis

ABSTRACT

Photoacoustic (PA) imaging is rapidly emerging as a promising clinical diagnostic tool. One of the main applications of PA imaging is to image vascular networks in humans. This relies on the signal obtained from oxygenated and deoxygenated hemoglobin, which limits imaging of the vessel wall itself. Giant cell arteritis (GCA) is a treatable, but potentially sight- and life-threatening disease, in which the artery wall is infiltrated by leukocytes. Early intervention can prevent complications making prompt diagnosis of importance. Temporal artery biopsy is the gold standard for diagnosing GCA. We present an approach to imaging the temporal artery using multispectral PA imaging. Employing minimally supervised spectral analysis, we produce histology-like images where the artery wall is clearly discernible from the lumen and further differentiate between PA spectra from biopsies diagnosed as GCA- and GCA+ in 77 patients.

1. Introduction

Giant cell arteritis (GCA) is the most common form of primary vasculitis in the elderly, presenting with symptoms including headache, jaw claudication, fever and fatigue [1–4]. If left untreated, GCA can result in significant morbidity, including blindness, or death. Early diagnosis and treatment are therefore of utmost importance [2,3]. The diagnosis of GCA is primarily based on clinical evaluation, and is confirmed by temporal artery biopsy, an invasive procedure where a segment of the temporal artery is surgically excised and examined histopathologically to identify inflammatory lesions in the vessel wall. Temporal artery biopsy is associated with several risks, such as injury to the facial nerve or the trigeminal nerve, hemorrhage, wound infection and scarring [5–7]. It would, therefore, be of great advantage to develop a non-invasive method capable of mapping the extracranial vascular network, as well as other blood vessels, in order to improve the diagnostic accuracy, while at the same time avoiding surgery and treatment with potentially harmful side-effects.

Non-invasive methods of diagnosing GCA have been investigated previously, although each has limitations. Color Doppler ultrasonography has insufficient diagnostic accuracy and correct diagnosis depends on the expertise of the operator [8–10]. Magnetic resonance imaging and positron emission tomography with fluordeoxyglucose have also been investigated, but have both been found to have insufficient specificity and sensitivity in diagnosing GCA [11]. These vascular imaging modalities require the use of a contrast agent, or exposure to ionizing radiation, have poor usability or low resolution, or a combination of both [12]. Although these techniques can support the diagnosis of GCA, they cannot replace surgical biopsy and histopathological examination [5,11,13,14]. Hence, there is to date no reliable non-invasive method of diagnosing GCA.

Photoacoustic (PA) imaging is a rapidly developing biomedical imaging technique, that combines the strengths of optical and ultrasound imaging to reveal the molecular composition of tissue at high resolution [15,16]. In PA imaging, energy from non-ionizing laser pulses is absorbed by endogenous chromophores, causing a thermoelastic expansion that generates acoustic waves, which are detected by an ultrasound transducer [17,18]. PA imaging can provide high-resolution three-dimensional (3D) images of the structure and function of tissues, including small blood vessels in animals [19], where in humans some examples involve vessels of the skin [20,21], coronary arteries [22], the radial artery [23,24], the tibialis posterior and dorsalis pedis arteries...
the carotid artery [26,27], the digital arteries [28,29], and the palmar digital arteries [30,31]. In most of these studies, imaging of the vascular network was based on detection of oxygenated and deoxygenated hemoglobin, which strongly absorb light of different wavelengths, thus providing good PA contrast. GCA is an inflammatory disease involving the infiltration of leukocytes into the vessel wall, which leads to a reduced lumen and thus restricted blood flow. The detection of hemoglobin inside the artery is therefore not sufficient for diagnosing GCA, and new methods of characterizing the artery wall are needed.

The aim of the present study was to use multispectral PA imaging for detailed spectral and spatial characterization of the temporal artery exc vivo. Temporal artery biopsies were investigated directly after surgical excision from 77 patients suspected of having GCA. Spectral analysis was used to generate histology-like images from the multispectral PA images of the temporal artery cross-sections, providing detailed spectral and spatial information on the architecture of the artery wall.

2. Materials and methods

2.1. Subjects

Patients with suspected GCA undergoing temporal artery biopsy at the Department of Ophthalmology at Skåne University Hospital, in Lund, Sweden, were included in the study from October 2017 to December 2020. PA imaging was performed ex vivo on the temporal artery specimens before the histopathological examination. Some of the specimens were also examined with high-frequency ultrasound center frequency shift (CFS), as described in our previous study [32].

Subjects were excluded if the biopsy was inconclusive (i.e. uncertainty of the presence of inflammatory changes in the vessel wall or inappropriate biopsy specimen) or if the signal-to-noise ratio in the PA excision from 77 patients suspected of having GCA. Spectral analysis was used to generate histology-like images from the multispectral PA images of the temporal artery cross-sections, providing detailed spectral and spatial information on the architecture of the artery wall.

There was some degree of variability in spectral characteristics between the different temporal artery biopsies. However, the limited study size made subgroup analysis impossible. It would be of great interest to assess the effect of medical conditions and treatments that may have affected the vasculature, such as diabetes, cardio- and cerebrovascular disease or smoking, or the duration of cortisone treatment, on the spectral signature, in the future.

| Table 1 | Patient characteristics. |
| --- | --- |
| | All patients (n = 77) | GCA+ biopsies (n = 36) | GCA- biopsies (n = 41) |
| Gender (female/male) | 57/20 | 25/11 | 32/9 |
| Median age (range) in years | 73 | 75 (61–90) | 72 |
| | (52–90) | (52–83) | |
| GCA treatment with corticosteroids prior to biopsy (n) | 68 | 32 | 36 |
| Eye complications due to GCA (n) | 10 | 10 | 0 |
| Systemic hypertension (n) | 37 | 13 | 24 |
| Diabetes mellitus, type 1 or type 2 (n) | 14 | 6 | 8 |
| Previous cardiovascular events (n) | 6 | 0 | 6 |
| Previous cerebrovascular events (n) | 8 | 5 | 3 |
| Diagnosis of polymyalgia rheumatica prior to temporal artery biopsy (n) | 10 | 3 | 7 |
| Current or previous smokers (n) | 40 | 18 | 22 |

2.2. Photocoustic image acquisition

Temporal artery biopsy was performed at the Department of Ophthalmology in Lund, Sweden, under local anesthesia according to local practice. The biopsy was then placed in balanced saline solution (BSS). The vessel lumen was first rinsed with BSS, using a hypodermic needle and a syringe, to remove blood from the vessel. Sutures were sewn to each end of the biopsy, and it was then placed in a 100 × 70 × 50 mm Perspex container filled with balanced salt solution (BSS), with a layer of black ultrasound-attenuating material in the bottom. The biopsy was held in place by the sutures. The setup and scanning of the temporal arteries are illustrated in Fig. 1a.

PA imaging was performed using a Vevo LAZR-X imaging system (FUJIFILM VisualSonics Inc., Toronto, ON, Canada) equipped with an ultrasonic linear array transducer and a tunable laser with nanosecond pulse duration. The ultrasound transducer (MX400) operates at a central frequency of 30 MHz with a bandwidth of 22–46 MHz, providing axial and lateral resolutions of 50 μm and 110 μm, respectively. The pulsed laser operates at 20 Hz, and the pulse duration is on the order of a few ns. The laser is spectrally tuned between 680 and 970 nm in steps of 5 nm to excite the sample with 59 unique wavelengths, which generates 59 unique PA images, or one multispectral PA image.

Two planar light beams, located on either side of the ultrasound linear array, illuminate the temporal artery biopsy (see Fig. 1a). A 10 mm thick Aquaflex Ultrasound gel pad (Parker Laboratories Inc., Fairfield, CT, USA) with protective plastic film was used to achieve an adequate distance between the laser fibers and the temporal artery, as described previously [33]. The transducer was fixed to an adjustable arm (Mounting Accessory, GCX Corporation, Petaluma, CA, USA) and the holder was driven by a linear stepper motor (VisualSonics Inc., Toronto, ON, Canada) with a step size of 0.5 mm, to allow multispectral PA images to be captured over a larger volume, resulting in a 3D stack of data [34].

A multispectral PA image contains a spectrum with 59 spectral elements in each pixel of the image. The length of the biopsy determined how many cross-sections we could measure, where on average we acquired a little more than three measurements per biopsy. In the collective analysis, we included all measurements from all biopsies, which is why we got a total 259 data points even though the number of patients was 77. When linear scanning was performed to image a volume, the number of spectral components was reduced to twelve in order to reduce the measurement time and the amount of data. Multiple excitation wavelengths provide detailed absorption spectra of different tissue chromophores, allowing a more robust analysis when determining the distribution of chromophores, as well as the identification of multiple chromophores with distinct spectral features. However, care must be exercised when reducing the amount of spectral information to avoid missing any unique spectral features in the data. Therefore, we reduced the spectral resolution while essentially maintaining the spectral range (680–940 nm in steps of 20 nm, excluding 920 nm). Fig. 1b shows the ultrasound image of the artery and the spectral absorption at different wavelengths in the PA images.

2.3. Data import and pre-processing

A flow chart describing the analysis process is shown in Fig. 2a, and examples of the results obtained in Fig. 2b–e. Raw data were exported from VisualSonics Vevo LAB 3.1.0 software and imported into MATLAB v.2017b (MathWorks Inc., Natick, MA, USA) where the analysis was performed. The signal from the artery was obtained by removing the background from the images as described previously [35]. The data were then prepared for spectral analysis, which involved extracting spectra on a pixel-by-pixel basis, or spatial averaging in order to obtain a spectrum from either an entire cross-section of the temporal artery or a smaller region within that cross-section. The spectra were normalized by dividing by the mean PA intensity of the entire image. This allowed...
spectra from one measurement to be compared to spectra from other measurements with less dependence on acquisition parameters such as laser power and signal gain.

2.4. Singular value decomposition

Fig. 2b shows an example of 500 randomly extracted PA spectra from individual pixels in a cross-sectional PA image of an artery similar to Fig. 1b. The red line represents the average PA spectrum, which clearly does not capture the two distinct spectral features at both shorter and longer wavelengths. To determine the number of spectral features contained in the spectral data set, singular value decomposition (SVD) was used to decompose the entire data set \(M\) into a set of eigenvectors \(V\), singular values \(\Sigma\), and linear coefficients \(U\) according to \(M = U\Sigma V^*\), where the asterisk indicates the transpose. The purpose of this is to construct a new coordinate system that better represents the spectral variance contained in the data set. In other words, the data set is sorted such that important spectral features become isolated making them more visible. These spectral features are represented by the basis vectors (or spectral components, SCs) that are ranked from the most to the least important. This means that all the important information in the data set is contained in the first few SCs, while the later ones do not contribute any useful information, and can be regarded as data noise.

The singular values provide a measure of the relative importance of each SC, and are used to determine how many important spectral features there are in the data set. Fig. 2c shows the singular values plotted for each spectral component in black dots. Using the last spectral components (excluding the last few points), which represent the noise in the data, the red dashed line is extrapolated toward the first spectral components in order to determine the “noise floor”. This serves as a reference point when determining the signal-to-noise ratio (SNR) of the singular value of each spectral component. The SNR can thereafter be used to determine how many spectral components are relevant in the description of the main spectral features of the data set (truncation). In the example data set, an SNR = 3 truncates the data set into four relevant spectral components, which are highlighted with red circles in Fig. 2e. For the sake of consistency, we use SNR = 3 in situations where SVD analysis is employed on spectra acquired as an average over one cross-section, and SNR = 2 when a single cross-section is examined pixel-by-pixel. The latter selection is to increase spectral variability.

2.5. Spectral clustering

Each measured spectrum in \(M\) has a linear coefficient for each of the spectral components, which in this example has been reduced to four. Thus, the spectral variance of all 500 spectra should be found within these four vectors. While it is mathematically possible to construct a 4D orthonormal coordinate system, it is challenging to represent this visually. Therefore, the presentation is limited to two dimensions represented by the linear coefficients for the first spectral component (SC1) on the x-axis, and the linear coefficient for the second spectral component (SC2) on the y-axis. Each of the 500 PA spectra is represented by a black dot in Fig. 2d (left). In this plot, the coordinates reveal how much of the two main spectral features each spectrum contains. It can be seen in this plot that the data are grouped in three directions, indicating that the data contain three main spectral features.

The highest density of data points is found closest to the origin of the coordinate system. Taking the average of all the PA spectra associated with the data points within the ROI close to the origin, results in a spectrum similar to the average PA spectrum for the entire data set in Fig. 2b. Repeating this process for the other three ROIs, shaded yellow, blue, and red in Fig. 2d, gives three distinct spectra, two of which (the red and yellow) show similar features to the trends observed in Fig. 2b. The third spectrum (blue) appears to be a combination of the two, which makes sense from the perspective of the new coordinate system since this corresponds to the distribution of data points in the middle of the
upper and lower clusters. In other words, this cluster (blue) primarily shows variance along SC\textsubscript{1}, while the other two exhibit variance in both SC\textsubscript{1} and SC\textsubscript{2}. SVD thus allows a more suitable coordinate system to be created in which different spectral features become more visible.

2.6. Spectral mapping

Instead of manually selecting ROIs as demonstrated in Fig. 2d, we employed k-means clustering, which divides the data points into a set number of clusters based on the relative distances between all the points. Since distance in the new coordinate system reflects spectral (dis)similarity, this allows for the implementation of a classification algorithm that assigns a class to each data point based on spectral features, and thereafter codes the corresponding pixel from which the spectrum was extracted with a unique color (Fig. 2e). It is thus possible to generate new images that show differences in PA spectral features in different colors. Spectral mapping can realistically only be applied when using pixel-by-pixel spectral extraction, while the steps before pixel classification can be applied when spatial averaging has been carried out.

2.7. Histopathological examination

After PA scanning, the temporal artery biopsy specimens were placed in formalin and sent to the pathology laboratory. The biopsies were cut into 3 mm sections, and these were then sectioned into 3 parts, each with a distance of about 200 µm between them. Three micrometer thick sections were cut from each part and stained with hematoxylin-eosin and elastica van Gieson for histological examination.

2.8. Statistical analysis

Calculations and statistical analysis were performed using GraphPad
Prism 9.2 (GraphPad Software Inc., San Diego, CA, USA). An Anderson-Darling test showed normal distribution of the data (p = N.S.). Thereafter, the statistical difference between the spectra in the wavelength range from 680 nm to 970 nm, from the cluster containing GCA- and GCA+ samples, was analyzed using two-way ANOVA with Bonferroni’s multiple comparisons test used for comparisons between groups. Significance was defined as p < 0.05.

3. Results

3.1. Histopathology

Of the 77 patients included in this study, 36 had biopsies with histopathological signs of GCA (GCA+), while 41 biopsies showed no signs of GCA (GCA-). Typical signs of GCA+ were infiltration of inflammatory cells (e.g. leukocytes), multinucleate giant cells, artery lumen reduction and fragmentation of the internal elastic lamina, visualized by elastica van Gieson staining (Fig. 3a).

3.2. The spectral signature

The spectral signature of temporal arteries was extracted as a spatial average over the cross-section of the artery (see schematic inset in Fig. 3b). Spectra from a total of 259 cross-sections were analyzed (a few per patient), each of which was tagged with their corresponding biopsy result, being either GCA- or GCA+. The spectral analysis outlined above and in Fig. 2 yielded five different clusters based on distinct spectral features. The percentages of spectra belonging to a cluster that was histopathologically diagnosed as either GCA- or GCA+ were then determined. We found one cluster containing 27 spectra, of which 74% were GCA-, while another cluster contained 83 spectra, 57% of which were GCA+. The spectra from the clusters containing most GCA- and GCA+ samples were significantly different (p < 0.0001, Fig. 3b).

3.3. Spatially resolved spectra

To obtain a more complete spectral picture of a single artery, multiple PA image stacks were acquired sequentially from different locations along the temporal artery (see schematic inset in Fig. 4c). A single PA spectrum was generated at each location, after which they were stitched together to generate a spatio-spectral heat map (Fig. 4a and b). This provides an image in which the spectral variation along the length of the artery is better visualized, allowing the spectral differences between GCA- and GCA+ arteries to be seen more clearly. There was significant variability in the spectra extracted from different locations along the long axis of the artery, which could potentially reflect the histopathological appearance of so-called skip lesions [36,37].

3.4. Pixel-by-pixel analysis in a cross-section of the artery

To further explore the extent to which the PA spectra may vary spatially, we performed pixel-by-pixel classification analysis of the cross-sectional PA images. Fig. 5 shows representative examples for GCA- and GCA+ arteries, where each pixel has been color-coded based on distinct spectral features. In both examples, the spectral features vary radially from the center, which is expected from an anatomical perspective considering the structures of the artery, such as the lumen, the vessel wall and the adventitia. In the GCA+ sample, the center region is primarily represented by PA spectra of low intensity. This is in contrast to the GCA- sample, where the central region of the artery exhibits a stronger signal and yields a PA spectrum that is visibly different from the corresponding region of the negative sample. These observations agree well with the histological images in Fig. 3a.

3.5. Pixel-by-pixel analysis in a longitudinal section of the artery

Spectral variation is also expected along the entire artery, and a single cross-sectional measurement may not capture all the relevant information. PA imaging enables analysis of the spectral information on a pixel-by-pixel basis in a longitudinal section of the artery. This provides a spatially resolved map of the architecture of the vessel showing the anatomical structures of the artery and the respective spectral features. Fig. 6 shows such examples for a GCA- and a GCA+ artery, together with the spectral features of the different regions of the sample.

4. Discussion

We have shown that photoacoustic imaging, in combination with advanced numerical analysis, can be used to obtain detailed spatially resolved PA spectra from temporal arteries, providing important information on the artery wall. The images produced by the spectral analysis and pixel-classification algorithm show strong resemblance to those observed histopathologically. This is promising for the clinical implementation of PA imaging as it enables visualization of the artery wall architecture. Although the spatial resolution of PA imaging cannot yet compete with that of histopathological examination, the spectral information provided by PA imaging reveals the molecular composition of tissue, surpassing that which can be obtained with histology.

We used 59 excitation wavelengths when acquiring and analyzing the multispectral PA images. Most previous PA imaging studies on human blood vessels [20,21,23,25] have used a limited number of excitation wavelengths in the analysis which may be sufficient, for
example, to assess oxygen saturation, but is only useful if the expected spectral variance is known prior to the measurements. In other words, if the chromophores that may be present in the sample are known, spectral unmixing at a few wavelengths can be utilized to determine the relative abundance of those chromophores. In cases where the spectral variation is unknown, the maximum number of available excitation wavelengths

Fig. 4. Spatio-spectral heat map showing the spectral variation along the length of a GCA- artery (a) and a GCA+ artery (b). The distance along the length of the artery is given on the x-axis, and the color-coded normalized PA intensity on the y-axis. Individual spectra were extracted from four different locations along the length of the GCA- (c) and the GCA+ (d) temporal artery (schematic inset), showing the variability in the spectra along the length of the artery.

Fig. 5. Cross-section of a GCA- sample showing (a) the ultrasound image, (b) color coded image generated from the pixel-classification algorithm in which each color represents a spectral feature that is shown in (c). (d-f) show the same results for a GCA+ sample. The schematic inset in panel (c) illustrates the measurement geometry. The scale bars represent 1 mm.
should be used in the spectral analysis. The only previously reported study on the artery wall, using a larger number of excitation wavelengths (25 in the spectral range between 1130–1250 nm), was performed on the carotid artery after excision from cadavers, which enabled the identification of fat and collagen, which are typical indicators of plaque [22]. The present study is a first study of its kind, mapping the temporal artery with full spectral range, without having any previous guidance of what spectral changes to expect in GCA- and GCA+ arteries.

The presented spectral analysis employed in this study revealed an interesting aspect besides the interesting spectral features of the temporal artery with high spatial resolution. In the analysis outlined in Fig. 2a, pixel-by-pixel analysis was first used to extract the PA spectra, after which spectral analysis was applied and finally the pixels were color-coded based on the spectral features. It should be noted that the spectra were analyzed without considering their spatial coordinates in the PA image. It is only after the spectral analysis that the pixels are color-coded, and it is therefore intriguing that we not only observe gradual spectral changes that correlate with neighboring regions in the image, but also that these reveal patterns that coincide with anatomical structures. The only instruction provided in the spectral analysis is the SNR that should be used to determine how many important spectral changes to expect in GCA- and GCA+ arteries.

GCA. Although the accuracy of the technique will probably be improved in the future, it is probable that PA imaging will have to be combined with clinical parameters obtained from patient examination to ensure diagnostic accuracy. Indeed, GCA is today mainly diagnosed based on temporal artery biopsy examination in combination with clinical parameters [39]. There are currently no specific diagnostic criteria or blood biomarkers for GCA [40,41]. The American College of Rheumatology (ACR) criteria for the classification of GCA [42], and the European League Against Rheumatism (EULAR) recommendations for the management of large vessel vasculitis may assist in diagnosing GCA, but these are intended to distinguish patients with GCA from those with other forms of vasculitis, and cannot be used to diagnose GCA [43,44]. The ACR and EULAR criteria require the fulfillment of 3 of 5 core features: age 50 years or older at onset, new onset of headache, clinical temporal artery abnormality, elevated erythrocyte sedimentation rate (ESR) of at least 50 mm/h and an abnormal temporal artery biopsy [42]. The importance of using clinical parameters is highlighted by the findings of the TABUL study (Temporal Artery Biopsy vs. Ultrasound in Diagnosis of GCA) [13]. One-third of the patients eventually diagnosed as having GCA had neither a positive ultrasound scan nor a positive temporal artery biopsy, emphasizing the importance of assessing clinical indicators to support the diagnosis.

The examination of temporal arteries ex vivo is a necessary step toward the ultimate goal of in vivo diagnostic examinations. However, a number of issues need to be addressed before the technique can be clinically implemented. The impact of absorbing chromophores in circulating blood and in tissue above the artery gives rise to “spectral coloring” and must be reduced. This is a phenomenon that occurs as photons travel deeper into tissue and undergo wavelength-dependent absorption [45]. Spectral coloring was not a problem in the current study, as we imaged the artery without any blood inside, or any tissue above. However, in in vivo measurements, melanin in the epidermis will strongly attenuate the fluence spectrum reaching the artery, which may affect the analysis. However, this mainly becomes an issue when performing supervised analysis methods, such as linear spectral unmixing, since these methods depend on there being a known spectral signature in the measured data. Spectral coloring produces increasingly more distorted spectra deeper into the tissue, which limits spectral unmixing.
The minimally supervised approach to spectral analysis in this study, where the procedure first identifies the relevant spectral features present in the data, and thereafter differentiates them in space, is more resilient to spectral coloring since it focuses on finding any difference, rather than a specific difference. In the present study, the temporal artery was not imaged in vivo because of the unresolved technical challenges of spectral coloring when imaging blood vessels that have an intraluminal signal, from hemoglobin. This generates spectral absorption that is presumably orders of magnitude higher than that from the actual vessel wall. Even though algorithms accounting for spectral coloring with the potential to improve the analysis have been developed, these are not sufficient to enable discrimination of spectral signal of the healthy artery, from that originating from biological inflammatory processes and leukocyte infiltration due to giant cell arteritis.

5. Conclusions

We have described how multispectral PA imaging can be utilized to map the architecture of the temporal artery wall providing detailed spectral information. A minimally supervised spectral analysis approach was described and shown to be useful in identifying and using unique spectral signatures in a sample without a priori knowledge of which chromophores may be present. Although the present results are encouraging, further technical development, including motion tracking and techniques to correct for spectral coloring, are needed to enable in vivo examination. A large clinical trial will then be required before PA imaging can be considered useful as a clinical diagnostic tool for GCA.

Funding information

This study was financed by Skåne County Council Research Grants, the Swedish Government Grant for Clinical Research (ALF), Lund University Grant for Research Infrastructure, Crown Princess Margaret’s Foundation (KMA), Skåne University Hospital (SUS) Research Grants, the Swedish Cancer Foundation, Friends of the Visually Impaired Association in the county of Gavleborg, a project grant from the Swedish Society of Medicine (SLS), the Foundation for the Visually Impaired in the County of Malmöhus, Lund Laser Center Research Grant, Carmen & Bertil Regnerus Foundation, IngaBritt and Arne Lundberg’s Research Foundation and the Swedish Eye Foundation, Cronqvist Foundation, Swedish Medical Association and Lund University grant for Research Infrastructure.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] W. Rahman, F.Z. Rahman, Giant cell (temporal) arteritis: an overview and update, Surv. Ophthalmo. 50 (5) (2005) 415–428.
[2] C. Dejaco, C. Dufner, F. Buttgerit, E.L. Matteson, B. Daugupta, The spectrum of giant cell arteritis and polymyalgia rheumatica: revisiting the concept of the disease, Rheumatol. (Oxf.) 56 (4) (2017) 506–515.
[3] M. Saleh, C. Turesson, M. Englund, P.A. Merkel, A.J. Mohammad, Visual complications in patients with biopsy-proven giant cell arteritis: a population-based study. J. Rheuma 43 (8) (2016) 1599–1565.
[4] A. Soriano, I. Maruho, N. Pipitone, B. Hamilton, S. Masters, B. McNaughton, M. Tellis, M. Guffey Johnson, H.E. Grossniklaus, C.E. Margo, P. Fouli, et al., Frequency of ultrasonography-derived edema of the temporal artery wall in giant cell arteritis: a second meta-analysis, BMC Musculoskelet. Disord. 11 (2010) 44.
[5] E.S. Valluru, J.K. Willman, Clinical photoacoustic imaging of cancer, Ultrasound 35 (4) (2016) 267–280.
[6] V. Ntziachristos, D. Razansky, Molecular imaging by means of multispectral optoacoustic tomography (MSOT), Chem. Rev. 110 (5) (2010) 2783–2794.
[7] Y. Zhang, H. Hong, W. Cai, Photoacoustic imaging, Cold Spring Harb. Protoc. 2011 (9) (2011).
[8] I. Steinberg, D.M. Huland, O. Vermesch, H.E. Frostig, W.S. Tummers, S.S. Gambhir, Photoacoustic clinical imaging, Photoacoustics 14 (2019) 77–96.
[9] S. Jain, H.B. Song, J. Kim, B.J. Lee, R. Managuli, J.H. Kim, J.H. Kim, et al., In vivo photoacoustic imaging of anterior ocular vasculature: a random sample consensus approach, Sci. Rep. 7 (1) (2017) 4318.
[10] H. Zafar, A. Breatnach, H.M. Subhash, M.J. Leary, Linear-array-based photoacoustic imaging of human microcirculation with a range of high frequency transducer probes, J. Biomed. Opt. 20 (5) (2015), 051021.
[11] D. Xu, S. Yang, Y. Wang, Y. Gu, D. Xing, Noninvasive and high-resolving photoacoustic dermoscopy of human skin, Biomed. Opt. Express 7 (6) (2016) 2385–2401.
[12] V. Daiichein, M. Wu, N. De Jong, A.F. van der Steen, G. van Soest, Frequency analysis of the photoacoustic signal generated by coronary atherosclerotic plaque, Ultrasound Med Biol. 42 (8) (2016) 2017–2025.
[13] A. Karlas, J. Reber, G. Diet, D. Boshko, M. Manaspinoupolou, T. Ibrahim, M. Schweiger, F. Hyafil, V. Ntziachristos, Flow-mediated dilatation test using optoacoustic imaging: a proof-of-concept, Biomed. Opt. Express 8 (7) (2017) 3595–3603.
[14] T.-H. Bok, E. Huyi, M.C. Kolios, Preliminary photoacoustic imaging of the human radial artery for simultaneous assessment of red blood cell aggregation and oxygen saturation in vivo, Proc. 2017 IEEE International Ultrasonics Symposium (2017).
[15] A. Taruttis, A.C. Timmermans, P.C. Wouters, M. Kacprowicz, G.M. van Dam, V. Ntziachristos, Optoacoustic imaging of human vascular: feasibility by using a handheld probe, Radiology 281 (1) (2016) 256–263.
[16] P. Kruitenga, A.F. van der Steen, N. De Jong, G. Springeling, J.L. Robertus, A. van den Lant, G. van Soest, Photothermal imaging of carotid artery atherosclerosis, J. Biomed. Opt. 19 (11) (2014), 110504.
[17] A. Dima, V. Ntziachristos, Non-invasive carotid imaging using optoacoustic tomography, Opt. Express 20 (22) (2012) 25044–25057.
[18] P. Hai, Y. Zhou, J. Liang, C. Li, L.V. Wang, Photoacoustic tomography of vascular distribution of oxygen saturation in an ischemia-reperfusion model in humans, Biomed. Opt. Express 12 (4) (2021) 2484–2495.
[19] Y. Matsumoto, Y. Asao, A. Yoshikawa, H. Sekiguchi, M. Takada, M. Furu, S. Saito, M. Aschwanden, S. Imfeld, D. Staub, T. Baldi, U.A. Walker, C.T. Berger, C. Hess, et al., Temporal artery compression sign-a novel ultrasound finding for the diagnosis of giant cell arteritis, Utrasound Med Biol. 35 (2 Suppl S9) (2011) S113–S5.
[20] C. Hauenstein, M. Reinhard, J. Jeiger, M. Markl, A. Hetzel, A. Treszl, P. Vaitl, A. B. A. Eley, Effects of early corticosteroid treatment on magnetic resonance imaging and ultrasonographic findings in giant cell arteritis, Rheumatol. (Oxf.) 51 (11) (2012) 1999–2003.
[21] C. Daikeler, T. Daikeler, The ultrasound compression sign to diagnose temporal giant cell arteritis shows an excellent interobserver agreement, Clin. Exp. Rheuma 33 (2 Suppl S9) (2011).
B. Persson, T. Erlov, R. Sheikh, M. Cinthio, M. Malmsjo, Comparison of photoacoustic imaging and histopathological examination in determining the dimensions of 52 human melanomas and nevi ex vivo, Biomed. Opt. Express 12 (7) (2021) 4097-4114.

[36] R.G. Klein, R.J. Campbell, G.G. Hunder, J.A. Carney, Skip lesions in temporal arteritis, Mayo Clin. Proc. 51 (8) (1976) 504-510.

[37] D.M. Albert, M.C. Ruchman, J.L. Keltner, Skip areas in temporal arteritis, Arch. Ophthalmol. 94 (12) (1976) 2072-2077.

[38] S. Tzoumas, V. Ntziachristos, Spectral unmixing techniques for optoacoustic imaging of tissue pathophysiology, philosophical transactions of the royal society a: mathematical, Phys. Eng. Sci. 375 (2107) (2017), 20170262.

[39] B. Dasgupta, F.A. Borg, N. Hassan, L. Alexander, K. Barraclough, B. Bourke, J. Fulcher, J. Hollywood, A. Hutchings, P. James, V. Kyle, J. Nott, M. Power, A. Samanta, Bee, G. Bhpr Standards, G. Audit Working, BSR and BHPR guidelines for the management of giant cell arteritis, Rheumatol. (Oxf.) 49 (8) (2010) 1594-1597.

[40] J.H. Smith, J.W. Swanson, Giant cell arteritis, Headache 54 (8) (2014) 1273-1289.

[41] T.A. Kermani, J. Schmidt, C.S. Crowson, S.R. Ytterberg, G.G. Hunder, S. Tzoumas, V. Ntziachristos, Spectral unmixing techniques for optoacoustic imaging and temporal arteritis. She is an ophthalmologist at the Department of Ophthalmology, Skåne University Hospital, Lund, Sweden, subspecialized in neuro-ophthalmology.

Magdalena Naumovska was born in Sweden, in 1985. She received her medical degree from Lund University, Lund, in 2010, where she is currently pursuing a Ph.D. degree in photoacoustic imaging and temporal arteritis. She is an ophthalmologist at the Department of Ophthalmology, Skåne University Hospital, Lund, Sweden, subspecialized in neuro-ophthalmology.

Aboma Mersedas received his M.Sc. in engineering physics (Lund University, 2010) focusing on optical detection of malaria infected blood cells without the need for chemical staining. He received his Ph.D. degree in chemical physics (Lund University, 2017) with his thesis topic on super-resolution optical microscopy of functional materials. After a two year post-doc at the Helmholtz-Zentrum Berlin working on spectroscopic characterization of energy materials, he is currently pursuing his research interest in biomedical physics employing a diverse range of spectroscopy and imaging characterization methods at Lund University and Skåne University Hospital.

Rafik Fauci is an Associate Professor and Senior Consultant in ophthalmology at Lund University and Skåne University Hospital. He was born in Visby, Sweden in 1980. He received his medical degree in 2009 and his Ph.D. in 2018 at Lund University, Lund, Sweden. His main areas of research interest are currently ocularplastic surgery, microvascular blood flow, neuro-ophthalmology and photoacoustic imaging. He works clinically as an ophthalmologist, specialized in cataract and vitreoretinal surgery.

John Albinsson received his M.Sc. degree in biomedical engineering and his Ph.D. degree in electrical measurements from Lund University, Lund, Sweden, in 2009 and 2017, respectively. He is currently employed as a research engineer at the Department of Ophthalmology, Lund University. His main research interest pertains to photoacoustic imaging, both concerning measuring and analyzing the data.

Ulf Dahlstrand received his medical degree from Lund University, Lund, in 2009, and his Ph.D. degree 2020, with a focus on the need for better noninvasive techniques for tumor margin delineation, both in the periorbital area as well as on the rest of the skin. He is an ophthalmologist at the Department of Ophthalmology, Skåne University Hospital, Lund, Sweden, specialized in oculoplastic and strabismus surgery.

Magnus Cinthio received his M.Sc. degree in biomedical engineering and his Ph.D. degree in electrical measurements from Lund University, Lund, Sweden, in 1999 and 2004, respectively. In 2010, he joined the Faculty of Engineering, Lund University, as an Associate Professor. He was a visiting researcher at Tohoku University, Sendai, Japan, in 2007, and at Florence University, Florence, Italy, in 2012. In 2013, he joined the Department of Biomedical Engineering, Lund University, as a University Lecturer. His research interests include the longitudinal movement and the resulting intramural shearing of the arterial wall, ultrasonic tissue motion measurements, photoacoustic imaging, as well as arterial, cerebral, and in-
Malin Malmsjö is an internationally recognized expert in oculoplastic surgery, an expertise that she has honored in her role as Professor and Senior Consultant in ophthalmology, focusing on cancer surgery at Skåne University Hospital, Lund, Sweden. She is currently the Head of the Department of Ophthalmology. She has written over 140 scientific publications and book chapters and is also the inventor and patent holder of award-winning medical devices for heart and vascular surgery. Her research in ophthalmology focuses on the development of novel noninvasive imaging techniques for tumor margin delineation and optimizing periorbital cancer surgery by monitoring blood perfusion.