Long-term cerebrovascular reactivity mediated by ozone autohemotherapy: a NIRS study

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***Abstract*—Ozone autohemotherapy is an emerging therapeutic technique. A validated and standard methodology to assess the effect of such therapy is still lacking. We used a near-infrared spectroscopy system (NIRS) to monitor the cerebral oxygenation of 8 subjects (6 neurological) before, during, and after ozone autohemotherapy. The oxygen concentration in brain tissue markedly increased about 1-2 hours after therapy. The time-frequency analysis of the NIRS signals revealed an increasing activity in the LF frequency band related to the vascular autoregulation. This preliminary study showed that NIRS could be useful to show the effects of ozone autohemotherapy at cerebral level, in a long term monitoring.**

***Keywords*—ozone autohemotherapy, near-infrared spectroscopy, cerebrovascular reactivity, time-frequency analysis.**

I. INTRODUCTION

THE autohemotherapy is gaining increasing importance in clinical practice. Recent studies showed that ozone autohemotheraphy could be very useful to treat vascular diseases [1], wounds [2], and to prevent limb ischemia in dialysed subjects [3]. The above referenced studies demonstrated the ozone capabilities of boosting the overall metabolism and, particularly, of enhancing peripheral tissue oxygenation.

Particular attention has been given to the possibility of utilizing ozone in neurology, in order to enhance brain oxygenation [1]. This in accordance to other important clinically studies on the vascular effects in neuropathology, such as the studies about the cerebral chronic venous insufficiency [4]. However, a uniformed and standardized evaluation protocol of such effects on the cerebral tissue is still missing.

Near-infrared spectroscopy (NIRS) is a non-invasive technique to monitor the changes in the brain concentrations of oxygenated (O_2Hb) and reduced (HHb) haemoglobin in real-time. We used NIRS to monitor the long-term effects of ozone autohemotherapy in neurological subjects. We coupled a time analysis to a time-frequency analysis, in order to evaluate the vascular effect of ozone.

II. METHODS

A. Experimental setup

After having signed a written informed consent, 8 subjects (6 neurological and 2 controls) underwent ozone therapy. During the therapy, we performed continuous monitoring by NIRS. The NIRS probe was placed on the patient's forehead 2 cm away from midline and 2 cm above the supraorbital ridge. The patient was asked to rest in supine position. The recordings were made using a NIMO tissue oximeter (Nirox Optoelectronics – EBNeuro, Firenze, Italy) and the sampling rate was set to 2 Hz.

The protocol consisted of *a)* baseline recording (average duration 258±58 s), *b)* blood drawing and ozonization (326± 154 s), *c)* reinjection (1520±804 s), *d)* new base line recording (longer than 2 hours). Two-hundreds and forty grams of blood were drawn from the subjects' antecubital vein and then 240 ml of O_2/O_3 mixture were added. This mixture was composed by O_2 at 50%, with an O_3 concentration equal to 50 µg/ml (M95, Multioxygen, Gorle (BG), Italy). The ozonized blood was then slowly reinjected and NIRS monitoring lasted for about 2 hours.

B. NIRS signal processing

We investigated the acquired signals in 6 different time intervals, lasting 256 s each. These 6 analysis windows were

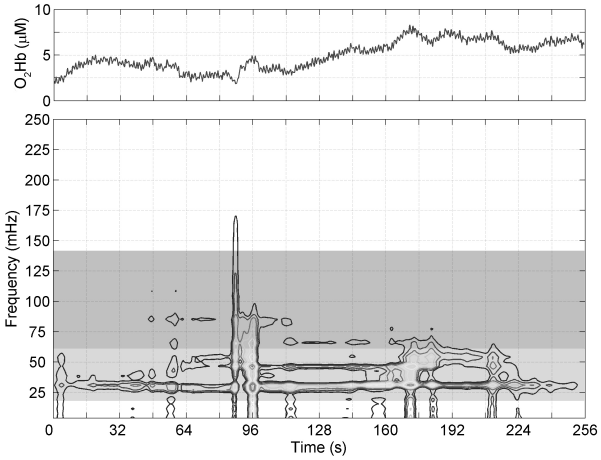

Fig. 1. An example of an observation window and its relative CW distribution. VLF and LF frequency bands are highlighted.

centred on *1)* baseline recording, *2)* blood drawing, *3)* middle of reinjection period, *4)* end (last 256 s) of reinjection, *5)* 1 hour after reinjection and *6)* 2 hours after reinjection.

For each window, on each patient we performed a time domain analysis and a time-frequency domain analysis (sample in fig. 1). In fact, it was already shown that NIRS cerebral signals are characterized by a marked non-stationarity [5]. The time analysis was made by averaging the signal amplitude in each of the observation windows, in order

to analyze the changes in the haemoglobin concentrations in the brain tissue.

The time-frequency analysis was made by means of the Choi-Williams distribution (CW) of the Cohen's class (with $\sigma = 0.5$). From the CWs, we measured the signals' power in two frequency bands: very low frequency (VLF: 20mHz - 60mHz) and low frequency (LF: 60mHz - 140mHz). Also, we measured the total signal power (P_{TOT}) for the O_2Hb and HHb concentrations. Finally, we computed the relative percentage powers P_{VLF}/ P_{TOT} and P_{LF}/ P_{TOT} for the O_2Hb and HHb concentrations, in the 6 analysis windows and for all the 8 subjects. The percentage powers were averaged over the sample population.

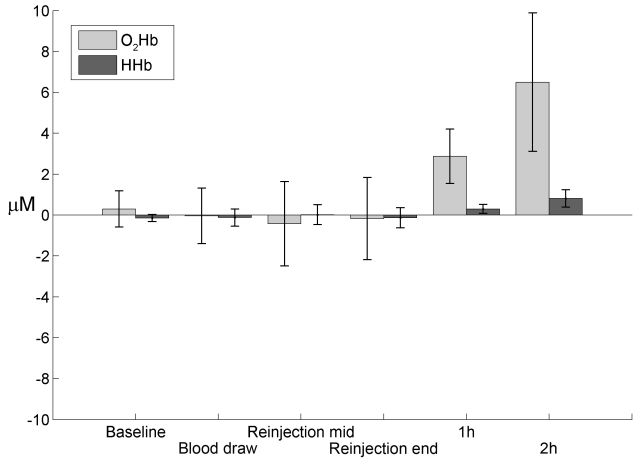

Fig. 2. Mean amplitude of O_2Hb and HHb signals on the 6 observed intervals. A rise of the mean amplitude has been recorded for both signals in the windows following the reinjection.

III. Results and Discussion

The mean values and standard error for each window are reported in fig. 2. A rise of the mean amplitude has been recorded for both signals in the observation windows following the reinjection of ozonized blood. However, the concentration increase is much more pronounced for O_2Hb relative concentration. This overt oxygen concentration increase in the brain tissue has been observed for all the subjects about 1 hour after reinjection of ozonized blood. Therefore, this metabolic boost occurring in the cerebral tissue is a long-term effect, which is in accordance with the subjective sensation reported by the patients undergoing autohemotherapy. Being the oxygen concentration increase far from the reinjection, this effect is not caused by a volumetric effect.

We performed a time-frequency analysis to evidence possible endothelial effect of ozone autohemotherapy. In fact, the spectrum of NIRS signals evidences the VLF and LF frequency bands, which are related to intracranial autoregulation and vasoreactivity, respectively [6]. The mean values and standard errors of the P_{VLF}/ P_{TOT} and P_{LF}/ P_{TOT} are represented in fig. 3. For both O_2Hb and HHb there is an increasing trend in the LF power and a decreasing trend in the VLF power. There is no statistical difference in the increase/decrease in the power levels between the O_2Hb and HHb concentration signals. Overall, therefore, comparing the *baseline* values to the post-reinjection values, we observed an increase in the gap between the P_{VLF}/ P_{TOT} and P_{LF}/ P_{TOT} values.

The results of the time-frequency analysis show that there is a marked vascular reactivity that follows the ozone injection. This reactivity is documented by the increased power in the LF band, which is observable in the spectra of both O_2Hb and HHb. The increased LF activity follows the autohemotherapy, but it lasts for 2 hours (*i.e.* the maximum of our monitoring recording). Such vascular activation lasts for hours, while the oxygen continues increasing (fig. 2). Hence, we believe that this could be the sign of a ozone-induced vascular effect, possibly affecting the microcirculation.

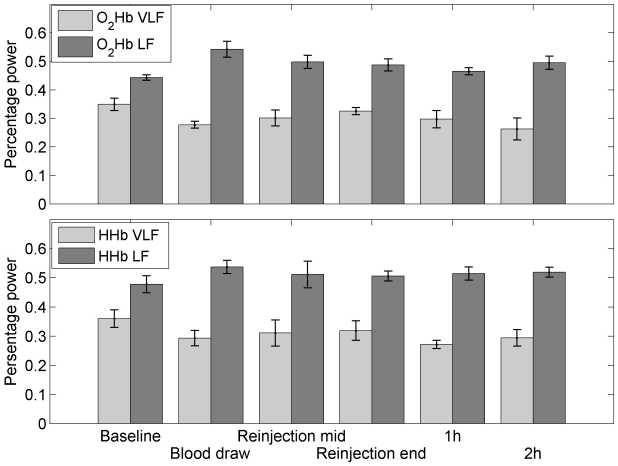

Fig. 3. Mean percentage powers in VLF and LF bands for O_2Hb and HHb relative concentration signals.

In conclusion, we performed a NIRS long-term monitoring of the cerebral effects of ozone autohemotherapy.
Results revealed that ozone triggers a vascular response that increases the metabolic exchange between blood and brain tissue. Even though this study is still preliminary, we believe that this assessment protocol could find its utility in clinical studies on large populations of neurological patients.

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