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Research Article

Hemodynamic Changes in Patients Who Underwent SARS-CoV-2 Infection: From A Quantitative to A Treatment Point of View Using Photobiomodulation

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Abstract

SARS-CoV-2 infection causes innate and acquired immune responses in the host. The immune response is crucial to eliminate the invading virus, however a persistent response might determine hyperinflammation and damage to host tissues. Although it was initially considered as a strictly respiratory disease, SARS-CoV-2 can disseminate to other organs, and symptoms are heterogeneous. In the brain, hyperinflammation and cytokine storm negatively affect cerebral oxygenation. Several symptoms may persist even months after infection. The need to find specific tools to alleviate SARS-CoV-2 post-infection symptoms has become urgent. In more than 10 years' experience in using Near-Infrared Spectroscopy (NIRS) as a functional analysis technique to detect hemodynamic variation in brain areas, our group succeeded in obtaining 3 different hemodynamic states in patients who had COVID-19 disease and underwent neuromodulation treatment to alleviate Neuroinflammation symptoms. From 2015 to 2019, 22 subjects, with different symptoms, aged between 43 and 76 were recruited to assess their cerebrovascular function with NIRS. Between 2021 and 2022, 16 of 22 subjects adhered to NIRS re-evaluation 6-12 months after COVID-19. Subsequently, they underwent rehabilitation protocols for Neuroinflammation with Photobiomodulation. At the end of the treatment cycle (10 sessions, twice a week), they were re-evaluated with NIRS. Data obtained from pre-COVID (T0), post-COVID (T1) and post-treatment (T2) NIRS evaluations were compared in terms of hemodynamic states variation. Results showed that all subjects presented an increase in T2 vascular exchange activity above initial T0. Even after the partition into symptomatology, age, and gender group the average values showed positive results. None of the subjects experienced a worsening after the treatment cycle. The encouraging results obtained from our study continues to show the PBM treatment efficacy, particularly in alleviating post-COVID symptoms and that NIRS technique is needs to be considered more as a promising tool for brain investigation.

Keywords: *SARS-CoV-2; Long COVID; Near-Infrared Spectroscopy; NIRS; Photobiomodulation; Neuromodulation Therapy*

Abbreviations

SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2; CNS: Central Nervous System; NIRS: Near-Infrared Spectroscopy; PBM: Photobiomodulation; NIR: Near Infra-red; COVID-19: Coronavirus disease 2019; IFN: Interferon; IL: Interleukin; CCO: Cytochrome C Oxidase; MeIG: Memory Impairment Group; MoIG: Motor Impairment Group; AIG: Attention Impairment Group

Introduction

In December 2019 the first case of pneumonia caused by an unknown infectious agent were reported in Wuhan, China. The infectious agent was later discovered to be a novel beta coronavirus (β CoV) that was called Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), due to its similarity with SARS-CoV. Coronavirus disease 2019 (COVID-19) has a higher severity and mortality rate in the elderly, in patients with underlying conditions like hypertension and diabetes, and in people with reduced immune activity [1].

Single-stranded RNA viruses, like SARS-CoV-2, colonize their target cells and activate innate immune system by engaging different intracellular pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs), Retinoic acid Inducible Gene-I (RIG-I)-like receptors and Melanoma Differentiation-Associated gene 5 (MDA5). Following PRR activation, molecular signalling cascades culminate in the activation of downstream transcription factors, such as Interferon Regulatory Factors (IRFs) and Nuclear Factor-κB (NF-κB). These transcription factors trigger the initial cellular antiviral defences by inducing the transcriptional activation of type I and III interferons (IFN-I and IFN-III) and interferon-stimulated genes (ISGs), as well as cytokines and chemokines [2].

Both the innate and the adaptive immune response have an essential role in recognizing and eliminating pathogens. The immediate immune response to infection by viruses, bacteria, or other microorganisms involves the mobilization of several cells and molecules including cytokines, transforming growth factor (TGF)β and chemokines. Key cytokines are involved in adaptive immunity (e.g. IL-2 and IL-4), as well as proinflammatory cytokines and interleukins (e.g. IFN-I, -II, and -III; IL-1, IL-6, and IL-17; and TNF-α); and anti-inflammatory cytokines (e.g. IL-10) [2,3].

Although the immune system plays an important role in fighting COVID-19, it may also be harmful. The immune response is crucial to eliminate the invading virus, however a persistent response might also cause massive production of inflammatory cytokines and damage to host tissues. Mechanistically, a stressed or infected cell, through receptor–ligand interactions, activates large numbers of white blood cells, including B cells, T cells, natural killer cells, macrophages, dendritic cells and monocytes. Their involvement leads to the activation of several signalling pathways which can lead to increased cell death, hyperinflammation, and "cytokine storm" [4,5]. Cytokine storm is defined as an unregulated immune response due to an auto-amplified cytokine production. It starts locally post-primary infection and spreads throughout the body via systemic circulation. Initially, the localized response is meant to eliminate the trigger and involves protective mechanisms. If the organ function is not gradually restored, healing occurs with fibrosis resulting in persistent organ malfunction [5,6].

In particular, the rapid and sustained increase in cytokines, chemokines, and other inflammatory cells lead to a disproportionate infiltration of these cells into the alveolar tissue causing lung injury and apoptosis of endothelial cells [7]. Histology studies have revealed that the lung central part is the most affected region, with pneumocyte injury and diffuse alveolar damage, oedema and alveolar haemorrhage, and the presence of interstitial inflammatory infiltrates. During this inflammatory phase involving the lungs, the disease can rapidly progress to severe illness characterized by Acute Respiratory Distress Syndrome (ARDS), a hyperinflammatory state and multi-organ dysfunction. While it was initially regarded as a strictly respiratory illness, the impact of COVID-19 on multiple organs is increasingly recognized. Complement activation, cytokine storm, dysregulated immune responses, coagulation dysfunction, and infiltration of inflammatory cells in SARS-CoV-2 infection can induce the multi-organ failure in these patients. The virus can spread to other organs and induce additional pathological conditions. Kidneys, brain, heart, liver and gut can be affected by SARS-CoV-2 infection, potentially leading to specific disease manifestations [2,8]. Myocarditis and myocardial infarctions have been reported [8,9], as well as thromboembolism and ischemic events, kidney dysfunction [10], gastrointestinal symptoms, Acute Liver Failure (ALF) [8,11] and neurological problems [12].

As far as the brain is concerned, most evidence suggests that immune activation and inflammation in the Central Nervous System (CNS) are the key point to neurological disorders in the acute phase. The most frequent complications observed in patients are confusion, stroke, and neuromuscular disorders. Symptoms such as lack of concentration, headache, sensory disturbances, depression, and psychosis may persist for several months after infection. Overall, the symptoms that remain after infection are called Long COVID [13,14]. Many neuroimmunological diseases, such as encephalopathy, encephalitis, myelitis, and acute disseminated encephalomyelitis (ADEM) have occurred more frequently after infections with SARS-CoV-2, which indicates a parainfectious or postinfectious association. The most likely underlying mechanisms include virus-triggered overactivation of the immune system with hyperinflammation and cytokine storm consequently affecting cerebral oxygenation [14].

Neuroinflammation

Neuroinflammation is a complex innate immune response induced by neural tissue to repair cellular damage, limit infection, or eliminate pathogens. Acute neuroinflammation, characterized by an early and short inflammatory response, is considered neuroprotective and is initiated by the activation of microglia. Prolonged activation is harmful to the Central Nervous System (CNS) [15].

Microglia are cells of the monocyte/macrophage lineage that act as the immune defence system in the CNS. Like other cells in the macrophage lineage, microglia can assume a diversity of phenotypes and retain the capability to shift their function to maintain tissue homeostasis [16,17]. In response to different kinds of neuronal damage, microglia is activated through the binding of the exogenous pathogen-associated molecular pattern to pattern-recognition receptors on their surface. Once activated, they shift their transcriptional profile to produce proinflammatory cytokines including TNF-α, IL-1β, and IL-6, as well as chemokines and reactive oxygen species (ROS) in order to neutralize the infection. In a similar way the astrocytes play a crucial role in regulating innate and adaptive immune response and contribute to the maintenance and permeability of the Blood-Brain Barrier (BBB). Activated astrocytes undergo various molecular and morphological changes depending on the context of stimuli. Inflammatory cytokines such as IL-1β, TNF-α, and interferon (IFN)-γ are major inducers of astrocytic activation. The activation of microglia and astrocytes is a key mediator of the neuroinflammatory response induced via SARS-CoV-2 infection [15]; in particular, microglial cells are considered a major source of cytokine production including interleukins (ILs) and INFs involved in the cytokine storm in the CNS [18].

Cytokine storm is directly correlated with multi-organ failure, lung injury, and other adverse prognoses of severe infection. The brain, however, is more susceptible to the cytokine storm partially due to "microglial priming". Microglial priming is an amplified microglial response in CNS that is more sensitive to minor stimuli, including peripheral cytokines. Consequently, the production of more cytokines and inflammatory mediators disrupts the CNS homeostasis with a major impact on synaptic plasticity and neuronal survival [15].

The overactivation of the immune system with hyperinflammation and cytokine storm affects cerebral oxygenation. The main goal of Near-Infrared Spectroscopy (NIRS) method is to measure changes in the local concentration of oxy- and deoxyhemoglobin as a correlate of functional brain activity.

We took advantage of our 10 years' experience in the NIRS field to determine the effective improvements of neuroinflammatory symptoms after transcranial Photobiomodulation treatment cycle by quantitatively determine the hemodynamic variation rate in each patient in a three-time evaluation period (pre-COVID, post-COVID and post PBM treatment).

Near-infrared spectroscopy (NIRS)

NIRS is a non-invasive monitoring method based on chromophore absorption of near infrared light with the capability of monitoring brain perfusion in the cortical area. Changes in regional tissue oxygenation reflect the delicate balance between oxygen delivery and con-

sumption. Specifically, low-intensity optical radiation measures changes in light absorption by cortical vascular tissue, in order to detect changes in the local concentration of oxy- and deoxyhemoglobin as a correlate of functional brain activity [19,20]. Due to the light diffusion properties of the tissue, a portion of the received light deeply pierce in the tissue structure, where it interacts with chromophores like hemoglobin. The penetration degree and the shape of the probing volume are determined by the distance between source and detector (3 centimetres from each other) and by the optical properties of local tissues [19].

NIRS is divided in 2 different categories: functional Near-Infrared Spectroscopy (fNIRS) and NIRS baseline. fNIRS mainly evaluates the oxygenation of specific brain areas during certain tasks and the results can be displayed in the form of a map over a specific area [21]. When a brain area is activated, neurons need energy and oxygen, that is transported in the cerebral area by blood combined with hemoglobin as HbO_2 [22].

Differently, NIRS baseline measures the oxygenation of the entire cerebral cortical surface at rest, without any action taking place. Precisely for this reason, our group of study used NIRX Sport 8x8 Channel device with detectors and sources placed on Prefrontal-Auditory-Occipital brain areas to assess the cerebrovascular function of resting patient. Spontaneous fluctuations of brain activity exist even in the absence of explicit tasks or stimuli. This phenomenon is detectable in hemoglobin oxygenation signals measured by NIRS for resting state in adults and elderly subjects, and for sleeping state in infants [23].

After NIRS baseline evaluation, patient underwent transcranial Photobiomodulation (PBM) treatment with Cerebro®'s NIR-Infrared device. Several studies have shown that non-invasive brain modulation techniques are effective in reducing Neuroinflammation and restoring brain function.

Photobiomodulation (PBM)

PBM is an innovative therapeutic approach that uses light in the red (with wavelengths usually in the range of 600 to 700 nm) or near-infrared region (780 to 1100 nm), at a relatively low power density to minimize tissue damage. PBM works on the main light-sensitive molecules called chromophores, like Cytochrome C oxidase (CCO). CCO, unit IV of the mitochondrial respiratory chain, appears to play a main role in this process, absorbing the red and near infrared wavelengths. This seems to promote an increase in the availability of electrons, in the mitochondrial membrane potential, in the production of the adenosine triphosphate (ATP) and calcium concentrations (Ca^{2+}). These processes activate several signalling pathways capable of influencing the cellular processes like proliferation, differentiation and inflammation [24,25].The therapeutic effects of PBM have been demonstrated in many studies on neurological diseases [26], peripheral nerve injuries, pain relief [27] and wound healing [28]. To date, no serious adverse events have been reported in the literature following PBM therapy [29]. Due to its specific characteristics, PBM can increase cerebral blood flow (CBF), enhance cellular metabolism, alleviate neuroinflammation symptoms and prevent neurodegeneration [17]. It is able to promote neurogenesis and elicit anti-apoptotic, anti-inflammatory and antioxidant responses. Several studies has demonstrated improvements in cerebral hemodynamics along with increased oxygenation, neurorehabilitation and restoring brain function. Precisely for this reason our research group has developed a PBM neurorehabilitation protocol for cognitive symptoms of Long COVID, in order to increase neuronal metabolism and relieve neuroinflammation. PBM is demonstrated to have positive impact on several other conditions such as stroke, traumatic brain injury [30], Parkinson Disease (PD) [31] and Alzheimer Disease [32]. Although this is an emerging technique and needs further investigation, previous results and those concerning our study are encouraging.

Materials and Methods

Between 2015 and 2019, before the COVID-19 pandemic, 22 patients (12 males and 10 females) aged between 43 and 76 years were recruited by San Celestino Institute in Milan and Polyclinic Giano in Cesena to assess their cerebrovascular function using NIRS.

The heterogeneous sample examined with NIRS is shown in table 1.

Symptoms	Number of subjects
Memory impairment	7
Attention impairment	6
Motor impairment	3
Post-stroke condition	2
Swallowing difficulties	1
Speech impairment	1
Ischaemia	1
Motor and memory impairment	1

Table 1: *Our sample included patients with cognitive, motor and speech impairment.*

All these patients were infected by SARS-CoV-2 during 2021-2022.

16 of 22 subjects accepted to undergo another functional reassessment with NIRS 6-12 months after SARS-CoV-2 infection. Subsequently, they underwent rehabilitation protocols for neuroinflammation with PBM using Cerebro®'s NIR Infrared, a non-invasive medical device. Cerebro®'s NIR Infrared device is a helmet consisting of 256 LEDs emitting at 810 nm and acting on the entire cerebral cortical surface. Rehabilitation program involves 10 sessions, twice a week. At the end of the treatment, patients were re-evaluated with NIRS.

Data obtained from pre-COVID (T0), post-COVID (T1) and post-PBM treatment (T2) NIRS evaluations were divided by category and compared.

NIRS evaluation was assessed with NIRX Sport 8x8 Channel device with detectors and sources placed on Prefrontal-Auditory-Occipital brain areas. The hemodynamic states obtained in T0, T1 and T2 were analyzed with NirsLab Software. Patients underwent transcranial Photobiomodulation treatment with Cerebro®'s NIR-Infrared device. Results were compared and statistically analyzed using Microsoft Office Excel.

Results and Discussion

Comparison of NIRS data obtained from hemodynamic assessment pre-COVID (T0), post-COVID (T1) and post-treatment (T2) showed a marked T2 improvement in patients with impaired T1 cerebral-vascular function. In particular, 13 out of 16 subjects presented an increase in T2 vascular exchange activity above initial T0, the remaining 3 reported a total restoration of initial hemodynamic function (Table 2 and figure 1).

By averaging the values at times T0, T1 and T2 we had a pre-COVID value (T0) of 816.6 (1/cm)/(mol/L), a post-COVID value (T1) of 786.9 (1/cm)/(mol/L) and a post-treatment value (T2) of 828.1 (1/cm)/(mol/L) (Table 2 and figure 2). In percentage terms, there was clear deterioration of 3.64% from pre-COVID (T0) to post-COVID (T1). The results after treatment showed a 1.41% improvement from T0 to T2, and a promising 5.24% increase from T1 to T2.

Age	Gender	Symptoms	Hb (1/cm)/(mo/L) pre-COVID (T0)	Hb (1/cm)/(mol/L) post-COVID (T1)	Hb (1/cm)/(mol/L) post-NIR treatment (T2)
43	M	Memory	820	790	840
55	F	Attention	820	760	830
57	F	Attention	800	780	820
58	M	Motor	820	810	830
60	F	Attention	840	810	830
60	F	Memory	830	790	830
61	F	Memory	800	790	830
62	M	Attention	810	770	830
62	M	Memory	810	780	820
63	M	Motor	810	790	810
64	M	Motor	810	780	820
65	F	Memory	800	770	830
66	M	Memory	835	780	840
68	M	Memory	810	800	820
71	F	Attention	830	820	850
76	M	Attention	820	770	820
Mean			816.6	786.9	828.1

Table 2: *Data from near-infrared spectroscopy (NIRS) evaluation. Age, gender, symptomatology, and hemoglobin evaluation (pre-COVID, post-COVID and post-treatment) are indicated. The mean of value is shown in the table.*

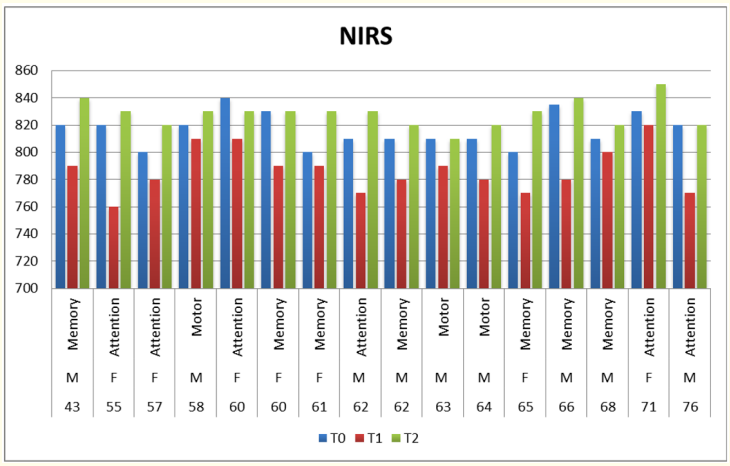

Figure 1: *NIRS data comparison. Age, gender, and symptomatology are shown on the x-axis. On the y-axis the hemoglobin values are indicated. 13 out of 16 subjects presented an increase in T2 vascular exchange activity above initial T0, the remaining 3 reported a total restoration of initial hemodynamic function.*

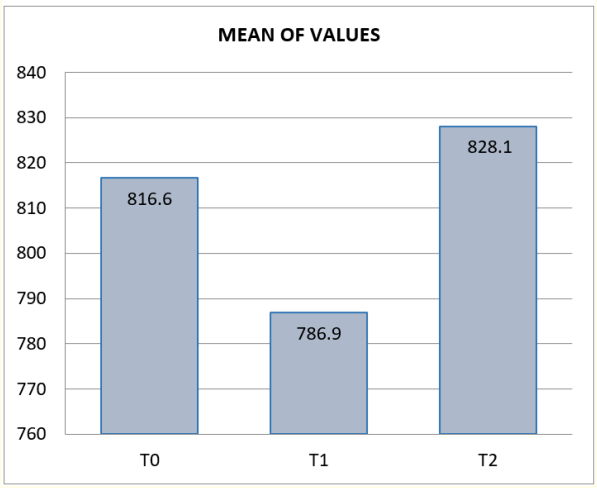

Figure 2: *Mean of Hb values at T0, T1 and T2 times.*

For the first analysis, the 16 subjects were divided according to their symptomatology: memory, motor, and attention impairment. None of the subjects experienced a worsening after the treatment cycle. For each group, only one subject reported a total restoration of hemodynamic function as a result of NIR treatment, the remaining subjects showed a significant increase in T2 compared to initial T0 condition (Figure 3-5).

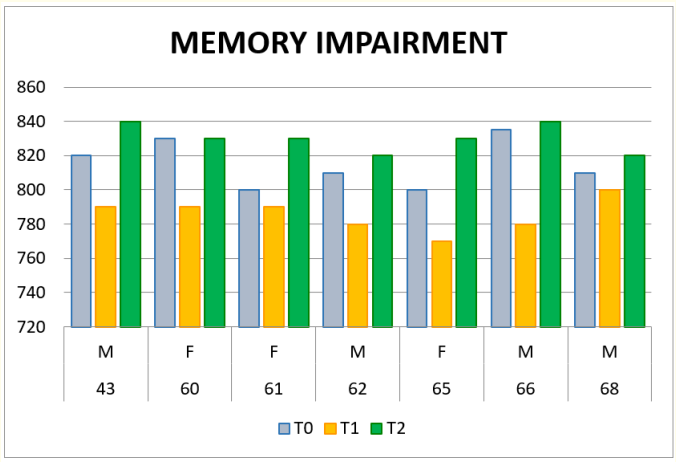

Figure 3: *NIRS data comparison of the 7 subjects with memory impairment. Age and gender are shown on the x-axis. On the y-axis the hemoglobin values are indicated. One subject reported a total restoration of hemodynamic function as a result of NIR treatment, the remaining subjects showed a significant increase in T2 compared to initial T0 condition.*

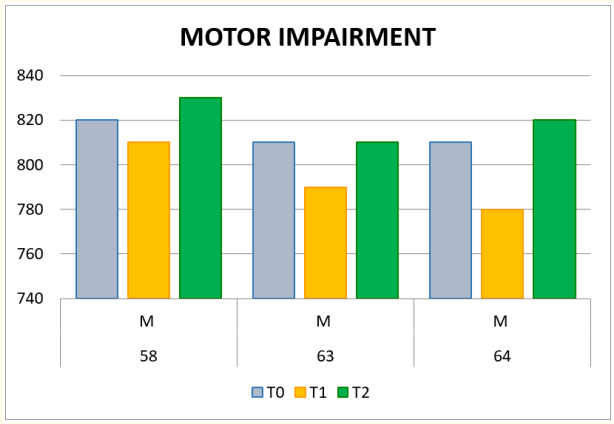

Figure 4: *NIRS data comparison of the 3 subjects with motor impairment. Age and gender are shown on the x-axis. On the y-axis the hemoglobin values are indicated. One subject reported a total restoration of hemodynamic function as a result of NIR treatment, the remaining subjects showed a significant increase in T2 compared to initial T0 condition.*

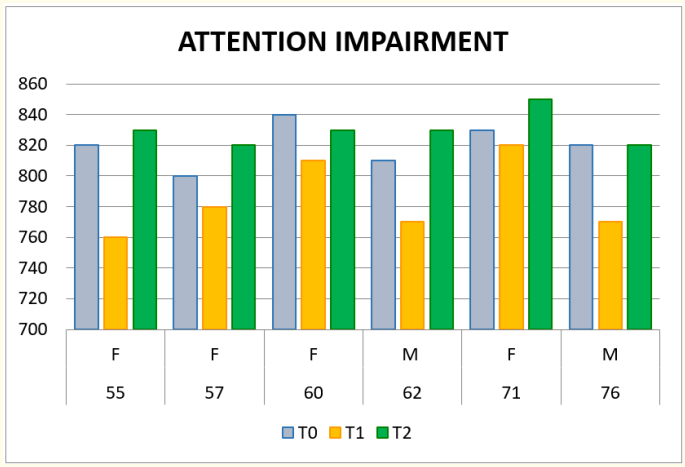

Figure 5: *NIRS data comparison of the 6 subjects with attention impairment. Age and gender are shown on the x-axis. On the y-axis the hemoglobin values are indicated. One subject reported a total restoration of hemodynamic function as a result of NIR treatment, the remaining subjects showed a significant increase in T2 compared to initial T0 condition.*

Subjects were divided by symptomatology (memory, motor and attention) comparing their mean of values. In all the categories, a post-PBM treatment improvement (T2) emerged in comparison with both post-COVID (T1) and pre-COVID condition (T0). The memory impairment group (MeIG) showed a pre-COVID hemoglobin value of 815 and a value of 830 post-treatment, the motor impairment group (MoIG) values went from 813.3 to 820 and in the attention impairment group (AIG) from 820 to 830. In percentage terms, the MeIG showed a 3.59% decrease from T0 to T1, a 1.84% increase from T0 to T2 and a 5.64% increase from T1 to T2. The MoIG showed a 2.46%

reduction from T0 to T1, a 0.82% increase from T0 to T2 and a 3.37% increase from T1 to T2. Similarly, the AIG showed a 4.27% worsening from T0 to T1, a 1.21% raise from T0 to T2 and a 5.73% increase from T1 to T2 (Figure 6-8).

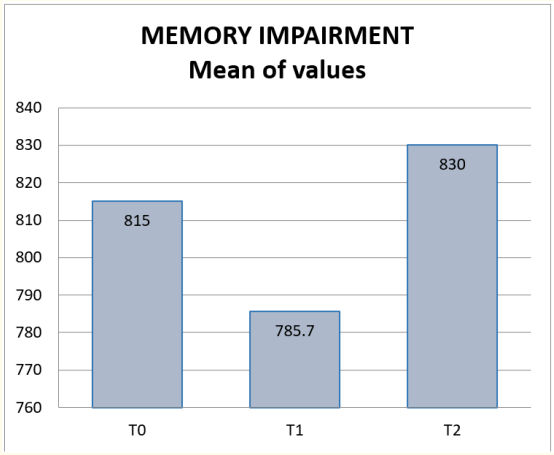

Figure 6: *IComparison between subjects with memory impairment. On the x-axis are shown pre-COVID (T0), post-COVID (T1) and post-PBM treatment (T2). On the y-axis the hemoglobin values are indicated. The average of each value is shown in the histogram.*

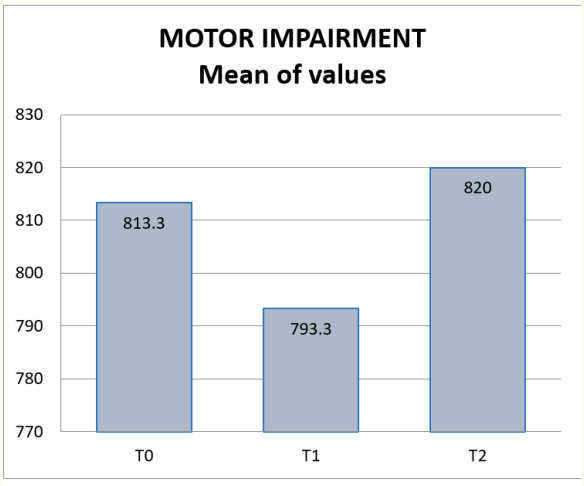

Figure 7: *Comparison between subjects with motor impairment. On the x-axis are shown pre-COVID (T0), post-COVID (T1) and post-PBM treatment (T2). On the y-axis the hemoglobin values are indicated. The average of each value is shown in the histogram.*

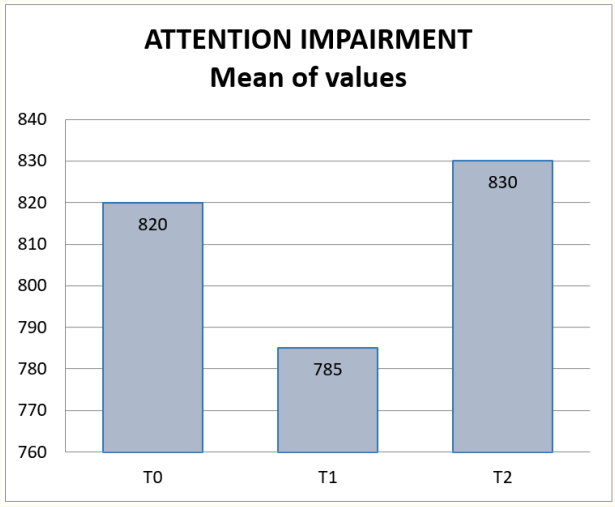

Figure 8: *Comparison between subjects with attention impairment. On the x-axis are shown pre-COVID (T0), post-COVID (T1) and post-PBM treatment (T2). On the y-axis the hemoglobin values are indicated. The average of each value is shown in the histogram.*

The 16 subjects were also divided according to gender, comparing their mean of values. In both groups, data showed a drastic drop in T1 values compared to the pre-COVID (T0) condition. Males started from a T0 value of 816.1 (1/cm)/(mol/L) to 785.6 post-COVID and females from 817.1 to 788.6 (1/cm)/(mol/L). The treatment resulted in values of 825.6 in males and 831.4 in females (Figure 9 and 10). In percentage terms, males showed a 3.73% decrease from T0 to T1, a 1.16% improvement from T0 to T2 and a 5.09% increase from T1 to T2. In a very similar way, females showed a 3.49% worsening from T0 to T1, a 1.75% improvement from T0 to T2 and a 5.43% from T1 to T2 (Figure 11).

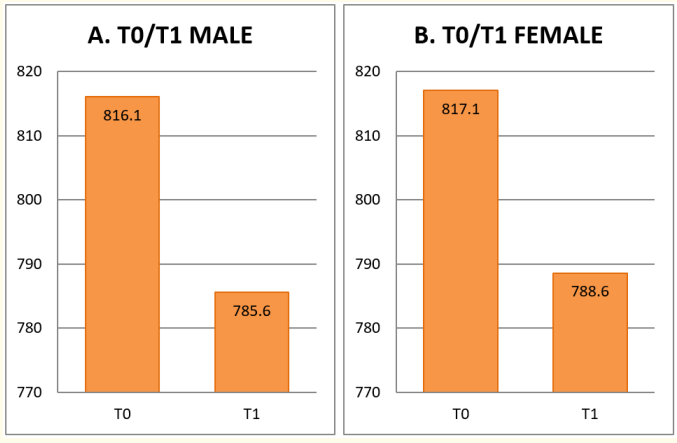

Figure 9: *A. Comparison between male subjects. On the x-axis are shown pre-COVID (T0) and post-COVID (T1). On the y-axis the hemoglobin values are indicated. The average of each value is shown in the histogram. B. Comparison between female subjects. On the x-axis are shown pre-COVID (T0) and post-COVID (T1). On the y-axis the hemoglobin values are indicated. The average of each value is shown in the histogram.*

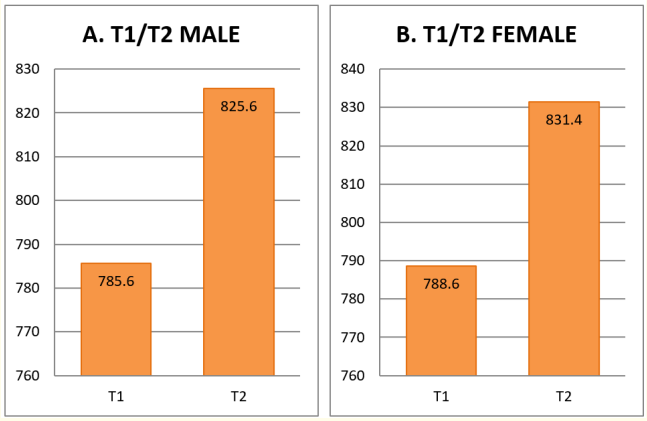

Figure 10: *A. Comparison between male subjects. On the x-axis are shown post-COVID (T1) and post-PBM treatment (T2). On the y-axis the hemoglobin values are indicated. The average of each value is shown in the histogram. B. Comparison between female subjects. On the x-axis are shown post-COVID (T1) and post-PBM treatment (T2). On the y-axis the hemoglobin values are indicated. The average of each value is shown in the histogram.*

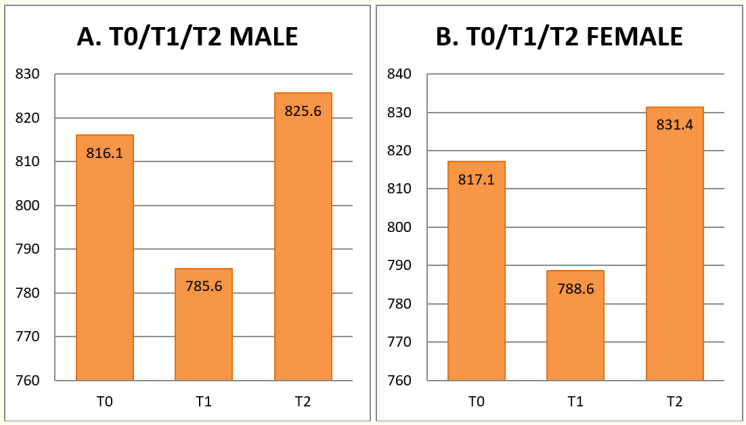

Figure 11: *A. Comparison between male subjects. On the x-axis are shown pre-COVID (T0), post-COVID (T1) and post-PBM treatment (T2). On the y-axis the hemoglobin values are indicated. The average of each value is shown in the histogram. B. Comparison between female subjects. On the x-axis are shown pre-COVID (T0), post-COVID (T1) and post-PBM treatment (T2). On the y-axis the hemoglobin values are indicated. The average of each value is shown in the histogram.*

The last comparison was made by dividing the subjects according to different age groups. The first group (41-60) showed a pre-COVID value (T0) of 821.7 (1/cm)/(mol/L) and a post-COVID value (T1) of 790 (1/cm)/(mol/L). After PBM treatment (T2), a value of 830 was reached (Figure 12). In percentage terms the first group showed a 3.86% worsening from T0 to T1, a 1.01% improvement from T0 to T2

and a 5,06% increase from T1 to T2. The second group (61-80) recorded a pre-COVID (T0) value of 813.5 and a drastic drop to a post-COVID (T1) value of 785. After NIR treatment a clear enhancement was recorded, reaching a value of 827 (Figure 13). In percentage terms the second group showed a 3.50% reduction from T0 to T1, a 1.66% improvement from T0 to T2 and a 5,35% increase from T1 to T2 (Figure 14).

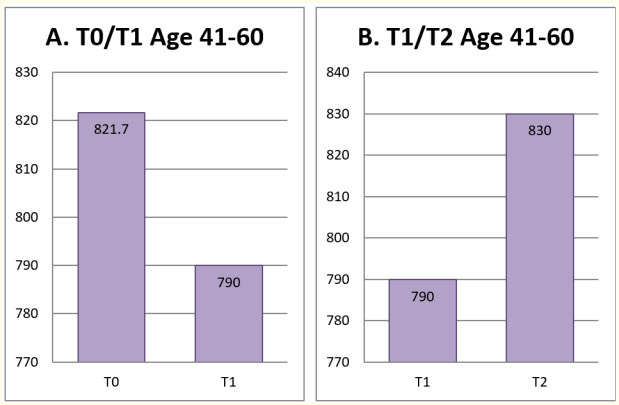

Figure 12: *A. Comparison between subjects from 41 to 60 years old. On the x-axis are shown pre-COVID (T0) and post-COVID (T1). On the y-axis the hemoglobin values are indicated. The average of each value is shown in the histogram. B. Comparison between subjects from 41 to 60 years old. On the x-axis are shown post-COVID (T1) and post-PBM (T2). On the y-axis the hemoglobin values are indicated. The average of each value is shown in the histogram.*

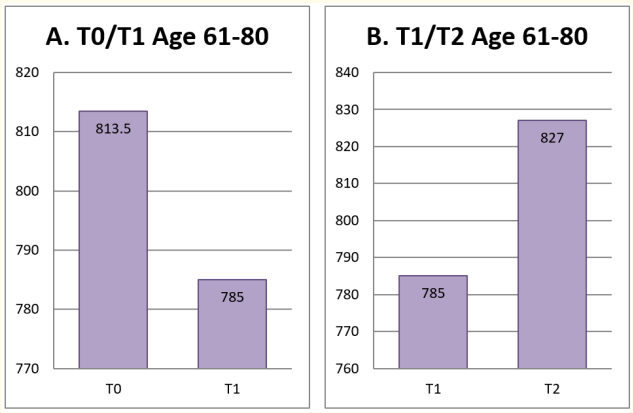

Figure 13: *A. Comparison between subjects from 61 to 80 years old. On the x-axis are shown pre-COVID (T0) and post-COVID (T1). On the y-axis the hemoglobin values are indicated. The average of each value is shown in the histogram. B. Comparison between subjects from 61 to 80 years old. On the x-axis are shown post-COVID (T1) and post-PBM treatment (T2). On the y-axis the hemoglobin values are indicated. The average of each value is shown in the histogram.*

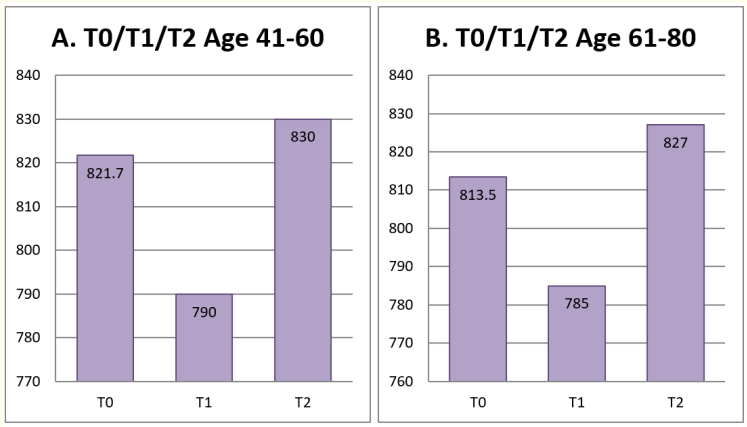

Figure 14: *A. Comparison between subjects from 41 to 60 years old. On the x-axis are shown pre-COVID (T0), post-COVID (T1) and post-PBM treatment (T2). On the y-axis the hemoglobin values are indicated. The average of each value is shown in the histogram. B. Comparison between subjects from 61 to 80 years old. On the x-axis are shown pre-COVID (T0), post-COVID (T1) and post-PBM treatment (T2). On the y-axis the hemoglobin values are indicated. The average of each value is shown in the histogram.*

Conclusion

SARS-CoV-2 causes innate and acquired immune responses in the host. The immune response is crucial to eliminate the invading pathogen, however a persistent response might also be dangerous. Complement activation, cytokine storm, deregulated immune responses, coagulation dysfunction and inflammatory cells infiltration can induce uncontrolled inflammation, cell death and multi-organ failure. While it was initially regarded as a strictly respiratory illness, the impact of COVID-19 on the other system is increasingly recognized and symptoms are heterogeneous. As far as the brain is concerned, virus-triggered overactivation of the immune system with hyperinflammation and cytokine storm, negatively affects cerebral oxygenation. The most common complications observed in patients are confusion, stroke, and neuromuscular disorders. Several symptoms may persist even months after infection. The need to find therapeutic tools to relieve Long COVID symptoms has become increasingly urgent. We took advantage of our 10 years' experience in the NIRS field to determine the effective improvements of neuroinflammatory symptoms after transcranial PBM treatment cycle by quantitatively determine the hemodynamic variation rate in each patient in a three-time evaluation period (T0, T1 and T2).

The heterogeneous sample examined with NIRS included 22 subjects pre infection, 16 of them adhered to the NIRS reassessment post-COVID. Subsequently, they underwent PBM rehabilitation protocols for neuroinflammation using Cerebro®'s NIR Infrared (10 sessions, twice a week). At the end of the treatment cycle, patients were re-evaluated with NIRS. Results were compared and divided by category. Comparison of data obtained from hemodynamic assessment pre-COVID (T0), post-COVID (T1) and post-treatment (T2) showed a marked T2 improvement in patients with impaired T1 cerebral-vascular function. In particular, 13 out 16 subjects presented an increase in T2 vascular exchange activity above initial T0, the remaining 3 reported a total restoration of initial hemodynamic function. In percentage terms, results after treatment showed a 1.41% improvement from T0 to T2 and a 5.24% increase from T1 to T2. None of the subjects experienced a worsening after the treatment cycle. The MeIG recorded a 1.84% improvement from T0 to T2 and a 5.64% increase from T1 to T2. Similarly, the AIG showed a 1.21% raise from T0 to T2 and a 5.73% increase from T1 to T2. Less impactful the MoIG, which registered a 0.82% increase from T0 to T2 and a 3.37% increase from T1 to T2. Male group showed a 1.16% improvement from T0 to

T2 and a 5.09% increase from T1 to T2. Even better, female group recorded a 1.75% improvement from T0 to T2 and a 5.43% from T1 to T2. Subjects aged 41 to 60 showed a 1.01% improvement from T0 to T2 and a 5,06% increase from T1 to T2. Surprisingly, those aged 61 to 80 recorded a 1.66% improvement from T0 to T2 and a promising 5,35% increase from T1 to T2. The encouraging results obtained from our study continues to show the PBM treatment efficacy, particularly in alleviating SARS-CoV-2 infection symptoms and Long COVID Syndrome.

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Conflict of Interest

Dr. Federica Peci declares that she is the administrator of Cerebro SRL, whose NIR helmet was used in this study.

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