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Clinical case series: the effect of MLS® treatment on 30 patients with lumbosacral sciatic pain.

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ABSTRACT

Lumbosacral sciatic pain is a condition associated to spine degeneration which is affecting people daily life and activities. In fact, often pain is not only affecting the lumbar zone, but it is also irradiating down to the lower limb and can influence movement flexibility and general physical function.

Conservative treatment involves the use of anti-inflammatory drugs and different physical therapy approaches. Nevertheless, most severe cases need to be treated with surgical intervention.

This case collection reports on the use of MLS® therapy in 30 cases of lumbosacral sciatic pain, where the goal was not only the management of the pain, but also the improvement in physical function with the aim of reaching a better quality of life for the treated patient.

All the patients were treated with 12 sessions of MLS® therapy.

Patients improved not only in terms of pain management, but also in function and therefore in every day activity comfort, i.e. better sleep and better walk ability. In terms of pain, before the treatment start, average VAS was 8, while at the end of the treatment cycle, average VAS was 1. The treatment was effective in keeping pain controlled between consecutive sessions.

In conclusion, MLS® therapy resulted a useful approach for the treatment of lumbosacral sciatic pain.

INTRODUCTION

Lumbosacral sciatic pain is one of the most common pathologies, affecting 8 people out of 10 in industrialized countries, causing not only patient discomfort but also economic loss due to work absence. This type of pain affects the lower back, irradiating in the lumbar and sacral portion, and sometimes reaching the gluteus and the lower limbs. It is common to have acute episodes of lumbosacral sciatic pain which can evolve, if not treated correctly acting on the causes of the pain, in a chronic condition. Spinal pain in the elderly is also a widespread and serious issue, as it affects general wellbeing and independence of this part of the population [1]. Additionally, considering population aging and the request for a longer active life, lumbosacral sciatic pain is a more and more significant health issue.

Kuslich et al [2] identified intervertebral discs, facet joints, ligaments, fascia, muscles, and nerve root as tissues capable of transmitting pain in the low back. The most common cause for this type of pain is the compression of nerve roots due to degenerated discs or herniated discs, spondylolisthesis or spinal stenosis in the lumbar area. Due to the anatomic conformation of the sciatic nerve, the pain associated to the compression irradiated to the gluteus muscle and down to the lower limb. In some cases, piriformis syndrome can also provoke sciatic pain, due to the compression of the nerve by the inflamed muscle. Piriformis syndrome is a relatively rare condition resulting in severe unilateral isolated buttock pain shooting in nature, non-discogenic in origin. Most of the times, the symptoms are monolateral, at the site of the affected root, and only some few patients report bilater sciatic pain.

Lumbosacral sciatic pain in most of the cases can be classified as neuropathic pain, namely a pain caused by a lesion or disease of the somatosensory system with high clinical incidence [3], whose pathophysiological mechanisms are not yet fully understood [4]. In many cases, beside pain, other symptoms associated to lumbosacral radiculopathy can include numbness, weakness, and loss of reflexes. About one-fifth of patients who report chronic pain have predominantly neuropathic pain [5,6].

Risk factors such as age, smoking, body weight, height, occupational load and mental stress contribute to lumbar radicular pain [7,8]. Current treatments involve the use of anti-inflammatory compounds either as traditional drugs i.e. NSAIDs and steroids in most severe cases. For example, epidural injection of corticosteroids is a commonly used intervention in managing chronic spinal pain [9]. Additionally, natural products, such as botanic extracts, appear to be promising sources of new drugs [10]. Other treatments used for sciatic pain relieve include chiropractic manipulation, acupuncture, therapeutic exercise and physical therapies. In the most severe cases, surgical intervention is required to solve or alleviate the pain acting on the pain cause.

MLS® Laser is a therapeutic device based on knowledge derived from experimental and clinical research which demonstrated the efficacy of the therapy in the treatment of many musculoskeletal diseases [11-14] and allowed to develop advanced treatment protocols. Previously, Viliani [15] used MLS® laserpuncture in the treatment of spinal pain,

based on the fact that, from the clinical point of view the laserpuncture seems equivalent to the classical acupuncture approach [16] and reported positive results in terms of safety and quality of life.

We also reported a case report, collected in our center [17], related to a patient presenting the regression of cervical radiculopathy after laser therapy treatment with MLS®. In this new paper, we have specifically reported our experience in the treatment of lumbosacral sciatic pain, which is a very common condition among our patients. In details, this paper reports on the use and results of MLS® laser treatment in 30 patients presenting lumbosacral sciatic pain, looking at aspects such as pain control and functional recovery related to everyday activities i.e. flexibility, sleep comfort, ect.

MATERIALS AND METHODS

Thirty patients presenting lumbosacral sciatic pain have been enrolled in the Centro Medico Adaptogeno, Bayamón, Stati Uniti. Demographic details (i.e. sex, size, age) were collected. Diagnosis was indicated for all the patients, specifying the status of the condition, i.e. acute, chronic or acute exacerbation of chronic pain. Imaging evaluations, such as CT scan, X-ray or MRI, were recorded whenever available. Additionally, patients were evaluated by the specialist performing the treatment before therapy start.

The treatment consisted in 12 MLS® Laser therapy sessions, performed thrice a week with M6 device (ASA Srl, Arcugnano (VI), Italy). MLS® Laser therapy is cleared by FDA and widely used in clinics. M6 laser is a class IV NIR laser with two synchronised sources, one is a pulsed 905nm laser diode (peak power 25W, duty cycle of 50 %), the second is a continuous 808 nm laser diode (peak radiant power 1.1 W). The two laser beams work simultaneously and synchronously with coincident propagation axes. During the treatment, patients and therapists wore safety glasses to prevent eye damage.

The protocol used for MLS® Laser therapy sessions interested the entire area from L2 to S2, covering from 155 cm^2 to 300 cm^2, according to the specific area to be treated. The MLS® Laser therapy sessions were either dedicated to lumbosacral arthritis or lumbar/sciatic pain specific treatment with the following parameters:

- Lumbar pain: 1 patient treated. Frequency: 700Hz, exposure time: 10 minutes, Intensity 100%. Robotised head was used to apply a total of 1035 J, with a dose 3.5 J/cm^2 in scanning mode. Additionally, the handpiece was used to treat 6 points, for 1'40" each, to apply a total of 315J with a dose 16J/cm^2. The anatomical points for the treatment were identified as follows: the spinal apophysis space from L2 to S2 was divide into three equal parts – top, medial, bottom – and the handpiece treatment was carried out on:
  · 2 points bilaterally above L4
  · 2 points bilaterally above the selected area
  · 2 points bilaterally below the selected area, on gluteus
- Lumbosacral arthritis treatment: 16 patients treated. Frequency: 1500Hz, time: 10 minutes,
  Intensity 100%.
  Robotised head was used to apply a total of 1090 J, with a dose 3.5J/cm^2. Additionally, the handpiece was used to treat 6 points repeated two times, for 43" each, to apply a with a dose 8J/cm^2 each, delivering a total of 285J. The anatomical points for the treatment were the same described above for lumbar pain.
- Sciatic pain treatment: 12 patients treated. Frequency: 900Hz, exposure time: 10 minutes, Intensity 100%. Robotised head was used to apply a total of 1050 J, with a dose 3.5J/cm^2. Additionally, the handpiece was used to treat 7 points, repeated two times, for 43" each, to apply a with a dose 7J/cm^2 each, delivering a total of 320J. The anatomical points for the treatment were the same described above for lumbar pain, with the addition of a point, homolateral to the pain, along the sciatic nerve, on the posterior face of the inferior limb painful point.

Moreover, one patient received 4 treatments using the lumbosacral pain parameters and 8 sessions with sciatic pain parameters.

Trigger points were treated in all patients with the following parameters: Frequency: 10 Hz, time: 23 s, Intensity: 25%. In the trigger point phase, the hand piece was perpendicular to the treated points.

Most of the involved patients were old and affected by multiple systemic pathologies, such as diabetes, therefore they have not discontinued their routine therapeutic regimen during MLS® treatment.

Pain evaluation was performed before and after each laser session using a Visual Analogue Scale (VAS) scale. It is a scale comprising 10 grades, with 10 representing 'unbearable pain' and 0 representing 'no pain'. It is a pain scale commonly used in the medical field, and it was shown to be a reliable and valid measure of pain [18]. Functional evaluation and global assessment were reported by the specialist as final comment to the treatment cycle.

RESULTS

The demographic characteristics of the 30 patients involved are summarized in Table 1, showing a good balance between males and females and confirming that most of the cases were related to people with 65 years or more. The diagnosis was associated to an acute stage in 26 cases, to a chronic stage in 3 cases, while condition stage was not specifically indicated in 1 case. Pain was present bilaterally in 2 patients.

Specific conditions that were reported in the study population were: radiculopathy, which was observed in 3 cases; diabetic neuropathy, present in 2 patients; degenerative disc disease/discogenic pain, that affected 6 patients.

When imaging results were available, they supported the lumbar vertebrae degeneration (i.e. lumbar spondylosis, vertebral space narrowing) status, presence of spondylolisthesis

Table I - Demographic characterization of patient population

Sex	17 Female 13 Males
Size	7 Small 23 Medium
Available evaluation	3 CT 1 X-Ray 6 MRI
Age	≥65 yrs – 20 patients Older than 40 and younger 65 years – 9 patients <40 years – 1 patient

Table II - Results according to the specific treatment

Treatment	Number of patients treated*	Average VAS before first treatment	Average VAS after last treatment
Lumbar pain	1	10	0
Lumbosacral arthritis	16	9.4	1.5
Sciatic pain	12	7.3	0.3

*1 patients received mixed treatment and has been excluded by this table

and canal stenosis and disc degeneration involvement, such as degeneration and protrusion. Lumbar lordosis was also evidenced, which is recognised to be one of the potential causes of sciatic pain. Some patients had also problems related to the cervical spine area, where osteopenia signs suggested a general degeneration of spine health.

Cauda equina inflammation was also observed in one chronic patient, as this was not an anatomical conflict but rather a tissue condition, beside application of laser therapy, the patient was also referred to a neurologist. After the treatment, the patient reported to be improved and the cauda equina inflammation was reduced.

No adverse effects have been observed during the treatment sessions.

Patients presented to the first treatment session with high level of pain. In fact, before the first treatment session, 29/30 patients reported pain scores >5 and average VAS was 8.

After the last treatment session, 28/30 patients reported pain scores < 3 and average VAS was 1.

The results corresponding to the specific type of treatment that was performed (i.e. lumbar pain, lumbosacral arthritis and sciatic pain) are reported in Table 2.

All patients reported immediately an improvement in VAS score after the first treatment. In general, after the treatment sessions it was possible to see a general improvement in VAS respect to the value before the laser treatment. Specifically, improvement was mostly evident comparing the pain before and after each session, reaching a control of pain in between treatment sessions.

A general improvement in flexibility of the treated area and in related anatomical sites (i.e. knee) was highlighted at the end of the treatment sessions by specialists and patients.

Some patients reported additional specific effects due to pain relief, such as better sleep, less muscle spasms and improvement in walking and in an overall increase in physical activity.

DISCUSSION

This case series reports the clinical outcome obtained by the application of MLS® laser therapy on 30 patients presenting lumbosacral and sciatic pain, a very common type of pain that patients complaint and for which they seek medical advice. The results further confirm the safety of MLS® laser therapy in the treatment of patients affected by lumbar and sciatic pain, even concerning complex patients presenting multiple pathologies and degenerative conditions, with no adverse effect that have been reported. Also, a patient with cauda equina inflammation, a severe condition that if left untreated can even degenerate into permanent paraplegia, reported a beneficial effect from MLS® treatment and the inflammation itself improved at the end of the laser therapy cycle.

Regarding pain management, our results indicate that laser therapy with MLS® has been beneficial for these difficult patients, providing immediate relief after the various treatments and maintaining stable pain level in between treatments, with a positive overall effect on quality of life. This is a remarkable result, considering that most of the patients in this collection were affected by degenerative conditions, which naturally evolve in progressive chronicity, and taking into account the age of the affected people which, as it could be expected, are mostly old people over 65 years of age. According to the results obtained by most of our patients including the ones affected by neuropathic conditions, such as diabetic neuropathy, systemic tissue pathology is likely to influence the clinical results and, in those cases, the treatment to control the chronic pathology (i.e. stabilization and containment of blood parameters) is an essential component of the therapeutic plan to obtain good results in terms of pain management too.

Beside pain evaluation, flexibility improvement was a key observation, which facilitated patient independence in routine activity. It is important to note that this flexibility improvement was underlined by the therapist even in patients reporting intermitting VAS improvement, suggesting the presence of an objective improvement, independent from patient's own evaluation. These patients' characteristics also played a role in the durability of the results obtained by the laser therapy, in light of the fact that the condition is degenerative, the mitigation of pain in the time between the consecutive treatment sessions is a very positive results. The most severe cases were anyway suggested to attend some additional treatment sessions in order to further improve and maintain the beneficial effects of MLS® therapy.

In a previous case report [17] treated in our center, we already reported the remarkable result obtained in a cervical radiculopathy patient, in relation to both pain and spinal cord narrowing and with this report on lumbosacral sciatic patients we further confirm the value of MLS® therapy in the treatment of back problems. Despite the mechanism of action of the laser in these pathologies had not been fully elucidated, it is suggested that the anti-inflammatory properties which are typical of MLS® therapy can play a key role in the alleviation of lumbar and sciatica pain. Recently, a publication by Kobiela Ketz et al [19] suggested that the reduction of hypersensitivity mediated by laser treatment in a model of neuropathic pain induced by spinal nerve injury could be exerted by modulating the activation of cells, such as macrophages and microglia components. Additionally, the well-known analgesic effect [20] plays an immediate role in relieving pain providing the patient with a positive feedback straight from the first treatment sessions, allowing patients to immediately gain confidence and improve everyday conditions.

CONCLUSION

The results obtained in our clinical practice and reported in this case series show that MLS® Laser therapy is a useful approach for the treatment of lumbosacral sciatic pain and it is able to promote flexibility. In a useful approach for the treatment of lumbosacral sciatic pain and it is able to promote flexibility. In

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Evaluation of Therapeutic Effects of MLS® on the Outcomes of Flapless Dental Implant Surgery in Posterior Maxilla of Post Menopause Women.

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ABSTRACT

The purpose of this study is to evaluate the therapeutic effects of MLS® on clinical outcomes of flapless dental implants using split mouth study in post menopause women age 50 years or over. This is a retrospective split mouth study involving the analysis of dental records of post-menopause patients undergoing bilateral implant surgery in the posterior maxilla. Sixty-five implants with no augmentative procedures were selected from 26 patients. Flapless implant technique was used for both sides of the jaw. The patients were divided into two groups: 32 implants in the sham group and, 33 implants in the MLS® group . Treatments were performed at day one, day 7 and day 28. Results were analyzed by: Satisfaction, Implant Survival, Visual Analogue Scale (VAS), Periotest, X-ray assessment. MLS® treatment had slightly better outcomes respect to the control side (survival rate: 100.0% and 96.9%), MLS® group had less pain and swelling and better overall satisfaction at one day and one week (*P<0.05). No difference was observed in bleeding and speech impairment. No significant difference in bone resorption at 3 months. After 6 months, bone change in the control group vs the test group was statistically significant [-0.56 (±0.52) vs +0.12 (±0.50), **P<0.05]. No statistical dissimilarity in Periotest Value (PTV). In flapless implant surgery, MLS® treatment is an adjunctive minimally invasive, and innovative method that can deliver a significantly superior early phase satisfaction, minimal bone loss, less pain, less complications, and similar PTV respect to the control side.

INTRODUCTION

Dental implants have become a household name in dentistry in the last twenty years [1]. Mentioning of dental implants one cannot ignore the term osseo-integration. Osseo-integration implies a series of events that happens directly after insertion of a dental implant into the jaw bone, comprises several steps that can be influenced by multiple elements such as patients' health status, implant sites, surgical techniques, systemic and local conditions, and remedy employed [3, 4, 5]. There are many propositions that survival rates of implant practices significantly reduced with age and certain health conditions, for instance post menopause osteoporosis [6,7]. Poor bone quality and quantity, for example those found in post menopause females, may have a negative result on osseointegration [5,7].]. Normally, in initial phase of osseointegration, radiographical imaging can detect a minute quantity of peripheral bone loss adjoining dental implants, and this is accepted as a norm [8].

Literature review of dental implants use in the posterior maxilla region illustrates that flapless surgery could be a practical and foreseeable therapy for dental implant insertion, showing both efficacy and clinical effectiveness with certain reserve [3,4].

Currently a novel technique is emerging for the management of post-operative complications in post-surgical dental implant placement, involving the use of Multiwave-Locked System (MLS®) laser devices. The distinctive attribute of MLS® Laser Therapy is a patented technology based on two synchronized wavelengths, one emitted from continuous source (808 nm) and the other pulsed (905 nm), which produces an efficient laser for handling pain and inflammation, particularly, in post-operative dental implant placement pain [2]. MLS® laser has several therapeutic applications including sprains, muscle tears, tendinitis, brachial neuralgia, craniofacial pain, bursitis, lumbago, arthritis, articular pain, edema, and hematoma [11-17]. MLS® Laser Therapy exerts its effect via anti-inflammatory and analgesic action [2]. These biological effects are exceptionally valuable in managing of complications such as pain in post dental implant surgery. Implants survival is an essential parameter of evaluation and it was recorded as the existence of the implants at the end of the study [4]. To quantify patient satisfaction, the study used McGill questionnaire on a visual analogue scale (VAS) [9].

The Periotest machine was used to establish the firmness of implants (Periotest Values or PTV) at implant laying stage [8]. Digital

Figure 1 - Overall implant and MLS® treatment procedure: Shining MLS® Mphi laser (lower right corner) at control (sham) and study site after flapless implant placement in Posterior Maxilla in Post Menopause Woman (top right corner)

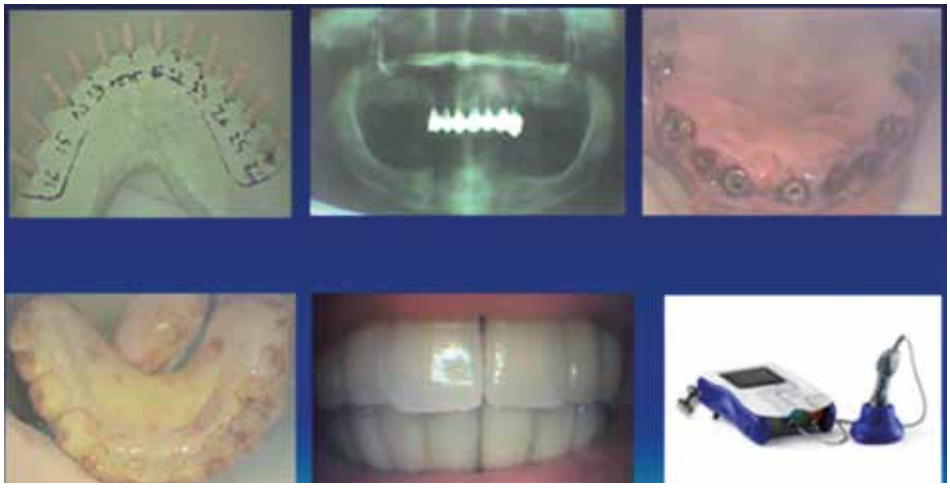
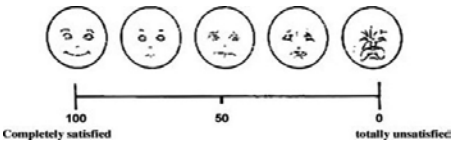


Figure 2 - Pain assessment using Visual Analogue Scale

Figure 3 - A measure of overall satisfaction



x-ray evaluation is the most frequent method for bone quantity or marginal bone height appraisal [4,10]. The purpose of this study was to evaluate the therapeutic effects of MLS® treatment on clinical outcomes of flapless dental implants placed using split mouth study and to measure patients' satisfaction using visual analogue scale and implant survival status in post menopause women age 50 years or over.

MATERIALS AND METHODS

This study is a retrospective split mouth study on the therapeutic effects of MLS® laser on the outcomes of flapless dental implant involving the study of dental records of 26 post-menopause patients undergoing bilateral implant surgery in the posterior maxilla. A total of 65 implants with no augmentative procedures were selected from 26 patients for the study. Flapless implant technique was used for both sides of the jaw. The patients were divided into two groups: the control group had 32 implants and had sham MLS® laser treatment, and the test group consisted of 33 implants treated with MLS® laser at day one, day 7 and day 28. Only those patients with complete dental record were involved in this study. The treatment results were assessed as follows: Satisfaction, Implant Survival, Visual Analogue Scale (VAS), Periotest, X-ray assessment.

MLS® laser therapy was applied with a Mphi D device (ASA S.r.l., Arcugnano (VI) Italy) and using the following protocols: upper posterior teeth region- 24 seconds for each implant site at an intensity of 50% and frequency of 1500 Hz, time used for each application is 6 seconds, and dosage of 3.25 J/cm^2 at 4 locations (buccal, lingual, distal and occlusal aspect of the implant sites). Total energy applied was 6.5 J (Fig. 1). The control group had sham laser treatment and standard management. The degree of postoperative pain and swelling, was recorded for both groups at day one, day 7 (one week) and day 28 (4 weeks).

A. Implant survival

Implants survival was registered as the existence of the implants at the conclusion of the studied interval (28 days).

B. Visual Analogue Scale (VAS) assessment

To determine patient satisfaction, the study used McGill questionnaire on a visual analogue scale (VAS) spans from 1 to 10 of which 1 as having no pain and 10 is the worst pain (Fig. 2). The patients were questioned to register their total satisfaction on sensation of discomfort on a visual-analogue-scale with 0% being totally unsatisfied and 100% being completely satisfied (Fig.3). The total satisfaction VAS scores were recorded for both sides at one day, one week, one month and three months follow up. The VAS scores obtained were analyzed for statistical significance.

C. Periotest values (PTV)

The Periotest device was employed to determine the stability of implants at implant placement stage as well as at subsequent recall appointments at one month and three months. The Periotest's scale varies from -8 to +50. The lesser the Periotest value, the greater is the stability / hampering effect of the test object (tooth or implant). At these assessing visits, healing abutments were connected to those implants which had no

healing posts, and the patient was placed so that the maxilla is in a horizontal position. The Periotest tip was pressed flat right angle to the implant post, and it was positioned as near to the alveolar crest as possible. The implants included in the study were appraised in lateral directions. Acceptable readings were attained only when the device recorded comparable results in three successive readings.

D. X-ray assessment for bone level

A digital periapical X-ray was performed for each implant by means of same holders to measure marginal bone height at the time of surgery, at one month, three months, and six months. The digital X-rays were calibrated to compute the changes in bone height and bone loss. The pertinent implant features such as: site, sizes, design, and other relevant characteristics were recorded. The X-rays were appraised by two experienced and unbiased assessors by means of a grid to determine the dimension of the implant and the proportion of bone loss in millimeters.

E. Statistical analysis

One-way analysis of variance was performed for statistical significance.

RESULTS

The results of this study are found in Table 1. The findings illustrated that MLS® treatment had a slightly better outcome in terms of survival rate (100.0% and 96.9%), respect to the control MLS® treated group reported less pain and swelling [*P<0.05] but no difference in bleeding and speech impairment [P>0.05]. Additionally, MLS® treated group had better overall satisfaction at one day and one week than the control side [*P<0.05]. No significant difference in bone resorption was observed at 3 months [P>0.05]. While, after 6 months, bone change in the control group vs the test group was statistically significant [-0.56 (±0.05) vs +0.12 (±0.02), **P<0.05]. No statistical dissimilarity in Periotest Value (PTV) [P>0.05] was observed.

Table I - Overall results

		Control group Sham laser treated	Test group MLS laser treated	Overall results
Number of implants placed		32	33	65
Number of implants failed		1	0	1
Survival rate (6 months)		96.9%	100.0%	98.5%
Visual Analogue Scale (0 = lowest and 10 = highest)	Pain	3.5 (±1.85)*	1.6 (±1.75)*	2.6 (±1.80)
	Swelling	4.8 (±1.86)*	1.6 (±1.48)*	3.2 (±1.88)
	Bleeding	1.8 (±1.80)	1.4 (±1.74)	1.6 (±1.77)
	Speech impairment	2.7 (±1.30)	2.1 (±1.24)	2.4 (±1.27)
Percentage (%) of Overall Satisfaction (0 = lowest and 100 = highest)	Day 1	71.6 (±7.53)*	95.0 (±8.68)*	83.3 (±8.1)
	Day 7	76.6 (±8.6)*	96.8 (±9.18)*	86.7 (±8.9)
	Day 28	82.2 (±7.4)	97.4 (±8.28)	89.8 (±7.84)
	Day 54 (3 months)	93.6 (±16.4)	98.8 (±17.8)	96.2 (±17.1)
Bone resorption at 3 months in mm (+ = gain and - = loss)		-0.69 (±0.10)	-0.56 (±0.08)	-0.63mm (±0.08)
Bone changes (6 months) in mm (+ = gain and - = loss)		-0.56 (±0.05)**	+0.12 (±0.02)**	-0.22mm (±0.04)
Periotest value [-8 (least mobile) to +20 (most mobile)]	Day 1	-3.40 (±0.84)	-3.68(± 0.89)	-3.574 (±0.87)
	Day 28	-3.40 (±1.18)	-3.62 (±1.54)	-3.51 (±1.36)
	Day 54 (3 months)	-5.18 (±1.46)	-5.48 (±1.56)	-5.33 (±1.51)

Statistical significance: *P<0.05 and **P<0.005

DISCUSSION

This study showed that the application of MLS® treatment after flapless dental implant surgery is a minimal invasive novel technique that can help to reduce pain and swelling after flapless implant placement. This is in line with MLS® laser anti-inflammatory, anti-edema and analgesic effects. Though implant survival rate was better in the laser group as compared to the control counterpart the sample size should be bigger to achieve better power of the study. The outcome of the study also confirmed that MLS® laser can offer an anticipated outcome with greater efficiency and efficacy even in poor quality bone, such as that found in post menopause women.

Visual analogue scale (VAS) is employed extensively for pain measurement, though it is subjective, but continue to be a valuable means for quantifying subjective data, if it is utilized correctly. In this study, it illustrated the greater satisfaction of study group as compared to the control group.

Periotest is useful in calculating the rigidity level of an implant. Though Periotest can identify terminal or unsuccessful implants, it has fundamental disadvantage in recognizing bone quantity in typical osseointegration. Thus, digital imaging seems to be a more reliable method of substantiating peri-implant bone loss. A standardized same designed parallel x-ray holder was used to improve consistency. Even so, digital periapical radiographs along with Periotest apparatus were found to provide the best reliable evaluation of implant condition.

In term of overall satisfaction, as expected the greater difference between the two groups (control and treated) of patient was found in the early stage of the MLS® treatment,

and not at the later stage where the implant wounds were almost healed, therefore, satisfaction rate appeared to be not significantly different. The encouraging results of this study using the state of the art MLS was in line with those found in previous studies [11-17].

CONCLUSION

The application of therapeutic MLS® Laser in dental implant flapless surgery in posterior maxilla of post menopause women, is a an adjunctive minimal invasive, efficacious, and innovative method that can deliver a significantly superior early phase satisfaction, minimal bone loss, less pain, less complications, and similar PTV respect to the control.

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Preliminary data on the efficacy of multi-wave (multi-wavelength) diode laser on bacteria in superficial canine pyoderma.

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INTRODUCTION

Superficial canine pyoderma is a common bacterial skin infection which affects the superficial portion of the hair follicle (Bajwa, 2016; Baumer, 2017). In dogs, this condition is associated with *Staphylococcus pseudintermedius* as predominant pathogen. Other bacteria can be also isolated, such as E. coli and others (Rantala, 2004). Antibiotic resistance is observed for *Staphylococcus pseudintermedius* for the presence of transposon-borne resistance gene which are incorporated into the chromosomal DNA; other mechanism of resistance in Staphylococci and Gram negative bacteria include plasmid-borne resistance genes. Resistance genes can be easily acquired and transferred in the bacterial population. Superficial bacterial folliculitis often tends to become a recurrent condition for inappropriate therapy (drugs used, duration of treatment), lack of diagnostics, development of methicillin resistance in the staphylococci population.

It is therefore of critical importance the application of a correct diagnostic protocol which include cytology, bacterial culture and antibiotic sensitivity evaluation.

Since the acquisition of antimicrobial resistance has become a common condition in the bacteria causing superficial canine pyoderma, therapeutic options usually require novel approaches.

At date, guidelines for antimicrobial therapy for canine superficial bacterial folliculitis include a combination of both topical and systemic antibiotic therapy (Hillier, 2014).

In the initial step of the therapeutic treatment, topical therapy is considered a good approach, when lesions are localized, or in the early stages of generalized superficial bacterial folliculitis (SBF) when lesions are mild. Moreover, local therapy can help prevent recurrence (Bajwa, 2016).

Laser therapy is an alternative treatment, since it has reported to be effective in the management of bacterial dermatitis. Laser therapy may help the homeostasis of host tissue, but beside that, a direct activity on pathogen survival, host inflammatory response and repair mechanisms have been demonstrated.

This study had a dual purpose: the first was to evaluate the ability of Near Infrared (NIR) laser emission (MLS® - Multiwave Locked System laser, Mphi, ASA Srl, Arcugnano, Italy) to decrease the bacterial load present in the skin lesions of the examined dogs, before and after the in vivo laser treatment; the latter was to evaluate the *in vitro* bactericidal activity of the laser treatment on the isolated bacterial strains, after direct irradiation of the bacterial suspension in log phase of growth.

MATERIAL AND METHODS

Experimental design

This preliminary study was carried out on a small group of patients (4 dogs). The study was conceived as a two-step work. In the first step, the bacterial load present in the superficial pyoderma lesions of the dogs was evaluated before and after laser treatment. Moreover, the bacterial agents collected from the superficial pyoderma lesions of the patients were identified. Skin swabs were collected before (T0) and after 30 minutes from multi-wave diode laser (MLS®) treatment (T30). Bacteriological culture, strain identification and CFU counts were performed. On each bacterial strain an evaluation of antibiotic sensitivity was performed by Kirby-Bauer agar disk

diffusion test.
The second step of the study was aimed to evaluate, on four of the previous bacterial isolates, the direct in vitro antimicrobial activity of NIR laser treatment (MLS®). This step was performed by evaluating the CFU count of a standardized bacterial suspension before and after laser irradiation.

Enrollment of patients
Dogs presenting clinical signs of superficial generalized pyoderma were examined by the clinician (Clinica Veterinaria Malpensa, Varese, Italy), who collected swabs from skin lesions to investigate the microbiological flora. Cytological examination of each lesion was also performed and only 4 patients with evidence of Gram positive cocci were included in the study.

Laser source
Details of the laser source have been previously described [Monici et al., 2013]. Briefly, the treatments were performed with a Multiwave Locked System (MLS®) Laser (Mphi, ASA Srl, Arcugnano, Italy), a commercial laser source built in compliance with EC/EU rules, cleared by the US Food and Drug Administration (FDA).
MLS laser is a class IV NIR laser with two synchronized sources (laser diodes). The two sources have different wavelength, peak power and emission mode. The first one is a pulsed 905nm laser diode with 25 W peak optical power. The pulse frequency may be varied in the range 1-2000 Hz, thus varying the average power delivered to the tissue. The second laser diode (808nm) may operate in continuous (power 1.1 W) or frequenced (repetition rate 1-2000 Hz, 550mW mean optical power) mode, with a 50% duty ratio independently of the repetition rate. The two sources emit radiation synchronously and the propagation axes of the two laser beams are coincident.

In vivo laser treatment and skin swabs collection
After collection of skin swabs (T0), each patient was treated by MLS® laser, using the specific program "contaminated wounds", with a radiant exposure of 2.05J/cm^2. The laser exposure in each of the 6 treated points was of 6 seconds. After 30 minutes from the laser exposure, other swabs were collected from the skin lesions for the microbiological cultures. All swabs were maintained at 4°C and conferred to the Infectious Diseases laboratory of the Department of Veterinary Science, University of Parma, within 24 hours.

Evaluation of antimicrobial activity of in vivo MLS® laser treatment
Each swab was vortexed in 1ml of sterile saline and this suspension further diluted 1:10 and 1:100. One hundred microliters of each dilution were plated on Columbia blood agar with 5% of bovine erythrocytes and MacConkey agar and incubated overnight at 37°C. After incubation, bacterial growth was evaluated and colonies were isolated and amplified when necessary. Identification of bacterial strains was based on colony morphology, Gram staining, catalase and oxidase reactions. Species identification was carried out using the API biochemical test system (bioMérieux, France), as well as conventional biochemical tests (Markey BK, 2013). Antimicrobial susceptibility test was performed by agar disk diffusion methods (Bauwe AW, 1966) on each isolated bacterial strain, according with the CLSI guidelines (Clinical Laboratory Standards Institute, 2015). Tested antibiotics were selected on the basis of the clinical use in the treatment of canine pyoderma. This list included Amikacin; Amoxicillin + Clavulanic acid; Ampicillin; Cephalexin; Cefovecin; Clindamycin; Chloramphenicol; Doxycycline; Enrofloxacin; Imipenem; Marbofloxacin; Oxytetracycline; Oxacillin; Sulfamethoxazole + Trimethoprim; Rifaximin; Rifampin.

To determine the antimicrobial activity of the MLS® laser treatment in vivo, samples collected before and after treatment were compared. For each sample and each isolated bacterial strain, CFU were counted.

Evaluation of antimicrobial activity of direct in vitro MLS® laser treatment
To evaluate the direct bactericidal activity of the MLS® laser treatment, in vitro tests were performed. Briefly, four of the isolated bacterial strains were inoculated in sterile Mueller Hinton Broth and incubated overnight at 37°C. The bacterial suspension was centrifuged 20 minutes at 2000 rpm and 4°C, then the pellet resuspended in phosphate buffer. The turbidity of the bacterial suspension was immediately measured and adjusted by spectrophotometry. At 600 nm absorbance, the OD range 0.08-0.13 was considered to correspond to a bacterial concentration of 108CFU/ml. The obtained suspension was further diluted 1:200 in phosphate buffer to obtain a final bacterial concentration of 5*105CFU/ml. Then, 500µl of the bacterial suspension were transferred in a 24 wells plate, according to Tab. 1. A black paper was placed between contiguous wells to avoid the transmission of laser radiation through the walls .
The bacterial suspensions were irradiated with two different exposure times (6 and 41 sec) with a radiant exposure of 2.05 J/cm^2. The laser source was placed in the biosafety cabinet and fixed by a mechanical arm, which maintained the laser source from a vertical distance of about 0.5 cm from the top of the plate well containing the bacterial suspension.
After the treatment, plates were incubated at room temperature for two different period (5 and 30 min). These incubation times were selected to understand if the direct application of laser treatment required some time to cause bacteria killing, depending on the bacterial structure and/or the parameters used in laser irradiation.

Then, for each incubation time, 10 µl of the irradiated bacterial suspension and its 1:10 dilution were plated on Mueller Hinton agar plates. Sterility and growth controls were also diluted and plated in the same way. Agar plates were incubated at 37°C overnight, then bacterial colonies (CFU) were counted. Each test was performed in triplicates and three independent experiments.

Table I - Scheme of the plate used for one experiment for the determination of the direct antimicrobial activity of MLS® laser on different bacterial strains. Each test was performed in three replicates and three independent experiments.

					Sterility control
	1st replicate		3rd replicate		1st growth control
		2nd replicate			2nd growth control
					3rd growth control

Table II - Bacterial isolates and CFU count before and after MLS® laser treatment.

		PRE- TREATMENT	POST- TREATMENT	Pre-treatment Total CFU count	Post-treatment Total CFU count	Overall inhibition percentage
Case 1	*Staphylococcus pseudintermedius*	59.8*10⁴ CFU/ml	3*10⁴ CFU/ml	**59,8*10⁴ CFU/ml**	**3*10⁴ CFU/ml**	**95%**
Case 2	*Staphylococcus pseudintermedius*	0,28*10⁴ CFU/ml	0.22*10⁴ CFU/ml	**6*10⁴ CFU/ml**	**0,56*10⁴ CFU/ml**	**90,6%**
	Proteus mirabilis swarming	4.5*10⁴ CFU/ml	0.035*10⁴ CFU/ml			
	Proteus mirabilis not swarming	1.1*10⁴ CFU/ml	0.28*10⁴ CFU/ml			
	Escherichia coli	0.125*10⁴ CFU/ml	0.02*10⁴ CFU/ml			
Case 3	*Staphylococcus pseudintermedius*	273*10⁴ CFU/ml	269*10⁴ CFU/ml	**2,75*10⁶ CFU/ml**	**2,69*10⁶ CFU/ml**	**2,2%**
	Pseudomonas fluorescens	2.29*10⁴ CFU/ml	0 CFU/ml			
Case 4	*Staphylococcus pseudintermedius*	28200*10⁴ CFU/ml	22700*10⁴ CFU/ml	**2,82*10⁸ CFU/ml**	**2,27*10⁸ CFU/ml**	**19,5%**

RESULTS

Evaluation of antimicrobial activity of *in vivo* MLS® laser treatment

Four clinical cases were collected.

Case n. 1

The first was a Bull Terrier male dog, eight years old, affected by a generalized superficial pyoderma with crusts and papules localized on legs, trunk and head. From each swab, a pure culture of *Staphylococcus pseudintermedius* was isolated.

Case n. 2

The second clinical case was a Great Dane male dog, seven years old, affected by a generalized superficial pyoderma. From each swab, *Staphylococcus pseudintermedius*, *Proteus mirabilis* (swarming and not) and *Escherichia coli* were isolated.

Case n. 3

The third case was a West Highland White Terrier male dog, four years old, affected by generalized superficial pyoderma with crusts and papules localized on legs, trunk and head. From the pre-treatment swab *Staphylococcus pseudintermedius* and *Pseudomonas fluorescens* were isolated, while from post-treatment swab only pure culture of *Staphylococcus pseudintermedius* was isolated.

Table III - Antimicrobial susceptibility test.

		Sensitive	Intermediate	Resistant
Case 1	*Staphylococcus pseudintermedius*	Amikacin; Amoxicillin + Clavulanic acid; Cephalexin; Cefovecin; Clindamycin; Chloramphenicol; Doxycycline; Enrofloxacin; Imipenem; Marbofloxacin; Oxytetracycline; Oxacillin; Rifaximin; Rifampin	Ampicillin	Sulfamethoxazole + Trimethoprim
Case 2	*Staphylococcus pseudintermedius*	Amoxicillin + Clavulanic acid; Cephalexin; Cefovecin; Chloramphenicol; Doxycycline; Enrofloxacin; Imipenem; Marbofloxacin; Oxacillin; Rifaximin; Rifampin	Amikacin; Ampicillin; Clindamycin	Oxytetracycline; Sulfamethoxazole + Trimethoprim
	Proteus mirabilis swarming	Amoxicillin + Clavulanic acid; Ampicillin; Cephalexin; Cefovecin; Chloramphenicol; Enrofloxacin; Imipenem; Marbofloxacin; Rifaximin; Sulfamethoxazole + Trimethoprim		Amikacin; Clindamycin; Doxycycline; Oxytetracycline; Oxacillin; Rifampin
	Proteus mirabilis not swarming	Amoxicillin + Clavulanic acid; Ampicillin; Cephalexin; Cefovecin; Chloramphenicol; Enrofloxacin; Imipenem; Marbofloxacin; Rifaximin; Rifampin; Sulfamethoxazole + Trimethoprim	Doxycycline	Amikacin; Clindamycin; Oxytetracycline; Oxacillin
	Escherichia coli	Amoxicillin + Clavulanic acid; Ampicillin; Cephalexin; Cefovecin; Chloramphenicol; Enrofloxacin; Imipenem; Marbofloxacin; Oxytetracycline; Sulfamethoxazole + Trimethoprim	Rifaximin	Amikacin; Clindamycin; Doxycycline; Oxacillin; Rifampin
Case 3	*Staphylococcus pseudintermedius*	Amoxicillin + Clavulanic acid; Ampicillin; Cephalexin; Chloramphenicol; Enrofloxacin; ; Marbofloxacin; Oxacillin; Rifaximin; Rifampin; Sulfamethoxazole + Trimethoprim	Amikacin; Cefovecin; Clindamycin; Imipenem	Oxytetracycline; Doxycycline
	Pseudomonas fluorescens	Enrofloxacin; Imipenem; Oxytetracycline	Amikacin; Amoxicillin + Clavulanic acid; Chloramphenicol; Marbofloxacin; Doxycycline	Ampicillin; Cephalexin; Cefovecin; Clindamycin; Oxacillin; Rifampin; Sulfamethoxazole + Trimethoprim
Case 4	*Staphylococcus pseudintermedius*	Amoxicillin + Clavulanic acid; Cephalexin; Cefovecin; Doxycycline; Enrofloxacin; Imipenem; Marbofloxacin; Oxacillin; Rifaximin; Rifampin	Amikacin	Ampicillin; Clindamycin; Chloramphenicol; Oxytetracycline; Sulfamethoxazole + Trimethoprim

Table IV - Antimicrobial activity of direct irradiation of bacterial isolates.

		Seconds of exposition	Minutes post- exposition	Laser (CFU/ml)	Growth control (CFU/ml)	% Inhibition
Bacterial strains	*Staphylococcus pseudintermedius* **Case 1**	6	5	1.17±0.20*10^5	1.31±0.18*10^5	11%
			30	1.05±0.30*10^5	1.05±0.30*10^5	0%
		41	5	1.04±0.05*10^5	1.13±0.13*10^5	8%
			30	1.51±0.99*10^5	1.51±0.99*10^5	0%
	Staphylococcus pseudintermedius **Case 2**	6	5	0.67±1.70*10^5	0.67±1.70*10^5	0%
			30	0.63±1.49*10^5	0.63±1.49*10^5	0%
		41	5	0.26±0.04*10^5	0.26±0.04*10^5	0%
			30	0.27±0.38*10^5	0.27±0.38*10^5	0%
	Escherichia coli **Case 2**	6	5	3.31±0.37*10^5	3.63±0.42*10^5	9%
			30	3.36±0.09*10^5	3.39±0.25*10^5	1%
		41	5	2.36±0.97*10^5	2.56±1.22*10^5	8%
			30	2.25±0.94*10^5	2.25±0.94*10^5	0%
	Pseudomonas fluorescens **Case 3**	6	5	1.64±0.17*10^5	1.67±0.14*10^5	2%
			30	1.59±0.45*10^5	1.59±0.45*10^5	0%
		41	5	2.28±1.63*10^5	2.35±1.71*10^5	3%
			30	2.05±1.61*10^5	2.21±1.56*10^5	7%

Case n. 4
The fourth case was a Poodle male dog, seven years old, affected by generalized superficial pyoderma with crusts and papules. From each swab *Staphylococcus pseudintermedius* was isolated.
CFU count was performed for each sample and each bacterial isolate. Results are shown in Table 2. and each bacterial isolate. Results are shown in Table 2.
Antimicrobial susceptibility test was performed on each isolated bacterial strain. Results are shown in Table 3.

Evaluation of antimicrobial activity of direct in vitro MLS® laser treatment
In the second step of the experimental design, four bacterial strains from clinical cases were selected for the evaluation of direct in vitro antimicrobial activity of MLS® laser, on the basis of their clinical importance (two Gram positive and two Gram negative).

Strain 1: *Staphylococcus pseudintermedius* isolated from Case 1. Five minutes after laser treatment (irradiation time of six seconds) growth inhibition percentage was 11%, while after thirty minutes inhibition was 0%. The same bacterial

strain subjected to a laser exposure of 41 seconds showed 8% of growth inhibition after incubation time of five minutes, while after thirty minutes inhibition was 0%.

Strain 2: *Staphylococcus pseudintermedius* isolated from Case 2. This strain did not show any growth inhibition, both after five minutes and thirty minutes of incubation from the irradiation step.

Strain 3: *Escherichia coli* isolated from Case 2. This strain showed growth inhibition after laser treatment of six seconds, both after five minutes and thirty minutes of incubation (9% and 1%, respectively), while laser irradiation of 41 seconds resulted in 8% inhibition of growth, only five minutes post-treatment.

Strain 4: *Pseudomonas fluorescens* isolated from Case 3. This strain showed an inhibition of growth equal to 2% in the 6 seconds laser-treatment mode, after five minutes incubation, while none inhibition was observed after thirty minutes. Furthermore, inhibition was of 3% and 7%, respectively, after five and thirty minutes of incubation from laser-treatment of 41 seconds.
All the results of the direct irradiation of bacterial strains are shown in Table 4.

DISCUSSION

As expected, from all the clinical cases of superficial pyoderma the most isolated strain was *Staphylococcus pseudintermedius*. Other bacterial isolates (Pseudomonas fluorescens, E. coli, Proteus spp.) can be considered opportunistic, co-infectious agents. Multi-drug resistance was observed mostly in Gram negative isolates (*Pseudomonas*, Proteus, E. coli).
Following in vivo laser treatment on the skin lesions, significant results in bacterial growth inhibition, demonstrated by CFU count, were observed on the bacterial flora load isolated after 30 minutes from laser application. In particular, a significant reduction of CFU count was observed for all isolates, except the three strains of *Staphylococcus pseudintermedius* isolated from cases 2, 3 and 4. A higher effectiveness on Gram negative isolates was observed, according to the literature, where it is reported that laser antimicrobial activity depends on bacterial species. This may be attributable to the peculiar structure of Gram positive and Gram negative bacterial membranes and wall (Schoop, 2004). Gram positive wall is thicker, being structured in an outer cytoplasmic lipid membrane, thick peptidoglycan layer, lipoteichoic acids and a smaller periplasm than in Gram negative bacteria. Other studies have confirmed that structural configuration of the cell wall affects bacterial susceptibility to laser irradiation. In different conditions, several cycles of laser irradiation are required to affect Gram-positive bacteria; whereas, Gram-negative bacteria are eliminated faster and more easily (Moritz et al, 2000). Moreover, it has been demonstrated that differences in the wavelengths, power, irradiation time, spot size and number of cycles are responsible for the variable efficacy of lasers reported in literature (De Paula, 2001).
The anti-bacterial effect of laser-therapy has been widely described in literature. IGAlAr laser emission was used against *Staphylococcus aureus* strains, and the results revealed reduction in the number of colonies (Wilson et al, 1995). In humans it was studied the effect of laser-therapy with the wavelengths 630, 660, 810 nm and 1-50 J/cm^2 fluence on *Staphylococcus aureus, Pseudomonas, and E. coli* which were collected from infected wounds (Nussbaum et al, 2002). A significantly reduction in the growth of bacteria was obtained in a study on diabetic wound healing in rats suffering from bacterial infection induced by *Staphylococcus aureus* (Ranjbar, 2016). A Diode laser source was used on dental implants in order to avoid microbial platelet aggregation (Kreisler et al, 2002). Moreover, an antibacterial effect on third-degree burn wounds was proved (Bayat et al, 2006). It has been also suggested that the anti-inflammatory action of laser therapy in wounds, skin lesions and infectious diseases could be partly due to the reduction of the bacterial load at the lesion site, thus reducing a major cause of inflammation (Meyerholz et al, 2009; Silva, 2013).
However, the results obtained in the second phase of the present study, where the antimicrobial effect of direct in vitro NIR laser (MLS®) treatment was evaluated, demonstrated a limited microbicidal activity. The growth inhibition was observed mostly with 6 sec exposure and 5 min incubation time. The findings suggest that there is no direct correlation between effectiveness and exposure time: in one case only a slight increase in growth inhibition was observed with increasing exposure time.
This result suggests that the damage produced by laser treatment on the bacterial population is slight, therefore it can be detected immediately after treatment but is easily diluted over a short time.
It was beyond the scope of this study to evaluate the effect of laser treatment on canine pyoderma from the clinical point of view. In fact, the clinical protocol for this application of MLS® therapy foresees several weekly sessions for a few weeks. Therefore, in this study, which was based on a single *in vivo* irradiation, the host biological response to laser treatment was not studied.
However, the higher antimicrobial activity observed *in vivo* in comparison with the in vitro irradiation suggests that *in vivo* laser irradiation induces a complex response which involves both microorganisms and host tissues. The final effects depend on the absorption of laser radiation by the host tissues and consequent biological response, which involves the immune cells

and their interaction with microrganisms (Clemente et al., 2015), the modulation of inflammation (Monici et al., 2013, Squarzoni et al, 2017) the enhancement of anabolic processes in the tissues and activation of repair mechanisms (Monici et al., 2013).

Beyond the complexity of the *in vivo* biological response, another explanation for the lower effectiveness of laser treatment in inhibiting bacterial growth *in vitro* could be the fluence (2.05J/cm^2), which had deliberately been kept the same as that used *in vivo*.

However, the absorption of laser radiation in the tissues is greater than that in bacterial suspension *in vitro*. So, higher fluences are probably required *in vitro*. This hypothesis is in agreement with what has been observed applying laser therapy to inhibit fungal growth. After trying different energy densities, the maximum effect in terms of mortality rate in *Candida albicans* suspensions treated *in vitro* was obtained with 7J/cm^2 fluence (Clemente et al., 2015). When considering the treatment of oral mucositis *in vivo*, very significant results are reported also with very low fluence (0.16J/cm2) (Squarzoni et al., 2017).

CONCLUSION

In conclusion, this study shows that a single application of laser therapy carried out by MLS device effectively reduced the microbial load of skin lesions in superficial canine pyoderma. The antimicrobial action was higher on the Gram negative bacteria than on the Gram positive ones.

This effect was not reproduced by *in vitro* irradiation of bacterial suspensions prepared from skin swabs collected before *in vivo* laser treatment of patients affected by superficial canine pyoderma.

In vitro, only a slight inhibition of bacterial growth was observed immediately after the treatment. The different effectiveness shown in *in vivo* and *in vitro* conditions could be explained with:

1) a higher absorption of laser radiation by the host tissues in comparison with bacterial suspensions (this implies that higher doses are required *in vitro*);

2) a more complex biological response induced by the *in vivo* irradiation, involving the interaction with the host tissues.

In conclusion, in-depth studies on the application of laser therapy in the management of canine skin infections are required and further investigations are necessary, considering a wider bacterial sample and varying lasers parameters to maximize their efficacy *in vitro*.

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Key words: CAPD, Auditory Event Related Potentials (AERP), Contingent Negative Variation (CNV), LASER, attention problems, respiratory chain, ATP, Wire and Fire, Learning disabilities

Hypothesis for a future application of a Laser-device in patients with symptoms of a developmental auditory processing disorder. Part II: Evaluation of clinical cases.

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ABSTRACT

The present study examined whether changes of electrophysiological late event related potential pattern could be used to reflect clinical changes from therapeutic intervention with a LASER-device in a group of patients with symptoms of central auditory processing disorder (CAPD). The contingent negative variation (CNV) and event related auditory cortical potentials (AERP) reflect a synchronization of together firing wired neural assemblies responsible for auditory processing, suggesting an accelerated neuromaturation process when applying a LASER device stimulation. This was discussed already in Part I of this article (1), where a model was presented explaining possible effects of LASER application of auditory neurons by inducing the respiratory chain of the mitochondria. Part II of the article now provides clinical data, that a LASER stimulation might be useful for the clinical improvement of attention (distraction) symptoms caused by auditory processing deficits.

Subjects consisted of 59 patients average age 14 years (range 7-53 years) with normal hearing threshold, learning disability and attention deficits caused by central auditory processing disorder. These patients were stimulated with a LASER-device system. Results after 10 LASER stimulation sessions indicated, that this type of LASER-device stimulation significantly improved auditory CNV and p200/p300 pattern morphology.

INTRODUCTION

It is well accepted that an acoustical environment (noise and reverberation) in classroom conditions is a critical factor in the educational achievement of many children. Such populations being at risk for academic failure encompass children with language impairment, dyslexia, attention deficits and general developmental delay (literature in 1). It is reasonable to assume, that poor neural acoustic representation will lead to serious problems in the maturation of the auditory pathways and hence the development of auditory process ability. Recent research suggests that neuroplasticity and neuromaturation are dependent on stimulation (11, 12). Therefore comprehensive management of CAPD should include auditory stimulation to achieve functional changes within the central auditory nervous system. Thus young children would be expected to benefit from a great degree of neuroplasticity. In another paper (2) we focused already on the use of late event related auditory potentials (AERP´s) in documenting changes in clinical status after direct stimulation with binaural FM-devices. These data were interpreted as indicating that neuroauditory maturation could be influenced by a specific intervention and could be distinctly objectified by means of late event-related potential measures.

Recording of the Contingent negative variation (CNV) and p200/p300 pattern requires the patient to pay active attention to a stimulus. Auditory event related potentials (AERP´s) allow the evaluation of brain activities and are presumed to be related to attention, recognition, and memory processes. The contingent negative variation (CNV) is a slow negative potential decrease, which will appear hundreds of ms before target stimulation. CNV is representing a large number of increasing synchronous self

regulatory excitatory activity of neuron populations and is preparing the brain for the following auditory stimulus. In this sense CNV and p200/p300 are related to the synchronous firing of wired neurons in order to provide the ability of reaction capacity of a certain brain task.

Studies of brain development show that sensory stimulation in the case of the visual centres of the brain is critically important, and influences the actual organization of visual brain pathways. Increase in visual stimulation results in morphological and functional alterations within the visual parts of the brain (4, 5). Strategies for stimulation of auditory processing disorder are usually direct remediation, environmental modifications and compensatory strategies. One of a possible new strategy for reducing the deleterious effects of auditory noise is the use of LASER light, providing discrete wavelengths (frequencies) to improve auditory clarity and avoidance of ear pressure, tinnitus and background noise (2,8). The purpose of direct stimulation of auditory processing on the level of neurostimulation is to maximize neural plasticity and possibly accelerate maturation, improving auditory performance.

The purpose of this article is to present data from patients with developmental auditory-perception problems when applying LASER light. A hypothetical model is already discussed in Part I of this article (1) to explain the results using electron modelling and proton exchange inside the respiratory chain. As this article is the second part of a two-part article it is referred to the first part for reasoning of this clinical evaluation. Most of the literature is already cited in Part I of this article (1).

METHODS

Subject selection criteria

59 subjects (47 males, 12 females) with normal hearing sensitivity participated in this study. Subject age ranged from 7 to 53 years with a mean age of 14 ± 10 years.

All subjects met the following criteria: Hearing sensitivity better or equal to 20 dB HL at 250 – 8000 Hz, German was the primary language. All subjects had a history of learning disability and attention deficits. 13 patients were additionally diagnosed with ADHD by DSM IV criteria and well controlled treated with stimulant medication. In these cases CAPD was considered as a comorbid condition of ADHD. Children with and without ADHD did not demonstrate a significant different distribution pattern of pathological auditory event related potential. This is in agreement with other literature (7). Only subjects were included with an IQ-level ≥ 90 measured with different appropriate instruments.

All subjects were administered with electrophysiological tests before and after 10 sessions of LASER stimulation 1 - 3 months after LASER stimulation.

Assessment battery

Prior to participation in the study each subject underwent a comprehensive audiologic evaluation to rule out any abnormalities of the auricle and auditory meatus. Pure tone air and bone conduction thresholds were assessed using a Maico ST-28 clinical audiometer (250-8000 Hz) demonstrating normal values. Tympanometry and acoustic reflex as well otoacoustic emission testing revealed normal values. All subjects were required to have normal ABR tracings in each ear.

Laser application

The Laser source was a Multiwave Locked System Laser (MLS®, ASA Srl, Vicenza, Italy). It is a commercially available laser source built in compliance with EC/EU rules, which received FDA clearance and is widely used in clinics. MLS® laser is a class of NIR laser with two synchronized sources (laser diodes). These emit at two different wavelengths, peak power and emission mode. The pulse frequency is for both wavelengths 1500 Hz (T-on mode) delivered to the auditory neurons. The first one is a pulsed 905 nm laser diode with 25 W peak optical power with a pulse duration of 100 ns. The second laser diode (808 nm) is operating with 1000 mW optical power with a pulse duration of 300 µs. The two laser beams are emitted synchronously and the propagation axes are coincident. The patients received the following energy dose on both ears for 1/2 hour separately: 164.43 J/cm^2 (according to A. Kaiser, personal communication).

Event Related Auditory Cortical Potentials (AERP)

Cortical potentials were recorded from 26 electrodes positioned on the human brain scalp according to the international electrode placement system (10-20). To maximize CNV, P200 and P300 amplitudes and stability, electrodes over the central (CZ, FZ, PZ), left (C3, F3, P3, F7, T3, T5, P3, O1) and right (C4, F4, P4, F8, T4, T6, O2) central, parietal and occipital cortex parts of the brain were used for evaluation.

Electrodes were referenced to linked earlobes. Vertical EOG provide control for blinks. Data were recorded with a 32–channel bio signal amplifier (Brainamps) with a frequency response 0.5 to 30 Hz and a A to D conversion rate of 1000 Hz. Trials were corrected for baseline and VEOG artefacts with the brain vision analyser software. These data were interpreted as indicating that neuroauditory maturation could be influenced by a specific intervention and could be distinctly objectified by means of late event related potential measures.

Auditory CNV (contingent negative variation):

Recording of the CNV potentials was

achieved by presentation of 50 tone burst sequences (25 high frequency/ low frequency 3 kHz/2 kHz and 25 low frequency/ high frequency 2 kHz/3 kHz tone burst sequences, 75 dB HL), respectively. Stimulus duration was 50 ms. The Interstimulus Interval (ISI) is offered with 5 sequences of 10, 20, 30 40 or 50 ms. Mean reaction time and number of errors of omission and commission were recorded separately (not shown). 1.5 seconds prior to the burst sequences a warning burst tone (1 kHz, 15 ms duration) was presented. Prior to recording the event related potentials subjects were given time to practice the tone discrimination task to become familiar with the paradigm while listening to the high/low frequency tone burst sequences presented. According to the number of errors of omission the ISI intervals were adapted to 50, 100, 150, 200 and 250 ms, respectively. Subjects were asked to answer either with "high" or "low" after the second tone burst was realized.

CNV reflects a slow potential drift towards negativity, which will take place several hundreds of ms before target condition, preparing the processing of the following target stimulus. One CNV epoch was scored separately for tone burst (target) trial from all electrode positions. From 1120 ms on before target stimulus, a significant CNV component can be detected, representing an excitatory activation of the corresponding brain area.

Auditory P200 and P300 components:

Recording of the potentials was achieved by presentation of frequent and infrequent tone bursts (75 dB HL) at a ratio of 3:1 in an "oddball paradigm". The children were asked to recognize the infrequent stimuli. Binaural tones were presented in a random sequence with a 2.2 kHz infrequent target tone and 4 kHz frequent target tone with a stimulus duration of 50 ms and an ISI of 3025 msec. Prior to recording the event related potentials subjects were given time to practice the tone discrimination task to become familiar with the oddball paradigm while listening to the rare and frequent tones presented. Each session consisted of 75 presentations of the frequent tone and 25 presentations of the rare tone in a random sequence. Subjects were asked to push on a mouse button whenever an infrequent tone was realized.

Latency and amplitude measures were averaged over three complete trials for the N1, N2, P2 and P3 components of the long latency response to the target tone. P2 was identified as the largest positive peak between 130 and 290 ms, following N1. P3 was identified as the highest positive peak between 250 and 600 ms following N2. Latency was measured to the highest peak on the wave. Amplitudes were measured at the highest wave peak relative to the prestimulus baseline: Artefact rejection was set to ignore any trial which the ongoing EEG exceed ± 140 µV. Averaging was carried out generally over all segments allowing comparison among several data sets with the same software.

This method (P300 "oddball paradigm") was specifically selected. In this case a series of paired stimuli has to be detected

Figure 1 - Composite Grand Mean CNVs before (red) and after (green) LASER application.

which reflects additionally aspects of attention, discrimination and memory.

Laboratory analysis

ATP levels were determined in an outsourced laboratory. Mitochondria are compartments of eukaryotic cells. They produce by means of the respiratory chain using oxygen, glucose and phosphate ATP. ATP determination is provided by measurement of the ATP production in mitochondria from living T-lymphocytes of the patient. Cells with adequate ATP production show after addition of a specific metabolic agent a different color spectrum compared to cells with decreased ATP production. Differentiation and quantification take place in a high sensitive fluorescence-activated cell sorter.

Statistical analysis

Statistical analysis was done with a temporal resolution t-Test belonging to the Brain Analyser software system. All analyses were performed at a $p < 0.05$ level of significance.

RESULTS

Electrophysiological results

CNV

Composite grand mean wave forms for the experimental groups at different time intervals are shown in Figure 1. The two waveforms represent the CNV locations in target condition (red: inital situation; green: after 10 binaural LASER sessions). Notice in the development of the CNV distribution pattern the increase in CNV area from the frontal to the parietal electrodes already after 10 LASER application sessions. A map view of CNV between the time interval – 300 ms to 0 ms (appearance of target stimulus) illustrates the increase in excitatory activation of neuron population after the course of 10 sessions of LASER device applications (Fig. 2 before and Figure 3 after LASER application). Table 1 shows the mean values of absolute CNV areas under curves calculation levels before and after 10 LASER applications, respectively.

Figure 2 - Composite Grand Mean CNVs (mapping view -300 ms to 0 ms) before

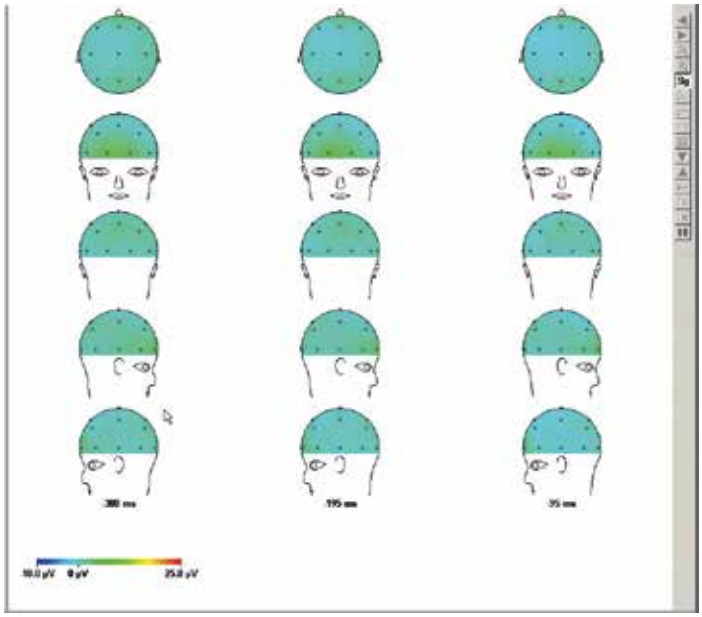


Figure 3 - Composite Grand Mean CNVs (mapping view -300 ms to 0 ms) after

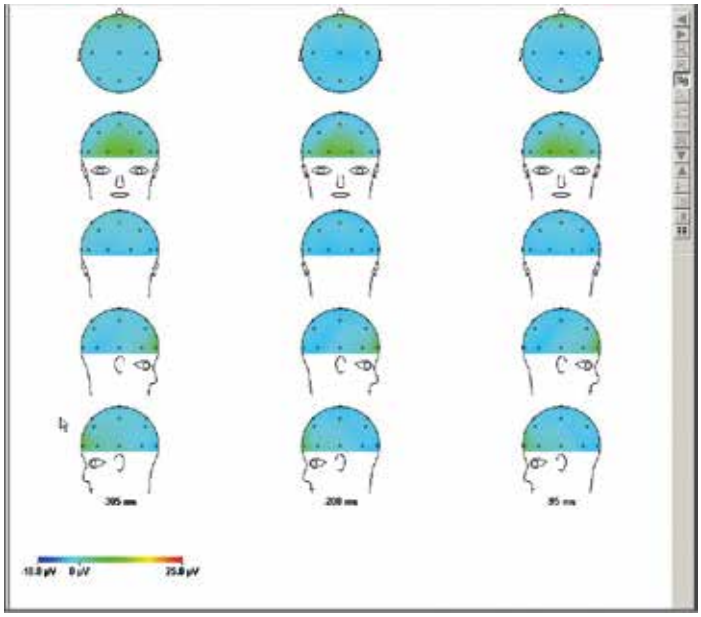


Figure 4 - P200/P300 Pattern (before)

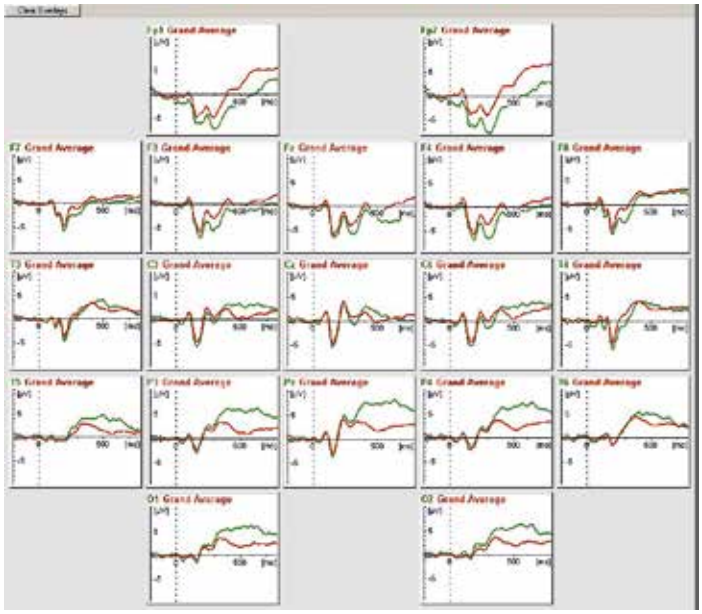


Figure 5 - P200/P300 Pattern (after)

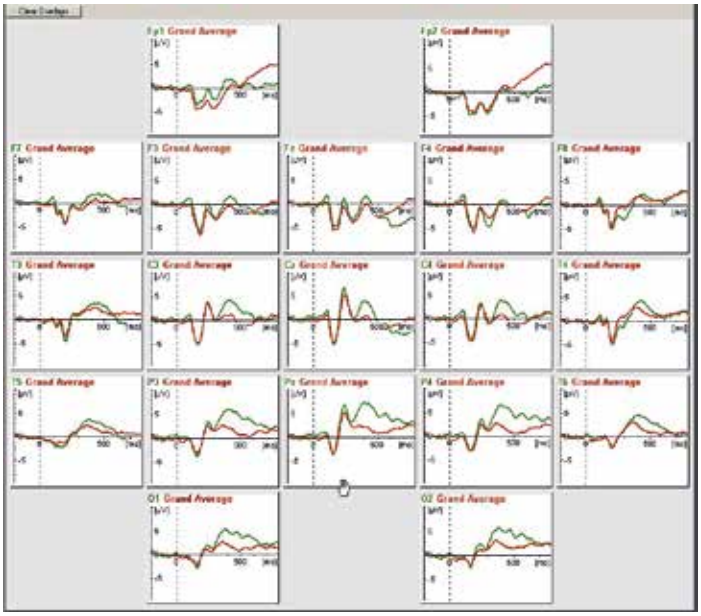


Figure 6 - C3,C4,Cz left, right and central electrode sites (before)

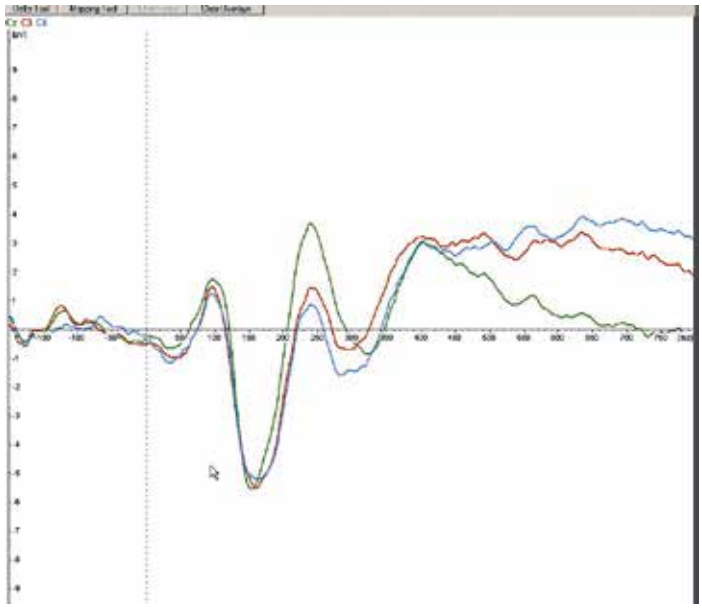
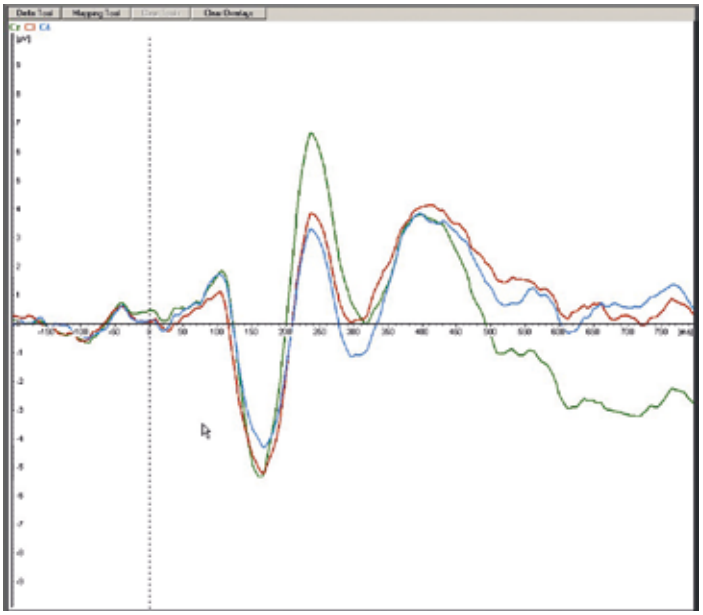


Figure 7 - C3,C4,Cz left, right and central electrode sites (after)



P200/P300

Analysis of the P200 and P300 components reveals a significant height and morphological discrepancy between the target condition (green line) and the non-target condition (red line) after the LASER application (Figure 5) compared to the inital situation (Figure 4) level reflecting an increased discrimination efficiency especially at the P300 level.

Composite grand mean wave forms for the experimental groups at different time intervals are shown in Figures 6, 7, 8 and 9. The three waveforms represent in Figure 6 the C3 (red), C4 (blue), CZ (green) and in Figure 8 the P3 (red), P4 (blue) and PZ (green) non-inverting locations before LASER application, while in Figure 7 and 9 the appropriate locations are shown after 10 sessions of LASER application. Notice in the case of infrequent tone responses the development of the classical P200/P300 distribution pattern with increase in P200 amplitude and more pronounced P300 pattern after LASER application reflecting an improved allocation of resources.

Table 1 shows the absolute PZ amplitude mean values. It should be noticed that the P3, P4, PZ pattern of the treated sample approached more pronounced waveform pattern after LASER application compared to the initial pattern giving a more convincing argument than only looking at the absolute PZ levels.

Table I - Pre- and post treatment mean area (μV*ms) and amplitude (μV) and latency (ms) measures for P200 and P300 of the patients taken from the graph

	CNV (CZ) Area (μV*ms)		P200	P300	P200	P300
			Peak height (μV)		latency (ms)	
Before	190	p <	4.84	7.22	240	400
After	4277	0.05	6.62	7.12	240	400

Figure 8 - P3,P4,Pz left, right and central parietal electrode sites (before)

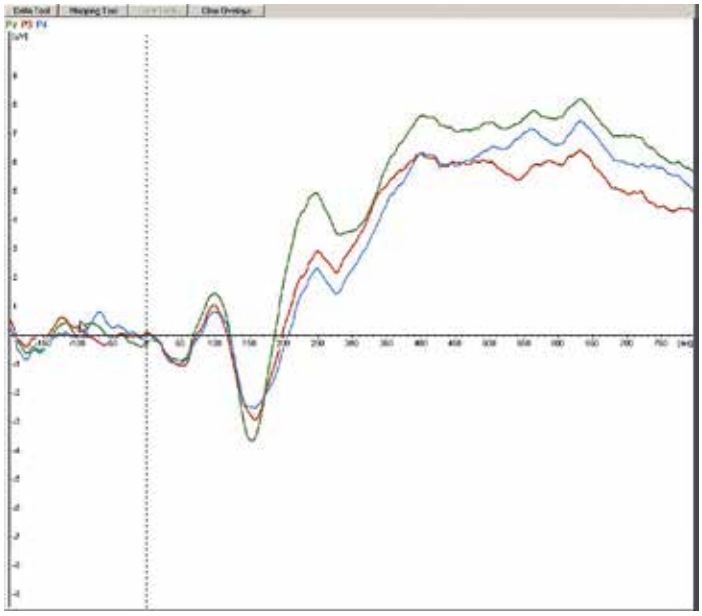
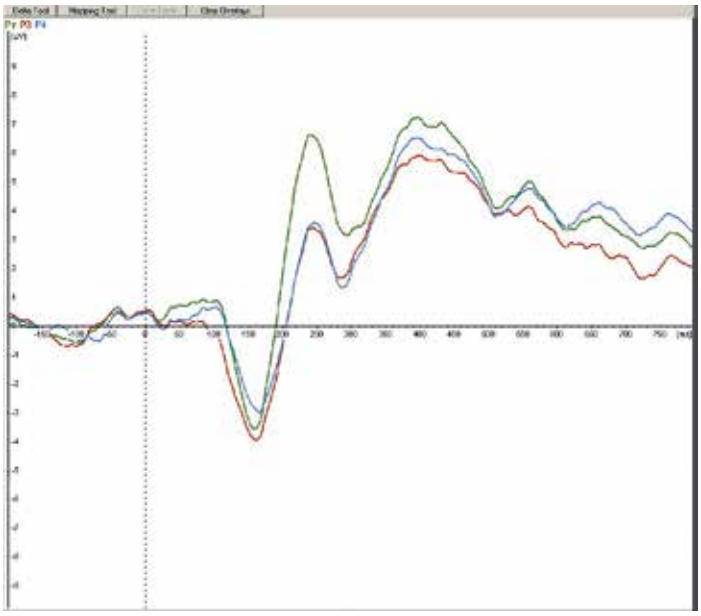


Figure 9 - P3,P4,Pz left, right and central parietal electrode sites (after)



ATP levels

ATP levels before and after intervention (% T cells):

Before	After	p
92.8 +/- 11.6	98.8 +/- 1.8	< 0,05
Range 46 - 100	95.0 – 99.9	

ATP levels before and after intervention were significantly different, reflecting a possible energy dependent reaction.

DISCUSSION

Only a few studies have been found in literature having focused on the use of CNV and P300 potential in documenting changes in clinical status (literature in 1). This study was designed to evaluate whether development of the event related auditory event related potential distribution pattern would reflect any changes of the auditory processing system resulting from LASER stimulation. Recording of the CNV requires the patient to pay active attention to a stimulus. AERP´s are presumed to be related to attention, recognition, and memory processes. Our results revealed a significant improved allocation of resources of patients with CAPD after 10 LASER stimulation sessions. This is reflected in the differential reaction on relevant and non relevant stimuli measured with the P200/P300 pattern as well as in CNV pattern before and after the course of 10 LASER stimulation sessions.

Although some of the electrophysiological changes might be attributable to normal maturation processes, the differences in CNV distribution pattern and P200/P300 from the pre-and post measures can be assigned to the treatment intervention. This can be interpreted as precise discrimination efficiency and as an optimization of the central auditory information processing. The improved activation pattern of the contingent negative variation (CNV) can be explained by a synchronous activation of a great amount of neurons population (assembly) which are necessary to prepare for course of action.

The organic living brain is quite the opposite of an engineered machine with hardwired circuits that can only perform a limited number of actions, but during the day the brain is forming / uniforming new flexible neuronal networks. A group of neurons will be used for different purposes at different times. Tasks can be performed using different coalitions (assemblies) of neurons (4, 5). Learning skills are encoded in the cumulative electrical patterns resulting from the neurons firing together (4, 5). The pattern, i.e. the population is interesting, not the individual cell. Cells that are, on whatever reason, chronically inflamed, are more sensitive to red and near-infrared light than are well-functioning cells. To heal, the body often needs to create new cells. The first step in cell reproduction occurs when DNA replicate itself. Specific wavelengths 404, 620, 680, 760 and 830 nm can activate DNA and RNA synthesis in cells by inducing the respiratory chain of sick neurons thus leading to an increased cell proliferation (literature in 1).

Wilden et al. (8) already reported, that LASER stimulation with distinct wavelenght may vitalize the cell by increasing the mitochondrial ATP (adenosine-tri-phosphate)-production. With regard to radiation phenomena and its inhanced electron flow in the cellular energy transfer (respiratory chain), these authors postulated already that the experimentally found increase of ATP-production could be explained by means of low-level laser light on a cellular level. Their investigations are mainly based on patients with Tinnitus and sudden hearing loss, while developmental hearing problems are not considered. Our data support this hypthosesis as ATP concentration significantly increased after LASER application. The model is extensively discussed in Part I of this article (1).

In most of the patients the CNV before LASER stimulation was abnormally evoked, severely reduced and in most of the cases absent in amplitude suggesting problems of the patients paying attention. As CAPD can be a distinct disorder or a comorbid condition of ADHD the success of this type of intervention seems to be independent of the type of CAPD: Children and adolescents applied with LASER stimulation did significantly and in different degrees benefit from this therapeutic approach whether ADHD was present or not. It seems reasonable to assume that changes in the amplitude and/or morphology of the P200/P300 waveforms correlate with changes of the clinical status.

Because maturation processes in highly plastic brains in childhood should be enhanced through sensory stimulation, expectation of improvement of auditory processing abilities could be confirmed in the current investigation. These findings support previous research from our group showing that children with various types of learning or cognitive disorders may be (i) differentiated from normal control subjects on the basis of event related potentials and (ii) stimulation by FM-devices could indeed improve auditory processing abilities (2). Data of the control subjects from this study could serve as a baseline definition. While trainings or FM-devices usually will be applied in the morning or the afternoon as additional educational support for the children, LASER stimulation can be applied independently from school schedule and may have a faster and a more sustainable success for the disabled children. Because auditory neuromaturation and

neural plasticity depend on distinctive auditory stimulation, "aggressive" management of CAPD (either with or without ADHD) should begin as early as possible. Studies of brain development show that sensory stimulation of the auditory centres of the brain is critically important, and influences the actual organization of auditory brain pathways (9). Increase in auditory stimulation may result in morphological alterations within the auditory parts of the brain (10, 13). The ability of the auditory cortex to reorganize continuously throughout life span reflects the ability to acquire new skills and behaviours. Several studies have focused on the use of the late auditory event-related potentials (AERPs) in documenting changes in clinical status. These studies emphasized the feasibility of using P300 event related potentials to document levels of auditory dysfunction (14, 15). There are several studies suggesting that P300 auditory event related potentials in children with CAPD showed longer latency times and smaller amplitudes compared to controls (16,18,19). Jirsa (16, 17) demonstrated a significant decrease in P300 latency time along with an increase in P300 amplitude in the evoked potentials obtained from children with CAPD following an intensive therapeutic 14 week intervention program. The children in the experimental group exhibited improvement on selected auditory tasks and positive changes in overall academic performance. These data were interpreted as indicating that neuroauditory maturation could be influenced by a specific intervention and could be distinctly objectified by means of late event related potential measures. In our case, no decrease in p300 latency time could be demonstrated. One explanation might the difference in maturation stage from our subjects, as the age range of our subjects was from 7 to 53 years. Further studies (including longterm follow up) have to address the question whether use of AERP´s may be more sensitive for prediction of treatment outcomes as it has been already suggested by Walsleben et al. (6). Studies on AERP measures performed at different maturation stages and with increased number of LASER simulation sessions are in progress, to predict already possible therapeutic advantages of such a LASER device very early. The degree and speed of improvement between different forms of interventions has still to be evaluated.

Although results of this type of investigation must be interpreted cautiously because of the limited number of subjects and possible interfering medication effects, they do suggest that event related potential measures are sensitive to changes in clinical status when applying a controlled use of LASER stimulation in children/adolescents with CAPD. Up to now there is still a great lack of consensus on precise definitions of what a processing disorder encompasses. It is not yet clear how to differentiate a CAPD from other processing disorders. Future research has to address these questions to enhance specifity of the clinical intervention tools and/or programs on auditory neuromaturation. Additionally it can improve our knowledge of the development of auditory function in children. Intrahemispheric and interhemispheric functional measurements may also give a more precise view into these questions. In summary our data support, that auditory event related potentials measures may be useful in the clinical assessment and treatment of populations with possible CAPD. Maturational effects cannot be totally excluded, but are not expected already after 3 to 4 months. Further research is warranted. Thus, our data support (a) the use of electrophysiological measures as a more sensitive parameter for the detection and follow up of auditory neuromaturation processes which was (b) induced by LASER stimulation.

Use of LASER stimulation does not replace other intervention measures as occupational therapies or other kinds of auditory training interventions although they may support and possibly accelerate these methods. In some cases LASER stimulation might not be helpful. As a result of this clinical evaluation patients and their parents need to be intensively involved in the decision making process using a LASER device very early to improve acceptance and the beneficial effect of such a tool.

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Conflicts of Interest

The author declares that he discloses any financial and personal relationships with other people or organizations that could inappropriately influence (bias) this work.

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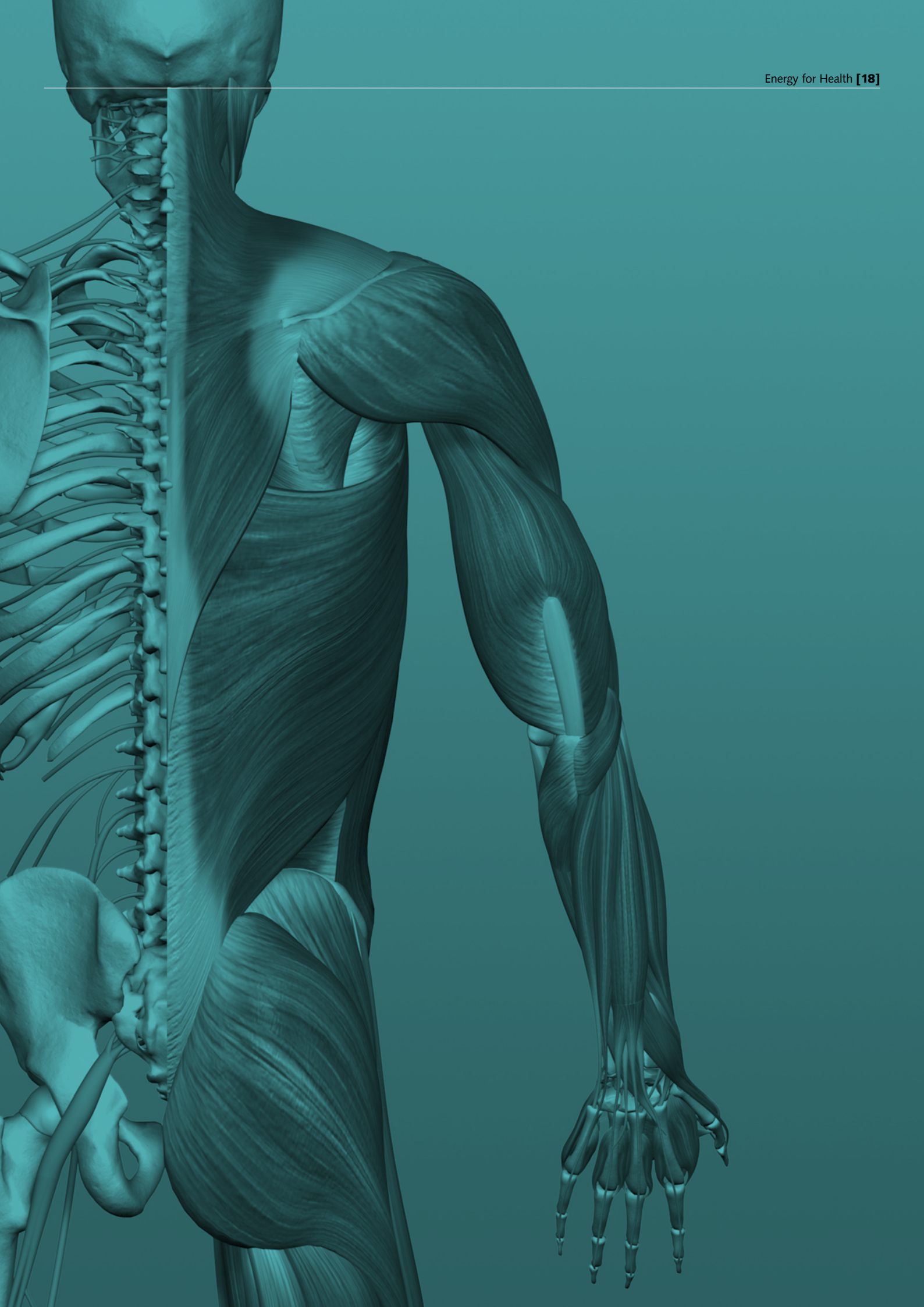

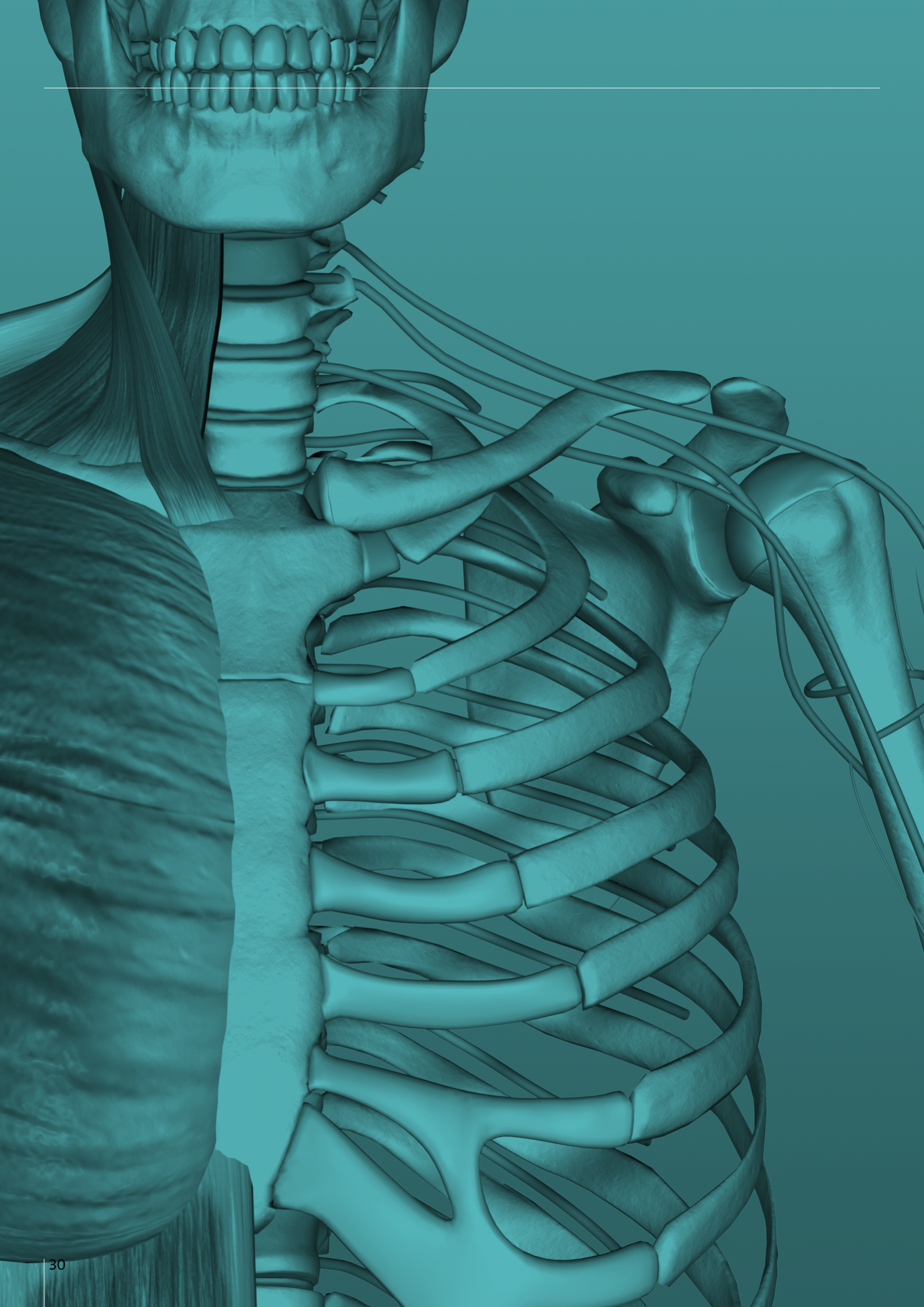

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ACKNOWLEDGEMENTS

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