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Papers

Risk of Preneoplastic and Neoplastic Events in Operated Oral Leukoplakias

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We retrospectively analysed 167 consecutive patients with oral leukoplakias operated on by CO_2 laser resection in the Day Hospital of the Istituto Nazionale Tumori in Milan from January 1981 to December 1988, with post-operative histological examination negative for cancer. Within 5 years there were 69 patients with at least one unfavourable event. First unfavourable events were: 31 local relapses, 27 new leukoplakias, 5 oral carcinomas and 6 other neoplasms elsewhere. To identify possible prognostic factors we recorded age, sex and history of previous oral leukoplakias or head and neck cancers; also number, site, size and type of lesion; as well as tobacco and alcohol consumption and oral hygiene. The Cox regression model was employed to compare disease-free survival between different patient groups, both by univariate and multivariate analysis. From this analysis it emerges that age of operated patients and size of resected lesion are significantly predictive for development of relapses, new leukoplakias and carcinomas.
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INTRODUCTION

ORAL LEUKOPLAKIAS are considered precancerous lesions, with a malignant transformation rate estimated to range from 0% to 20% over 20 years [1–7], although clinical and pathological correlations are still uncertain. Classification of oral precancerous lesions is still based on the clinical picture, while precancerous lesions of the cervix uteri and larynx, which are similar to oral precancerous lesions both clinically and pathologically [7], are now classified histologically. The present definition of oral leukoplakia is in fact a negative one: "... white patch or plaque that cannot be removed by scraping and cannot be classified clinically or histologically as another disease entity, ... use of this term is unrelated to the absence or presence of dysplasia" [1].

There is consensus that surgery is the most effective therapy for leukoplakia and that laser surgery is the technique of choice [7–10]; however, many surgeons vaporise these lesions leaving no specimens for post-operative histological examination.

Criteria for judging the malignant potential of an oral leukoplakia have not been established, although some authors report that non-homogeneous, nodular lesions often show dysplasia and have a higher canceration rate than those with a homogeneous clinical picture [1, 5, 11]. Prognostic factors in general are not well-known, although many studies have been published, and establishing these would be useful for planning preventive measures aimed both at the general population and GPs.

Since January 1981 we have been systematically treating all operable leukoplakias observed at the Istituto Nazionale Tumori of Milan (INT) by CO_2 laser excision. This paper analyses a series of uniformly treated and followed patients. The aim was to evaluate the hazard ratio of subsequent preneoplastic and neoplastic events according to a number of putative prognostic factors.

MATERIALS AND METHODS

We retrospectively analysed 167 patients (128 males, 39 females, median age 57, range 27–79 years) with oral leukoplakias, operated on consecutively by CO_2 laser resection in the INT's Day Hospital from January 1981 to December 1988, and with post-operative histological examination negative for dysplasia and cancer. Median follow-up was 52 months (75% had at least 78 months of follow-up and 25% at least 29 months). Preoperative evaluation included careful clinical examination and photograph of lesion; two types of oral leukoplakias were distinguished: 1. Homogeneous, with keratinised mucosa; 2. Non-homogeneous: (a) speckled red and white lesions; (b) leuko-erythroplasia, reddish ulcerated lesions.

Operations were performed under local anaesthesia using the laser in continuous wave mode (9–12 W power output) and in conjunction with an operating microscope of 200 mm focal length. Lesions were stained with toluidine blue after Mashberg [12] to delineate margins. The laser was used as a knife to remove the whole lesion with at least 0·5 cm margins in healthy tissue (in depth and laterally); in this way the

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pathologist was able to evaluate the specimens. Chronic inflammatory infiltrate in the lamina propria, plus epithelial hyperplasia with extensive surface keratinisation (hyperkeratosis) and/or incomplete keratinisation (parakeratosis), without dysplasia, were always observed on histopathological examination.

History of previous oral leukoplakias or head/neck cancers, number, site, size and type of lesions, tobacco and alcohol consumption habits, and oral hygiene were recorded (Table 1). The 20 cigarette/day threshold divided heavy from light smokers [13] and 1 l of wine/day (or equivalent) divided heavy from light drinkers [14]. Oral hygiene was rated according to the state of teeth or prostheses and the presence of tartar or plaque: as good (good teeth without plaque or tartar), sufficient (plaque or tartar present, good teeth) or poor (badly-fitted denture or broken teeth and presence of plaque and tartar). Data were not recorded systematically at the beginning of the study and items are missing in 3–11% of patients. Some patients had been treated previously for oral leukoplakia (10.2%) or a head and neck cancer (11.4%). Most (124 = 74.2%) had a single lesion when leukoplakia was diagnosed. Non-homogeneous leukoplakia was observed in 115/167 (68.9%) patients. The buccal mucosa (99 = 59.2%), tongue (27 = 16.2%) and lip (23 = 13.8%) were the most frequently involved sites. Many patients were drinkers (74.3%), smokers (59.3%), or had poor oral hygiene (39.5%).

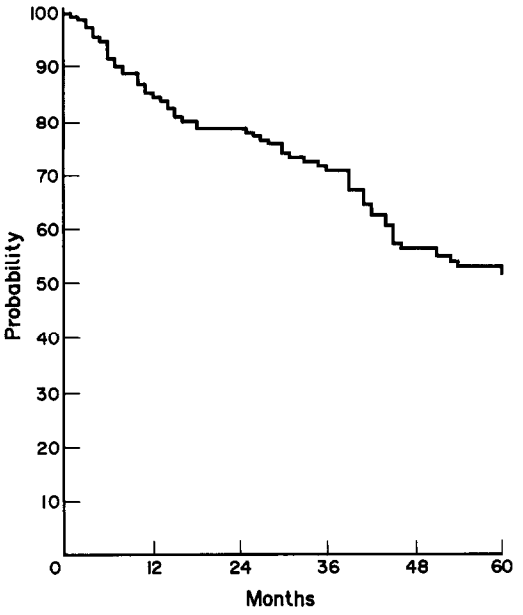

Fig. 1. Disease-free survival.

Table 1. *Summary of the clinical characteristics of* 167 *patients treated by* CO_2 *laser excision*

Sex	*N*	%	Site	*N*	%
Male	128	76.6	Lip	23	13.8
Female	39	23.4	Tongue	27	16.2
			Floor of the mouth	5	3.0
Age			Buccal mucosa	99	59.2
< 45 years	23	13.8	Gingiva	9	5.4
46–65 years	108	64.6	Palate	4	2.4
> 65 years	36	21.6			
			Smoking habits: smoker		
Previous leukoplakias			Not recorded	11	6.6
Not recorded	5	3.0	No	36	21.6
No	145	86.8	Yes	99	59.2
Yes	17	10.2	Ex	21	12.6
Previous head and neck cancers			Tobacco consumption		
Not recorded	5	3.0	Not recorded	11	6.6
No	143	85.6	No	57	34.1
Yes	19	11.4	< 20 Cigarettes/day	76	45.5
			> 20 Cigarettes/day	23	13.8
Size					
< 1 cm	24	14.4	Drinking habits: drinker		
1–2 cm	77	46.1	Not recorded	13	7.8
2–3 cm	44	26.3	No	29	17.4
> 3 cm	22	13.2	Yes	124	74.2
			Ex	1	0.6
Number of leukoplakias					
1	124	74.2	Wine consumption		
2	39	23.4	Not recorded	13	7.8
3	3	1.8	No	30	18.0
4	1	0.6	≤ 1 l/day	116	69.4
			> 1 l/day	8	4.8
Type of leukoplakia					
Homogeneous	52	31.1	Oral hygiene		
Non homogeneous			Not recorded	17	10.2
Speckled	94	56.3	Good	57	34.1
Leuko-erythroplasia	21	12.6	Sufficient	27	16.2
			Poor	66	39.5

Table 2. Univariate analysis: hazard ratio in 167 operated patients, according to prognostic factors

Variables	Unadjusted hazard ratio	95% Confidence interval	Wald statistic	*P*
Sex				
Females vs. males*	1.101	0.627–1.932	0.11206	0.7378
Age				
46–65 vs. ⩽45*	2.123	0.909–4.961	3.02405	0.0820
>65 vs. ⩽45*	2.144	0.821–5.600	2.42390	0.1195
Previous leukoplakias				
Yes vs. no*	0.810	0.292–2.244	0.16457	0.6850
Previous head and neck cancers				
Yes vs. no*	1.126	0.558–2.275	0.11022	0.7399
Size of lesion				
1–2 cm vs. <1 cm*	1.760	0.824–3.760	2.12884	0.1446
>2 cm vs. <1 cm*	2.180	0.964–4.929	3.50354	0.0612
Number of lesions				
>1 vs. 1*	0.978	0.565–1.692	0.00623	0.9371
Type of lesion				
Homogeneous vs. non homogeneous*	0.983	0.593–1.631	0.00416	0.9485
Site of lesion				
Anterior oral cavity vs. posterior*	0.844	0.528–1.350	0.49959	0.4797
Lateral oral cavity vs. median*	1.179	0.644–2.158	0.28357	0.5944
Smoking habits				
Smokers vs. non smokers*	0.692	0.395–1.215	1.63945	0.2004
Ex smokers vs. non smokers*	0.711	0.295–1.716	0.57492	0.4483
Tobacco consumption (no. of cigarettes/day)				
⩽20 vs. 0*	0.828	0.468–1.463	0.42390	0.5150
>20 vs. 0*	0.826	0.410–1.664	0.28472	0.5936
Alcohol habits				
Drinkers vs. non drinkers*	1.015	0.517–1.994	0.00186	0.9656
Alcohol consumption (l/day)				
⩽1 l vs. 0*	0.805	0.417–1.553	0.41885	0.5175
>1 l vs. 0*	1.944	0.717–5.273	1.70654	0.1914
Hygiene				
Poor vs. good*	0.967	0.594–1.575	0.01833	0.8923

* Reference category.

Patients were checked every 6 months. When a local relapse or new precancerous lesion was diagnosed, another laser excision was proposed. Patients who refused surgery underwent vitamin A therapy or simple check-up if they did not want therapy. If cancer developed, patients were treated according to INT therapeutic protocols.

In the analysis of the disease-free survival and unfavourable events, patients with multiple lesions were considered as one unit.

Statistical methods

Disease-free survival (DFS) was calculated as the time from surgery either to first relapse (local failure, new precancerous lesion), occurrence of tumour (oral or elsewhere), or date of last check up if neither relapse nor tumour was observed. DFS curves were estimated according to the product-limit method [15].

Preliminary graphical analysis suggested that the proportional hazard assumption was tenable, therefore the Cox regression model [16] was employed to compare disease-free survival experience between different patient groups, both by univariate and multivariate analysis. In this model the regression coefficient corresponds with the logarithm of the ratio between hazards for the unfavourable event. For patients grouped into two putative prognostic categories, and under the null hypothesis of having the same DFS experience, the hazard ratio is expected to be 1.0. When the DFS of patients in a given category is lower (higher) than that of patients in the reference category, the hazard ratio is greater (less) than 1.0.

Univariate analyses were performed on each putative prognostic factor and the corresponding "unadjusted" hazard ratios calculated. Prognostic factors with an unadjusted hazard ratio significantly different from 1.00 (at the 20% significance level or less) were subjected to multivariate analysis to evaluate their combined effects and to estimate, for each variable, the "adjusted" hazard ratio. The variables selected by this procedure were: age, smoking, alcohol consumption

Table 3. Multivariate analysis: hazard ratio in 154 operated patients (with information on all variables available) according to prognostic factors significant at 20% level in univariate analysis

Variables	Adjusted hazard ratio	95% Confidence interval	Wald statistic	*P*	Likelihood ratio test	*P*
Age						
46–65 vs. ⩽45*	2.067	0.868–4.919	2.692	0.1008		
>65 vs. ⩽45*	2.648	0.957–7.325	3.519	0.0607		
					4.199	0.1225
Size of lesion						
1–2 cm vs. <1 cm*	1.958	0.856–4.479	2.534	0.1114		
>2 cm vs. <1 cm*	2.253	0.944–5.374	3.351	0.0671		
					3.982	0.1366
Smoking habits						
Smokers vs. non smokers*	0.785	0.409–1.506	0.531	0.4660		
Ex smokers vs. non smokers*	0.875	0.345–2.217	0.079	0.7778		
					0.531	0.7670
Wine consumption (l/day)						
⩽1 l vs. 0*	0.881	0.439–1.767	0.127	0.7211		
>1 l vs. 0*	1.885	0.651–5.459	1.366	0.2425		
					2.443	0.2948

* Reference category.

Table 4. Multivariate analysis: hazard ratio in 154 operated patients (with information on all variables available) according to age and size of lesion

Variables	Adjusted hazard ratio	95% Confidence interval	Wald statistic	*P*
Age				
46–65 vs. ⩽45*	2.259	0.958–5.325	3.466	0.0626
>65 vs. ⩽45*	2.866	1.059–7.750	4.304	0.0380
Size of lesion				
1–2 cm vs. <1 cm*	2.004	0.887–4.524	2.797	0.0945
>2 cm vs. <1 cm*	2.466	1.042–5.835	4.217	0.0400

* Reference category.

and size of lesion. The multivariate analysis was only carried out on the 154 patients with complete data.

Starting from a model including these four selected variables, a parsimonious model was obtained by means of a backward procedure in which a factor was eliminated whenever the corresponding significance level (obtained from the likelihood ratio test) was higher than 5%. The final model thus obtained included only age and size of lesion as prognostic factors.

RESULTS

At the time of analysis 69 patients had developed at least one unfavourable event (31 local relapses, 27 new leukoplakias, 5 oral carcinomas and 6 cancers elsewhere). 16 of these developed a second unfavourable event (6 local relapses, 9 new leukoplakias and 1 oral carcinoma). And 3 of the 16 developed a third unfavourable event (one oral carcinoma and two cancers elsewhere). Overall 15 patients developed cancer (7 oral cavity, 4 lung, 2 skin, 1 colon, 1 pancreas); 1 of these developed two tumours (oral cavity and lung) and 2 developed three cancers (three oral cavity and three skin). Furthermore, 3 of these 15 patients had already been treated for previous head/neck squamous cell carcinoma.

25 patients (15%) had a follow-up of less than 6 months: when contacted by phone and invited to come for a check up they refused.

DFS is shown in Fig. 1. We considered all the unfavourable events noted above, even if they developed outside the oral cavity. Three year DFS is 70.7% and 5 year DFS is 51.5%.

Table 2 shows the unadjusted hazard ratios (HRs) for the putative prognostic variables considered. DFS experience for patients aged >45 years is poorer than that for younger patients and HRs for patients over 45 (2.123 and 2.144 for 46–65 and >65, respectively) are about double those for younger patients (under 45, reference category) and hence significantly different from 1 at the 20% level.

DFS experience for patients drinking >1 l of wine a day is poorer that than that for patients who did not drink alcohol (reference category), while DFS for "light drinkers" was similar to that for the reference category. The HR of patients drinking >1 l of wine/day was 1.94 times that of patients who

did not drink alcohol (significant at 20% level) while the HR for patients drinking < 1 l/day was very similar to that for the reference category.

Non-smokers have a shorter DFS experience than smokers. Considering non-smokers as the reference category, the HRs of the variables tobacco consumption and habit of smoking (non- ex- and smoker) were less than 1, but not significantly different at the 20% level.

Patients with < 1 cm, lesions had a better DFS, and HRs for larger lesions were 1.76 and 2.18 times higher than those of the reference category: both significantly different from 1 at the 20% level.

The remaining variables considered do not emerge as prognostic since their HRs were close to 1.

The joint effect of age, alcohol consumption, smoking, and size of lesion was evaluated by a multiple regression model. The adjusted HRs obtained from this model (full model) are given in Table 3. They are similar to the four corresponding unadjusted HRs but only age and size of lesion retain a prognostic value.

Adjusted HRs from the parsimonious regression model are reported in Table 4. For two variables, the adjusted HR is similar to the unadjusted HR reported in Table 3: patients > 60 years vs. < 45 years and lesions > 2 cm vs. < 1 cm are significantly different from 1 at the 5% level. The contributions of alcohol consumption and smoking to the full model are not significant; the likelihood ratio test is: $\chi^2 = 2.864$ (4 DF) $P = 0.5808$. From this it emerges that age and size of lesion are the most important prognostic factors in our series.

DISCUSSION

It is known that patients with oral leukoplakia are a high risk population [2–6]. The present study confirms this, revealing a higher cancer incidence in patients operated on for oral leukoplakia than the general population [17]. Furthermore, these patients remain at risk after the operation for developing further leukoplakias and for developing cancers inside and outside the oral cavity. Some authors consider that this risk is related to the presence of dysplasia in the treated leukoplakia [1, 5, 11]; but our experience is that new lesions and cancer after operation are also associated with leukoplakias without dysplasia [4]. We therefore analysed a series of patients homogeneous for therapy (all underwent laser excision) and post-operative histology (hyper-parakeratosis without dysplasia) in order to identify prognostic factors for the development of unfavourable events following the surgery. These factors may easily differ in such a population from those operating in an unselected population.

We considered all available information: (a) host factors—sex, habits (alcohol and tobacco consumption, oral hygiene) and their modification after surgery; (b) field factors—site and type of lesion; and (c) biological factors—history of previous leukoplakias and cancers, age, size and number of lesions.

Of the host factors, being female seems to have a higher HR than being male, though the difference is not significant. Hygiene does not seem to influence the development of new events. Non-drinkers and non-smokers appear to be at risk if alcohol and tobacco consumption are considered alone (Table 2), but smoking and drinking emerge as not significant when evaluated by multivariate analysis (Table 3). Most patients did not change their smoking or drinking habits after surgery (only 2 patients reduced alcohol consumption and stopped smoking before the development of a new leukoplakia) and these factors are not significant in our series; although Banoczy, analysing 320 patients with leukoplakia, found that cancer developed more frequently in those who did not stop smoking or drinking [2]. It is known that recording such habits accurately is difficult because patients tend to under-report [18]; some of our patients disclosed different alcohol and tobacco consumption patterns at different interviews. Some authors report that leukoplakia in non-smokers has a higher risk of canceration than in smokers (in the latter the leukoplakia can disappear if smoking stops) [1, 5, 8].

Field factors do not show significant HRs. This indicates that the clinical picture is not predictive of lesion evolution, and points to the need for a histological classification of oral leukoplakias, as established for leukoplakias in other head and neck areas (e.g. larynx) [7]. We evaluated anterior vs. posterior oral cavity HRs because of the diagnostic and therapeutic difficulties of treating posterior lesions, and also median vs. lateral sites because of the higher incidence of canceration on the floor of mouth and the tongue [5, 11, 12, 18]. However HRs showed no significant differences in either analysis.

Of the biological factors, history of previous leukoplakias and cancers, and number of lesions are not significant in our series, whereas age and size of lesion are significant (Table 4). The risk factors usually considered to predispose for oral precancerous lesions (smoking and drinking) do not emerge as significant in our series. We conclude that while these habits probably favour the appearance of precancerous events, their subsequent evolution depends on other mainly biological factors. In fact our series was selected on the basis of an initial precancerous event and within this selected group, the majority were smokers, drinkers, had poor oral hygiene or all three. We have identified additional biological factors which are prognostic for the development of new preneoplastic and neoplastic events in patients treated surgically for oral leukoplakias.

This result points to the need for action in three areas: (a) continuing research on drugs which may protect high risk patients operated on for oral leukoplakias (to date retinoids seem the best chemopreventive therapy); (b) investigation of biological factors and clinico-histological correlations in order to identify factors or agents (viruses, aploidia, labelling index, markers such as cytokeratins, . . .) which have an aetiological or prognostic significance; (c) multiplication of efforts to inform the population and GPs of the risk of leukoplakias, emphasising that habits considered to be correlated with the first appearance of leukoplakias (drinking alcohol, smoking and poor oral hygiene) should be avoided.

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