"CAROL DAVILA" UNIVERSITY OF MEDICINE
AND PHARMACY BUCHAREST

DOCTORAL SCHOOL
FIELD OF MEDICINE

PHD THESIS SUMMARY

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PhD Candidate:
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CLINICAL-THERAPEUTIC IMPLICATIONS OF THE TNF-α LEVEL IN PATIENTS WITH SEVERE PSORIASIS VULGARIS AND / OR PSORIATIC ARTHRITIS

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Introduction

Psoriasis vulgaris belongs to the chronic inflammatory diseases that are extremely difficult to manage. It is a common condition, having a 4.99% prevalence in Romania [1]. A challenge to achieving a correct management of this disease is the fact that psoriasis vulgaris is influenced by numerous trigger factors that at some point induce a loss in the therapeutic response despite correct treatment administration. Lack of a pathognomonic biomarker further increases the risk of confusion, with the doctor lacking a physiopathological explanation for the phenomenon.

A firm psoriasis vulgaris diagnosis is not always easy to make. For the so-called classic forms a clinical assessment of the lesions usually suffices. Issues arise when the patient only presents with lesions on difficult-to-treat areas and which in accordance with the new definitions and classifications may represent severe forms of the illness. The greatest confusion arises in cases of palmar or plantar psoriasis, where differential diagnosis against eczema lesions is sometimes very difficult. Developing paraclinical diagnosis methods becomes absolutely necessary.

Managing a patient with psoriasis vulgaris is highly dependent on the proper fitting within a severity degree. This presents a new challenge since until recently this was only done through an evaluation of the affected surface, more or less correlated with the aspect of the plaque. The PASI score was calculated for this purpose. In light of the new recommendations, the severity can also be expressed through the lesions that only appear on special areas. For these areas we use special scores, namely ESIP, PSSI, NAPSI. However, no correlation was made between these different ways to calculate severity and no biomarker was used to see whether the mechanism of disease production is the same[2]. This is because in the physiopathology of psoriasis vulgaris we describe type I inflammation (predominantly TNF-α), type III inflammation (predominantly the IL23/IL17 axis) and of course the innate immunity involved.

The next step for correct management is treatment choice. This should be a personalised treatment for each patient and should have a high prediction rate. As was stated in the global 2016 WHO report on psoriasis “there is an urgent need for rapid intervention and evidence-based treatment of psoriasis to avoid long-term suffering, disease progression and CLCI escalation” [77]. The lack of a biomarker to guide us towards the right choice of treatment leads to the fact that the patient does not always benefit from the most appropriate therapy, even though nowadays we have an important therapeutic arsenal on hand.

I have only presented a handful of the reasons why identifying a prediction biomarker for psoriasis vulgaris becomes more and more pressing. Several attempts have been made for this purpose. However, the majority of them, although important, were extremely

difficult to put into practice. From my point of view one should validate a biomarker that can be relatively easy to apply in medical practice, available to each doctor and of course at a reasonable cost.

Working hypothesis and specific objectives

In the past years, perhaps as a consequence of the misunderstanding of over specialisation, doctors' attention has been focused more on the disease than on the patient. Treating skin lesions was the main objective of the doctor, sometimes transmitted to the patient as well, regardless of the consequences of the treatment for the patient.

As the first obstacle to adequate patient management, it is extremely important to make a correct and complete diagnosis, including patient status, i.e. understanding the additional risks that the psoriasis generates, the associated diseases and their comorbidities.

In the last few years the approach for a patient with psoriasis has changed in the sense that strong emphasis is placed upon lesions located in special areas, even without significant damage to the body surface. Here however arises the issue of differential diagnosis that is difficult to make (psoriasis vs eczema for palmar lesions). When we must use highly advanced therapies certainty in diagnosis becomes crucial. Until now only skin biopsy was used with its advantages and disadvantages. From the need to validate other methods as well we initiated **Study 1 – Diagnostic value of ultrasound imaging of the skin for particular forms of psoriasis. Correlation with TNF-α serum value** and to some extent **Study 2 – Defining severe forms of psoriasis vulgaris. Correlations between severity scores and TNF-α serum value for correct classification of the patient with psoriasis vulgaris** to determine the diagnostic value of skin ultrasound imaging, to which TNF-α serum levels can be added.

Psoriasis vulgaris has a different therapeutic approach depending on its fitting within a severity degree. For a long time, the severe form of the illness involved a significant bodily damage by surface area. With the introduction in the past few years of the concept of special illness areas the perception of the severe form of psoriasis vulgaris has shifted. Area scores have been developed to quantify severity. However, the way to employ them is not very clear and particularly the physiopathological pathway of these forms of psoriasis vulgaris. For these reasons I have initiated **Study 2 – Defining severe forms of psoriasis vulgaris. Correlations between severity scores and TNF-α serum value for correct classification of the patient with psoriasis vulgaris** as well as **Study 3 - Correlations between the**

TNF-α serum level and various particular forms of psoriasis vulgaris for initiating personalised therapy.

Correct initiation of a personalised and predictive treatment is another objective that must be fulfilled in order to obtain quality management for the patient with psoriasis vulgaris. Complete control of the disease is desired, along with quick, long-term remission of lesions as well as control over comorbidities and associated diseases. For these reasons we have initiated **Study 4 - Dynamic correlation between the TNF-α serice values and the evolution of severity scores for patients with psoriasis vulgaris undergoing systemic treatment** and **Study 5 - TNF-α serum levels for patients with a severe form of psoriasis vulgaris with or without previous systemic therapy**. We have on hand numerous biological therapies anti TNF-α, anti IL17, anti IL17 receptor, anti IL12/23 (p40), anti IL23 (p19) or with small molecules with an intracellular acting mechanism acting on different physiopathological pathways. Nevertheless the unanimous opinion is that the patient with a severe form of psoriasis vulgaris is still treated suboptimally. Today the choice of treatment is made mostly based on subjective criteria while lacking a valid biomarker. A holistic approach to the patient with psoriasis vulgaris is taken especially with respect to the associated diseases or comorbidities of the patient, eventually based on the professional experience of the dermatologist, which as I said implies an important dose of subjectivism. The therapeutical challenge arises both in the choice of initial treatment and in the change of therapy in the case of non-responder patients. Without a biomarker we have no solid scientific arguments to initiate or change treatment in a certain therapeutic class.

One of the causes for these problems is the lack of a pathognomonic biomarker by which one could correctly evaluate the status of the patient with psoriasis vulgaris. Knowing the role that cytokine TNF-α plays in physiopathological mechanisms of vulgar psoriasis, I decided to assay and dynamically follow its serum value for patients with various forms of illness severity, bio-naïve or bio-experienced. This way I will attempt to prove if the TNF-α serum value can be validated as an important marker for patients with psoriasis vulgaris and in which situations it is of value to the dermatologist.

General research methodology

I initiated five studies through which I attempted in a logical flow to validate the role of the pro-inflammatory cytokine TNF-α as a pathognomonic sign in psoriasis vulgaris, along with other manoeuvres and diagnostic instruments. The consulted patients presented with a psoriasis vulgaris in various forms of severe illness. I have completed the consultation

consent form and the GDPR form and I had the agreement of the ethical committee for these. The entire activity undertaken for these studies was in agreement with the Declaration of Helsinki on the ethical principles surrounding medical research on human subjects. I have studied the current literature on the subject, particularly by following papers published in indexed journals, using the PubMed platform as a tracking engine.

The objective of the work being the detection and validation of a pathognomonic biomarker for psoriasis vulgaris in its severe form, I focused on determining the serum value of the pro-inflammatory cytokine TNF-α associated with other diagnostic manoeuvres and instruments to evaluate psoriasis vulgaris.

In **Study 1 – Diagnostic value of ultrasound imaging of the skin for particular forms of psoriasis. Correlation with TNF-α serum value** I aimed to obtain a firm diagnosis for particular forms of psoriasis vulgaris, especially palmo-plantar forms, where in many cases the differential diagnosis against atopic dermatitis or other forms of eczema with a strict palmo-plantar localisation. I initiated a cross-sectional cohort observational study. I randomised the patients into two groups depending on diagnosis, I performed the skin ultrasound imaging for potential imagistic differences between the two conditions and I attempted a correlation to the cytokine TNF-α serum levels.

In **Study 2 – Defining severe forms of psoriasis vulgaris. Correlations between severity scores and TNF-α serum value for correct classification of the patient with psoriasis vulgaris** I continued the logical thread for a correct management of the patient with psoriasis vulgaris in a severe form. I followed a correct fitting within a degree of illness severity by separately using each possible score for each of the evaluated patients. Afterwards I determined the cytokine TNF-α serum levels for each patient regardless of lesion placement – on the scalp, palmar, plantar, nails or trunk.

In **Study 3 - Correlations between the TNF-α serum level and various particular forms of psoriasis vulgaris for initiating personalised therapy** I continued this attempt at a personalised and predictive approach of the patient with psoriasis vulgaris. Thus I assayed the cytokine TNF-α serum levels for all patients with a severe form of the illness. I evaluated three groups of patients depending on the PASI score. The first one only included patients with a PASI score ≥10 without damage to the special areas, the second one included patients with PASI ≥10 with severe damage to special areas (palmo-plantar, scalp or nails) and the third one included patients with the PASI score <10 with severe damage to special areas (palmo-plantar, scalp or nails). I aimed to validate the serum value of TNF-α as a marker for personalised initiation of biological therapy for each of the three forms of severe psoriasis

vulgaris. At the same time I tried to explain therapeutic failure or slower response to treatment for several affected areas on these patients.

In **Study 4 - Dynamic correlation between the TNF-α serice values and the evolution of severity scores for patients with psoriasis vulgaris undergoing systemic treatment** I attempted to highlight the correlation or lack thereof between the cytokine TNF-α serum levels and the evolution of the severity scores (as a consequence, the management of the disease) in patients with severe psoriasis vulgaris. For this purpose I performed clinical and laboratory evaluations at the beginning, at 6 months and at 12 months after beginning systemic therapy. I aimed to validate cytokine TNF-α as a biomarker for favourable evolution or therapeutic failure of the patient with severe psoriasis vulgaris.

In **Study 5 - TNF-α serum levels for patients with a severe form of psoriasis vulgaris with or without previous systemic therapy** I aimed to evaluate the cytokine TNF-α serum levels in patients with severe psoriasis vulgaris, this time from the perspective of a bio-naïve or bio-experienced patient. There were two groups of patients for which the TNF-α serum value was assayed. I attempted to validate the role of this cytokine in choosing a therapeutic class, given the numerous innovative drugs having appeared on various physiopathological pathways. I have evaluated each individual patient but I also tried to establish if we can also have a general prediction for each type of patient (naïve or experienced).

To conclude I attempted to illustrate both the value and the limits of this pro-inflammatory cytokine which is extremely important in psoriasis (TNF-α) as a marker for diagnosis and/or treatment choice for the patient with psoriasis vulgaris so that we can truly speak of a personalised and predictive management for them.

Conclusions and personal contributions

1. Study 1 – Diagnostic value of ultrasound imaging of the skin for particular forms of psoriasis. Correlation with TNF-α serum value

Psoriasis vulgaris is a chronic disease for which a firm diagnosis is mostly easy to make through a local clinical exam. However particular situations arise in which this diagnosis is extremely difficult to make. I refer particularly to forms of psoriasis located only in certain areas termed in the literature “difficult-to-treat” [2]. An eloquent example is the palmar form of psoriasis very often mistaken for atopic dermatitis or other types of eczema. Lack of a correct diagnosis leads to serious mistakes in illness management, especially when we deal with a severe illness form requiring a systemic therapeutic approach.

In this situation a skin biopsy with a histopathologic exam is conducted. The manoeuvre implies additional costs, waiting time and is traumatising, especially when considering the areas (palmar, plantar, nails, scalp) that might predispose to infections or even certain degrees of functional impotence. For these reasons I have decided to perform and attempt to validate a non-invasive investigation method, namely high-resolution skin ultrasound imaging which I would associate with the serum levels of the pro-inflammatory cytokine TNF-α. I performed a cross-sectional cohort observational study. I included 56 patients in this study whom I separated into two groups. There were 32 patients with palmar psoriasis and 24 patients with atopic dermatitis with palmar lesions. Each patient had already been diagnosed with the respective condition. The aim of the study was to highlight potential obvious ultrasound imaging differences, that would allow me to make a firm diagnosis when this is not possible clinically. An additional mention is the fact that the patients with psoriasis vulgaris who presented with palmar lesions and also had disseminated lesions had a PASI score < 10. For the imagistic investigation I used a special device for skin ultrasound imaging DermaScan C version 3, manufactured by the company Cortex Technology, which has an extremely high resolution. It comes with a special software which allows high fidelity analysis focusing only on skin lesions. I used a 20 MHz transducer having a variable resolution with values between 60-150 microns and a depth of approximately 12.1 mm. With this device we can perform real-time scans in mode A or mode B. The device can take up to 8 frames per second which confers an additional degree of acuity. Following skin ultrasound I detected in the patients with psoriasis vulgaris a hypoechoic band situated under the basal membrane (SLEB) [eco]. This hypoechoic area was missing in all the patients in the group with atopic dermatitis with palmar lesions. The correlation with the histopathological exam indicates the fact that this band represents the dilated capillaries inside the crests of the dermal papillae, a pathognomonic sign for psoriasis vulgaris.

For each of the two patient groups I performed a descriptive analysis on gender and age. In the group with psoriasis we have a roughly even gender distribution (slightly higher for the male gender) which differs slightly from the prevalence study in Romania [1]. This can also induce diagnostic confusion. For statistical analysis and result validation I used the Skewness (asymmetry) and Kurtosis (tailedness) tests, the Levene test for the age variable, the Shapiro-Wilk normality test and the Fisher's Exact test to compare the presence of the band between the patients with a diagnosis of psoriasis (Pso) and those with atopic dermatitis (DA). The study showed a sensitivity (True Positive Rate) and a specificity (True Negative Rate) of 100%. These are percentages rarely encountered in real life but perhaps the group

size and the lack of previous therapy for each of these patients have determined these values. The values obtained following SLEB measurements (nm) fit within the estimated parameters as shown in the table below.

SLEB measurements (mm)

- Average: 0.421 mm
- Median: 0.412 mm
- Mode (the most frequent value): 0.418 mm
- Standard deviation: 0.038 mm
- Minimum: 0.378 mm
- Maximum: 0.551 mm

The conclusion of this study is that we can successfully use high-resolution skin ultrasound imaging for diagnosing special forms of psoriasis vulgaris, since the hypoechoic band situated under the basal membrane plays a crucial role in differential diagnosis between psoriasis and eczema. Moreover, as I have also presented in the *in extenso* work, the histopathological exam in these areas is not always suggestive of psoriasis and, as results from the second study, the TNF-α cytokine serum level is normal if the patients only have lesions at the palmo-plantar level.

2. Study 2 – Defining severe forms of psoriasis vulgaris. Correlations between severity scores and TNF-α serum value for correct classification of the patient with psoriasis vulgaris

Psoriasis vulgaris is a condition which determines an extremely high disease burden. This is directly correlated to the degree of severity of the disease. In the past, quantifying the severity degree was considered to be directly related to the affected body surface. As more and more quality of life questionnaires named PRO (Patient Reported Outcomes) were developed and implemented it was observed that not only this surface parameter is important but also the affected areas. Therefore, new scores were developed, dedicated to the lesions in these areas (ESIF, PSSI, NAPSI). A dilemma appears however when we must choose a score to classify the severity of the patient's illness, since some of these areas are also included in the PASI score. For example, total scalp affection is a severe form of the illness (having a value of 72) when calculated through the PSSI score but it is a moderate form of the illness (having a score of 8) when calculated through the PASI score. This impacts treatment choice and justification. Do we only use local treatment, possibly a conventional systemic one (the moderate form) or a biological one (the severe form)?

Starting from these realities I decided to conduct a study by which I attempt to correlate the TNF-α cytokine serum level with severe forms of the illness regardless of the score used. In this manner I attempted to determine whether lesions in the special areas represent a severe form of the illness through the high TNF-α level or through the anatomic particularities of the area or through a severe damage of the patient's quality of life, respectively. Sometimes these patients cannot perform their activities due to lesion placement (stomatologist with strictly palmar lesions).

I assessed three groups of patients presenting palmo-plantar psoriasis vulgaris lesions, scalp lesions and nail psoriasis vulgaris lesions. For each patient I calculated the degree of illness severity using the possible severity scores (see appendix 5-8). I assayed the TNF-α cytokine serum level for each patient. Sampling was performed fasting or postprandial, in a specialised laboratory. The chemiluminescence method was used. The advantage is that this method is already a routine one with acceptable costs. I then compared for each patient the TNF-α serum value with the severity score, in fact with the affected area to see whether the patients with lesions in the special areas have the same physiopathological mechanism as those with classic damage.

The SPSS v.29 was used as a method to perform statistical analysis on the variables of interest.

I used various descriptive methods, such as the graphical representation in the form of a histogram, the calculation and the creation of absolute and relative frequency charts.

Continuous variables were reported as their average with a confidence interval (CI) of the mean for a 95% confidence interval. At the same time a series of statistical indicators were presented completely describing the variables (parameters) analysed: standard deviation, minimum value, maximum value, median, quartile (Q25 – the 25% quartile, Q75 – the 75% quartile).

I also checked the type of values distribution (normality) and series homogeneity regarding statistical variances between series variances through the Levene test (Levene Test of Homogeneity of Variances). Afterwards, to compare groups I used the t-student test, ANOVA or the Mann-Whitney U test for continuous variables.

I also used the Pearson test for correlations, and I performed group comparisons based on the results from M-L, Yates or Pearson Chi-square nonparametric tests.

The data analysis showed the correctness of using all severity scores in order to correctly fit the patient with psoriasis vulgaris. Furthermore, it showed the fact that for these

patients the cytokine serum value is generally with normal values, for this reason the IL23/IL17 physiopathological axis is most likely involved here. Particular mention should be made for forms of psoriasis vulgaris with nail damage where TNF-α serum values were generally increased.

The correct fitting within a severity degree, along with the correct approach of the patient with psoriasis vulgaris also allows a prediction towards developing eventual associated diseases. Therefore for example nail damage in psoriasis is oftentimes a clue for ulterior occurrence of nail damage. Labelling as a severe illness form allows a precocious innovative treatment with limitation of this risk. Labelling as a light illness form delays correct treatment and increases the risk of articular disease appearing.

In the future, it would be interesting to perform a study dosing cytokines involved in the other physiopathological pathway of psoriasis, namely Th17 (IL23/IL17). This way we would determine the reasons why these types of psoriasis are severe forms of the illness.

3. Study 3 - Correlations between the TNF-α serum level and various particular forms of psoriasis vulgaris for initiating personalised therapy

The choice of treatment for the patient with psoriasis vulgaris is a significant challenge, ever since the appearance of a high number of advanced therapies and especially while lacking a pathognomonic biomarker. While in the previous study I evaluated the correlation between the level of TNF-α and each affected area, particularly in order to understand the physiopathological mechanism for each severity form, now I endeavoured to evaluate the relationship between the TNF-α serum value and patients with a severe form of the illness in order to try and personalise the initiated treatment.

To carry out this study, I consulted a number of 86 patients with various forms of psoriasis vulgaris – classic, palmar, plantar, scalp or ungual. For this study I divided the patients as shown below into three groups, namely:

a. group 1 – patients with a PASI score ≥10

b. group 2 – patients with severe area scores and a PASI score ≥10

c. group 3 – patients with severe area scores and a PASI score <10

For the last two groups the assessment of lesions in special areas was included in the calculation of the PASI score. I was not interested in a specific area but all patients in groups 2 and 3 had severely affected special areas. Some of them even had multiple lesions on their body (group 2), others did not (group 3).

For the future it would be interesting to conduct a study with the dosage of cytokines involved in the other pathophysiological pathway of psoriasis, namely Th17 (IL23/IL17). In this way we would determine the reasons why these types of psoriasis represent severe forms of the disease. The method used for the serum assay of the pro-inflammatory cytokine TNF-α was CLIA, meaning immunochemical or immunoenzymatic assay method with immunofluorescence detection, or chemiluminescence method for short. The collection was performed in a specialized laboratory fasting or postprandial.

Correlational analysis indicated a significant correlation between nominal PASI values and TNF values ($r=0.5286$, $p<0.001$).

Statistical analysis performed with all correction methods showed a strong, statistically significant correlation for TNF-α cytokine values and PASI severity score values. Thus, for patients in the first two groups (those with a PASI score ≥10 regardless of the damage to special areas), the TNF-α value was increased. For patients in group 3 (those with a PASI score <10) the values of the cytokine TNF-α were on average lower, although the patients according to the new classifications were in a severe form of the disease, that is, having special areas that were affected. Another interesting observation is that these values are not influenced by the age or gender of the patients.

These results give us a clearer picture for certain situations encountered in patients with psoriasis vulgaris. First of all, we can consider the TNF-α cytokine to be a very important player for patients with PASI score ≥10 and we have the scientific argument for the initiation of anti TNF-α therapy in these patients. In addition to personalized therapy we also have an important financial gain because this therapeutic class involves lower costs, considering the biosimilar products it includes. Secondly it explains the better results observed in clinical trials that the other therapeutic classes (anti IL17 or anti IL23) obtain compared to the anti TNF-α class in the treatment of psoriasis vulgaris lesions located in difficult-to-treat areas.

In the future I believe that, just as for the other studies in this work, research can also be developed here by evaluating the other important pathophysiological pathway in psoriasis vulgaris, namely the IL23/IL17 pathway. The clinical form of strictly ungual psoriasis vulgaris would also deserve more in-depth research, where things are a little more complex, on the one hand because these lesions are not found in the PASI score, on the other hand for the increased risk of developing arthropathic psoriasis through the anatomical connection that it has with the distal phalanx.

4. Study 4 - Dynamic correlation between the TNF-α serice values and the evolution of severity scores for patients with psoriasis vulgaris undergoing systemic treatment

Psoriasis vulgaris is an illness for which long-term management is required. An early implemented treatment is needed for rapid control of skin lesions, comorbidities and associated illnesses. Of equal importance is also the patient persisting on the treatment. For this reason, proper choice of treatment from the very beginning is extremely important.

Psoriasis vulgaris is one of the diseases whose evolution is frequently influenced by external trigger factors. Oftentimes, the dermatologist is faced with a dilemma regarding the therapeutic value of the medication, not always having arguments to assess the aggravation of the psoriasis vulgaris under treatment by the lack or loss of patient response to the product used. This situation is mainly generated by the lack of a valid biomarker.

If in the previous study we identified the TNF-α cytokine as a biomarker with its value and limits for choosing a predictive and personalised treatment for the patient with a severe form of psoriasis vulgaris, in the current study I tried to dynamically evaluate the concordance between the TNF-α serum level (through repeated dosage at regular time intervals) and the illness severity scores, namely PASI, PSSI, ESIF, and NAPSI.

For this purpose I consulted a number of 86 patients for whom I initially ran a descriptive statistical analysis through which I calculated the average, mean, standard deviation, maximum value, minimum value, variance, skewness and kurtosis. In order to compare the values I employed two established tests, the T test (for a normal distribution) or the Mann-Whitney U test (for distributions that differ from a normal distribution). I also used the Wilk-Shapiro Test and the Friedman Test with post-hoc analysis, for which the Bonferroni correction was applied.

To determine the TNF-α serum values, the samples were collected and processed under normal working conditions in a laboratory in Bucharest. Samples were collected both fasting and postprandial, since determination of the TNF-α serum values does not require special conditions. Three determinations of the TNF-α VALUE were made for each patient. The determinations were made at fixed dates which coincided with the moment of clinical-biological efficacy and patient safety assessment. This means the initial assessment (T0), the six-month assessment (T6) and the twelve-month assessment (T12). In the evaluated group 64% of the patients were male and the average patient age was mostly situated in the fifth decade, being equal to 45.99 ± 12.69 years. There was no statistically significant difference

between the male and female gender in terms of age. Interestingly, by statistically analysing the serum level of the TNF-α cytokine, I did not detect significant variations of its value in relation to gender or patient age. Regarding severity score values, although I noticed slightly higher values of the PASI score and lower values of the NAPSI score in males, in the end there were no statistically significant differences regarding the PASI, PSSI, ESIF and NAPSI values. Another important aspect is the correlation between these severity scores and age. If for the PASI, ESIF and NAPSI scores there is no statistically significant correlation between their values and age, for scalp damage (PSSI) a tendency for the PSSI score to decrease with increasing age is observed, the linear correlation being a negative (r=-0,56), statistically significant one (p=0,042).

The dynamically tracked TNF-α cytokine serum values decreased from 12.99 ± 13.04 at the T0 time point towards average values of 7.55 ± 2.60 at the T6 time point and towards average values of 6.51 ± 1.53 at the T12 time point since having started treatment.

Using all the aforementioned tests I reached the conclusion that TNF-α cytokine serum values have been statistically significantly different in three time points when they were evaluated, namely T0, T6 and T12 $\chi 2(2) = 71.56$, $p<0.001$. The values decreased dynamically, the lowest value being recorded at time T12 (the one year clinico-biological evaluation).

By tracking the evolution of the severity scores I noticed that they have also progressively decreased, starting from the highest values at the initial time point T0 and reaching the lowest value at the T12 time point (the one-year clinical-biological evaluation) with an intermediate value at the six-month clinical-biological evaluation (the T6 time point).

By making a direct correlation for each assessed time point (T0, T6, T12) between the TNF-α cytokine serum level and the value of each severity score I noticed a strong direct correlation between the cytokine serum value and the PASI, PSSI and ESIF scores. I also noticed a direct correlation with a high degree of statistical significance, but not as strong between the TNF-α cytokine serum values and the ESIF palmo-plantar severity score.

The conclusion of this study is that the dynamically predictive value of the TNF-α vs severity scores relationship is a statistically significant one. It represents an important biomarker to prove the role of this cytokine in the development of psoriasis vulgaris and particularly the therapeutic value of anti TNF-α biological medication. The assessment is highly valuable because it has been proven that the TNF-α value is influenced neither by age nor gender. Another important gain arising from this study is the fact that we can explain

therapeutic failure for anti TNF-α biological medication and our choice of another therapeutic class. Without this biomarker we might be tempted to continue with the same therapeutic class, while only changing the drug, since it is known that there are differences even between products from the same class ($T_{1/2,}$ affinity, binding site, dissociation constant, etc). Moreover, the sometimes slightly slower response to anti TNF-α biological therapies administered to patients with palmo-plantar psoriasis vulgar lesions can be explained. In the case of some patients with disseminated lesions on the body who also show palmo-plantar lesions, a topical or systemic treatment can be associated in the case of the latter without needing to change the biological therapy. We therefore have an objective overall picture of the evolution of the disease along with the possibility that based on scientific evidence we can approach the patient with a severe form of psoriasis vulgaris in a personalised and especially predictive manner.

5. Study 5 - TNF-α serum levels for patients with a severe form of psoriasis vulgaris with or without previous systemic therapy

The choice of adequate therapy for each patient with psoriasis vulgaris presents a significant challenge to any dermatologist. At present, numerous innovative therapies have been developed that act on several physiopathological pathways. This relates to anti TNF-α, anti IL12/23 (p40), anti IL17 or anti IL23(p19)z biological therapies. In the absence of a predictive biomarker for treatment choice for severe forms of psoriasis vulgaris, medication choice is mostly based on subjective criteria.

There is a great difference between therapeutic approaches for bio-naïve patients compared to bio-experienced patients. A correct initial choice in therapy enables a faster response as well as more significant lesion remission. In the case of patients having to change a therapy that has since become ineffective, positive results are obtained with greater difficulty and usually with less significant remission.

For these reasons I have initiated this study, trying to validate the TNF-α serum level as a predictive biomarker for therapy choice. Evaluated patients were divided into two groups, some who had not had any systemic therapy and others who had been using a systemic treatment to which they had become unresponsive. Of these, 51 were therapeutically naïve while the other 35 had been using systemic medication. For each of them, laboratory samples were drawn in order to assay the TNF-α cytokine. The laboratory method employed was immunoenzymatic assay with immunofluorescence detection. I

attempted to establish the TNF-α cytokine level for each of these groups in order to be able to determine the therapeutic class to be used from the very beginning.

The analysis and data processing in the study were of a descriptive statistics type. I used histograms or box-plot charts as well as several statistical tests. All of them showed that the two groups had statistical significance. However, no statistically significant difference for the TNF-α cytokine level between the two patient groups could be obtained in order to certify the initiation of anti TNF-α biological therapy for certain types of patient (bio-naïve and bio-experienced, respectively).

Nevertheless, from this assessment I have noticed that the average value of TNF-α cytokine was high in both groups. Interestingly, the TNF-α cytokine level was higher in patients with previous treatment (14.44 ± 16.32) compared to those without any treatment (12.002 ± 10.28). This could be explained by the fact that previous therapies were predominantly focused on the IL23/IL17 axis while the physiopathological process was transferred through the TNF-α pathway. Therapeutic failure could also be explained in this manner, with all the patients in the study having a severe form of the illness, practically all with previous therapies being unresponsive.

Although in general we do not have a predictive value for TNF-α cytokine serum levels for various patient categories (bio-naïve vs bio-experienced), its individual value could be ascertained from this study. By assaying TNF-α, we can choose a biological therapy with scientific arguments so that we can truly talk about customization and therapeutic prediction. Regardless of whether we discuss initial therapy (bio-naïve) or treatment change (bio-experienced). Finally, this is what is meant by proper management of the patient with a severe form of psoriasis vulgaris.

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