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PhD THESIS

PSORIASIS: CHALLENGES IN DIFFERENTIAL DIAGNOSIS WITH A RARE DISEASE, PARTICULARITIES OF BIOLOGICAL TREATMENT AND COL9A1 AND PDCD1 GENE POLYMORPHISMS.

- A B S T R A C T -

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GENERAL PART

Psoriasis is a complex disorder that not only impacts the skin but also has systemic implications and can significantly affect patients' quality of life. In recent years, the understanding of psoriasis has evolved to view it as a comprehensive condition that affects various organs and systems, the current concept framing it as psoriatic disease. Understanding the epidemiology, clinical characteristics, coexisting medical conditions, and treatment approaches of psoriasis is essential for delivering comprehensive care to patients impacted by this persistent inflammatory condition. Psoriasis is a multifactorial disease with multiple clinical manifestations, in which genetics and environmental factors have an essential role either by triggering the disease or exacerbating it. Simultaneously, extensive research on this condition has resulted in the advancement of the pharmaceutical sector. In recent years, this has led to the introduction of groundbreaking treatments for psoriasis, such as biological therapies. Biological molecules have significantly transformed the way diseases are treated, and the current goal of therapy is the complete disappearance of skin lesions. Nevertheless, biological therapies are not exempt from adverse responses and susceptibility to certain infections, given the immunosuppressive nature on which they rely. In present thesis, we have specifically examined the clinical characteristics that coincide with the rare condition known as Mycosis Fungoides. Additionally, we have investigated certain genetic variations that have not been previously explored in the European population, as well as the potential risk of latent tuberculosis infection associated with biological treatments.

EXPERIMENTAL PART

The thesis is structured in three studies:

- Clinical aspects overlap between a common disease such as psoriasis vulgaris and a rare disease like MF.
- The evaluation of patients with psoriasis on treatment with biological therapies and risk of latent tuberculosis.
- Implications of PDCD1 and COL9A1 genes in psoriatic disease.

First study.

Purpose and objectives

In this study, we chose two different diseases, MF as a rare disease and Psoriasis vulgaris, a common condition in dermatology services. The incidence of the two diseases, clinical aspects and the overlapping stages until the MF diagnosis were the questions we wanted to answer through this study.

Materials and methods

We collected the medical records of hospitalized patients who were assigned a PCTCL diagnosis at the University Clinic of Dermatology and Venereology Timisoara during 13 years from 1st of January 1, 2010 to December 31, 2023. The diagnostic codes which we have taken into account for describing a PCTLC were: C84.0 or C84.1 (Mycosis Fungoides, Sezary Syndrome). After selecting patients according to ICD-10 codes, we gave unique identification codes to each subject and searched for histopathological examination of skin tissue performed to establish certainty of diagnosis.

Results

There were admitted 56 patients with diagnosis code CTLC including primary and additional, 43 men and 13 women.

We observed the admission of 39 patients with initial suspicion of CTCL. Of these, 11 were certified by histopathological and immunohistochemical examination, while in 4 patients these examinations were not presented and they were excluded from the study. 17 patients were initially diagnosed with another dermatological condition among chronic erythematous and squamous dermatoses. Of these, 15 received a histopathological and immunohistochemical diagnosis of Mycosis fungoides and 1 was diagnosed with parapsoriasis and another with psoriasis vulgaris.

Histopathological and immunohistochemical investigations confirmed the diagnosis of Mycosis Fungoides in 27 patients at different stages.

MF was the most frequent in the patch stage and only a pattern of folliculotropic MF and tumoral MF.

Intermediate diagnoses were parapsoriasis for 3 cases, endogenous eczema for 3 cases, nonspecific lichenified dermatitis for 2 cases, psoriasis vulgaris for 3 cases, and one case of nodular prurigo. The average time to confirm the diagnosis of MF by histopathological examination was 1.34 years (min<1 year, max=10 years).

An average of 1.55 (between 1 and 3 examinations) histopathological examinations were performed until the histopathological diagnosis of MF, and the time period between the onset of symptoms and the physician's decision to biopsy was from several months to 10 years.

The main reason why the doctor decided to do a skin biopsy was the chronic evolution without healing tendency under topical treatment with corticoid and emollient.

We followed the clinical aspects of the patients and considered hospitalized patients with a presumptive diagnosis of psoriasis vulgaris but whose histopathological and immunohistochemical examination supported the diagnosis of MF and, conversely, hospitalized patients with suspected MF but whose histopathological examination was psoriasis vulgaris .

Clinical features of erythematous squamous plaques with pearly white scales were present in 20 patients with confirmed MF and 16 of them had marked pruritus as their chief complaint. At the same time, 13 subjects had generalized skin lesions, 10 subjects had lesions limited to the trunk and limbs, 3 subjects had lesions in large skin folds, and one had lesions only on the cephalic extremity.

The reasons why the 9 patients diagnosed by histopathological examination with psoriasis vulgaris had suspicion of MF were lymphnodules, erythrodemia, non-specific skin lesions, rapid evolution and no response to topical therapy.

One of these patients was diagnosed with psoriasis vulgaris about 32 years ago, treated over the years with topical and systemic therapies, including biologics. Biologic treatment was performed with 3 molecules for a total of 9 years and the unfavorable evolution of erythroderma despite the three-fold change of the biomolecule led to the suspicion of MF.

To compare the period prevalence of the two diseases, patients admitted to the Department of Dermatology were searched in the computer system with ICD-10 codes for MF and Sezary syndrome (C84.0, C84.1) and psoriasis (L40.0, L40.5 +, L40.1, L40.4, L40.8, L40.9). As expected, the prevalence of MF (0.3%) is much lower than that of psoriasis (7.5%).

Discussions and conclusions

The present study demonstrated that although psoriasis is a common disease that can be easily diagnosed through a clinical examination, sometimes it can overlap with diseases that are rarely encountered in medical practice. We advocate for the necessity of increasing knowledge regarding the importance of histopathological investigation and its integration into ordinary medical practice, even for common disorders.

Second study.

Purpose and objectives

The purpose of this study is to determine the risk of developing LTBI in psoriatic patients treated with biological therapy and the implications for continuity of therapy. In addition, we sought to assess the impact of prophylactic treatment for LTBI on the evolution of the disease and the incidence of active infection with Mycobacterium tuberculosis.

Materials and methods

The composition of the study group was performed by retrospective observation of patients whose data were stored and monitored at the University Clinic of Dermatology and Venereology Timisoara. Periodic monitoring of patients at initiation of treatment was noted every 12 months for a minimum period of 1 year and a maximum of 17 years (mean = 6.9 years). The study group included 97 patients aged between 28 and 78 years with an average age of 57.81 who were diagnosed with moderately severe psoriasis. The diagnosis was established based on clinical evaluation and confirmed by histopathological examination.

According to inclusion and exclusion criteria, 97 patients diagnosed with moderately severe psoriasis according to clinical and histopathological evaluation were registered. Data were collected on gender, age of patients, age of disease onset, age at initiation of biological therapy, disease severity by PASI score, type of psoriasis with special area involvement. At the same time, data were recorded related to systemic therapy used, both conventional and biological and patients' comorbidities.

PASI score considers parameters such as erythema, desquamation, and induration, as well as the percentage of body surface area affected in different regions of the body. The PASI score ranges from 0 to 72 and is calculated by summing the scores for erythema, induration, and desquamation in each region, multiplied by the area of involvement in that region. A 50% reduction in the PASI score (PASI50) during treatment is considered a meaningful improvement, while a ≥75% improvement in PASI score from baseline (PASI 75) indicates a good treatment response. PASI improvement is often expressed as a percentage change from baseline, with failure to achieve PASI 50 indicating no response to treatment.

According to the Romanian National Protocol Guide for the use of biological treatments in moderate to severe psoriasis, the evaluation of patients during therapy is carried out periodically every six months through blood tests and annual pulmonological evaluations, so we recorded comorbidities occurred during follow-up and were evaluated the safety profile and effectiveness of therapy.

Following the medical reports issued at the regional dispensary for the diagnosis and treatment of tuberculosis, attached to the files of the monitored patients, the vaccination with the Bacillus Calmette-Guerin was recorded by the presence of a post-vaccination scar. Similarly, data related to the interpretation of chest X-rays, Mantoux tests and treatments performed for LTBI prophylaxis were collected from the analysis of medical reports of pulmonologists.

The Mantoux test was carried out with 5 units of purified protein derivative (PPD) and 1 mL serum injected intradermally into the ventral forearm. The intradermal reaction of tuberculosis (IDR) was assessed 72 hours after injection and if the induration was equal or greater than 10 mm as measured along the longest axis, the test was considered positive. Patients with positive Mantoux tests but no active lesions in the chest X-ray and no symptoms of TBI were diagnosed with LTBI.

The data were collected in an Excel spreadsheet, and descriptive statistics include the mean value of continuous variables, the standard deviation (SD), the standard error (SE) and the percentage of categorical variables. Chi square analysis and t tests were conducted as appropriate. The differences observed during the study were of 2 types:

a) non-significant differences are differences that have been attributed to chance (sampling variability);

b) significant differences are differences that arise as a result of a certain cause.

Highlighting the significance of the observed differences was achieved by applying the procedures from the category of statistical tests that calculate the parameter p, which represents the probability that the observed differences occur by chance.

Results

We collected data for 97 patients with moderately severe psoriasis, some patients had psoriasis in specific areas such as scalp (34%), genitals (2%) and nails (5%). In 5% of patients, the relationship between the scalp and the genitals is observed, while the correlation between the scalp and the nails is 10%.

In all patients, there was moderate to severe psoriasis, some patients had only plaque psoriasis while others had associated arthropathic psoriasis. Psoriasis has been found to be more common in men and less common in women similar to joint involvement.

In the 60-69 age group, arthritic forms and plaque psoriasis were more common.

The scalp was most commonly involved as a single special area and most patients had more than one specific area affected.

The average period between the diagnosis of psoriasis and the start of biological treatment was 18.3 years.

Data on various infections occurring during biological therapy were recorded, the most common infections were gastrointestinal infections, followed by pharyngeal infections, urinary tract infections, lung infections, LTBI, pneumonia and viral hepatitis with virus B and virus C. Fifty percent of subjects who had infections during antipsoriatic therapy developed two or more infections.

LTBI was encountered in 24.74%, however, none of the patient's required discontinuation of psoriatic therapy to treat the infection, nor did they suffer serious complications as a result of these infections.

At the initial evaluation for the start of biological therapy, the Mantoux test and chest X-ray were performed and 30 patients with LTBI were detected. They were treated with methotrexate before the evaluation.

All patients diagnosed with LTBI at baseline were on prophylactic antituberculosis therapy for 1 or 2 months before initiation of biologic therapy. PASI scores before and after chemoprophylactic treatment were recorded to assess the impact of LTBI on the efficacy of biological treatment. For statistical analysis, patients detected with LTBI before biological treatment were excluded. The Chi-square test was performed to compare these data and we observed an increased PASI score in patients with LTBI ($p = 0.022$).

We identified 25 patients who were treated with MTX and biologics for a satisfactory therapeutic response and found that 10 of them had positive IDR at first evaluation and 10 were diagnosed with LTBI during follow-up.

Of the 72 monotherapy patients, 20 were diagnosed with LTBI at baseline and 14 during follow-up.

A Chi-square test was performed for comparisons between combination therapy and monotherapy patients to determine whether there were differences in the frequencies of LTBI in the two groups. The results showed that patients treated with MTX and biologics had more frequent LTBI.

The LTBI present in relation to the period of administration of the therapy had a mean time to the occurrence of the event of 3.45 years.

Thus, the frequency of patients with LTBI was higher among patients treated with anti-TNF-α (87.5%).

Follow-up of patients treated for LTBI was performed for 1 year (+/- 3 months) after full chemoprophylactic treatment and 8 of them were found to have a positive Mantoux test (IDR>15 mm) 1 year after diagnosis. None of them showed signs of active disease on chest X-rays, and further tests (sputum examination by optical microscopy with a Ziehl-Neelson colored immersion lens and MTB molecular testing by the GenExpert MTB test) were performed to rule out active TB. In these patients, it was established that the intradermal reaction to tuberculin is hyperreactive.

During the retrospective follow-up of patient files, there were patients who switched biological therapy to another molecule (n=31), but none had LTBI during treatment with the modified biological molecule. None of the study subjects was diagnosed with active tuberculosis during the entire follow-up period.

Discussions and conclusions

In the present study, we observed a higher incidence of LTBI under biological treatment, especially anti-TNF-α and the fact that MTX can give LTBI or false positive Mantoux test results.

Third study.

Purpose and objectives

We chose as the topic for this study the evaluation of polymorphisms of two PDCD1 and COL9A1 genes and their implications in psoriatic disease. The determination of genotypes and allele frequency of the two rs10204525 for PDCD1 gene and rs550675 for COL9A1 genes in psoriasis and correlations with clinical forms, severity of skin tissue damage and comparisons between a healthy study group and the patient population was the main purpose of this study.

Materials and methods

The study group consisted of patients who presented to the University Clinic of Dermatology and Venerology Timisoara diagnosed by clinical examination and histopathological with plaque psoriasis. The recruitment of the batch took about 1 year (2022-2023) and followed the steps described in the following figure.

DNA Isolation

2 mL of peripheral blood was collected from all participants in the study in vacutainers containing ethylenediaminetetraacetic acid (EDTA). The genomic DNA was extracted from blood samples using the MagCore® Extractor System and the MagCore® genome DNA complete blood kit (RBC Bioscience, New Taipei City, Taiwan) according to the manufacturer's protocol. DNA samples were stored at - 20°C.

Genotyping

Allelic discrimination was carried out using the following TaqMan genotyping tests: C___2989110_1_ (rs550675) and C___172862_10 (rs10204525).

The experiments were conducted with QuantStudio 7 Real-Time PCR systems (Thermo Fisher Scientific, Waltham, Massachusetts, USA) following manufacturer protocols.

The genotype was determined by measuring the fluorescence of specific alleles using the Allele Discrimination software (Applied Biosystems, Foster City, CA, United States).

For quality control, we repeated the analysis of approximately 5% of the samples randomly selected. The results did not show any discrepancies.

Results

This study was conducted on 45 patients with plaque psoriasis who presented to the University Department of Dermatology in Timisoara between April 2022 and April 2023. The study also involved 43 healthy subjects with similar ages, gender, and BMI to obtain more relevant data in the study.

The evaluation of the rank of the C/T alleles for rs550675 (COL9A1 gene) showed statistically significant values in the group of patients with psoriasis ($p = 0.026$), while the comparison of the G/A alleles for rs10204525 (PDCD1 gene) between the two groups had values statistically insignificant ($p = 0.450$).

Comparisons between allele frequencies and genotypes of the PDCD1 and COL9A1 genes were without statistical significance between the form of mild psoriasis and severe psoriasis.

Statistical differences were observed for the frequency of genotypes of the COL9A1 gene between the control group and patients, and the results for the PDCD1 gene were not statistically significant.

Analysis of rs550675 and rs10204525 variants in the patient group and the control group.

Genotype	Cases (*n* = 45)		Controls (*n* = 43)		X^2 Test	*p*
	n	%	*n*	%		
COL9A1 gene rs550675						(C/T)
TT	3	6.66	8	18.6	4.2418 (CC/TT)	0.039
CT	24	53.3	25	58.1	1.6817 (CT/TT)	0.194
CC	18	40	10	23.2	1.6618 (CC/CT)	0.197
C allele	42	93.3	35	81.3		
PDCD1 gene rs10204525						
GG	35	77.7	32	74.4	0.6558 (GG/GA)	0.418
GA	7	15.5	10	23.2	0.0541 (GA/AA)	0.816
AA	1	2.2	1	2.3	0.0038 (GG/AA)	0.950
G allele	42	93.3	42	97.6		

X2 = Chi-square test, p-value < 0.05.

Comparative tests for each type of psoriasis according to disease severity showed that CC vs CT, CC vs TT genotypes and C vs T allele frequencies were more frequent in plaque psoriasis.

Comparative tests for each type of psoriasis depending on the severity of the disease.

	Plaque Psoriasis	Arthropathic Psoriasis	Palmoplantar Psoriasis
	p-Value	*p*-Value	*p*-Value
GG/GA	0.5692	0.8231	0.3173
GG/AA	0.1266	0.6547	0.5127
GA/AA	0.1797	1	-
	0.5868	0.8317	0.3415
CC/CT	**0.0246**	0.3865	0.8864
CC/TT	**0.0070**	-	0.2568
CT/TT	0.4096	1	0.5637
	0.0154	0.6522	0.4431

For the PDCD1 gene, GA and GG genotype were more associated with plaque psoriasis than with arthropathic psoriasis, and for the COL9A1 gene CT and TT genotypes were more common in arthropathic psoriasis than palmoplantar psoriasis.

Statistical analyses according to models of inheritance.

Psoriasis Type	Plaque Psoriasis versus Arthropathic Psoriasis (*p*-Value)	Plaque Psoriasis versus Palmoplantar Psoriasis (*p*-Value)	Arthropathic Psoriasis versus Palmoplantar Psoriasis (*p*-Value)
GA + AA/GG	0.5972	0.5116	0.3375
GA + GG/AA	0.0009	0.1069	0.4028
CC + CT/TT	0.3808	0.2960	0.4669
CT + TT/CC	0.2817	0.0648	0.0280

Discussions and conclusions

This study revealed correlations between PDCD1 and COL9A1 gene polymorphisms with psoriasis or its many clinical manifestations. These results mark the commencement of further investigations that will be conducted based on this subject.

GENERAL CONCLUSIONS

In conclusion, in this doctoral thesis:

We demonstrated that in clinical practice, there can be instances where there are similarities in clinical characteristics between Mycosis Fungoides, a rare disease, and psoriasis vulgaris, a common condition.

- We emphasized the significance of conducting histopathological analysis, even in common diseases and at the early stages of skin conditions.
- We demonstrated the importance of upholding LTBI screening in patients undergoing biological therapy and the necessity of doing more targeted testing, such as the INF-γ relay assay.
- We demonstrated that the use of Methotrexate can result in either latent tuberculosis infection LTBI or false positive results.
- We analyzed PDCD1 and COL9A1 genes in patients with psoriasis by comparison with healthy subjects. Patients exhibited a greater frequency of the C/T alleles for rs550675 (COL9A1 gene) compared to the control group, and comparisons of CC/TT genotypes showed the association of psoriasis with the TT genotype.
- We compared genotypes and alleles of COL9A1 and PDCD1 gene polymorphisms for each type of psoriasis according to disease severity. The results showed differences of the COL9A1 gene between CC/CT and CC/TT

genotypes and the frequency of C/T alleles in plaque psoriasis in favor of severe disease.

- We performed the comparisons based on inheritance patterns in plaque psoriasis, arthropathic psoriasis and palmoplantar psoriasis and observed that GA/GG genotypes are more associated with plaque psoriasis than with arthropathic psoriasis and CT/TT genotypes are more common in arthropathic psoriasis than palmoplantar psoriasis.

FUTURE RESEARCH DIRECTIONS

- Conducting INF-γ relay assay tests to screen patients taking Methotrexate and assessing the association with the risk of latent tuberculosis infection.
- Conducting investigations on many variations of the PDCD1 and COL9A1 genes in psoriasis.
- Analyzing the alterations in the epigenetic profiles of the COL9A1 and PDCD1 genes after various psoriatic therapies.