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DOCTORAL THESIS ABSTRACT

CUTANEOUS LYMPHOMAS.

CLINICO-PATHOLOGICAL AND GENETIC CORRELATIONS

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Contents

I.INTRODUCTION.....	1
1. GENERAL PART. Current state of knowledge	2
1.1 CLINICAL AND HISTOPHENOTYPICAL ELEMENTS. SKIN LYMPHOMA AND BENIGN LYMPHOPROLIFERATIONS.....	2
1.2 GENETIC EVENTS IN THE ONCOGENESIS OF SKIN LYMPHOMA. GENETIC INSTABILITY, COPY NUMBER VARIATIONS AND ALTERATIONS	3
2. SPECIFIC PART	4
2.1 MATERIAL AND METHOD.....	4
2.2 RESULTS.....	4
2.2.1 CONTRIBUTIONS ON CLINICAL AND PARACLINICAL ASPECTS. PARTICULARITIES AND CORRELATIONS	5
2.2.2 CONTRIBUTIONS ON THE ANATOMO-PATHOLOGICAL AND IMMUNOPHENOTYPICAL ASPECTS	6
2.2.3 CONTRIBUTIONS ON THE GENETICS OF SKIN LYMPHOMAS AND BENIGN LYMPHOPROLIFERATIONS.....	7
2.3 DISCUSSIONS.....	8
3. CONCLUSIONS.....	9

I. INTRODUCTION

Progressive clonal proliferations of B and T lymphocytes or NK cells in the skin, which bring together a group of diseases with different evolution and prognosis, are known as Cutaneous Lymphomas (LC). From a morphological point of view, the skin tissue can be both the initial or unique tissue affected or secondary to a previous organ. The topic of the present doctoral thesis is directed on primary cutaneous non-Hodgkin's lymphomas, without systemic damage at the time of diagnosis. These diseases, which fall into the niche of dermato-oncology, are extremely difficult to address, due to the small number of cases, their complexity, and the lack of trained specialists in this field.

Depending on the cellularity of the infiltrate found at the skin level, cutaneous lymphomas are divided into cutaneous B or T cell lymphomas. Most primary cutaneous lymphomas originate from the T lymphocyte (65% of cases), the number of cutaneous B cell lymphomas being lower.

T and B cell lymphoproliferations in the skin fall into two broad categories: malignant and benign lymphoproliferations. Differentiating the two pathologies is sometimes extremely difficult, due to the multiple common features, both clinically, histologically and immunophenotypically.

The tumor microenvironment is complex and difficult to understand, composed of elements that are expressed by objective symptoms, blood tests, pathological and genetic analysis. Taken individually, these elements are insufficient for the correct approach of the case and its understanding. Taken as a whole and integrated into the context, they are of great clinical and scientific value.

For a correct clinical approach and a targeted therapeutic action, it is imperative to recognize the different subtypes of lymphoma. To frame them, clinical, biological, histological, immunophenotypic and genetic factors are used.

This paper aims to bring together patients with primary cutaneous lymphomas and benign lymphoproliferations, for clinical, histological, molecular, and genetic analysis. The aim is to highlight factors that change the evolution of the disease, the response to treatment and distinct and common patterns that predispose the individual to a certain histological subtype. This doctoral study aims to draw the line between a benign and a malignant lymphoproliferation and to try to highlight possible premonitory processes that lead the status of benign to malignant. Clinical, immunohistochemical, histological and genetic analysis is performed in order to highlight possible interconnections, specificities, or deviations from the notions known in the literature so far on the subject of skin lymphomas.

Keywords:

cutaneous lymphomas, clinical correlations, immunophenotypic correlations, tumor suppression genes, oncogene, copy number alteration

1. GENERAL PART. Current state of knowledge

1.1 CLINICAL AND HISTOPHENOTYPICAL ELEMENTS. SKIN LYMPHOMA AND BENIGN LYMPHOPROLIFERATIONS

Cutaneous T cell lymphomas are sometimes difficult to diagnose, especially in the early stages, due to the heterogeneity of presentation and the common appearance of the lesions with those of Parapsoriasis. This leads to a postponement of the diagnosis by about 6 years.

Mycosis fungoides is characterized by polymorphic lesions with indolent evolution that progress from the stage of macules to plaques and placards with an infiltrative character, and then in advanced stages even tumors. Characterized as a Th2 pathology, although the affected skin regions express a Th1 profile, it is frequently associated with serum eosinophilia.

Sezary syndrome is a form of aggressive CTCL that reveals the presence of Sezary cells (atypical T lymphocytes with cerebriform nuclei) in skin tissue, lymph nodes, hematogenous marrow and peripheral blood. Clinically, it manifests itself in the form of a generalized erythematous induration, intensely pruritic, with scales and lichenification, which are associated with ectropion, alopecia along with palmoplantar and subungual hyperkeratosis.

Histologically, the bioptic samples have many features in common with those in MF, although the epidermotropism of the cells is less intense than that in MF.

CD30 + primitive cutaneous lymphoproliferative disorders include Primary Anaplastic Large Cell Skin Lymphoma (ALCL), Lymphomatous Papulosis (LyP) and borderline cases, comprising approximately 30% of cutaneous lymphoma cases.

The processes on the surface of T lymphocytes are typically CD3 +, CD20-, CD45 +, and those of B lymphocytes are CD3-, CD20 + and CD45 +.

The group of benign lymphoproliferations includes a subcategory of controversial pathologies in terms of nomenclature and classification. The two variants of Parapsoriasis are considered benign diseases by some specialists, and by others as incipient forms of Mycosis fungoid or pre-Mycotic eruptions. The last category includes especially Large Plaque Parapsoriasis which in 35% of cases can progress to MF after 6-10 years.

From a clinical point of view, parapsoriasis in small plaques is characterized by a monomorphic, asymptomatic rash, in plaques of about 2.5-5 cm, round-oval, erythematous to brown, with fine scales, disposed on the trunk and limbs which may be more obvious in the cold season. The lesions have an insidious onset and a persistent character.

Large Plaque Parapsoriasis has a chronic evolution and permanent character. The specific lesions are represented by plaques and placards larger than 6 cm, erythematous to brown, with atrophic appearance disposed on the trunk and sometimes on the limbs. The change in the appearance of the lesions, polymorphism and pokiloderma accompanied by pruritus, suggests the malignant transformation towards MF.

From an immunohistochemical (IHC) point of view, Parapsoriasis lesions retain the properties of T lymphocytes that have pan-T antigens: CD2, CD3, CD5, CD7. These functional receptors are present on the surface of mature T lymphocytes.

1.2 GENETIC EVENTS IN THE ONCOGENESIS OF SKIN LYMPHOMA. GENETIC INSTABILITY, COPY NUMBER VARIATIONS AND ALTERATIONS

The growing number of genetic studies and advances in genome analysis technologies promise a better understanding of the molecular events responsible for the clonal transformation of cutaneous lymphomas. Also, highlighting genomic changes has the potential to support the classification, prognosis, and response to treatment of these pathologies.

The high-resolution SNPs concretized the identification of copy number variations (CNV) and copy number alterations (CNA). The analysis of these copy numbers and the identification of genomic regions that alter the individual susceptibility to cancer is underestimated.

In the neoplastic process, the advantage offered to the tumor cell by its growth potential is the consequence of genetic changes. The early development of the tumor is based on genetic instability which accelerates the sequence of events leading to malignant cell transformation. Suppression of apoptosis genes and mechanisms of cell growth control, increased signals to stimulate replication and duplication of protooncogenes, changes in cellular metabolism, accelerated mitotic rate with replicative immortality, induction of angiogenesis, inflammation and then invasion and dissemination, are tumor traits. The identification of molecular changes specific to neoplasms can lead to targeted therapies, sometimes even personalized ones. This aspect is related to the medicine of the future in the era we have already entered.

The skin was among the first organs that benefited from microarray investigative technologies, due to its high accessibility and complex neoplastic pathologies. Increasing the degree of interest in this direction and at the same time, directly proportional, the research at this level, more and more frequently we meet the term skinomics. This term defines transcriptional studies on gene expression in the skin. In the field of skin lymphomas, the study of the transcriptome involves the study of gene expression and genetic profile.

In the pathogenesis of CTCL, gene mutations that have been identified to be involved in cellular activation and apoptosis, or genes with a role in DNA response to injuries are: NFkB signaling, TP53, DNMT3A, FAS, ARID1A, ZEB1, CDKN2A, CDKN2B, MTAP, MYC.

In CBCL, important roles of BCL-2, MALT1, CDK4, GIL1 and MDM2 have been identified.

Tumorigenesis is a complex process that includes alterations of the genetic material in the cascade, thus accumulating, over time, more and more genetic defects that will result in increasingly aggressive behavior in the evolution of this pathology.

2. SPECIFIC PART

2.1 MATERIAL AND METHOD

The patients were selected from the University Clinic of Dermatology and Venereology Timișoara. We selected cases clinically correlated with CL for both B-and T-cell lymphomas alongside with LPP and SPP.

Due to the low incidence of the group of pathologies studied, this research is focused on the prospective collection of bioptic samples, starting from October 2015, associated with retrospective research related to the biobank of the discipline. The collected samples contain fresh fragments of cryopreserved skin tissue at -80 ° C. Skin biopsies were collected using standardized techniques.

All patients underwent a history, an objective and detailed laboratory examination. Emphasis was placed on significant personal pathologic history, exposure to harmful environmental factors, previous / concomitant treatments, presence / absence of pruritus, description of rash and skin lesions, affected body surface area %, presence of lymphadenopathy, history of the disease (months) and blood tests : red blood cells no. (million / dl), HB, HT, leukocytes no. (thousand / dl), Ne (%), Eo (%), Ly (%), Mo (%), ESR, CRP, Fibrinogen, ALAT, ASAT, urea, uric acid, creatinine.

The collected specimens were then diagnosed by histological, immunohistochemical and morphological features by two independent specialists in skin anatomopathology. Cases were considered eligible when they met the clinical, histopathological and immunohistochemical (IHC) criteria for the group to which they belonged (LC, SPP, LPP).

From these samples, we extracted DNA using the single nucleotide polymorphism (SNP) method. In regions where poor SNP coverage was observed, whole exome (WES) sequencing was used, but SNP remained the gold standard.

Data related to alterations in the number of copies (CNA) were then imported into the oncogenomic online archiving system: arraymap. Quantitative and qualitative changes were analyzed and mapped at 1Mb genomic intervals. The distribution of changes in the number of copies (gain / loss) was then plotted on a graphical map of the entire genome, in order to highlight the changes.

2.2 RESULTS

Following the evaluation of the inclusion and exclusion criteria, 60 patients (24 women and 36 men) were selected for this study, from which 73 skin samples were collected, some of the patients requiring a rebiopsy over time, due to the changing of the rash appearance. For 11 of these patients the histological, immunohistochemical and genetic analyzes were repeated (for 9 of them 2 samples were collected during the study and for 2 of the patients 3 samples were collected).

The study group includes 16 samples of small plaque parapsoriasis (PPS), 21 samples of large plaque parapsoriasis (LPP), one sample of Sezary Syndrome (SS), 2 samples of primary anaplastic large cell lymphoma (ALCL), 2 samples of Lymphomatoid papulosis (LyP), 5 primary cutaneous peripheral T cell lymphomas not otherwise specified (CTCL NOS) and 4 cutaneous B cell lymphomas (CBCL). We had a higher incidence of cutaneous T-cell lymphomas compared to those with B cells, a fact easily explained from a statistical point of view, CTCL, being variants of cutaneous lymphoma more frequently encountered in this spectrum of pathologies.

2.2.1 CONTRIBUTIONS ON CLINICAL AND PARA CLINICAL ASPECTS. PARTICULARITIES AND CORRELATIONS

Anamnesis. Personal disease history.

The personal pathological antecedents of each patient in the study group were analyzed, following the incidence of associated chronic pathologies. It is observed that Type 2 Diabetes and Cardiovascular Diseases are predominant in the studied group. This can be explained by the increased incidence of these pathologies in the general population and the fact that our group mostly includes patients who are over 50 years old (62%). The group most affected by chronic pathologies is LPP and MF group, which can be explained by the higher number of patients in this diagnostic category.

Patients with Psoriasis Vulgaris are considered to be a group of patients at higher risk of developing CTCL, a theory we wanted to analyze. In our study, we found a case of moderate to severe Psoriasis vulgaris followed by the diagnosis of LPP, a possible causal relationship between these two pathologies should not be neglected.

Continuing in the same spectrum, we analyzed the association of other types of neoplasms, both benign and malignant, among our patients, starting from the theory that patients with cutaneous lymphoma have a higher risk of developing other cancers. The associated oncological pathologies were 8 in number, 6 of them being in the group of LPP (3 benign, 3 malignant) and 2 (1 benign, 1 malignant) in the group of MF.

Paraclinical evaluation of inflammatory markers.

We analyzed the existence and behavior of serum inflammatory factors in the studied groups. According to the etiopathogenic theory of physico-chemical factors, chronic inflammation is associated with the progression and development of neoplasms. Targetting inflammation in cancer prevention and treatment is intensively studied. Three good acute phase indicators are erythrocyte sedimentation rate (ESR), fibrinogen and C-reactive protein (CRP).

Of our groups, 12 patients had accelerated ESR at the time of evaluation, LPP being the most affected group. Fibrinogen was increased in 12 cases; the group of MF being affected the most. In chronic inflammatory processes, the level of fibrinogen remains constantly increased, demonstrating the relationship between tumor growth and metastasis, being also correlated with poor prognosis and advanced stage of cancer. In the group of MF, CRP and ESR were increased equally but less than Fibrinogen levels. CRP was predominantly within normal limits even in CBCL, known as a lymphoma with a high degree of malignancy. Studies have shown that normal levels of serum CRP, in patients with incipient malignancies, led to better survival and even a more favorable prognosis in advanced or metastatic diseases but with low CRP values.

Individual assessment and correlation of pruritus and eosinophil levels.

Itching, although a subjective, variable, and indeterminate sign, is extremely important in assessing the disease. Itching can occur before the lesions appear or before the disease turns or worsens, being a discreet but valuable sign for the clinician and an extremely debilitating symptom for the patient. Eosinophilia in cutaneous lymphomas is associated with advanced or turning stages occurring at the time of a predominantly Th2 cytokine profile.

Among our samples, pruritus is present in the group of LPP and MF, along with SS. Eosinophilia is present in half of MF cases and almost $\frac{1}{4}$ in LPP cases. In the future, it would be appropriate to evaluate over time the evolution of patients diagnosed with LPP who associates pruritus and / or eosinophilia with the possibility of CTCL transformation.

Correlating the presence of pruritus with eosinophilia, we observed that 5 out of 6 patients with eosinophilia in the LPP group have associated pruritus, usually discrete to moderate, and in the MF group, half of those with paraclinically associated eosinophilia also associated pruritus.

2.2.2 CONTRIBUTIONS ON THE ANATOMO-PATHOLOGICAL AND IMMUNOPHENOTYPICAL ASPECTS

Histopathological changes in all groups of cutaneous lymphomas are difficult to assess and require clinical experience and integration. The difficulty is even greater in MF, especially in the plaque stage and in situations where it must be differentiated from a parapsoriasis, especially LPP.

In the present research, immunohistochemical tests (IHC) were performed for several antigens: T cell markers (CD2, CD3, CD4, CD5, CD7, CD8), B cell markers (CD20, CD79a), Ki67 proliferative index, markers for Langerhans cells (CD1a), marker of dendritic follicular cells (CD21), marker of histiocytes (CD68), protein S100, MUM-1, bcl-2, bcl-6, pax-5, granzyme B, perforin and marker for EBV.

Assessment of the disposition and presence of atypical lymphocytes.

Analyzing the disposition and presence of atypical lymphocytes, we noticed that in the group of LPP and MF their number was considerably higher. In SPP, atypical lymphocytes were observed in a small number of cases only in the epidermis. In MF, atypical lymphocytes were present in most cases in the dermis and epidermis and in LPP they were predominantly in the epidermis.

Assessment of the disposition and presence of Pautrier's microabscesses.

We have also noticed the absence of Pautrier's microabscesses in SPP and their presence in LPP, but to a lesser extent than MF. The same thing was highlighted in the case of epidermotropism.

Evaluation of the presence of parakeratosis, perianexial and eosinophilic infiltrate.

Next, we have compared in the same 3 groups the presence of parakeratosis, perianexial and eosinophilic infiltrate. Parakeratosis was predominantly present in LPP (50%), perianexial infiltrate predominates in a large percentage in MF, and eosinophils were present in dermal infiltrate in a close percentage in all 3 groups.

Evaluation of T lymphocyte markers.

The CD4 / CD8 ratio increases from 1/1 in LPP to 3-4 / 1 in the MF group and becomes 1 in the case of MF with CD30 transformation. It should be noted that the CD4 / CD8 ratio may also be 1 or even in favor of CD8, suggesting a transition from inflammatory to neoplastic (LPP → MF).

2.2.3 CONTRIBUTIONS ON THE GENETICS OF SKIN LYMPHOMAS AND BENIGN LYMPHOPROLIFERATIONS

CNA in CTCL

By including all CTCLs in one group (32 samples), we have generated a map that highlights the losses and duplications of the number of CNA genetic copies along the genome. Reviewing this histogram, we have found that the most common CNAs, i.e. genomic regions with frequent imbalances, consisted of gains in the number of copies on chromosome 1p, 16q, 17.19, while the deletions were punctiform. As expected, it has been found that each of these regions hosts oncogenes or tumor suppression genes, with critical roles in the evolution and development of these oncological entities.

The spike deletions observed at 9p21.3 correspond to the CDKN2A, CDKN2B and MTAP genes, which encode tumor suppressor proteins such as p14 (role in p53 protection) and p16.

CNA in CBCL

In the CBCL histogram, gains house loci such as 2p, 3q, chromosome 7, 11q, 18q, and losses on 1p, 8p, 10q, 15q, 16q, and on chromosome 17 and 19. TP53 is located at 17p13.1 and its deletions are easily observed on the CBCL histoplot. CBCL is characterized by a broad spectrum of imbalances, including gains on chromosome 18 containing the proto-oncogene BCL-2.

CNA in MF versus LPP

In the present doctoral thesis, the LPP group and the MF group are homogeneous groups that contain almost equal number of samples LPP = 21, MF = 22, also comprising most of the samples of our study.

After examining each group, we have noticed that the histograms generated along chromosome 1-22 are remarkably similar, having a lot of CNAs in common. As described above, duplications are more common than deletions, both for MF and LPP. Duplications are seen on chromosomes 1p, loci on chromosomes 3, 4p, a spike on 6p, 9qter, some on chromosome 10, 12qter, 18p and 18qter, 21, but by far the most affected are chromosomes 16, 17, 19, 20 and 22.

Deletions are rarely seen as limited portions of DNA.

T receptor rearrangements (TCR)

TCR clonality testing was performed to facilitate the diagnosis of these two pathologies. TCR rearrangements affected 13 (10 affecting TCRB and 3 TCRA) samples in the MF group and 5 (excluding TCRB) in the LPP group. We can observe an important difference between these very similar pathologies. TCR clonality tends to be more present in MF samples, rather than in LPP.

CNA in SPP

We have observed duplications of the number of copies on the short arm of chromosome 1, the cytogenetic locus p31-p33 being affected in greater proportions than in the LPP group.

On chromosome 4, we have observed CNA losses along the entire length of the chromosome, with a spike at the 4p16.1 location, which affects over 50% of cases. This deletion is common to that of LPP, affecting MF in the opposite direction, through duplications of this DNA locus. It is a contribution that can differentiate benign from malignant lymphoproliferations of the CTCL spectrum. To our knowledge, studies researching this theory were not found in the literature before.

In the SPP histogram, we can also observe deletion CNAs on chromosome 6, 12p and duplications of genetic material on chromosome 4q13.3, 6p, 9qter, 16qter, 17, 19, 20, 21q, 22.

CNA in CTCL NOS

In contrast to other types of cutaneous T-cell lymphoma such as ALCL, CTCL NOS, has a more severe prognosis and an aggressive course. The group of patients in this doctoral thesis includes 5 samples of CTCL NOS. Although the number of samples is limited, we have noticed significant changes in CNA. CNAs are predominantly gains of genetic material, affecting chromosome 1p, 16q, 4p, 5q, 10q, chromosomes 17, 19 and 20 being affected entirely. Deletions are small and limited, affecting chromosomes 1, 5, 7qter and 9p.

CNA in LyP

Lymphomatoid Papulosis group included 2 patients, where we observed a few and punctiform CNAs, predominated by deletions and some duplications. Apart from the small number of cases in this category of cutaneous lymphomas, the few CNAs observed in our analysis can also be explained by the fact that LyP is a chronic pathology with a varied clinical presentation but a nonaggressive evolution.

2.3 DISCUSSIONS

The oncological mechanisms are not only the result of an uncontrolled proliferation but are also determined by factors from the cutaneous micro-environment and from the one to which each individual is exposed.

The complexity of things is given by the fact that a single focal variation or alteration of a locus or a gene is often not necessary or sufficient in triggering or evolving a pathology. A multitude of losses or duplications in multiple loci, with direct or indirect influences, lead to that interindividual genetic variation and also to copy number alterations (CNA), which have a role in triggering cancer.

The challenge is to determine which of the genes involved are sensitive to these variations or alterations in the number of copies, in order to initiate and support the neoplasm and at the same time, to inhibit the defense mechanisms. The seemingly random changes, which add up to the process of tumor initiation and growth, must be studied on sufficient number of samples to allow a relevant conclusion to be drawn.

3. CONCLUSIONS

Advances in the field of gene expression profiling and next-generation sequencing have led to new mechanisms for understanding the pathogenesis and molecular pathways of skin lymphomas.

CNAs occur frequently in cutaneous lymphomas, as they are present in cancers in general, affecting not only by deletions tumor suppression genes, or by duplications oncogenes, but indirectly, more complex mechanisms, signaling pathways and feedback, through intermediate genes.

The complexity is given by the fact that a single focal variation or alteration of a locus or a gene is often not necessary or sufficient in triggering or stimulating a pathology evolution. A multitude of losses or duplications in multiple loci, with direct or indirect influences, lead to that interindividual genetic variation and also to alterations in the DNA copy numbers, which play a role in triggering cancer. The challenge is to determine which of the genes involved are sensitive to these copy number variations or alterations, in order to initiate and support the neoplasm and at the same time, to inhibit the defense mechanisms. The seemingly random changes, which add up to the process of tumor initiation and growth, must be studied intensively to draw a relevant conclusion and then lead to the creation of a comprehensive database of all these copy number alterations in different types of cancer.

The results of this doctoral study are in line with previous reports, emphasizing once again that duplications are CNAs that predominantly affect cutaneous T-cell lymphomas and deletions are the predominant prerogative of B-cell lymphomas. It was expected that important loci with CNA damage would host important tumor suppression or oncogenes. In the studied groups, CNA changes involved loci that host genes such as: CDKN2A, CDKN2B, MTAP, TP53, BCL-2, MALT1, GLI1, MDM2, mTOR, PIK3CD, ZAP70, MLF1, SP100, TNF, NOTCH1, FAS, CDKN1B, NUMB, XRCC3, STAT, SOCS3, RARE, BIRC5, NDC80, CD70, JAK3, GDF15.

Skin lymphomas are characterized by complex CNA profiles, according to their complex pathogenesis. In addition, it is important not only the number of CNAs, but also the location of chromosomal aberrations. Identifying specific genomic imbalances could provide critical insight into these underlying molecular events. Moreover, genomic changes may contribute to a more accurate classification of this spectrum of pathologies, which is still a topic of debate. All these results lead to the development of more specific treatments, that through targeted action, towards clear molecules, can change the course of patients' lives and their life expectancy, while avoiding the adverse effects of conventional treatments.

The scientific community must undertake continuous efforts to reveal the order in the chaos which characterizes the neoplastic process. Molecular biology requires the identification and direct signaling of the phenomena that lead to the resistance and adaptation of the neoplastic cell. In response to these needs, innovative therapeutic and prophylactic solutions can be revealed, for what is known to be the most prominent, constant, and full of pathological challenges: cancer.