Lasers in Surgery and Medicine 37:29–36 (2005)

Cytologic and DNA-Cytometric Follow-Up of Oral Leukoplakia After CO_2- and Er:YAG-Laser Assisted Ablation: A Pilot Study

Frank Schwarz, Dr. Med. Dent., DDS,[1]* **Dimitra Maraki,** DDS,[2] **Sebnem Yalcinkaya,** PhD, DDS,[3] **Katrin Bieling,** DDS,[4] **Alfred Böcking,** Prof. Dr. Med.,[5] **and Jürgen Becker,** Prof. Dr. Med. Dent., DDS[6]
[1]*Department of Oral Surgery, Heinrich Heine University, Düsseldorf, Germany*
[2]*Department of Oral Surgery, Heinrich Heine University, Düsseldorf, Germany*
[3]*Department of Oral Surgery, Heinrich Heine University, Düsseldorf, Germany*
[4]*Department of Oral Surgery, Heinrich Heine University, Düsseldorf, Germany*
[5]*Chairmen of the Institute of Cytopathology, Heinrich Heine University, Düsseldorf, Germany*
[6]*Chairmen of the Department of Oral Surgery, Heinrich Heine University, 40225 Düsseldorf, Germany*

Background and Objectives: The aim of the present pilot study was to determine therapeutic responses to Er:YAG- and CO_2-laser ablation in patients with oral leukoplakia as evaluated by means of exfoliative cytology (EC) and DNA-image-cytometry (DNA-I).
Study Design/Materials and Methods: Ten patients exhibiting a total of 16 lesions affecting a variety of intraoral sites were randomly treated with either (1) an Er:YAG laser (300 mJ/pulse, 10 Hz, defocused mode) (ERL), or (2) an CO_2 laser (4–6 W, 20–50 Hz, focused mode) (CO). Brush (B) and incisional (I) biopsies were obtained from the respective lesions immediately before treatment (B, I) as well as 24–96 weeks postoperatively (B). In cases, in which EC revealed suspicious cells, nuclear DNA-contents were measured using a TV image analysis system.
Results: Both treatment approaches resulted in a complete (C) or partial (P) remission of all investigated lesions. In particular, ERL exhibited C(3), P(5), and CO C(5), P(3). However, in the CO group, two of eight lesions showed a recurrence 32–48 weeks following treatment. Among all investigated lesions, both histological and EC/DNA-I diagnosis revealed no sign of malignancy or dysplasia before or following laser assisted ablation.
Conclusions: Within the limits of the present study, it may be concluded that both treatment approaches seem to have limitations to achieve predictable eradication of oral leukoplakia. Lasers Surg. Med. 37:29–36, 2005.

Key words: oral leukoplakia; exfoliative cytology; DNA-image-cytometry; DNA-aneuploidy; Er:YAG laser; CO_2 laser; pilot study

INTRODUCTION

Oral leukoplakia is defined as a white patch or plaque on the oral mucosa that cannot be removed by scraping and cannot be classified clinically or microscopically as another disease entity [1]. It has been reported to be the most common precancerous lesion of the oral mucosa. The risk of neoplastic transformation varies from 0.3% to 25% dependant on location, clinical features, degree of dysplasia, and etiological factors [2]. Based on these findings, implementation of early diagnostic tools combined with predictable eradication of oral leukoplakia seems to be a prerequisite in the management of oral cancer. Until now, scalpel biopsy has been the only reliable and accepted method for the examination and diagnosis of suspicious oral mucosal lesions, although inter- and intra-observer variability of histological diagnoses often yield insufficient results [3–7]. Recently, exfoliative cytology (EC) has been introduced as a screening tool for the non-invasive examination of suspicious oral leukoplakias and erythroplakias. Yet it is neither proposed for screening nor for the assessment of urgently suspicious lesions but to investigate leukoplakias and erythroplakias in which malignancy cannot be ruled out [8–13]. It is principally based on the method of Papanicolaou, which is accepted worldwide as a successful screening method for epithelial dysplasias and in situ or invasive carcinomas of the uterine cervix. Additionally, DNA-image-cytometry has been introduced as a tool adjuvant to the cytological diagnosis for the very early diagnosis of malignant transformation of squamous epithelial cells [8,12–14]. Most recently, the combination of both EC and DNA-image-cytometry has been reported to be a highly sensitive, specific, and non-invasive method for the early diagnosis of oral epithelial neoplasia, showing excellent compliance among patients [8,12,13]. The main objective in the treatment of oral leukoplakia is to completely remove potentially neoplastic cells, due to the possibility of recurrence and/or malignant transformation from those cells. In recent years,

*Correspondence to: Dr. Frank Schwarz, Department of Oral Surgery, Heinrich Heine University, Westdeutsche Kieferklinik, D-40225 Düsseldorf, Germany. E-mail: info@frank-schwarz.de
Accepted 8 April 2005
Published online 13 June 2005 in Wiley InterScience (www.interscience.wiley.com).
DOI 10.1002/lsm.20188

various treatment approaches such as surgical removal (e.g., surgical excision, laser surgery, cryotherapy), photodynamic therapy, and topical medical treatments (e.g., anti-inflammatory agents, anti-mycotic agents, carotenoids, retinoids, and cytotoxic agents) have been proposed and are considered to be equally effective in ablating these lesions [15–22]. Because of a high absorption of its wavelength (10,600 nm) by water, the CO_2 laser has been reported to be very effective for the surgery of soft tissues exhibiting a high water content [23–27]. The cure rate following CO_2 laser assisted ablation of oral leukoplakia has been reported to be within the range of conventional treatment approaches varying from 57% to 97% [28–32]. The absorbed energy causes vaporization of intra- and extra-cellular fluid and destruction of cell membranes [33]. Since ablation is mainly due to the action of heat generation, carbonization may occur easily on the irradiated surface. Therefore, the CO_2 laser produces a relatively thin layer of thermally changed tissue around the ablated lesion [34]. Indeed, several studies have pointed out that epithelial regeneration is delayed following laser ablation, and wounds take longer to re-epithelialize than following excision surgery with sutures [19,24]. Among all lasers emitting in the near- and mid-infrared spectral range, absorption of the Er:YAG laser in water is the greatest because its 2,940 nm wavelength coincides with the large absorption band for water. Indeed, the Er:YAG laser theoretically has a 10 times higher absorption coefficient of water than the CO_2 laser [35,36]. Recent histologic findings investigating the morphological, histochemical, and immunocytochemical changes of the human oral mucosa after CO_2- or Er:YAG-laser irradiation have shown that the Er:YAG laser may be routinely used in surgery because of its minimal damage of the epithelial tissue, its low inflammatory reaction, its quicker healing process, and its lower risk of scarring [37]. Based on these findings, it may be hypothesized that the Er:YAG laser may also be suitable for the ablation of oral leukoplakia. However, until now no studies were available evaluating the influence of an Er:YAG on the cure rate of leukoplakia. Therefore, the aim of the present pilot study was to determine therapeutic responses to Er:YAG- and CO_2-laser ablation in patients with oral leukoplakia as evaluated clinically and by means of histology, EC and DNA-image-cytometry.

MATERIALS AND METHODS

Subject Selection and Study Design

Ten patients, partially or fully edentulous, each of whom displayed at least one leukoplakia lesion were included. The patient population comprised five men and five women (mean age 55 ± 12 years). Three of them reported a history of cigarette smoking (<10 per day). The average duration of these lesions, as estimated on patients anamnesis, ranged between 6 and 24 months. In all cases, laser treatment was not preceded by other treatment modalities. Each patient was given a detailed description of the procedure and signed consent prior to participation. The study protocol was in accordance with the Helsinki Declaration of 1975, as revised in 1983. Ten patients with a total of 16 lesions were randomly treated using either (1) an Er:YAG laser device ($n = 5$ patients/8 lesions), or (2) an CO_2 laser device ($n = 5$ patients/8 lesions).

Baseline Clinical Examination

Baseline clinical examination consisted of determining the localization and extension of oral leukoplakia. All lesions were accurately measured and photographed for further evaluation. Usually, lesions were small, homogenous and plaque like. Some, however, were more extensive, reaching even 2–4 cm in diameter. A detailed description of each treated patient is presented in Table 1.

Histologic, Cytologic, and DNA-Cytometric Examinations

Cytologic (EC) and DNA-cytometric (DNA-I) examinations were performed at the baseline examination (before treatment), as well as 24–96 weeks postoperatively. To obtain a smear, a Cytobrush cell collector (Cytobrush GT, Med-Scand Medical, Malmö, Sweden) was rolled at the same place of the mucosal lesion at least five times with gentle pressure [38]. The brush was turned around its own axis on four different positions of a glass slide in order to transfer the cells, which were immediately fixed with Merckofix-spray (Merck, Darmstadt, Germany). Additionally, incisional biopsies were taken of the respective oral lesions at baseline and prepared for histo-pathological evaluation. The examination of the slides and the biopsy specimens were carried out in the institutes of cytopathology and pathology, University of Duesseldorf, Germany, respectively.

Staining of the Smears

The glass slides were stained according to Papanicolaou and examined according to accepted cytological criteria for dysplasia and malignancy [39]. Boecking [40] has defined the following categories of cytological diagnoses: "insufficient" for specimens without any or with exclusively autolytic cells, "tumor cell negative" (1) for inconspicuous, reactive or inflammatory cellular images, "doubtful for tumor cells" (2) in cases with slight atypical cellular changes (e.g., with mild or moderate dysplasia), "suspicious for tumor cells" (3) if only sparse abnormal or severe dysplastic cells were seen or the diagnostic criteria for malignancy were only vague and "tumor cell positive" (4) for smears containing unequivocal malignant cells. In cases of a doubtful, suspicious (2, 3) or tumor cell positive (4) cytological diagnosis, the nuclear DNA-contents of the respective cells were measured after Feulgen restraining of the slides, using a TV image analysis system. For that purpose the slides were uncovered in xylene, destained, and restained with Schiff's reagent [41–44]. If necessary, restaining of Feulgen stained slides according to Papanicolaou was possible.

Measurement of DNA-Contents

The AutoCyte QUIC DNA-workstation (AutoCyte, Burlington, NC, Zeiss, Jena, Germany) was used for the

TABLE 1. Individualized Description of the Study Population and Therapeutical Remissions

Patient	Gender	Age	Location	Lesion size (cm)	CD(1)	Laser	N	Therapeutical remission	Residual patches (%)	Follow-up time (weeks)	Recurrence	Recurrent patches (%)	CD(2)	Smoker
1	Male	36	FM	1.5 × 0.5	neg.	CO	1	Complete	0	28	no	0	neg.	Yes
2	Female	40	GM regio 14–15	3.0 × 0.5	neg.	ERL	2	Complete	0	54	no	0	neg.	No
			GM regio 24–27	3.0 × 0.5	neg.	CO	1	Complete	0	36	no	0	neg.	
3	Male	62	GM regio 46–48 buccal	3.5 × 1.0	neg.	ERL	2	Partial	5	64	no	0	neg.	No
			GM regio 46–48 lingual	3.0 × 0.5	neg.	ERL	2	Partial	13	24	no	0	neg.	
4	Male	69	GM regio 11–13	2.5 × 0.5	neg.	ERL	2	Partial	12	24	no	0	neg.	No
5	Female	69	GM regio 23–27	1.0 × 0.5	neg.	CO	1	Complete	0	56	no	0	neg.	No
			GM regio 13–17	2.5 × 0.5	neg.	ERL	3	Partial	4	56	no	0	neg.	
6	Female	59	FM	1.5 × 0.5	neg.	CO	2	Complete	0	24	no	0	neg.	No
7	Female	53	GM regio 15–17	4.0 × 1.5	neg.	CO	2	Partial	15	32	yes	60	neg.	No
			GM regio 24–26	3.5 × 1.0	neg.	CO	2	Partial	15	32	yes	60	neg.	
8	Male	42	LM right	4.0 × 1.0	neg.	ERL	2	Partial	8	56	no	0	neg.	No
			LM left	4.0 × 0.5	neg.	CO	2	Partial	10	48	no	0	neg.	
9	Male	67	AM right	1.0 × 0.5	neg.	CO	1	Complete	0	96	no	0	neg.	Yes
			AM left	1.5 × 0.5	neg.	ERL	1	Complete	0	96	no	0	neg.	
10	Female	45	FM	2.0 × 0.5	neg.	ERL	1	Complete	0	92	no	0	—	Yes

AM, angle of mouth; FM, anterior floor of the mouth; GM, gingival mucosa; LM, lateral lingual margin; CD, cytologic and DNA-cytometric examination before(1), and after 24–96(2) weeks; N, number of treatment sessions; ERL, Er:YAG laser; CO, CO_2 laser; neg., negative for dysplasia or DNA-aneuploidy.

measurements of the nuclear DNA-contents in the Feulgen stained slides; it consists of a conventional light microscope adapted to a TV black-white camera of a computer-based TV image analysis system [45]. The European Society for analytical Cellular Pathology (ESACP) task force on standardization of diagnostic DNA-image-cytometry [41,43,46] has defined standards for the performance of these systems.

A lesion has been classified as DNA-diploid, if there was only one DNA-stem-line between 1.80c and 2.20c. A lesion was characterized as DNA-polyploid if there were DNA-stem-lines between 1.80c and 2.20c and between 3.60c and 4.40c. DNA-aneuploidy was assumed, if there were abnormal stem lines <1.80c and >2.20c, or <3.60c and >4.40c and/or 9c exceeding events (9cEE) >0 [47]. A DNA-stem-line was defined as the G_0/G_1 cell-phase fraction of a proliferating cell population (with a first peak and a second doubling one, or nuclei in the doubling region) [43,48].

Treatments

In both groups, laser treatment was performed under local anesthesia according to a combined parallel group/split-mouth design. An Er:YAG laser device (KEY 3®, KaVo, Biberach, Germany) emitting a pulsed infrared radiation at a wavelength of 2.94 μm was selected for laser treatment of the test group. Laser parameters were set at 300 mJ/pulse and 10 Hz, according to the recommendation of the manufacturer. The laser beam was guided in a defocused mode (spot size 2–4 mm) under water irrigation (handpiece 2060, KaVo). In the control group, treatment was performed using a CO_2 laser (Smart US20D, DEKA, Freising, Germany) at 4–6 W and 20–50 Hz in focused mode (12.5 mm), according to the recommendation of the manufacturer. In both groups, ablation of the lesions was performed up to the layer of the subepithelial connective tissue, recognizable as a slight bleeding, including a safety margin of clinically normal tissue of about 3 mm around each lesion. The amount of time needed for ablation depended on the extension of the respective lesion, varying from 5 to 15 minutes in both groups. All treatments were performed by the same experienced operator. The postoperative care consisted of mouth rinses with a 0.2% CHX solution (Corsodyl®, GlaxoSmithKline Consumer Healthcare, Bühl, Germany) twice a day for 2 minutes over the first 8 postoperative days. All patients were reviewed at 1-week intervals for 4 weeks following treatment. Additional treatment sessions were provided according to individual needs.

Evaluation of Therapeutic Remissions

Complete remission following treatment was defined as total disappearance of leukoplakia patches on clinical inspection and confirmed by negative cytologic and DNA-cytometric examinations. Partial remission was defined as incidence of residual leukoplakia patches, which failed to respond on additional treatment approaches. In these cases, cytologic and DNA-cytometric examinations were used to exclude malignancy or DNA-aneuploidy.

Follow-Up Observation

All patients were considered to be routinely re-examined at 12-week intervals following treatment. However, only four patients joined the recall sessions meticulously due to an insufficient compliance. The follow-up observation period ranged from 24 to 96 weeks (mean: 51.1 ± 25.4, median: 51). Recurrence was defined as reappearance of leukoplakia at areas previously showing complete remission. At each follow up session, cytologic and DNA-cytometric examinations were performed to exclude DNA-aneuploidy at both previously irradiated areas exhibiting complete or partial remission, and areas exhibiting recurrence of leukoplakia.

Morphometric Assessment of Residual/Recurrent Leukoplakia Patches

Digital images of all lesions were evaluated using a software program (Image J®, Scion Corp., Frederick, Maryland). Residual/recurrent leukoplakia patches were measured as a percentage of the respective lesion. All measurements were performed by one blinded and calibrated examiner.

RESULTS

The postoperative healing was uneventful in all cases. In both groups, there were no signs of any adverse effects that could be associated with the specific treatment procedure. However, wound healing seemed to be improved following irradiation with ERL. In 14 of 16 leukoplakias, EC revealed no sign of malignancy or dysplasia immediately before treatment, whereas 2 of 16 cases revealed atypical regenerative epithelial cells. Both cases revealed inconspicuous, reactive or inflammatory cellular images, which were classified as (2) "doubtful for tumor cells" [40]. However, in both cases, ICM revealed DNA polyploidy. Among all investigated lesions, EC revealed no sign of malignancy or dysplasia following laser-assisted ablation. Histological examination was negative for malignancy in all lesions before treatment. The results are summarized in Tables 1 and 2 as well as in Figures 1, 2, and 3.

DISCUSSION

The results of the present study have shown that both ERL and CO may successfully be used for the ablation of oral leukoplakia. However, both treatment modalities seemed to be limited with regard to complete remission and recurrence following irradiation. In particular, ERL assisted ablation resulted in most of the cases in a partial remission but without any recurrence of the lesions, whereas the use of CO was mainly associated with a complete remission but with recurrence of leukoplakia patches in two cases. In this context, it is important to point out that the present study does not have the statistical power to rule out the possibility of a difference between the two groups. Further prospective, controlled, randomized, clinical studies of higher power are needed to support equivalence or superiority [49]. On the other hand, it needs to be pointed out that these are the first clinical data evaluating the use of an ERL for the ablation of oral

TABLE 2. Details on Histologic (H), Cytologic, and DNA-Cytometric Examinations (CD) Before (1), and After 24–96 (2) Weeks

Patient	Location	CD(1)	H(1)	CD(2)
1	FM	Hyperkeratosis, no dysplasia	Hyperplasia, parakeratosis, no dysplasia	Normal squamous epithelial cells
2	GM regio 14–15	Hyperkeratosis, no dysplasia	Hyperkeratosis, no dysplasia	Normal squamous epithelial cells
	GM regio 24–27	Hyperkeratosis, no dysplasia	Hyperkeratosis, no dysplasia	Normal squamous epithelial cells
3	GM regio 46–48 buccal	Hyperkeratosis, no dysplasia	Akanthosis, hyperkeratosis, no dysplasia	Hyperkeratosis, no dysplasia
	GM regio 46–48 lingual	Hyperkeratosis, no dysplasia	Akanthosis, hyperkeratosis, no dysplasia	Hyperkeratosis, no dysplasia
4	GM regio 11–13	Atypical regenerative epithelial cells DNA polyploidy	Hyperkeratosis, no dysplasia	Hyperkeratosis, no dysplasia
5	GM regio 23–27	Hyperkeratosis, no dysplasia	Hyper-parakeratosis, no dysplasia	Normal squamous epithelial cells
	GM regio 13–17	Hyperkeratosis, no dysplasia	Hyper-parakeratosis, no dysplasia	Hyperkeratosis, no dysplasia
6	FM	Atypical regenerative epithelial cells DNA polyploidy	Ortho-hyperkeratosis, no dysplasia	Normal squamous epithelial cells
7	GM regio 15–17	Hyper-parakeratosis, no dysplasia	Hyperplasia, parakeratosis, no dysplasia	Reactive changes in squamous epithelial cells with slightly altered maturity of cells
	GM regio 24–26	Hyper-parakeratosis, no dysplasia	Hyperplasia, parakeratosis, no dysplasia	
8	LM right	Hyperkeratosis, no dysplasia	Akanthosis, hyper-parakeratosis, no dysplasia	Hyperkeratosis, no dysplasia
	LM left	Parakeratosis, no dysplasia	Akanthosis, hyper-parakeratosis, no dysplasia	Hyperkeratosis, no dysplasia
9	AM right	Hyperkeratosis, no dysplasia	Hyperplasia, hyperkeratosis, no dysplasia	Normal squamous epithelial cells
	AM left	Hyperkeratosis, no dysplasia	Hyperplasia, hyperkeratosis, no dysplasia	Normal squamous epithelial cells
10	FM	Hyperkeratosis, no dysplasia	Ortho-hyperkeratosis, no dysplasia	—

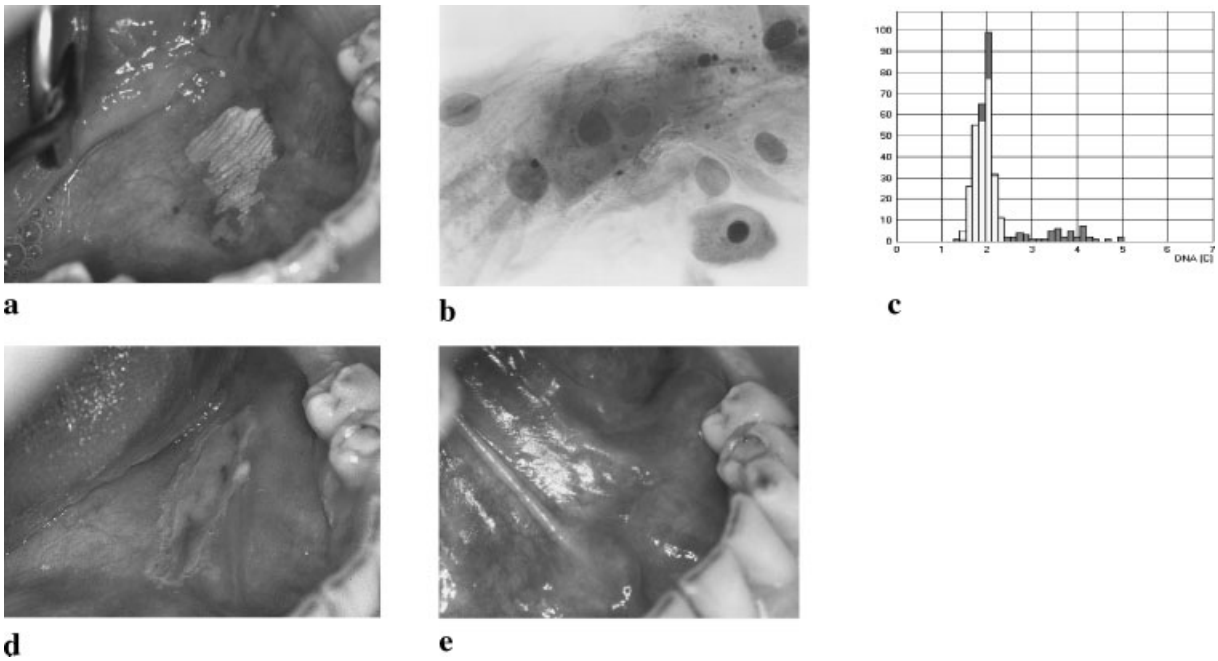

Fig. 1. Baseline situation of Patient 10 **(a)**. Nucleated and anucleated keratinized cells with keratohyaline granules were seen on a clean background (PAP×63) **(b)**. DNA-histogram revealed DNA-polyploidy **(c)**. Clinical situation 3 days **(d)** and 92 weeks **(e)** following treatment with ERL exhibited full remission and no recurrence of the leukoplakia lesion.

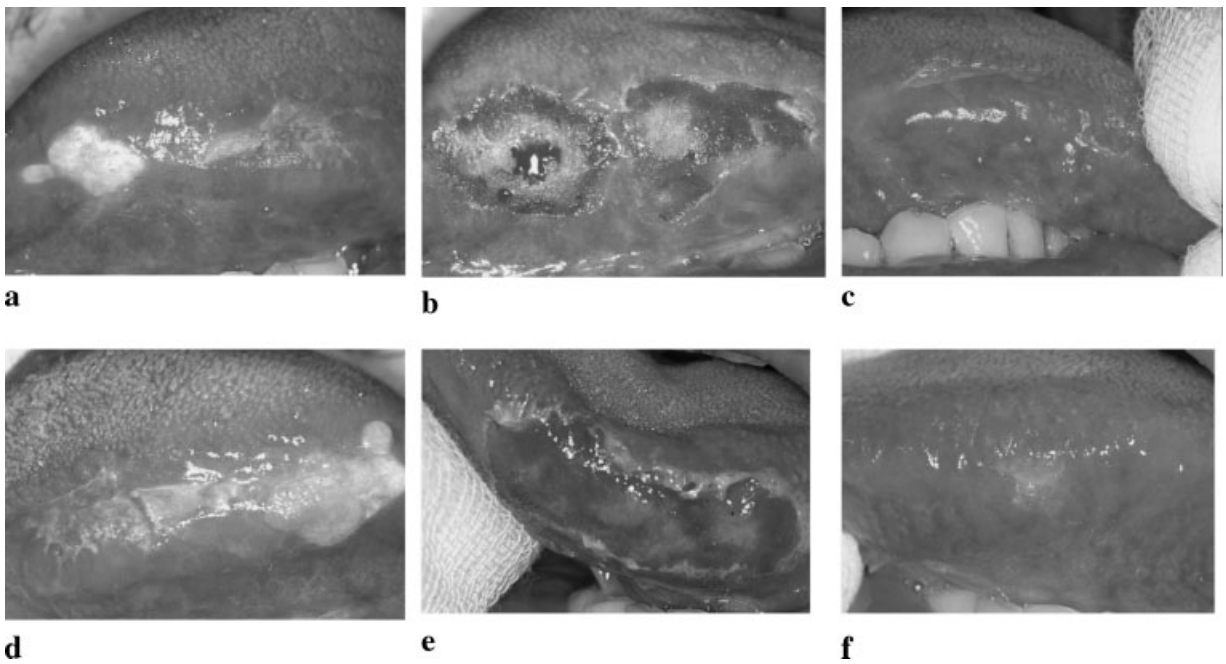

Fig. 2. Extensive contralateral leukoplakia at the lateral lingual margins of Patient 8 **(a**, **d)**. Clinical situation 3 days following treatment with ERL **(b)** showing improved wound healing compared to CO **(e)**. Partial remission of the lesions without any signs of recurrence 56 weeks following treatment with ERL **(c)**, and 48 weeks following treatment with CO **(f)**.

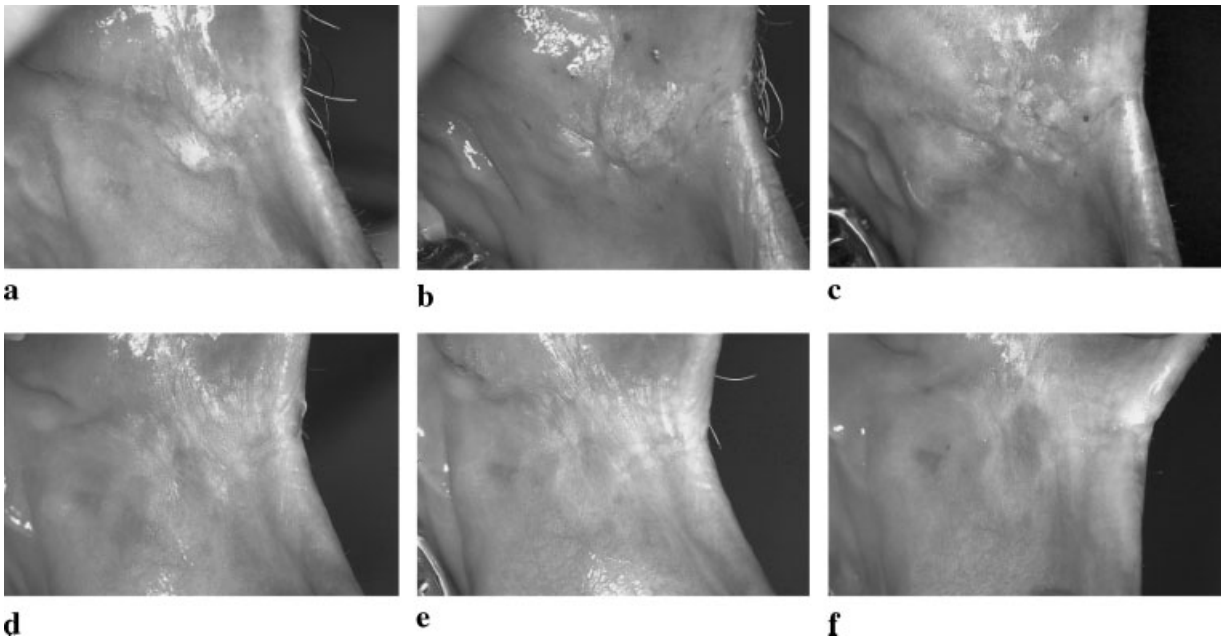

Fig. 3. Sequence of clinical healing of a leukoplakia lesion at the left angle of mouth following treatment with ERL (Patient 9). Situation at baseline **(a)**, and after 1 **(b)**, 2 **(c)**, 6 **(d)**, 12 **(e)**, and 96 **(f)** weeks following treatment. Note that full remission was noted 6 weeks following treatment.

leukoplakia. As mentioned above, recent histologic findings investigating the morphological, histochemical, and immunocytochemical changes of the human oral mucosa after CO- or ERL-irradiation have shown that the ERL may be routinely used in surgery because of its minimal damage of the epithelial tissue, its low inflammatory reaction, and its quicker healing process [37]. These findings are in agreement with the present observation that wound healing generally seemed to be improved following irradiation with ERL. However, postoperative healing was uneventful in both groups, outlining that there were no signs of any adverse effects that could be associated with laser treatment. The finding that ablation of oral leukoplakia using CO may be associated with recurrence is in agreement with previous studies [28–32]. Chisea et al. [30] reported that the probability of developing recurrences or new occurences ranged between 40% after 3 years and 23% within 1 year following irradiation. Similar findings were also reported by Horch et al. [28] with 22% of local recurrences within an average follow-up period of 37 months. However, Roodenburg et al. [32] observed a cure rate of 90% during a mean observation period of 5.3 years. In this context, it was observed that new patches of leukoplakia mainly develop adjacent to the margins of perviously treated sites [50]. Based on these findings, recurrence of leukoplakia patches may be explained by the fact that new epithelium migrates from the periphery to cover the wound may originate from areas of potentially altered mucosa [50]. This suggests that the origin of cells in recurrence of oral leukoplakia may be located in adjacent epithelia, visualized clinically as normal oral mucosa before and during treatment [51]. Indeed, it has been observed that an inadequate margin, as evaluated by means of molecular profiles, may in part be responsible for the high rate of recurrence, especially in high-risk lesions [52]. However, recent findings have also pointed out that the margin status of initial oral leukoplakia resection had no relation to the development of oral cancer [10]. Nevertheless, from a clinical point of view, it seems to be important to ascertain the border of the respective lesion inclusive a safety margin on the one hand, and a sufficient ablation depth of the oral mucosal epithelium on the other hand [51]. However, despite complete ablation of the respective lesion as evaluated clinically, the adjacent or peripherial epithelium may proliferate in the recurrence phenomenon, since these epithelial tissues have been reported to consist of highly proliferating cells which are probably abundantly widespread in the basal cell layer [51]. In this context, it must be pointed out that "field cancerization" of oral mucosal cancer has been shown to be very important in explaining the presence of dysplastic cells adjacent to squamous cell carcinoma, and recurrence following laser assisted ablation [53]. Therefore, implementation of very early diagnostic tools seems to be a prerequisite in the management of oral cancer. In the present study, brush biopsies with additional cytological/DNA-cytometric examination have been chosen for microscopic evaluation of oral leukoplakia. Among all investigated lesions, there were no signs of malignancy or dysplasia before or following laser-assisted ablation. As mentioned above, the combination of both EC and DNA-image-cytometry has been reported to be a highly sensitive, specific and non-invasive method for the early

diagnosis of oral epithelial neoplasia, showing excellent compliance among patients [8,12,13]. In this context, however, it must be emphasized that the present study has also some drawbacks. One key limitation was the lack of consistent follow-up by patients. Secondly, it has to be noted that only two out of the lesions revealed atypical regenerative epithelial cells. Furthermore, all lesions investigated were classified as homogeneous leukoplakias. Since non-homogeneous leukoplakias and lesions with signs of dysplasia may carry a higher degree of risk of transformation [54,55], further research is needed to verify the efficacy of both treatment approaches in the treatment of such lesions.

Within the limits of the present study, it may be concluded that both treatment approaches seem to have limitations to achieve predictable eradication of oral leukoplakia.

REFERENCES

1. Kramer IR, Lucas RB, Pindborg JJ, Sobin LH. Definition of leukoplakia and related lesions: An aid to studies on oral precancer. Oral Surg Oral Med Oral Pathol 1978;46(4):518–539.
2. Hansen LS, Olson JA, Silverman S, Jr. Proliferative verrucous leukoplakia. A long-term study of thirty patients. Oral Surg Oral Med Oral Pathol 1985;60(3):285–298.
3. Abbey LM, Kaugars GE, Gunsolley JC, Burns JC, Page DG, Svirsky JA, Eisenberg E, Krutchkoff DJ. The effect of clinical information on the histopathologic diagnosis of oral epithelial dysplasia. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998;85(1):74–77.
4. Pindborg JJ, Reibel J, Holmstrup P. Subjectivity in evaluating oral epithelial dysplasia, carcinoma in situ and initial carcinoma. J Oral Pathol 1985;14(9):698–708.
5. Fischer DJ, Epstein JB, Morton TH, Schwartz SM. Interobserver reliability in the histopathologic diagnosis of oral pre-malignant and malignant lesions. J Oral Pathol Med 2004;33(2):65–70.
6. Abbey LM, Kaugars GE, Gunsolley JC, Burns JC, Page DG, Svirsky JA, Eisenberg E, Krutchkoff DJ, Cushing M. Intraexaminer and interexaminer reliability in the diagnosis of oral epithelial dysplasia. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1995;80(2):188–191.
7. Karabulut A, Reibel J, Therkildsen MH, Praetorius F, Nielsen HW, Dabelsteen E. Observer variability in the histologic assessment of oral premalignant lesions. J Oral Pathol Med 1995;24(5):198–200.
8. Maraki D, Becker J, Boecking A. Cytologic and DNA-cytometric very early diagnosis of oral cancer. J Oral Pathol Med 2004;33(7):398–404.
9. Sudbo J, Kildal W, Risberg B, Koppang HS, Danielsen HE, Reith A. DNA content as a prognostic marker in patients with oral leukoplakia. N Engl J Med 2001;344(17):1270–1278.
10. Sudbo J, Lippman SM, Lee JJ, Mao L, Kildal W, Sudbo A, Sagen S, Bryne M, El-Naggar A, Risberg B, Evensen JF, Reith A. The influence of resection and aneuploidy on mortality in oral leukoplakia. N Engl J Med 2004;350(14):1405–1413.
11. Sciubba JJ. Improving detection of precancerous and cancerous oral lesions. Computer-assisted analysis of the oral brush biopsy. US Collaborative OralCDx Study Group. J Am Dent Assoc 1999;130(10):1445–1457.
12. Remmerbach TW, Weidenbach H, Hemprich A, Bocking A. Earliest detection of oral cancer using non-invasive brush biopsy including DNA-image-cytometry: Report on four cases. Anal Cell Pathol 2003;25(4):159–166.
13. Remmerbach TW, Weidenbach H, Pomjanski N, Knops K, Mathes S, Hemprich A, Bocking A. Cytologic and DNA-cytometric early diagnosis of oral cancer. Anal Cell Pathol 2001;22(4):211–221.
14. Duesberg P, Li R, Rasnick D. Aneuploidy approaching a perfect score in predicting and preventing cancer: Highlights from a conference held in Oakland, CA in January, 2004. Cell Cycle 2004;3(6):823–828.
15. Einhorn J, Wersall J. Incidence of oral carcinoma in patients with leukoplakia of the oral mucosa. Cancer 1967;20(12):2189–2193.
16. Lodi G, Sardella A, Bez C, Demarosi F, Carrassi A. Interventions for treating oral leukoplakia. Cochrane Database Syst Rev 2004;3(CD001829) DOI: 10.1002/14651858.CD001829.pub2.
17. Epstein JB, Gorsky M, Wong FL, Millner A. Topical bleomycin for the treatment of dysplastic oral leukoplakia. Cancer 1998;83(4):629–634.
18. Shah JP, Strong EW, DeCosse JJ, Itri L, Sellers P. Effect of retinoids on oral leukoplakia. Am J Surg 1983;146(4):466–470.
19. Ben-Bassat M, Kaplan I, Shindel Y, Edlan A. The CO_2 laser in surgery of the tongue. Br J Plast Surg 1978;31(2):155–156.
20. Mincer HH, Coleman SA, Hopkins KP. Observations on the clinical characteristics of oral lesions showing histologic epithelial dysplasia. Oral Surg Oral Med Oral Pathol 1972;33(3):389–399.
21. Poswillo DE. Cryosurgery of the oral mucous membranes. Proc R Soc Med 1975;68(10):608–609.
22. Sieron A, Adamek M, Kawczyk-Krupka A, Mazur S, Ilewicz L. Photodynamic therapy (PDT) using topically applied delta-aminolevulinic acid (ALA) for the treatment of oral leukoplakia. J Oral Pathol Med 2003;32(6):330–336.
23. Carruth JA. Resection of the tongue with the carbon dioxide laser: 100 cases. J Laryngol Otol 1985;99(9):887–889.
24. Frame JW. Removal of oral soft tissue pathology with the CO_2 laser. J Oral Maxillofac Surg 1985;43(11):850–855.
25. Loh HS. A clinical investigation of the management of oral lichen planus with CO_2 laser surgery. J Clin Laser Med Surg 1992;10(6):445–449.
26. Pecaro BC, Garehime WJ. The CO_2 laser in oral and maxillofacial surgery. J Oral Maxillofac Surg 1983;41(11):725–728.
27. White JM, Chaudhry SI, Kudler JJ, Sekandari N, Schoelch ML, Silverman S, Jr. Nd:YAG and CO_2 laser therapy of oral mucosal lesions. J Clin Laser Med Surg 1998;16(6):299–304.
28. Horch HH, Gerlach KL, Schaefer HE. CO_2 laser surgery of oral premalignant lesions. Int J Oral Maxillofac Surg 1986;15(1):19–24.
29. Silverman S, Jr., Chu FW, Dedo HH. CO_2 laser treatment of oral leukoplakia. Laryngoscope 1988;98(2):125–130.
30. Chiesa F, Tradati N, Sala L, Costa L, Podrecca S, Boracchi P, Bandieramonte G, Mauri M, Molinari R. Follow-up of oral leukoplakia after carbon dioxide laser surgery. Arch Otolaryngol Head Neck Surg 1990;116(2):177–180.
31. van der Hem PS, Nauta JM, van der Wal JE, Roodenburg JL. The results of CO_2 laser surgery in patients with oral leukoplakia: A 25 year follow up. Oral Oncol 2005;41(1):31–37.
32. Roodenburg JL, Panders AK, Vermey A. Carbon dioxide laser surgery of oral leukoplakia. Oral Surg Oral Med Oral Pathol 1991;71(6):670–674.
33. Rossmann JA, Brown RS, Hays GL, Lusk SS. Carbon dioxide laser surgical therapy for the management of oral leukoplakia: A case report. Tex Dent J 1994;111(12):17–19, 21.
34. Arashiro DS, Rapley JW, Cobb CM, Killoy WJ. Histologic evaluation of porcine skin incisions produced by CO_2 laser, electrosurgery, and scalpel. Int J Perio Rest Dent 1996;16(5):479–491.
35. Hale GM, Querry MR. Optical constants of Water in the 200 nm to 200 É m wavelength region. Appl Optics 1973;12:555–563.
36. Robertson CW, Williams D. Lambert absorption coefficients of water in the infrared. J Opt Soc Am 1971;61:1316–1320.
37. Zaffe D, Vitale MC, Martignone A, Scarpelli F, Botticelli AR. Morphological, histochemical, and immunocytochemical study of CO_2 and Er:YAG laser effect on oral soft tissues. Photomed Laser Surg 2004;22(3):185–189.
38. Ogden GR, Cowpe JG, Green M. Cytobrush and wooden spatula for oral exfoliative cytology—A comparison. Acta Cytol 1992;36(5):706–710.

39. Koss L. Diagnostic cytology and its histopathologic bases, 4th edn. Philadelphia: Lippincott Company; 1992. pp 865–879.
40. Bocking A. Standardisierte befunderstellung in der extragenitalen zytologie, standardisierung der zytopathologischen diagnostik (in german). Pathologe 1998;19:235–240.
41. Böcking A, Giroud F, Reith A. Consensus report of the ESACP task force on standardization of diagnostic DNA image cytometry. European Society for Analytical Cellular Pathology. Anal Cell Pathol 1995;8(1):67–74.
42. Chatelain R, Willms A, Biesterfeld S, Auffermann W, Bocking A. Automated feulgen staining with a temperature-controlled staining machine. Anal Quant Cytol Histol 1989; 11(3):211–217.
43. Haroske G, Baak JP, Danielsen H, Giroud F, Gschwendtner A, Oberholzer M, Reith A, Spieler P, Bocking A. Fourth updated ESACP consensus report on diagnostic DNA image cytometry. Anal Cell Pathol 2001;23(2):89–95.
44. Ogden GR, Cowpe JG, Wight AJ. Oral exfoliative cytology: Review of methods of assessment. J Oral Pathol Med 1997; 26(5):201–205.
45. Sanchez L, Regh M, Biesterfeld S, Chatelain R, Bocking A. Performance of a TV image analysis system as a microdensitometer. Anal Quant Cytol Histol 1990;12(4):279–284.
46. Giroud F, Haroske G, Reith A, Bocking A. 1997 ESACP consensus report on diagnostic DNA image cytometry. Part II: Specific recommendations for quality assurance. European Society for Analytical Cellular Pathology. Anal Cell Pathol 1998;17(4):201–208.
47. Bocking A, Motherby H. Abklärung zervikaler dysplasien mittels DNA-Bildzytometrie (in german). Pathologe 1999; 20:25–33.
48. Haroske G, Giroud F, Reith A, Bocking A. 1997 ESACP consensus report on diagnostic DNA image cytometry. Part I: Basic considerations and recommendations for preparation, measurement and interpretation. European Society for Analytical Cellular Pathology. Anal Cell Pathol 1998;17(4): 189–200.
49. Gunsolley JC, Elswick RK, Davenport JM. Equivalence and superiority testing in regeneration clinical trials. J Periodontol 1998;69(5):521–527.
50. Frame JW. Removal of oral soft tissue pathology with the CO_2 laser. J Oral Maxillofac Surg 1985;43(11):850–855.
51. Ishii J, Fujita K, Komori T. Laser surgery as a treatment for oral leukoplakia. Oral Oncol 2003;39(8):759–769.
52. Zhang L, Poh CF, Lam WL, Epstein JB, Cheng X, Zhang X, Priddy R, Lovas J, Le ND, Rosin MP. Impact of localized treatment in reducing risk of progression of low-grade oral dysplasia: Molecular evidence of incomplete resection. Oral Oncol 2001;37(6):505–512.
53. Grant WE, Hopper C, Speight PM, Macrobert AJ, Bown SG. Photodynamic therapy of malignant and premalignant lesions in patients with 'field cancerization' of the oral cavity. J Laryngol Otol 1993;107(12):1140–1145.
54. Lumerman H, Freedman P, Kerpel S. Oral epithelial dysplasia and the development of invasive squamous cell carcinoma. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1995;79(3):321–329.
55. Schepman KP, van der Meij EH, Smeele LE, van der Waal I. Malignant transformation of oral leukoplakia: A follow-up study of a hospital-based population of 166 patients with oral leukoplakia from The Netherlands. Oral Oncol 1998;34(4): 270–275.