

**UNIVERSITY OF MEDICINE AND PHARMACY OF CRAIOVA
FACULTY OF MEDICINE**

DOCTORAL THESIS

SUMMARY

**CLINICAL, HISTOPATHOLOGICAL AND
IMMUNOHISTOCHEMICAL STUDY OF THE EPITHELIAL
PRECANCEROUS LESIONS PRECURSORS OF THE
SQUAMOUS CELL CARCINOMA, ON PHOTOEXPOSED SKIN**

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KEY WORDS: carcinogenesis, apoptosis, precancerous keratinocytic lesions, squamous cell carcinoma, tumor suppressor gene p53, Bcl-2, Ki67, E-cadherin, CD4+, CD8+, CD68.

STATE OF THE KNOWLEDGE

Keratinocytic carcinogenesis evolves in four consecutive stages: initiation, promotion, premalignant progression and malignant conversion, events that involves oncogenes and antioncogenes. Most carcinomas, in particular squamous cell carcinomas (SCC), develop from dysplasia to carcinoma in situ and then to invasive carcinomas. In the areas of skin cancers, the group with mixed pathogenesis, genetic and environmental, is the most important, standing out correlations between the incidence of carcinomas and skin phototype. Genetic factors occur through the mechanism of somatic mutation, the cancer being promoted by an alteration of the genetic apparatus of the cell of origin of the tumor in question.

Recently, the following events necessary for carcinogenesis have been considered: genome instability, disruption of cell cycle, induction of a mechanism for maintaining telomere length and tumor angiogenesis. The transformation of a solid tumor involves a variety of growth factors, associated with angiogenesis stimulation and an early recurrence of visceral cancers, and also having an immunosuppressive role, inhibiting the maturation of dendritic cells. Particular attention is given to stem cells of the skin and the matrix-metalloproteinases, with the assumption that these will become important therapeutic targets for skin therapy.

The studies regarding to oncogenes have focused on the regulation of cell proliferation and the negative control of cell growth: growth and apoptosis blocking.

The development of various carcinomas has been linked to signaling pathways involving any of the tumor suppressor genes p53 and Ras, Myc and Erb - B family of tyrosine kinase receptors. Among the extrinsic factors involved in carcinogenesis, we confer special attention to carcinogenic process induced by ultraviolet radiation, by viruses, by chemical agents, detailing the mechanisms involved. Immunodeficiency is also a risk factor for cancer and its evolution toward metastasis.

The present study focuses on precancerous keratinocytic lesions, precursor of squamous cell carcinoma, located on chronic sun exposed areas, namely: actinic keratoses, actinic cheilitis, cutaneous horn, Bowen's disease and keratoacanthoma.

In recent years many authors tried to redefine actinic keratoses and keratoacanthomas as malignant neoplasms, considering them superficial

intraepithelial squamous cell carcinoma in evolution and particular forms of SCC, respectively. This paper details the mechanisms by which UV radiation is involved in the development of the keratinocytic precancerous lesions and possibly SCC, and presents recent data on cancer related molecular alterations (mutations of p53, overexpression of telomerase, similar chromosomal aberrations) claiming their genetic relationship with SCC and possible malignant nature of these lesions, previously considered as precancerous lesions.

Numerous clinical aspects encountered correspond to a variety of histopathological types, which confirms the importance of the histopathological examination in the diagnosis of these lesions and their malignant potential. Other controversial issues are the evolutionary pathway, the possibility of regression of the lesions, and metastatic potential for some of them.

Most studies show increased levels of p53 and p63 in lesional cells, and also have reported increased expression of the pro apoptotic and cell proliferation markers, as bcl-2 and Ki67, increased expression of cyclooxygenase-2, E-cadherin, and PCNA. Immunoperoxidase stains can be used to distinguish the type of the precancerous lesion and differentiation from the SCC, although there is still much controversy regarding the utility of the immunohistochemical markers.

PERSONAL RESEARCH

Clinical study of the keratinocytic precancerous lesions

The study group included 557 patients hospitalized for a 5 years period, from 1 January 2006 to 31 December 2010, in the Dermatology Clinic of Craiova; only 224 epithelial precancerous lesions localized on chronic sun exposed skin and precursor of SCC were histopathologically diagnosed, in 87 cases malignancy being noticed. Statistical data obtained show that actinic keratoses represented 39.29 % of cases, followed by keratoacanthoma (36.16%), cutaneous horn (10.71%), actinic cheilitis (9.37%), Bowen's disease (4.47%). Age was the most important risk factor for the development of the keratinocytic precancerous lesion and SCC; most cases were diagnosed between the 7th and 8th decades of life. The highest incidence of the malignant transformed cases was recorded in the eighth decade of life. There was no predisposition regarding the sex, although actinic keratoses, cutaneous horn, keratoacanthoma and Bowen's disease were observed more frequently in women, but without statistical significance, while actinic cheilites were diagnosed more frequently in men (68.42%).

The role of the cumulative exposure to ultraviolet radiation in the etiology and the pathogenesis of the keratinocytic precancers is supported by the location of these lesions on chronically sun exposed skin, the location of the cephalic extremity representing 85.22% of the cases of actinic keratosis, 83.33% of the cases of cutaneous horn, 87.65% of the keratoacanthomas and 80% of the lesions of Bowen's disease. The analysis of phototype of the skin revealed the presence of phototype I, II and III in 65-90% of patients. Keratinocytic precancerous lesions had a relatively equal distribution between the urban and rural areas, excepting for actinic cheilitis which occurs in 90.47% of cases in patients from rural areas ($p < 0.001$); malignant actinic cheilitis were diagnosed in 91.66% of the cases in rural areas. Hence, rural environment is a significant risk factor for the occurrence of the actinic cheilitis and its malignancy, but not for the other precancerous lesions, which demonstrates that the residence is only one of the factors involved in their development. The accuracy of the clinical diagnosis of actinic keratosis was 21.90%, 57.10% of misdiagnosed cases being considered as basal cell carcinomas. For actinic cheilitis clinical diagnostic accuracy was only 32%, most commonly being diagnosed as SCC. Accuracy of clinical diagnosis of cutaneous horn, keratoacanthoma and Bowen's disease was 83.33%, 72.84% and 50% respectively. Clinical suspicion of malignancy for cases in which histopathological examination reveals malignant transformation was 47.12%.

Regarding clinical aspects encountered in the studied group we have noticed increased frequency of the hypertrophic type of actinic keratosis (71.59%), and increased its polymorphism explains many presumptive clinical diagnoses encountered. From clinical forms of actinic cheilitis we observed predominantly the intermediate keratosis type (50%), followed by the appearance of chronic descumative cheilitis (39.47%) and abrasive cheilitis Anzilotti-Manganotti (10.47%). Bowen's diseases with single lesions have been met in 90% of cases and of particular clinical forms we encountered only one case of Bowen's disease with multiple lesions.

Keratoacanthomas present a multitude of clinical aspects, remarking increased frequency of solitary forms (98.76%), and prevalence of the typical form (86.25%). Progression of keratinocytic precancerous lesions to squamous cell carcinoma is mainly due to chronic exposure to ultraviolet radiation, particularly UVB; we noted the appearance of the precancers transforming to SCC in 64.19% of the

keratoacanthomas, 57.14% of the actinic cheilitis, 20.45% of the actinic keratoses, 20% of Bowen's disease, and 12.5% of the cases of cutaneous horn. Clinical suspicion of progression to malignancy was 27.77% for actinic keratoses, 75% for actinic cheilitis, 66.66% for cutaneous horn, 50% for Bowen's disease and 17.34% for keratoacanthomas.

Histopathological study of the epithelial precancerous lesions

Histopathological study included 224 cases; in most of the cases multiple histological sections were performed in order to form an image of the tumor as a whole. Histological study focused on the following objectives: to assess the degree of the cell differentiation; to evaluate the structural polymorphism compared with the clinical aspects; to assess the degree of the invasion of the surrounding structures; host tissue reaction to the presence of the tumor; the role of peritumoral immune barrier; detailed analysis of peritumoral area.

For the 88 cases of actinic keratoses (AK) the histopathological subtypes (table no. 1) classification was based on the appearance of the tumor, the presence of the melanin hyperpigmentation of cells from the deeper layers of the epidermis, the spinous layer atrophy and dermo-epidermal junction layout, the presence of slots or acantholytic gaps, presence of acanthosis, papillomatosis, anaplastic cells.

Histopathologic variants of AK	No. of cases	Percentage (%)
Hypertrophic AK	58	65,93
Bowenoid AK	1	1,15
Acantholytic AK	2	2,28
Atrophic AK	4	4,55
Pigmented AK	3	3,41
Inflamed AK	2	2,28
AK with areas of in situ carcinoma (CIS)	4	4,55
AK with areas of microcarcinoma	7	7,98
AK with areas of CIS and microcarcinoma	3	3,41
AK with areas of moderately differentiated SCC	4	4,55

Table 1.
Distribution of actinic keratoses (AK) by histopathological aspect

In 18 cases (20.45%) histopathological examination reveals malignant transformation of the actinic keratoses: AK with areas of carcinoma in situ (CIS) - 22.22%, AK with areas of CIS and microcarcinoma – 16.68 %, AK with microcarcinoma areas - 38.8%, AK with areas of moderately differentiated SCC - 22.22 %. In malignant cases, we noticed an intense hyperkeratosis, presence of the dyskeratosis, the presence of the atypia and invariable presence of an intense lymphoplasmocitar inflammatory infiltrate most abundant and dense in the dermis.

The malignant transformation of actinic cheilitis, also in the SCC, was confirmed in 57.15% of cases: actinic cheilitis with in situ carcinoma (25.01%), actinic cheilitis with areas of microcarcinoma (58.33%), and actinic cheilitis with well differentiated invasive SCC (16.66%). Performing multiple sections allowed revealing the area of the carcinoma in situ. Reactionary inflammatory infiltrate at this level is denser and marks the imminent rupture of the basement membrane. All malignant cases were characterized by intense inflammatory infiltrate, which is a marker for an adjacent squamous cell carcinoma. 12.5% of the lesions of cutaneous horn were transformed into squamous cell carcinoma; the possibility of a skin cancer at the base of the cutaneous horn should be considered especially in elderly people with chronic exposure to the sun. Overall lesion length correlates with the presence of dysplasia, an abundant chronic inflammatory infiltrate and an evolution trend toward malignancy.

In cases of Bowen's disease, both in cases with single lesions as well as with multiple lesions, histopathologically identical appearance was suggestive for the diagnosis of carcinoma in situ. Dermal invasion was noticed in 20% of cases, occurring initially in a limited area, highlighting the area of carcinoma in situ and microcarcinoma, and in one case the image is of an invasive SCC, and the inflammatory infiltrate became abundant.

Keratoacanthoma was diagnosed in 81 cases, of which only 29 cases (35.80%) without malignancy. The lesions have a tendency to persist and progress toward an invasive squamous cell carcinoma. Thus, we observed progression of the neoplastic process in keratoacanthoma to keratoacanthoma with dysplasia, keratoacanthoma with areas of carcinoma in situ (3.84%), keratoacanthoma with microcarcinoma (46.15%), keratoacanthoma with well-differentiated invasive SCC (26.92%), moderately differentiated (19.25%) or acantholytic (3.84%). Increased level of invasion correlates with increased stromal inflammatory infiltrate, sometimes seeing a giants-cell inflammatory reaction similar with a foreign body reaction.

Immunohistochemical study of the epithelial precancerous lesions

Immunohistochemical study was performed on 28 precancerous cutaneous lesions precursor of the squamous cell carcinoma, located on photoexposed skin (actinic keratosis, actinic cheilitis, cutaneous horn, keratoacanthoma, Bowen's disease). The major objectives of the immunohistochemical study were: assessment of the expression of p53, bcl2, Ki67, PCNA, COX-2 protein, associated with

apoptosis and cell cycle in order to establish their role in tumor progression and malignant transformation of the cutaneous epithelial precancers; assessing cell adhesion protein expression E-cadherin; the utility of these antibodies in the differentiation of the squamous cell carcinoma (SCC); highlighting the involvement of the immune response using anti-CD4 antibody (helper T cells), anti-CD8 (cytotoxic T lymphocytes), anti-CD45RO (memory T cells), anti-CLA (common leukocyte antigen), anti-CD68 (macrophages), in intratumoral and peritumoral stroma of the epithelial precancerous lesions. Immunohistochemical method used in the study was of the soluble enzyme immunoassay methods, based on complex called LSAB/HRP (Labelled streptavidin biotin). Grout used was DAKO LSAB 2 System HRP (DAKO Labelled Universal Biotin 2 System Horseradish Peroxidase Streptavidin).

P53 expression was found in all actinic keratoses, higher in the hypertrophic type, which can support a greater number of cells with p53 mutations, and greater resistance to this type of keratosis cells to apoptosis. All actinic cheilitis express p53, the intensity of the expression increasing in malignant forms, suggesting the involvement of the p53 in carcinogenesis, but not as a marker of malignant transformation. In the cutaneous horn the strength of immunostaining was weak, which correlates with low grade dysplasia in these lesions. Keratoacanthomas p53 immunostaining intensity correlates with the degree of the dysplasia and the presence of the malignancy. Utility of the p53 in differentiating keratoacanthoma from SCC is controversial, but emphasizes a keratinocyte population that may have a potential for aggressive growth. In Bowen's disease I have noticed a moderate positive nuclear immunostaining for p53 in the two thirds of the lower epidermis, and sometimes in the whole thickness of the epidermis. 80% of the studied actinic keratoses, regardless of the histological subtype, expressed bcl-2 with diffuse distribution in 80% of the cases. Distribution pattern found maximum cytoplasmic positivity in basal keratinocytes and sometimes in the upper layers of the epidermis. Cutaneous horn found similar pattern of expression of bcl-2 with the hypertrophic actinic keratoses, and in actinic cheilitis I have noticed the absence of the immunostaining for bcl-2. In keratoacanthoma a positive cytoplasmic immunostaining for bcl-2 was highlighted in 70% of the studied cases. In Bowen's disease we have noticed low intensity cytoplasmic immunostaining positivity for bcl-2. Increasing bcl-2 expression in suprabasal layers was associated with epidermal

hyperproliferation and apoptosis resistance, which may promote tumor progression and tumor invasion.

All epithelial precancers, excepting for the actinic cheilitis, were cytoplasmic positive for Cox-2, the intensity of the immunostaining being weak or moderate. The intensity of the immunostaining of the COX-2 is a useful marker in distinguishing keratoacanthoma from SCC. Immunoexpression of the E-cadherin was studied in 22 precancerous lesions and positivity was detected in 63.63% of the cases; the intensity and the proportion of cells expressing the marker, and also the distribution of the pre-cancerous cells varied depending on the type of the precancerous lesion. Ki67 expression was detected in all the studied epithelial precancers. Increased occurrence of dysplasia and malignancy correlates with an intensely positive immunoexpression, increased number of positive cells, and diffuse distribution of immunostaining in all cases, suggesting a higher proliferative activity. Ki67 expression showed a peripheral pattern in keratoacanthoma, unlike squamous cell carcinoma, in which the expression is more diffuse.

PCNA expression was detected in all the studied epithelial precancerous lesions, all of them showing positive nuclear immunostaining. In hypertrophic actinic keratoses the intensity of the immunoexpression and the number of the positive cells is higher than in atrophic actinic keratosis; in malignant forms a diffuse positivity in area of microcarcinoma was noticed. Most of the atypical epidermal keratinocytes were PCNA-positive, noticing a positive correlation with the p53 and Ki67 expression. In cases of malignant actinic cheilitis there was a close correlation between the expression of PCNA, Ki67 and p53. PCNA immunostaining in cutaneous horn was moderately intense and diffusely positive in less than 50% of cells. We have noticed a similar expression of Ki67, which could suggest a potential correlation between the two markers.

In keratoacanthoma, PCNA marking intensity was moderate in 70% of cases, and poor in the remaining cases. I have noticed a peripheral pattern of expression in mild dysplasia shapes and forms with moderate dysplasia and pseudoepitheliomatous hyperplasia the proportion of affected cells increases, affecting diffuse between $\frac{1}{2}$ and $\frac{2}{3}$ of the epidermis. In the presence of the malignancy a diffuse distribution of the immunostaining in the carcinoma area was noticed. In Bowen's disease, positive nuclear PCNA was expressed in all cases, noticing a diffuse distribution marking,

with moderate to intense positivity; the percentage of the positive cells was above 50% in all of the cases. PCNA expression was correlated with Ki67 expression.

Immunostaining for CD45-common leukocyte antigen (CLA), was moderately or strongly positive in all of the studied precancers, suggesting a predominantly lymphocytic inflammatory infiltrate, plus histiocytes, neutrophils, eosinophils. Distribution was particularly sublezioneal diffuse, a denser infiltrate being noticed in inflamed forms or with presence of malignization.

Positive membrane immunostaining for CD45RO was observed in 95.45% of the studied cases, this highlighting the inflammatory infiltrate of mature T lymphocytes. CD4+ positive immunostaining was observed in 50% of the studied cases. In actinic keratoses malignancy correlates with the presence of the T helper population growth in the dermal inflammatory infiltrate. In actinic cheilitis and Bowen's disease CD4+ immunostaining was negative.

CD8+ positive immunostaining was seen in the 91.66 % of the studied cases, at the surface of the membrane. In actinic keratoses CD8+ positive immunostaining was observed in 83.33% of the cases, the intensity of immunostaining being weakly positive in lymphocytes isolated from the superficial dermis; in malignant forms immunostaining with moderate intensity distribution was diagnosed in isolated cells from the superficial dermis. A similar moderately positive marking was noted in actinic cheilitis, with distribution in rare interstitial lymphocytes and focal distribution in malignant forms. In Bowen's disease a moderately positive immunostaining, denser in common epithelial - stromal junction lymphocytes in whole thickness of the epidermis, was noted. We have noted in this study a predominance of the T cytotoxic cells population in the reacted inflammatory infiltrate.

CD68 positive immunostaining was observed in 81.81% of the studied cases; the staining pattern was diffuse or granular cytoplasmic. In actinic keratoses moderately positive CD68 immunostaining was observed in isolated subepidermal macrophages, in all the studied cases. The presence of the malignancy in two cases correlated with increased influx of macrophages, immunohistochemical proved through moderately positive immunostaining in common subepidermal macrophages. In all cases of the actinic cheilitis we have noticed a moderate positive CD68 immunostaining in rare subepithelial macrophages. In Bowen's disease we did not reveal a positive immunostaining for CD68.

Conclusions

1. Actinic keratoses (39.29%) and keratoacanthomas (36.16%) are the most common precancerous epithelial lesions encountered in the studied group. Keratoacanthoma's frequency is 3-4 times higher than previously reported by other authors.
2. Age was the most important risk factor for the development of the precancerous keratinocytic lesions; frequency of precancers and their subsequent malignancies increases with age, people aged between 51 and 90 years representing 90% of the cases. There was not a predisposition about sex or area of origin, although the incidence was higher in women (excluding actinic cheilitis) and rural areas (except for Bowen's disease).
3. More than 80% of the precancerous lesions are located on the cephalic region or on photoexposed skin. The analysis of the phototype revealed the presence of the phototype I, II and III in 65-90% of patients, depending on the type of the precancer.
4. The accuracy of the clinical diagnosis of all epithelial precancers was only 49.55% and a strong clinical suspicion of malignancy was present in 47.12% of the cases. Actinic keratoses had the lowest diagnostic accuracy due to the lesions polymorphism.
5. As clinical forms, the studied group was dominated by the hypertrophic actinic keratosis (71.59%), intermediate keratosis cheilitis (50%), Bowen's disease with single lesions (90%) and the typical solitary keratoacanthoma (86.25%).
6. We have found the malignant transformation of keratoacanthoma in 64.19% of the cases, 57.14% of the actinic cheilitis, 20.45% of the actinic keratoses, 20% of the Bowen's disease, 12.5% of the cases of cutaneous horn; the transformation was made only in squamous cell carcinoma. No clinical features 100% advocate for benign or malignant nature therefore histopathologic examination should be used in order to confirm the diagnosis of the epithelial precancers, especially because in many cases the clinical appearance of the precancer corresponds to SCC revealed histopathologically.
7. For actinic keratoses the highest risk of transformation has thicker lesions, hyperkeratotic and ulcerated. The appearance of the malignancy was positively correlated with age, with the infiltration of the lesion, the occurrence of pain (in 50% of cases correlates with malignancy), and the size of the lesion.

8. In actinic cheilitis malignant transformation correlated with the presence of ulcerative or erosive keratosis lesions (malignant alarm signals). All malignant cases were characterized by intense inflammatory infiltrate, which is a marker for an adjacent squamous cell carcinoma.
9. The cutaneous horn lesion length correlates with the presence of dysplasia and chronic inflammatory abundant infiltrate and evolution trend toward malignancy.
10. In Bowen's disease ulceration and infiltration of the lesion represented clinical signs of alarm for malignant transformation. Dermal invasion was noticed in 20% of the cases.
11. Keratoacanthoma has a tendency to persistence and progression to an invasive squamous cell carcinoma; we observed progression of the neoplastic process from keratoacanthoma to keratoacanthoma with dysplasia, keratoacanthoma with areas of carcinoma in situ (3.84%), keratoacanthoma with microcarcinoma (46.15%), respectively well-differentiated invasive SCC (26.92%), moderately differentiated SCC (19.25%) or acantholytic (3.84%).
12. The presence of the epithelial precancers indicates a long time solar injury and allows the identification of a population group at high risk of developing of SCC, BCC or melanoma. In 33% of the studied precancers we have observed association with skin cancers, predominantly carcinomas.
13. P53 expression was found in all the studied precancers, more intense in hypertrophic actinic keratoses, actinic cheilitis with malignization and keratoacanthoma; p53 immunostaining intensity correlate with the degree of the dysplasia and the presence of the malignancy. P53 immunostaining is useful in highlighting a population keratinocytes that may have an aggressive growth potential.
14. I have not noticed significant differences in the intensity of the immunoexpression and proportion of bcl-2 positive cells between malignant and non-malignant cases. Increased expression of bcl-2 in Bowen's disease in the suprabasal layers is associated with epidermal hyperproliferation and apoptosis resistance, which promote tumor progression and tumor invasion.
15. COX-2 immunoexpression intensity is weak or moderate in all epithelial precancers studied. We did not observe significant differences in the intensity of the COX-2 immunoexpression and the proportion of the positive cells between malignant and non-malignant cases, but in malignant cases we have found a moderate positive

cytoplasmic immunostaining in the area of dysplasia and in rare cells of basal cell layer.

16. Imunoexpression of E-cadherin was negative in all actinic keratoses and most actinic cheilitis; keratoacanthoma in 50% of the cases showed positive immunostaining for E-cadherin with cytoplasmic distribution on focal areas; immunostaining was negative in areas of dysplasia and carcinoma.

17. Ki67 expression was detected in all of the studied epithelial precancers. All actinic keratoses, regardless of the histological type, expressed Ki67, the intensity of the expression being higher in the malignant cases. We have noticed a positive correlation of p53 expression with Ki67 immunoreactivity in areas with increased proliferative activity of actinic keratoses and cutaneous horn.

18. Ki-67 expression in keratoacanthoma shows a peripheral pattern, unlike squamous cell carcinoma in which is more diffuse; the number of the Ki67 positive cells in keratoacanthoma is lower than in squamous cell carcinoma (IHC distinguishing features). In keratoacanthomas with pseudoepitheliomatous hyperplasia we have observed an increase in positive cells up to one third of the epidermis; the distribution is diffuse. In keratoacanthomas with malignant transformation immunostaining is diffuse in the carcinoma area in approximately 30% of the tumor cells.

19. Bowen's disease showed diffuse positive nuclear immunostaining for Ki67 in more than 50% of the cells, showing nuclear atypia and atypical mitoses specific to carcinoma in situ.

20. In hypertrophic actinic keratoses PCNA immunostaining intensity is higher and with a diffuse distribution to two thirds of the epidermis, unlike atrophic forms. Most of the atypical epidermal keratinocytes were PCNA-positive, noticing a positive correlation with the p53 and Ki67 expression.

21. Actinic cheilitis without malignization expressed PCNA less than cases with malignant transformation. In malignant cases there is a close correlation between the expression of PCNA, Ki67 and p53.

22. In keratoacanthoma, I have noticed a peripheral pattern of PCNA expression in forms with mild dysplasia and in moderate dysplasia and with hyperplasia; in pseudoepitheliomatous forms increase the proportion of the affected cells, affecting diffuse between $\frac{1}{2}$ and $\frac{2}{3}$ of the epidermis. In the presence of the malignancy, I have noticed a diffuse distribution of the immunostaining in the carcinoma area.

23. In Bowen's disease, positive nuclear PCNA was expressed in all cases, noticing a diffuse distribution marking, with moderate to intensely positivity; the percentage of the positive cells was above 50% in all cases. PCNA expression was correlated with Ki67 expression.

24. CD45 immunostaining (CLA) was denser in the inflamed forms or with the presence of the malignancy. Malignant keratoses expressed moderately or strongly positive CD45RO lymphocytes in common inflammatory infiltrate in the dermis, predominantly peritumoral.

25. Positive membranar immunostaining for CD45RO was observed in 95.45% of the studied cases. In actinic cheilitis, with or without malignancy, polymorphic inflammatory infiltrate with plasma cells are frequency; lymphocytes and PMN leukocytes are rare. In malignant keratoacanthomas and Bowen's disease positive immunostaining was moderate in frequent interstitial lymphocytes, being particularly abundant sublesional.

26. In precancerous keratinocytic lesions immune response of epithelial tissue is still effective, proved by the maintained capacity of the keratinocytes to activate the CD4+ helper T cells; altering mechanisms of immunodefense representing an important role in transformation in SCC. In malignant actinic keratoses positive immunostaining was in frequent helper T lymphocytes which are the main lymphocyte population of dermal reacted inflammatory infiltrate. In actinic cheilitis and Bowen disease CD4+ immunostaining was negative.

27. Immunostaining for CD8+ was more intense and diffuse in malignant forms, both for actinic keratoses and actinic cheilitis and Bowen's disease, showing the predominance of cytotoxic T population in the abundant inflammatory infiltrate, and an altered immune response.

28. The presence of the actinic keratoses malignancy was correlated with increased influx of macrophages, immunohistochemical materialized through a moderately positive CD68 immunostaining in frequent subepidermal macrophages. In actinic cheilitis in all cases we observed a moderate positive CD68 immunostaining, in rare subepithelial macrophages.

29. Actinic keratoses, keratoacanthoma and Bowen's disease are strong predictors of the risk of developing skin carcinoma and melanoma. Although these lesions are considered clinically benign, epidemiological, molecular, and histological arguments

highlights the close connection between them and the risk of developing a SCC; as a consequence therapeutic attitude should require prompt and careful monitoring.