HABILITATION THESIS

Researches in Skin Pathology:
from Mechanism to Therapy

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SECTION I

PROFESSIONAL, SCIENTIFIC AND ACADEMIC CONTRIBUTIONS

THESIS ABSTRACT

My Habilitation Thesis focuses on the main achievements of my professional-scientific activities carried out in Dermato-Venereology during 2004 till present. It reflects my interest constantly to contribute, through concrete results, to the current development of knowledge in the area of research in Dermatology, pursuing in the same time the alignment as personal academic training, operational European standards in superior medical education.

Solid professional activity in the specialty of Dermato-Venereology integrating component of educational level in licence and residency with current medical practice, created the prerequisites for accumulation of specific interdisciplinary skills and translational research. I had the opportunity to be involved in numerous clinical trials, randomized, double-blind, multicentre or international, as principal investigator, and to coordinate, as director, a grant won through national competition. I have always promoted new research ideas generated by pathogenic relationship between the pathogenic foundation, the efficacy of diagnostic and the necessity to identify modern major therapies centered on increasing the quality of life.

Consecutively, this Thesis has a character of clinical and fundamental research, completed with elements aimed at biomedical ethics applied to the field of Dermato-venereology.

My Habilitation Thesis is divided into two main sections according to the criteria recommended and approved by CNATDCU.

SECTION I, entitled Professional, scientific and academic contributions, is organized into five chapters, each devoted to a priority research directions for my professional portfolio. This section includes the most relevant scientific results obtained in the research, the presentation of which is connected to the existing information in the flow of main publications. Thus, each chapter is supported by a brief overview of known and accepted in the literature data which motivated the studies and later include presentation of the
methodology used and the results obtained, followed by interpretation, critical and constructive discussions.

Chapter 1, Research in psoriasis: from diagnosis to therapy, has as its starting point the current data that focuses on the study of psoriasis, referring to epidemiological data - the incidence and prevalence, the main factors involved in etiology and new theories relating to the inclusion in this list of modern factors such as stress. Comorbidities of the disease are presented in this chapter, and here are detailed the most common problems associated with different clinical forms of psoriasis. One of the themes of my research has been the metabolic syndrome and its association with psoriasis, and the results were published and presented extensively in the habilitation thesis. As in any other condition, the pathology approach changes with time and the emergence of new therapeutic options bring new perspectives to the patient. This chapter illustrates the emergence, evolution and new trends of biological therapies and results in a clinical study on the subject.

Chapter 2, Histopathological aspects in dermatological pathology is built on specific morphology of microscopic records in cutaneous manifestations. Building on the major role of skin biopsies as well as on immunohistochemistry techniques in order to complete the diagnostic panel, this chapter focuses on results obtained in the study of rare entities such as lymphomatosid papulosis and multiple clear cell acanthoma in cutaneous manifestations associated with infection with hepatitis C, as well as the analysis of pilo-sebaceous unit after analysis of antiandrogenic treatment of hirsutism in women.

Chapter 3, Ultrasonography in skin pathology, brings to the fore the role of modern imaging techniques, the values of applicability in pathology of melanoma, benign melanocytic lesions, benign tumors, inflammatory diseases, in lipoablation being accentuated by valences of repeatability. This chapter also includes the presentation of our results in investigation of histiocytomas cutaneous lesion as well as the role of an integrative analysis of skin tumors ultrasonography.

Chapter 4, Dermatology and rare diseases, submit the side of Dermatology associated with genodermatoses which are a broad group of rare genetically transmitted diseases, whose recognition is important not only to establish an appropriate therapy but also to identify anomalies that may be often associated with multisystemic diseases. Actual contribution to the current state of knowledge lies in the full diagnostic and therapeutic monitoring of cases with rarely seen pathology in current dermatological practice: Bloch-Sulzberger syndrome, pahidermodactilia and Ekbom syndrome.

Chapter 5 Ethic and legal in dermatology, brings to the fore the intense preoccupation to connect ethical premises with specialty of Dermato-venereology, respecting the national legal framework and specific international bioethics, as a systematic, pluralistic and interdisciplinary direction addressing moral theoretical and practical issues applicable to medical practice. Analysing the ethical values of the physician-patient relationship, the construction of this chapter concerns as major directions the study of the legal and ethical approach of sexually transmitted infections, psoriasis and skin tumors pathology.
SECTION II entitled *Plans of career development and future evolution of research*, presents the main projects I have in mind for future work, which are directed primarily on continuing study psoriatic disease under different clinical manifestations (vulgar, arthropathy, pustular), clinical and histological correlations on ultrasound and pathology of melanocytic or nonmelanocytic mucocutaneous lesions and the study of nail pathology, focusing on diagnostic imaging techniques.

SECTION III, *References, bibliography* includes supporting existing information in this Habilitation Thesis.
REZUMATUL TEZEI

Teza de abilitare concentrează principalele realizări ale activității mele profesional-științifice desfășurate până în prezent, în domeniul dermato-venerologie. Lucrarea reflectă interesul meu constant de a contribui, prin rezultate concrete, la dezvoltarea stadiului actual al cunoașterii în arie de cercetare specifică dermatologiei, urmărind în același timp alinierea, ca formare academică personală, la standardele europene operaționale ale învățământului superior medical. Activitatea profesională solidă în specialitatea dermato-venerologie, integrând componenta de învățământ, instructiv-educativă, la nivelul studiilor de licență și de rezidențiat, cu practica medicală curentă, a creat în timp premisele acumulării abilităților specifice cercetării interdisciplinare și translaționale. Am avut astfel oportunitatea de a fi implicată în numeroase studii clinice, randomizate, de tip dublu orb, multicentrice sau internaționale, în calitate de investigator principal, precum și de a coordona, în calitate de director, un grant câștigat prin competiție națională. Am promovat permanent idei de cercetare noi, generate de relația dintre fundamentul patogenic, eficacitatea diagnostică și necesitatea identificării unor terapii moderne, centrate major asupra creșterii calității vieții pacienților.

Consecutiv, prezenta teză de abilitare are un caracter de cercetare clinicofundamentală, completată cu elemente care vizează etica biomedicală aplicată domeniului dermatologiei și venerologiei.

Teza de abilitare este structurată în două secțiuni principale, respectând criteriile recomandate și aprobate de CNATDCU.

SECȚIUNEA I, intitulată Contribuții profesionale, științifice și academice, este organizată în 5 capitole, fiecare dedicat unei direcții de cercetare prioritare pentru portofoliul meu profesional. Această secțiune include cele mai relevante rezultate științifice obținute în activitatea de cercetare, a căror prezentare este conectată la informația existentă în fluxul principal de publicații. Astfel, fiecare capitol este susținut de o scurtă trecere în revistă a datelor cunoscute și acceptate în literatură care au motivat studii proprii, și include ulterior prezentarea metodologiei utilizate și a rezultatelor obținute, urmate de interpretarea acestora, prin discuții critice și constructive.

Capitolul 1, Cercetări în psoriazis: de la diagnostic la terapie, are ca punct de plecare datele actuale ce vizează studiul psoriazisului, cu referire la datele epidemiologice – incidența și prevalența, principalii factori implicați în etiopatogenie cât și noile teorii referitoare la includerea în această listă a unor factori moderni precum stresul. Comorbiditățile acestei afecțiuni sunt prezentate în acest capitol, aici fiind detaliate cele mai frecvente afecțiuni asociate diferitelor forme de psoriazis. Una din temele mele de cercetare a fost sindromul metabolic și asocierea sa cu psoriazisul, iar rezultatele au fost publicate și prezentate pe larg
În teza de abilitare. Ca în orice altă afecțiune, abordarea patologiei se modifică cu timpul și apariția de noi opțiuni terapeutice aduce noi perspective pentru pacient. În acest capitol sunt ilustrate apariția, evoluția cât și noile tendințe ale terapiilor biologice, dar și rezultatele obținute în cadrul unui studiu clinic pe acest subiect.

**Capitolul 2, Aspecte histopatologice în patologia dermatologică**, este construit în baza evidențelor de morfologie microscopică specifice manifestărilor cutanate. Pornind de la rolul major al biopsiei cutanate, precum și al tehnicilor de imunohistochimie în completarea panelului diagnostic, acest capitol concentrează rezultatele obținute în studiul unor entități diagnostice rare – anume papuloza limfomatoïdă și acantomul multiplu cu celule clare, în manifestările cutanate asociate infecției cu virus hepatic C, precum și în analiza unității pilo-sebacee după tratamentul antiandrogenic la femeile cu hirsutism.

**Capitolul 3, Ultrasonografia în patologia cutanată**, aduce în prim plan rolul tehnicilor moderne de imagistică cutanată, și valoarea aplicabilității în patologia melanomelor, a leziunilor melanocitare benigne, a tumorilor benigne și a bolilor inflamatorii. Capitolul include prezentarea rezultatelor noastre în investigarea histiocitomului cutanat, ca și leziune particulară, precum și o analiză integrativă a rolului ultrasonografiei în tumorile cutanate.

**Capitolul 4, Dermatologia și bolile rare**, supune atenției latura dermatologiei asociată cu genodermatozele – grup larg de afecțiuni rare, cu transmitere pe cale genetică, a căror recunoaștere este importantă nu numai în vederea instituirii unei terapii adecvate, dar și pentru identificarea unor anomalii ce pot fi asociate acestor maladii frecvent multisistemice. Contribuția efectivă la stadiul actual al cunoașterii constă în monitorizarea completă, diagnostică și terapeutică, a unor cazuri clinice cu patologie rar întâlnită în practica dermatologică curentă: sindromul Bloch-Sulzberger, pahidermodactilia și sindromul Ekbom.

**Capitolul 5, Etic și legal în dermatologie**, aduce în prim plan preocuparea intensă de a conecta premisele etice la specialitatea de dermato-venerologie, cu respectarea cadrului legal național și internațional specific bioeticii, ca direcție de studiu sistemică, pluralistă și interdisciplinară care abordează probleme morale, teoretice și practice, aplicabile la practica medicală. Analizând valorile etice ale relației medic-pacient, construcția acestui capitol vizează, ca direcții majore, studiul cadrului legal și etic în abordarea patologiei infecțiilor cu transmitere sexuală, a psoriazisului și a tumorilor cutanate.

**SECȚIUNEA II**, intitulată *Planuri de dezvoltare a carierei și evoluția viitoare a cercetării*, prezintă principalele proiecte pe care le am în vedere pentru activitatea viitoare, și care sunt orientate prioritar asupra continuării studiului bolii psoriazice sub diferitele manifestări clinice (vulgar, artropatic, pustulos), pe stabilirea corelațiilor clinicohistologice și ultrasonografice în patologia leziunilor cutaneo-mucoase melanocitare și nemelanocitare, precum și pe studiul patologiei unghiale, cu accent pe tehnicile diagnostice imagistice.

**SECȚIUNEA III, Referințe Bibliografice**, include bibliografia care susține informația existentă în prezentă Teză de abilitare.
CHAPTER I
RESEARCHES IN PSORIASIS: FROM DIAGNOSIS TO THERAPY

1.1. INTRODUCTION

Psoriasis is a chronic non-transmissible inflammatory-mediated autoimmune affection of the skin, produced by a keratinisation disorder, relatively frequent among people. Recent studies have demonstrated that psoriasis can have a big impact on the quality of life even in patients with limited illness. Additionally, psoriasis involves important financial costs for patients as well as health system. Although the cause for this illness remains unknown, research suggest that psoriasis is a complex affection determined by the interaction of numerous genes, immune system and environment factors (Bolognia et al., 2007; Heredi, Vegh et al., 2016).

Skin lesions in psoriasis are a "cascade" of disorders characterized by a dense infiltrate of inflammatory cells, massive proliferation, alterations of epidermal differentiation, neoformation vessels and alterations of lumphocytic structures. Several studies have been conducted on the the epidemiology of psoriasis. Epidemiology generally represents the science that studies distribution (incidence, prevalence) and determinants (risk factors) of an illness in different human populations.

THE DERMATOEPIDEMIOLOGY OF PSORIASIS

Incidence represents the proportion of individuals at risk in total population, individuals who develop a certain disease in a certain length of time. In contrast to prevalence which indicates the total of present cases, incidence quantifies the number of new cases of illness which appeared in a population at risk, over a given time.

The incidence of psoriasis in the world is estimated between 2 and 3% (Fotiadou, Lazaridou et al., 2016).

A populational study conducted by Mayo Clinic in the USA has shown a yearly incidence of psoriasis of 57.6 from 1.000.000 people (54.4 from 100.000 for men and 60.2 from women). It is important to mention that not all the patients suffering from psoriasis see the doctor, especially those with a mild form of the illness, this way the real incidence of psoriasis could be underquantified. In terms of age, the highest rate of incidence was recorded in the group 60 and 69 years of age (112.6 from 100.000 people). Most psoriasis cases have been diagnosed in this study (58% from 132 cases, over a period of 4 years) were mild forms (with under 10% of the cutaneous surface affected).
Psoriasis can appear at any age, from childhood up to the senior age. However, in any study determining the onset age, this is a real problem as research is based on patients’ statements, on their memory, which cannot always be exact. On the other hand, studies which take into consideration the first check up or the diagnosis moment can be irrelevant because patients ask for medical intervention after variable time periods from the beginning of the illness. In contradiction to these problems regarding the correct evaluation, larger studies have shown that the onset age for psoriasis (meaning incidence) has a bimodal distribution with maximum values somewhere in the first youth (20s-30s) and then between 50 and 60 years of age. A hypothesis was formulated that this bimodal distribution represents, in fact, two different clinical versions of psoriasis - the so-called type I and type II. Type I psoriasis means an onset before turning 40 years, being a more severe illness, having positive family history and association with certain (HLA) (Cw6) antigens and it represents 75% of cases. In patients with type II psoriasis, the onset of the illness is after 40 years of age and has no association with HLA-Cw6. There are also psoriasis cases which do not fit into any of these two types of illnesses. Additionally, there are no studies that could confirm these possible differences as far as the response to various therapies is concerned (Krueger, 2002).

Prevalence is the proportion of individuals from a population, who have a certain illness. The proportion is reported either at a certain time or according to a specified time. Moreover, studies of psoriasis prevalence use different versions for the definition of psoriasis case (confirmed by the patient or diagnosed by the doctor) in different populations of different ages, etc. All these elements influence the obtained results regarding the calculation of prevalence rates.

In most publications, the prevalence of psoriasis in general population is estimated between 0.6- 4.8%. The biggest incidences in Europe have been reported in Norway (4.8%), Denmark (2.9%) and Faeroe Islands (2.8), with an average of almost 2% for the Northern Europe. Rates of psoriasis prevalence have been calculated in the USA between 2.2% and 2.6%, with approximately 150.000 newly diagnosed cases each year. In Africa, prevalence is bigger in the Eastern part of the continent in contrast with the Western part, thing that could explain the low prevalence (of 1.3% only) of psoriasis among the Afro-Americans. Arabs have similar psoriasis prevalence rates as North Europeans. Asians from China and Far East present low rates of psoriasis prevalence, of almost 0.7%; and among the South American Indians and aborigines from Samoa Islands psoriasis is considered non-existent (Fry, 2004).

The literature review based on an assessment of three electronic databases in July 2011 with a total of 385 scientific works and 53 studies show a prevalence of psoriasis in children between 0% (in Taiwan) and 2.1% (Italy) and in adults between 0.91% (United States) and 8.5% (Norway). Also, it indicated that psoriasis appearance varied according to age and geographic region, being more frequent in the more distant equator countries (Parisi et al., 2013). However, although most of these reports can be combated, having just an indicative importance, the general impression is that in contrast with average prevalence of 2%, psoriasis is more frequent in North Europeans and less frequent in people from East Asia.
Brandrup and Green report that two thirds of patients have mild forms of psoriasis while a third has a sever affection. In a large group of patients with psoriasis (1728) 79% have been found with ungual affection. Psoriatic arthritis is probably up to 5-30% of patients with cutaneous lesions. This variability can partially establish the criteria that can be used (e.g. radiologic detection of articular erosions). In general, cutaneous lesions appear before the psoriatic arthritis becomes clinically detectable. Anyway, 10-15% of patients initially state occurrence of psoriatic arthritis, which is followed by the appearance of skin lesions (Christophers, 2001).

Although the age for psoriasis onset is smaller in women than in men, the natural evolution is similar, being chronic with remissions. In an epidemiologic study at Stanford, 39% of patients stated that they had remissions which lasted between 1 and 54 years (Neimann et al., 2006). Remissions are common, occurring either spontaneously or with treatment and have maintenance periods difficult to quantify.

In comparison with atopic dermatitis, psoriasis is less common in children. For example, at National Skin Centre in Singapore 1.1% of patients under 16 years old have been diagnosed with psoriasis. In a study involving 8298 school children between 12 and 16 years of age, 0.5% had psoriasis. During childhood, the most frequent type of psoriasis is guttate psoriasis and appears in association with a streptococcic infection. By treating the underlying infection may appear complete resolution of the lesions.

**Risk factors and determinants:**

**Risk factors:**

Various studies have tried to identify the risk factors in developing psoriasis. To reduce errors and establish a temporal relationship, controlled populational studies are necessary on patients with new cases of psoriasis. In this his way potential risk factors could be identified for the development of this illness.

A controlled study is an observational analytic study, in which subjects are selected depending on the presence (cases) or absence (control) of the studied illness. These two groups are then compared regarding the proportion of individuals which present a medical history connected to the exposure to certain events or characteristic of the illness (risk factors). In a populational study, the analysed cases are highly representative for all the cases from community, and control group comes from the same population in which cases have been identified, so that errors are minimized.

Family medical history (genetics) is the best documented risk factor for the development of psoriasis. There are also studies that have investigated infections, smoking, alcohol etc. as potential risk factors for psoriasis. Several studies have examined even diet, but results are contradictory.

Risk factors for this condition include the following categories: genetic factors, trigger , respectively, immunological factors.

1. Genetic factors aimed at family history and presence of the following loci:
   - PSORS-1 (psoriasis susceptibility) locus situated on the major complex of histocompatibility 6p21.3 chromosome
2. Trigger factors are represented by environmental factors (external): trauma, climatic factors, professional and systemic factors: age, gender, occurrence of infections, including HIV, metabolic or endocrine (hormonal) factors, mental stress (psychological component), medications, lifestyle (alcohol, smoking, diet) and associated diseases.

3. Immunological factors include acquired immune deficiencies (autoimmune thyroiditis, vitiligo, lupus, etc).

1. Genetic factors:

   There are undisputable proofs that psoriasis has a significant genetic component, as has long been known that the disease occurs with increased frequency in some families. Depending on the study group, family history of psoriasis is reported in 35-90% of patients. The classical epidemiological study on psoriasis of Lomholt in Faeroe Island, in which he examined 10,000 inhabitants, showed that the incidence of psoriasis is higher among first- and second degree relatives of patients than the rest of control subjects (Bolognia et al., 2007).

   A metaanalysis has established that deletion of gene 2LCE, LCE3C and LCE3B represents a susceptibility factor for psoriasis in different populations. Data from 9389 patients with psoriasis and 9477 control subjects were used to determine the contribution of LCE3B and LCE3C gene deletion, which is involved in psoriasis’ susceptibility in different populations. The study confirms that the deletion of these genes (LCE3C and LCE3B) is a common genetic factor in psoriasis patients in European populations (OR (global) = 1.21 (1.15-1.27)), demonstrating directly the association of erasing those loci in Chinese people with psoriasis (OR = 1.27 (1.16-1.34)) and Mongols (OR = 2.08 (1.44-2.99). (Riveira, He et al., 2011).

   Following a big study done in Sweden confirmed this data, showing that the prevalence of psoriasis is 7.8% in first grade relatives of patients, comparing to 3.14% in the similar control group and comparing to 1.97% in general population. Based on this data from populational studies many researchers have calculated the risk for a child to suffer from psoriasis. A study in Germany concluded that if both parents have psoriasis the risk for the child to develop this anomaly is 41%, if one of the parents developed the disease, the risk is 14%, and if one of the children is affected the risk for the others is 6%.

   The analysis of the concordance rate of psoriasis among the monozygotic and dizygotic twins is another method to analyze the influence of genetic factors on the illness, method that probably offers the best data regarding the genetic basis of psoriasis. Farber and Nall revised the data published referring to the analysis of studied pairs of twins with psoriasis. 82 pairs of monozygotic twins out of 141 pairs were concordant for psoriasis, and 59 were discordant; from 155 of dizygotic twins, only 31 were concordant for psoriasis, while 124 were discordant. This data shows that genetic factors play an important role in the pathogenesis of psoriasis. It is interesting that when monozygotic twins are concordant, onset of psoriasis, the distribution and severity are similar, while these issues differ in pairs of
dizygotic twins. These observations suggest that genetic factors play a role in the determinism of these parameters, as well and in the clinical evolution of psoriasis (Barker, 2001).

On the other hand, it is obvious that the rates of concordance do not reach 100% in the case of older twins, which shows that the environment also plays a key role in the expression of the disease. Based on the transmission variability within families, some authors have suggested that psoriasis represents a spectrum of genetic diseases. To one extreme end, we find the families in which changes at the level of one gene can be enough to trigger the illness. The second extreme end is represented by the situation in which the family medical history is missing. For these individuals, for the expression of the illness, genetic changes are probably needed, which are interacting with each other, as well as with environmental factors.

Interestingly also, that if one parent is affected by psoriasis, the risk of children developing the disease is higher if the affected parent is the father unless it would be the mother. In addition, children will develop the disease at a lower age than their parents (genetic anticipation). So gene/genes expression responsible for psoriasis is influenced by sex of the parent from which is derived (genetic imprinting) (Barker, 2001).

A polygenic model for psoriasis means that no single gene is neither sufficient, nor necessary, for the development of the disease. Polygenic transmission hypothesis is based on the transmission of a greater number of genes from parents. There is also the hypothesis that psoriasis would represent a spectrum of genetic diseases with rare monogenic forms and to the polygenic / multifactorial which are more common (Campalani, Barker, 2005). Rare cases in which changes in a single gene may be sufficient to cause the disease explains why some families seem to convey an autosomal dominant psoriasis. In these families, there is a strong genetic heterogeneity, individual families being associated with different loci. The majority of patients with psoriasis are in the situation that multiple genes interacting with each other (epistasis) are involved and / or with the environment (multifactorial etiology).

All the submitted studies refer to chronic plaques psoriasis, the most common form otherwise. Little is known about the epidemiology of other clinical psoriasis types. For example, guttate psoriasis is invariably associated with HLA and regarded as closely related to plaque psoriasis in plaques from the pathogenic point of view.

2. **Trigger factors:**

There are certain external trigger factors (which interact directly with the skin), as well as systemic factors, which in genetically predisposed individuals can trigger psoriasis.

External trigger factors:

**Traumatisms**

Isomorphic response, for example after the onset of psoriatic lesions in skin trauma, is observed in approximately 25% of patients with psoriasis. This phenomenon was first observed by a physicist called Koeber. Although 1/3 of patients with psoriasis develop skin lesions after trauma (Koeber are positive), the remaining 2/3 are Koeber-negative. Koeber phenomenon abiding "all or nothing", e.g. patients who are positive Koeber in a region of the skin they are positive all over the body and those who are negative are negative everywhere. This suggests that psoriasis is a systemic disease that can be triggered locally on
the skin. A patient may be "Koebner-negative" at some point in the course of the disease and later become "Koebner-positive" or vice versa, which indicates that there is a central factor influencing the development of psoriasis and that this factor is variable.

Psoriasis lesions can be induced by injury and other forms of cutaneous injury: sunburn, drug morbilliform rashes, physical, chemical, electrical, surgical, infectious (viral exanthema) trauma. The nature of the injury is not important. Koebner himself described the simultaneous development of psoriatic lesions in the same individual at a tattoo of a flea bites, impetigo lesions as well as of the level of excoriations produced by riding the horse. Latency between the trauma and lesions of psoriasis is usually 2-6 weeks. Psoriasis lesions can occur within existing dermatoses such as contact dermatitis and leprosy.

It has been shown that trauma which induces psoriasis in a Koebner-positive patient must produce positive Koebner-epidermal lesions. These lesions probably initiate a cascade of cytokines that trigger psoriasis. Inhibitory factor which stops the development of psoriasis is part of an immune response, probably in connection with regulatory T-lymphocytes.

A Koebner-inverse phenomenon has been recently described. In this case, removing a part of a psoriasis plaque by peeling the scales off up to the superior dermis; lead to that certain region being replaced by normal tegument unaffected by psoriasis. The Koebner-inverse phenomenon and the real one exclude each other which means that patients who are Koebner-positive, they are positive all over the body and cannot express the Koebner-inverse phenomenon and vice versa.

It should be noted, however, that the isomorphic answer cannot be considered pathognomonic for psoriasis. It also may appear in lichen planus, lichen nitidus, vitiligo and other skin disorders.

**Climatic factors**

Although solar radiation are generally beneficial for psoriasis, there is a small proportion of patients to whom psoriasis can be triggered by UV radiation and who are accusing summer exacerbations of the photo exposed regions. In a study based on questionnaires to patients in Sweden, photosensitivity prevalence among patients with psoriasis was estimated at 5.5% (2000 cases). Almost 40% of these patients reported a history of polymorphous light eruption, psoriasis lesions appearing as secondary phenomenon. Photosensitivity in psoriasis patients is associated with skin phototype I, with older age and female gender as well. Paradoxically, in these cases photo-chemotherapy (PUVA) may be beneficial.

In addition, psoriasis seems to be improving in regions with warm climate and get worse in those with cold climate. This effect is independent of UV light and could explain the high incidence of psoriasis in northern European countries.

**Professional factors (occupation):**

Occupational psoriasis represents only 1.2% of all occupational diseases. It can be found particularly in individuals with mild disease as those with severe disease avoid spontaneous traumatic activities. Identifying the signs and symptoms of disease, as well as
individuals prone to trauma are important for the recognition of professional psoriasis, but also for vocational guidance.

**Systemic factors:**

*Age and gender*

Men and women are equally affected by psoriasis vulgaris. Various studies indicated that the age of onset is lower in women. Thus, a german study has shown for early psoriasis age of onset peaks at 22 years for men and at 16 years for women. However, the results of the studies are highly dependent on the techniques used and are therefore variable. There is no evidence that the disease is phenotypically different between sexes.

**Infections:**

Infections, particularly those bacterial can induce or worsen psoriasis. Over 45% of patients with psoriasis have been recorded as a trigger factor infections with B-hemolytic streptococcus, especially pharyngitis are most commonly involved. Streptococcus can be isolated from other locations - dental abscesses, perianal cellulitis, impetigo. These streptococcal infections often lead (probably via activation of T cells by superantigens) to a rash of guttate psoriasis, especially in children. They can also trigger a pustular psoriasis or exacerbate classic lesions of psoriasis. More rarely, may be responsible for triggering the sinus, respiratory, gastrointestinal or genitourinary tract infections.

The scales on the surface of the psoriasis plaques are highly colonized with pathogenic Staphylococci (50% of cases). Therefore it is recommended that before any elective surgery and especially before any elective surgical intervention and especially orthopedics, psoriasis lesions should be cured or at least improved to reduce the risk of postoperative infection and the risk of new injuries at the scar (Camisa, 2004).

In addition, a number of other bacterial, fungal or viral could be related to psoriasis. Unfortunately, there are no large epidemiological studies on these associations, especially regarding plaque psoriasis. Most data regarding the presence of infections in patients’ psoriasis come from case reports. This way, the hypothesis that besides the subclinical streptococcal infection, a role in exacerbating chronic plaque psoriasis might have similarly skin infections such as Staphylococcus aureus, Candida albicans and Malassezia furfur is launched. Viral infections can be also sometimes implicated in the aetiology of psoriasis because it has been observed that the lesions are either precipitated or exacerbated by influenza-like illness. It remains to be determined whether this effect is due to a virus or bacterial superinfection can not be evidenced by routine bacteriological tests.

Psoriasis also appears on varicella lesions or herpes zoster, but this is due to Koebner phenomenon rather than the direct effect of varicella-zoster virus.

**HIV:**

It has been shown that HIV infection worsens psoriasis. The frequency of psoriasis is higher in HIV positive patients (approximately 1-3%), but the severity of the disease is higher in these individuals. Sudden onset of a psoriasiform rash or exacerbation of a preexisting psoriasis must suggest the necessity of a screening for HIV infection, particularly in patients with risk factors. If this occurs in a patient with known HIV infection, it is recommended to
assess the number of CD4 + T lymphocytes and viral load and should be considered the option of starting a highly active antiretroviral therapy (HAART).

HIV-positive patients, regardless of psoriasis severity (mild or severe disease, localized or generalized) have a particular tendency to be resistant to conventional topical therapies. There is also an increased tendency to develop inverse psoriasis clinically similar with seborrheic dermatitis (in the armpits and groin folds) and acral palmoplantar involvement with hyperkeratotic plaques or pustules. In the latter case, if there is asymmetric polyarthritis as well, patients are diagnosed with psoriatic arthritis or with Reiter syndrome. Reiter's syndrome incidence in HIV-positive patients is 0.5-10% and 75% of patients are HLA-B27-positive (Henseler, Christophers, 1995).

**Psychological factors:**

The psychological stress is a well-known systemic factor which triggers psoriasis. It is associated both with the development of psoriasis lesions of the first and subsequent eruptions of a preexisting psoriasis. Usually, exacerbations of psoriasis lesions appear after few weeks or months following a stressful event.

However, the general concept of patients that psoriasis is a "nervous substance" disease is not correct. It is difficult to estimate accurately the proportion of patients with psoriasis in which trigger stress factor is important because there are different definitions of stress. In addition, various stressful situations affect individuals differently, depending on their personality.

The mechanism by which stress aggravates or induces psoriasis is not yet known. Stress hormones act on the immune and autonomic nervous system. Currently there is already evidence that the immune system is involved in the expression of disease and that it can produce alterations in homeostatic systems that control the proliferation of epidermal characteristic element in psoriasis. Also, it has been recently demonstrated that neuropeptides secreted by the nerve endings can influence the immune cells in psoriasis skin, and the function of keratinocytes. This could be another mechanism by which stress affects psoriasis.

On the other hand, there is no doubt that psoriasis has a negative effect on the quality of life of patients and psychosocial therapies for stress management significantly reduce the need for the standard therapies.

**Drugs:**

Several drugs have been incriminated in the induction of psoriasis in particular the lithium, interferon, β-blockers, inhibitors of the angiotensinogen converting enzyme (ACE) inhibitors and antimalarials. The risk of these drugs has not been precisely assessed in controlled epidemiological studies. The adverse effects of β-blockers are based on substances used in the past, practolol, although the data on those found in use today do not exist. Instead, the effect of lithium or antimalarials in psoriasis can be severe.

Quickly reducing the dose of corticosteroids may induce pustular psoriasis, and rashes of plaque psoriasis. There are isolated reports of exacerbation psoriasis following administration of NSAIDs, but in most patients this class of medicines has a minimum impact or even a non-existent one (Traub, Marshall, 2007).
3. Immunological factors:

In the literature, the association between severe psoriasis, psoriatic arthritis and HIV infection is well known. In a study on 13 patients with HIV-positive psoriasis, who were followed for 2.5 years it was observed that at the same time with the development of the typical elements of the HIV infection, psoriasis lesions either get worse or new eruptions appear but in severe forms. AIDS prognosis in patients with psoriasis is also poor as 9 of the 13 patients died during the study. The mechanism of worsening psoriasis in this situation is unclear. Furthermore, although on the one hand psoriasis is aggravated by AIDS, a disease whose main target are T helper lymphocytes, on the other hand there is still evidence of improvement of psoriasis treated with cyclosporine, a substance that acts just by inhibiting T lymphocyte helper. This is a paradox that remains to be investigated and explained.

When trying to find an organ specific antigen responsible for triggering psoriasis, the molecular type similarities between streptococcal protein and keratinocyte are an interesting hypothesis. For this reason, infectious pathogens express similar peptides as structure or sequence with a particular self-element inducing immunological cross-reactions between host and pathogen. Classic prototype of such reactions is rheumatic fever induced by hemolytic streptococcus β.

However, they were described common epitopes between streptococcal antigens and keratinocyte proteins. Keratin 6, as well as other keratins, has been described as psoriatic autotigens as they present in their structure common fragments with aminoacids chain of streptococcal M protein. Therefore, the M proteins are able to divert the T lymphocyte responses, anti-streptococcal initial, to similar peptide fragments from keratin structure.

Integrated into a pathogenic concept, psoriasis can be interpreted as a skin reaction triggered by molecular mimicry. At some patients at least, it might be mediated by a special population of regulatory T cells which are cross reacting. These lymphocytes were initially primed against bacterial antigens, and they are reenabled in the skin when they recognize similar peptide in the keratinocyte proteins.

Accentuated epithelial hyperplasia with intense desquamation can be considered an expulsion mechanism of the epithelial surface in order to combat microbial invasion. It remains to be determined whether the association of psoriasis with HLA-Cw6 capacity is especially due to peptides which are common to M proteins and keratins.

Since psoriasis is not long recognized as a condition mediated by T lymphocytes, presenting as an autoimmune disease is still a subject of debate. Studied carefully psoriasis presents several criteria for autoimmunity: has a hereditary background, is associated with HLA class I, the onset can be triggered by bacterial infections and T lymphocytes are essential in clinical manifestations.

Given the position of the disease in the heart of the current multiple research centers, the plurality of opinions regarding incidence, prevalence, triggers, pathophysiological mechanisms and the constant association with a multitude of other diseases related data, justifies my concern to research further in this domain.
1.2. COMORBIDITIES: RELATIVE OR ABSOLUTE RISK

1.2.1. INTRODUCTION

In general, any disease can coexist with psoriasis. Probable pathogenic diseases linked with psoriasis are psoriatic arthritis, Reiter's syndrome, palmo-plantar pustulosis, subcorneal pustular dermatosis, Crohn's disease and ulcerative colitis, and it is relatively simple to explain pathogenic relationship between them. Between psoriasis and other diseases such as obesity, atherosclerosis, diabetes, hypertension, myocardial infarction, stroke, dyslipidemia and metabolic syndrome exist certain associations (Heredi, Vegh et al., 2016), and similar to other immune mediated diseases cardiovascular mortality in patients with psoriasis is higher than the general population (Maradit, Crowson et al., 2005).

In many patients with psoriasis has been reported an association of disease with autoimmune bullous dermatoses. Psoriasis is always the one that preceded the autoimmune bullous diseases. Most frequently, it has been reported an association with bullous pemphigoid. Since psoriasis appeared first, it looked for a link between antipsoriatic treatments (UVB, PUVA, tars, cignolin etc) and bullous pemphigoid onset. Bullous lesions may appear on the affected or apparently unaffected skin. Bullous pemphigoid treatment in these cases is problematic because in psoriasis doctors try to avoid the systemic corticotherapy. Before the general corticotherapy is applied, the therapy with methotrexate, cyclosporine, mycophenolate mofetil or acitretin needs to be applied (Burns et al., 2004). Also blisters in pemphigus foliaceous could be confused with pustular psoriasis lesions, the scalp involvement being remarked in both disorders and therefore clinical differential diagnosis being difficult. Skin biopsy and immunofluorescence allow differentiation between autoimmune bullous dermatosis and psoriasis. Candidiasis and urticaria have been reported more frequently in patients with psoriasis compared to the general population.

Patients with psoriasis have a higher risk of developing malignancies, particularly skin cancer. The risk is even greater as the disease is more extensive and probably has connection with phototherapy systemic therapies. There is convincing evidence that psoriasis itself is associated with an increased incidence of cancer (Morgolis, 2001).

The relationship between alcoholism and psoriasis is possible primarily due to the psychological impact of psoriasis on patients. Alcoholics usually have extensive psoriasis, with intense swelling. Also, the excess alcohol can cause failure of treatment and low therapeutic compliance. Increased alcohol consumption is recognized as a way of response to stress. The relationship between alcoholism and psoriasis is possible primarily due to the psychological impact of psoriasis on patients. There are also studies showing that alcohol may have a role in the exacerbation of preexisting psoriasis but it can not be incriminated in triggering de novo the disease. The effect appears to be stronger in men than in women. Remissions have been reported in case of alcohol withdrawal. On the other hand, another association between psoriasis and non-alcoholic liver pathology (NASH - non-alcoholic
Hepatic steatosis is mentioned in the literature. Narayanasamy and his collaborators studied in a Department of Hepatology in India in Madras Medical College prevalence of NASH in patients aged over 18 diagnosed with psoriasis. The study enrolled 250 patients in middle age (average: 44.74 ± 11,989 years), overweight (body mass index average (BMI): 24 772 ± 3611 kg / m2) and male (68%, n = 170). The overall prevalence of non-alcoholic fatty liver was evaluated in the amount of 45.2%. So this comorbidity is commonly associated with psoriasis vulgaris and it is necessary to be aware of this association for early assessment and diagnosis of non-alcoholic hepatic steatosis in patients with psoriasis in order to stop the progression of liver disease (Narayanasamy, Sanmarkan et al., 2016).

Some triggers include systemic endocrine and metabolic factors. Regarding endocrine (hormonal) factors, stands the early onset of psoriasis in women with peak of incidence around puberty. Changes during pregnancy and causing psoriasis by administering large doses of estrogen indicate factors with a likely endocrine role in this condition. A study based on questionnaires answered by 65 women who had one or more pregnancies after being diagnosed with psoriasis was conducted. In about half of all pregnancies, psoriasis has improved, and in 14% of cases worsened. Instead, in the first three months postpartum, 11% of cases had improved and 54% worsened. Therefore, the development of psoriasis is clearly influenced by pregnancy. The disease often improves during pregnancy, while usually postpartum psoriasis gets worse.

In addition, pregnant women can develop pustular psoriasis, known as impetigo herpetiformis of the association likely to hypocalcaemia (Mallon et al., 2000).

Among metabolic factors is included hypocalcaemia (e.g. accidental post-parathyroidectomy) and was reported as a possible trigger factor for severe forms of psoriasis, especially for generalized pustular psoriasis. Although active analogues of vitamin D3 improve the psoriasis lesions, it was not observed that abnormal levels of vitamin D3 induce psoriasis. Other metabolic disorders involved in the etiology of psoriasis could be B12 vitamin and folate deficiencies. Also, obesity, diabetes type 2 and metabolic syndrome are considered risk factors for psoriasis, being proved that they predispose to its occurrence. Lifestyle is also considered to be a trigger of the disease. So obesity, increased alcohol consumption and the incidence of smoking were associated with psoriasis. In a study conducted in Rochester, women recently diagnosed with psoriasis were smokers in greater proportion than the general population, compared to men, whose proportion of smokers was comparable to control groups. But most important is the association between smoking and forms of pustular psoriasis, especially palmo-plantar pustulosis, disease most commonly observed among women. A recent report shows that smoking appears to play a role in the onset of psoriasis, while obesity seems to be a consequence of it. Diet and obesity can still contribute to the development and severity of psoriasis. On average, patients with psoriasis have a 15% body weight greater than ideal value. The risk of psoriasis increases with increasing body mass index (BMI) and decreases with the consumption of carrots, tomatoes and fresh fruit, compared with control subjects who had other skin disorders. After
eliminating the influence of socio-economic status, alcohol consumption and smoking, the association between psoriasis and increased BMI remains significant.

Vitiligo occurs more frequently in patients with psoriasis than in general population. Psoriasis was more common in patients with certain metabolic syndromes, including gout, one of the explanations being that gout occurs due antipsoriatic systemic therapy (Bosmansky, Trnavsky, 1983).

Keller and Lin have investigated by using a cohort study subsequent risk for psoriasis having established a diagnosis of chronic periodontitis of 115365 patients from Taiwan and 115 365 patients without chronic periodontitis in the comparative group. Each patient was followed for 5 years and results showed a higher incidence (1.88 / 1000 person-years) of chronic periodontitis in patients who have been diagnosed with psoriasis reported to incidence of 1.22 / 1,000 person-years, respectively in patients without chronic periodontitis. Also a slightly higher risk of developing psoriasis in patients who have undergone surgery and gingivectomy was detected (Keller, Lin, 2012).

Our concerns regarding the comorbidities that may be identified in patients with psoriasis have resulted in the publication of the following articles submitted in detail in habilitation thesis:

|---|

1.2.2. MATERIALS AND METHODS

In research of this type of pathology and comorbidities associated with psoriasis vulgaris we conducted two studies. The first study is a retrospective analysis based on medical existing records of psoriasis patients hospitalized in Dermatological Clinic between January 2004 and December 2008. The diagnosis of psoriasis was established primarily based on clinical appearance, but with most cases of the disease a pathological confirmation of skin biopsies was performed. They were noted and monitored clinical forms of psoriasis patients and also the associated diseases. In our research we considered important comorbidities and fungal infections present in large numbers in patients with psoriasis. They were diagnosed in all evolutionary stages of psoriasis and clinical manifestation in different forms. Based on these specific issues, the combination of these conditions, we initiated a study on psoriasis and nail mycosis with focus on nail lesions as common target lesions for the 2 disorders. Thus, the second one was a retrospective observational analytical study of the information contained in the medical records of patients with nail psoriasis who were hospitalized in Dermatological Clinic in Iasi, from 2004 to 2008. The diagnosis of psoriasis was also established primarily based on clinical appearance and in patients who presented subungual hyperkeratosis or total
or partial onycholysis phenomena at hand or foot nails suggestive for onycomycosis directly mycological examination was performed. In this paper we proposed the following objectives: assessing the incidence of fungal infections in patients with psoriasis nail lesions admitted in Dermatological Clinic in Iasi; and highlighting the clinical and evolutionary morphology nail in patients with psoriasis and onychomycosis.

1.2.3. RESULTS

Data from the first study suggest that smoking and obesity are two factors that increase the risk of developing psoriasis. Psoriasis can be an independent risk factor in the development of diabetes, atherosclerosis and myocardial infarction. Therefore, life expectancy of patients with psoriasis is lower with 3-4 years than general population. Alcohol consumption chronically in high or moderate amount can increase the risk of developing psoriasis lesions or may aggravate existing lesions. For this reason, patients should avoid excessive alcohol consumption and smoking.

In the studied period 2004-2008 in Dermatological Clinic in Iasi, 360 psoriasis patients have been hospitalised, almost 72 per year (Tabel 1.1.). Along those 5 years, admissions of patients with psoriasis represented 3.5% of all admissions into hospital. Approximately two 3rds of the cases have been represented by men with psoriasis (61.9%) and 9% of patients with psoriasis were children. From a total of 360 patients hospitalised in the Dermatological Clinic in Iasi, the majority had psoriasis vulgaris (67%), being followed by palmo-plantar pustular psoriasis (10) and arthropathic psoriasis (8%). 22 cases of inverted psoriasis, 24 cases of guttate psoriasis and only 6 (2%) of patients with erythrodermic psoriasis have been hospitalized (Fig.1.1.).

Family psoriasis as well as sporadic form can start at any age. In the studied group, the majority of cases have been diagnosed in adults with age groups 40-49, 50-59, 60-69. Although specialised literature suggests an incidence peak in the young adult (30-40 years old) the economic-financial status, stress and quality of life probably expand the area to mature age groups. Table 1.2. summarizes the data obtained, showing that the increased percentage of patients with psoriasis who declare increased alcohol consumption in 2004 even 28.9% of admitted patients declaring themselves daily consumers. The relationship between alcoholism and psoriasis is possible primarily due to the psychological impact of psoriasis on patient. It is known that alcohol may have a role in the exacerbation of preexisting psoriasis, and is recognized as a response to stress. Certainly not all patients were sincere when they said the amount of alcohol consumed daily so it is possible that the actual percentage is higher. The high percentage of smokers among patients with psoriasis admitted in Dermatological Clinic in Iasi in 2004-2008 indicate the involvement of this risk factor in the onset or exacerbation of psoriasis lesions. Health education and awareness of the smoking involvement in psoriasis are defining elements in improving or maintaining remission periods. Progressive decrease in the percentage of smokers from 55.4% in 2004 to 34.9% in 2008 is encouraging and shows that patients are starting to realize the risks of exposure to smoking.

<table>
<thead>
<tr>
<th>Clinical forms</th>
<th>2004</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Psoriasis vulgaris</td>
<td>65</td>
<td>54</td>
<td>27</td>
<td>39</td>
<td>55</td>
<td>240</td>
</tr>
<tr>
<td>Arthropathic psoriasis</td>
<td>9</td>
<td>6</td>
<td>2</td>
<td>9</td>
<td>5</td>
<td>31</td>
</tr>
<tr>
<td>Psoriasis palmo-plantar</td>
<td>3</td>
<td>10</td>
<td>5</td>
<td>9</td>
<td>10</td>
<td>37</td>
</tr>
<tr>
<td>Inverse psoriasis</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>8</td>
<td>5</td>
<td>22</td>
</tr>
<tr>
<td>Guttate psoriasis</td>
<td>5</td>
<td>8</td>
<td>2</td>
<td>2</td>
<td>7</td>
<td>24</td>
</tr>
<tr>
<td>Erythrodermic psoriasis</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Hospitalised patients with psoriasis</td>
<td>87</td>
<td>81</td>
<td>40</td>
<td>69</td>
<td>83</td>
<td>360</td>
</tr>
<tr>
<td>Total number of patients</td>
<td>2300</td>
<td>2050</td>
<td>2125</td>
<td>1908</td>
<td>1859</td>
<td>10242</td>
</tr>
</tbody>
</table>

Fig.1.1. Clinical forms of psoriasis (2004-2008).
Fig. 1.2. The distribution of patients with psoriasis by age group

<table>
<thead>
<tr>
<th>Tabel 1.2. Comorbidities in patients with psoriasis</th>
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<tr>
<td></td>
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<tr>
<td>Alcoholism</td>
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<td>Smoking</td>
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<td>Hypertension</td>
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<td>Cardiovascular diseases</td>
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<td>Type 2 diabetes</td>
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<tr>
<td>Hepatitis</td>
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<tr>
<td>Neoplasia</td>
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<tr>
<td>Psychiatric disorders</td>
</tr>
</tbody>
</table>

Within the second study during 2004-2008 in Dermatological Clinic Iasi were admitted 260 patients with psoriasis with an average of 72 per year. 32 patients had also nail changes with variable degrees of discoloration, nail hyperkeratosis and onycholysis, clinical appearance suggesting other nail pathologies such as onychomycosis. Direct mycological examination was performed for 21 patients. At 12 patients there were identified mycelial filaments which enabled the diagnosis of dermatophytic onychomycosis. We noticed that 70% of patients were presenting onychomycosis and tinea pedis skin lesions confirmed by positive mycological examination of scales taken from the interdigital spaces. For other 9 patients mycological examination revealed numerous yeasts of the genus Candida albicans.

Nail changes in onychomycosis were showing varying degrees:
• 10% of patients had discolored from 1 to 5 nails;
• 50% of patients associated discoloration and important subungual hyperkeratosis from 1 to 4 nail;
• 40% of patients have revealed phenomena of partial or total onycholysis from 1 to 5 nails.

For all these patients was established systemic and local antifungal treatment for a period of three months and individual preventive measures. 50% of patients received systemic antifungal treatment associated with methotrexate for their cutaneous and nails psoriasis lesions. After a period of 3 months of treatment a significant improvement at 35% of patients with important nail changes (subungual hyperkeratosis and onycholysis) was remarked. In the remaining patients characteristic lesions persisted.

1.2.4. DISCUSSIONS

Various epidemiologic research papers confirmed that patients with psoriasis present a higher risk to develop cardiovascular diseases (heart attack, angina, atherosclerosis, peripheral cardiovascular diseases and cerebral vascular accidents) (Mallbris et al., 2006; Ludwig et al., 2007; Hansson, 2005). Taking into consideration the fact that patients were over 50s, it is difficult to demonstrate that cardiovascular affections of these patients are caused by psoriasis. The percentage of patients with cardiovascular comorbidities is similar to data that Gelfand and collaborators have obtained (Gelfand et al., 2006). In the studied group 19.4% of patients presented HTA and 10% of them had cardiovascular diseases.

The idea that psoriasis is regarded as a risk factor for patients with diabetes type 2 is more common, but the association between the two affections has been statistically significant only in patients over 45 years old. It would be extremely useful to propose a future study to find out the degree of involvement of cortisone topics used for chronic therapy in psoriasis and induction of diabetes type 2.

Hepatic diseases represented 7.2% of cases in the studied group. Clinical manifestations were such as chronic hepatitis (5.1%), hepatic cirrhosis (2.3%), and hepatosteatosis (1.7%).

Neoplasia cases discovered during hospitalizations were very rare, the percentage being similar to the one recorded in general population.

The presence of various psychiatric disorders in patients with psoriasis has been often mentioned in the speciality literature as psoriasis may become a traumatising disease for patients as well as for their peers due to the its consequences on self-image and self-esteem (Gheucă Solovăstru et al., 2008).

Regarding the pathology of psoriasis and nail, onychomycosis and psoriasis are common conditions in the population and it is possible that they coexist in the same patient. Psoriasis is a cause of disturbance of morphology nail and nail changes spectrum in psoriasis is very wide. Nail disorders have been reported in 10-80% of patients with psoriasis and may be associated with all clinical forms of the disease. It has been suggested that the nail dystrophy in patients with psoriasis lead to the loss of the natural protective barrier and...
increase the vulnerability of fungal infections. Most authors report that the prevalence of onychomycosis in psoriasis patients is no higher than in the general population (Szepiotowski, Salomon, 2007).

In literature is still controversial whether psoriasis nail dystrophy is a predisposing factor for fungal infection. It seems that there is a direct link between the linear growth rate of the nail and nail matrix kinetics. Some authors consider that the growth rate of nails with depressions in psoriasis is significantly higher than the "normal" one (125 versus 109 μm / day) (Bolognia, et al., 2007). Both were significantly higher than the growth rate of the nail from normal individuals (98 μm / day) (Camisa, 2004). During psoriasis treatment changes occur in the rate of nail growth. Some drugs such as systemic cytotoxics (methotrexate, azathioprine) significantly reduce the rate of nail growth. Reducing the rate of nail growth in patients with psoriasis nail fungal infection is favoured. Etretinate has an effect of accelerating the rate of fingernail growth in psoriatic patients. In some particular forms of psoriasis, such as pustular psoriasis or palmo-plantar the growth rate of the nail is not significantly different from that of the normal control subjects (Camisa, 2004).

Recent studies have shown that psoriasis nail lesions are more severe in patients with early onset psoriasis and with family history of psoriasis (Gonzaga et al., 1996). The nails of the hands are more frequently affected than those of the feet and there is no predisposition related to gender or age. A number of other nail changes such as pinpoint depressions in the nail plate were observed in other diseases such as alopecia areata, atopic eczema, lichen planus, trauma. In psoriasis depressions are wider, deeper and more spread out than those seen on nail blade in alopecia areata. The appearance of lichen planus and eczema pinpoint depressions is usually accompanied by periungual cutaneous changes. Onycholysis is a frequent lesion and occurs in infections (fungal, bacterial and viral), trauma, contact dermatitis, the use of chemicals in nail care or atopic dermatitis. Onycholysis associated with subungual hyperkeratosis appears in psoriasis and fungal tests are needed to establish the diagnosis with certainty. Longitudinal striae remarked in nail psoriasis were also observed in diseases accompanied by peripheral circulatory disorders, and they may be physiological (especially at elderly) or may be associated with collagen diseases or trauma. Bleeding "in the chip" can be caused by trauma, vascular infarcts, eczema, onychomycosis or scurvy. Leuconichia occurs in trauma, areata alopecia, zinc deficiency, heavy metal poisoning. Appearance of "oil stain" can be found in onychomycosis (candidiasic or dermatophytic).

Even though the prevalence of onychomycosis is similar to that of the general population, however, nail fungal infection in patients with psoriasis is also possible and relatively frequent. Predisposing factors for onychomycosis are: age, male gender, genetic predisposition, comorbidities (diabetes, immunodeficiency, peripheral arterial disease) and not least psoriasis. Systemic therapies are problematic in people with advanced age due to possible side effects, these patients having various comorbidities and following other therapies. Topical therapies do not have these disadvantages, but these patients often require assistance in topical treatment (Murdan, 2016).
Nail disorders represent a therapeutic challenge and can be often disappointing for both patient and clinician since the time required to see results is long and patients interrupt their treatment alone (Iorizzo, 2015).

The majority (90%) of the nail fungal infections are caused by dermatophytes (Trichophyton rubrum and mentagrophites), the rest being produced by yeasts and other fungi.

Morphological alterations observed in the nail unit are suggestive for various pathologies, but their presence in association with psoriasis skin lesions allows the diagnosis of nail psoriasis. Literature reports cases of nail psoriasis without characteristic cutaneous lesions, and similarly onychomycosis and nail psoriasis. Constellation of nail changes taken together with other lesions is strongly suggestive for the diagnosis of psoriasis. Psoriasis affects matrix, nail bed and hyponychium. Differential diagnosis is sometimes difficult in the absence of skin lesions. All onychopathy can erroneously be attributed to psoriasis without a mycological investigation or a general medical examination (Klaus, Goldsmith et al., 2007).

Nail psoriasis lesions are evocative: nail plate depressions, leuconichia, loss of nail transparency, onycholysis, "oil stain", bleeding nail, transverse striae, longitudinal striae, subungual hyperkeratosis. Many age-related changes that occur in toenails can be confused with psoriasis: nail bed thickening and yellowing, nail dystrophy and longitudinal striae. Onycholysis may be secondary to trauma, inadequate footwear, bone deformities, vascular insufficiency and dermatophytoses. In a study about the importance of changes in nail psoriasis, the authors state that 79% of patients with psoriasis have nail touch, 52% accusing pain associated and 14% declaring major restriction in daily activities due nail changes. Another conclusion of the study is that patients more frequently develop joints damage. Thus it can be said that reaching the nail bed in arthropathic psoriasis is more common than in other clinical forms of psoriasis (Bolognia et al., 2007).

Morphological alterations observed in the nail unit are nonspecific and do not represent diagnostic elements for disease unless they are associated with psoriasis of the cutaneous proximal and lateral nail fold. The final result of psoriasis on the nail matrix cells occurs by changing the nail plate surface texture while the nail bed lesions are generally transmitted through the blade nail discoloration or onycholysis. Thickening or nail lifting and subungual hyperkeratosis result from accumulation of scales in the nail bed or hyponychium psoriasis lesions.

Nail psoriasis severity index (Nail Psoriasis Severity Index, NAPSI) assess changes:

- nail bed (0-4) - onycholysis, splinter haemorrhages, discoloration and subungual hyperkeratosis.
- nail matrix (0-4) - pitting, leuconiquia, red spots on lunula and friable nail plate (a sign or all).

NAPSI score ranges from 0 to 8 (per nail). For all the nails, the score ranges from 0 to 80 (only hands) or between 0 and 160 (and legs) (Baran, 2004; Parrish et al., 2004). Making NAPSI score is very laborious and very time consuming and therefore has not found its place in practice. Other methods must be implemented faster in the future (Kaçar et al., 2008).
Treatment of nail psoriasis may be local, systemic, radiotherapy or phototherapy. Local treatment can be done with topical corticosteroids. The problem of this therapeutic approach is penetration in the affected area. Superpotent occlusive steroids applied for two weeks have been successful, but the risk of tachyphylaxis and atrophy of periungual skin is important. Chemical avulsion of the nail with occlusive 40% urea seven days in combination with systemic therapy had a 50% success rate in the growth of a normal nail. Intrallesional corticosteroids - administered periungual - can lead to promising results. The disadvantage of this procedure is significant pain. The procedure must be repeated every 1-2 months until it reaches the desired results. Chemotherapy - mechlorethamine known by dermatologists as an therapeutic alternative in T-cell lymphomas – is also effective in nail psoriasis. 5-fluorouracil administration, another chemotherapy applied topically in nail psoriasis, brings potential benefits, but adverse effects are quite important (Jong et al., 1996; Gupta et al., 1997). The best treatment for nail psoriasis is methotrexate, although its administration in cases with exclusive nail touch is controversial. Benefits are visible when it is administered to patients with psoriasis vulgaris or extended arthropathic psoriasis associated with nail changes. Also, synthetic retinoids appear to have a beneficial effect on the psoriasis nail changes. In case of failure of methotrexate and retinoids, cyclosporine combined with topical application of superpotent corticosteroids can be tried (Klaus, Goldsmith et al., 2007).

Superficial radiotherapy with doses of 160 cGy split has led to improvement in nail psoriasis affection for 10-15 weeks after treatment. UVB therapy is not effective in nail psoriasis as nail filters ultraviolet and is allowing transmission of trace amounts of UVB to the nail bed (like a window of glass). PUVA therapy has proved to be effective in nail psoriasis, but at doses of 2-2.5 times higher than those used for cutaneous affection. It is necessary to protect the skin when using these higher doses. The risk of these procedures is inducing photo-onycholysis or photo-hemolysis (bleeding followed by onycholysis) (Piraccini et al., 2001).

1.2.5. CONCLUSIONS

Psoriasis may be associated with any disease. Current data suggests that smoking and obesity increase the risk of developing psoriasis. Psoriasis may be an independent risk factor for developing diabetes, atherosclerosis and myocardial infarction in the end. Therefore it is concluded that patients with psoriasis die 3-4 years earlier than general population. Further studies are needed to clarify the link between the severity of skin lesions, the followed treatment and evolution of related diseases.

The association between nail psoriasis and onychomycosis is possible and frequent, probably being underdiagnosed. Direct mycological examination is a noninvasive test that is easily and quickly performed, and is recommended for all patients who present nail changes suggestive of onychomycosis. Subungal hyperkeratosis process is a common element in terms of both pathophysiological conditions. Increased reactivity of the nail bed to inflammatory process induced by fungal infection is similar to response of inflammatory
process in psoriasis. Early diagnosis and appropriate therapy of any nail changes will have a conservative effect protecting architectural nail changes.

Remission of skin lesions and psoriasis nail lesions is the therapeutic purpose in management of moderate to severe psoriasis and of arthropathic psoriasis, with or without onychomycosis. Biological therapy now provides the most effective remission of nail psoriasis (Lawry, 2007) and is further associated with the administration of systemic antifungals in case of proven fungal infection. Regarding antifungal treatment should be emphasized that it improves the appearance of the nail but still should be prescribed with caution because antifungal substances such as terbinafine may exacerbate psoriasis.

Knowing what diseases may be associated with psoriasis, we can identify the particularities of each patient. This will avoid the application of a standard therapy and we will promote a multidisciplinary approach to the modulation of treatment options based on patient individual needs.

1.3. PSORIASIS, METABOLIC SYNDROME AND LEPTIN

1.3.1. INTRODUCTION

Metabolic syndrome is a common and complex disease in which insulin resistance is the main trigger and mediator. From abdominal fatty deposits constantly encountered in this condition, proinflammatory cytokines are releasing in bloodstream.

Although the influence of environmental factors on psoriasis is not clearly demonstrated, Body Mass Index (BMI) is recognized as an important factor, and a significant association between BMI and psoriasis is proven (Aktan et al., 2007).

Leptin is a 16 kDa molecular weight protein secreted by adipocytes, which plays an important role in the regulation of appetite and metabolism, its values being correlated with BMI (Naldi et al., 2005; Naldi et al., 1996). This cytokine presents OB-R type receptor having a role in signal transmission in the central nervous system, body fat being in a negative feedback stage (Huang, Li, 2000). Leptin is also involved in inflammatory processes by increasing the activity of macrophages, the release of TNF and IL-6 which then causes the release of CRP (Chen, 2008).

Metabolic syndrome represents the simultaneous presence at the same patient of three of the following disorders: abdominal obesity, high blood pressure, hyperglycemia, hypertriglyceridemia and reduced HDL serum cholesterol levels. It is known the existence of a direct relationship between psoriasis and metabolic syndrome, which is often diagnosed in patients with plaque vulgaris psoriasis (Sommer et al., 2006; Gisondi et al., 2007).

Our concerns regarding this problem have resulted in the publication of the following articles submitted in detail in habilitation thesis:

1.3.2. MATERIALS AND METHODS

The study has been done on 78 patients who had been diagnosed with plaques vulgaris psoriasis and metabolic syndrome. They were divided into three groups such as: the first group formed by 26 patients with psoriasis vulgaris and systemic metabolic syndrome who benefited from topical therapy only; the second group of 22 patients with psoriasis vulgaris and metabolic syndrome with systemic treatment (except biological therapy) and the third group of 30 patients with psoriasis vulgaris and metabolic syndrome who begun various biological therapies. Assessments were carried out in week 0 (before starting any treatment) and after 24 weeks of treatment for each of the three groups.

The diagnosis of plaque psoriasis vulgaris was established by clinical and histopathological examination. Disease severity was assessed by PASI score.

The diagnosis of metabolic syndrome was based on WHO criteria including: impaired fasting plasma glucose, impaired glucose tolerance or confirmed diabetes and at least two of the following criteria: hypertension (> 140/90 mmHg); microalbuminuria, obesity (ratio waist / hip > 0.90 in men or > 0.85 in women, BMI > 30 kg / m²); dyslipidemia (triglycerides ≥ 150 mg / dl and HDL cholesterol ≤ 35 mg / dl in men or ≤ 38.6 mg / dl in women).

Body mass index was calculated by the ratio between weight and waist measured in kg [BMI = G (kg) / T² (m)], according to the BMI value settling the class of obesity (BMI = 30-34,9 kg / m² => class I; BMI = 35-39,9 kg / m² => class II = BMI > 40 kg / m² class III). In group 1 were 6 patients with obesity class I, 18 patients with obesity class II and 2 with obesity class III. In group 2 were 6 patients with obesity class I, 13 patients with obesity class II and 3 patients with obesity class III. In group 3 were 8 patients with obesity class I, 18 patients with obesity class II and 4 patients with obesity class IV.

All patients were clinically evaluated and PASI score was calculated to determine the severity of psoriasis form. Blood samples were taken (in vacutainer venous blood without anticoagulant, followed by centrifugation and separation of ~ 2ml serum collected for each vacutainer). Serum leptin levels were determined by ELISA immunoassay method (biological parameters interpreted according to BMI).

1.3.3. RESULTS

78 patients clinically diagnosed and histologically confirmed with plaque psoriasis and metabolic syndrome, aged between 18 and 72 years were enrolled in this study. The distribution by sex includes 36 female patients and 42 male, with provenance from urban areas - 55 patients, and rural - 23 patients (Fig. 1.3., Fig. 1.4).

Patients were divided into three groups (Table 1.3.) as follows: the first group with 26 patients with psoriasis and metabolic syndrome, including 16 females and 10 males; they received topical treatment for their skin disease. Group two with 22 patients including 8 females and 14 males with psoriasis and metabolic syndrome, systemic therapy (methotrexate 7.5 mg / week or PUVA three sessions / week).
Fig. 1.3. Skin lesions before treatment: scaly, erythematous, well-defined plaques disseminated on the trunk and upper limbs.

Fig. 1.4. Skin lesions after treatment: erythematous plaques accompanied by residual hyperpigmented macules localized on the upper back and upper limbs.

The third group contains 30 patients, 12 women and 18 men with psoriasis and metabolic syndrome, and biological therapy was initiated, as follows: 11 patients with Etanercept (Enbrel) sc. 50mg x2 / week, 12 weeks and then 50 mg / week 12 weeks; 14 patients with Infliximab (Remicade) 5mg / kg piv in weeks 0, 2, 6 and then every 8 weeks; 4 patients with Adalimumab (Humira) sc 40mg / 2 weeks interval; 1 patient with Ustekinumab (Stellara) sc. 45mg at week 0, week 4 and every 12 weeks. For each group was calculated PASI score at week 0 and after 24 weeks of treatment. Changes in PASI score were used as a clinical marker of the disease evolutivity, and comparative values between the initial scores and those after 24 weeks of therapy established the disease activity and response to therapy.

Serum leptin level was reported to BMI for both determinations. It was noted the maintaining of its high level for the patients in the group with topical treatment even if they have achieved the PASI 50 (Table 1.4.). For the second group, patients with systemic therapy, serum leptin level decreased after 24 weeks of treatment compared to the initial determination (Table 1.5.) more significant decrease was observed in the case of those who have reached PASI75. For those with PASI 50 serum leptin is slightly reduced compared with the original. For group 3, those who were treated with biologic therapy, plasma levels of leptin decreased significantly after 24 weeks of treatment, reaching biological parameters even in patients who have achieved PASI 50 (Table 1.6.).

<table>
<thead>
<tr>
<th>Tabel 1.3. Distribution in groups by gender</th>
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<tbody>
<tr>
<td><strong>Group 1</strong></td>
</tr>
<tr>
<td>Females</td>
</tr>
<tr>
<td>16</td>
</tr>
</tbody>
</table>
### Tabel 1.4. Leptin values in PASI score in group 1

<table>
<thead>
<tr>
<th>Group 1</th>
<th>LEPTIN reported to BMI</th>
<th>Week 0</th>
<th>Week 24</th>
</tr>
</thead>
<tbody>
<tr>
<td>PASI&lt;50</td>
<td>10</td>
<td></td>
<td>↑</td>
</tr>
<tr>
<td>PASI 50</td>
<td>14</td>
<td></td>
<td>↑</td>
</tr>
<tr>
<td>PASI 75</td>
<td>2</td>
<td></td>
<td>↑</td>
</tr>
</tbody>
</table>

### Tabel 1.5. Leptin values in PASI score in group 2

<table>
<thead>
<tr>
<th>Group 2</th>
<th>LEPTIN reported to BMI</th>
<th>Week 0</th>
<th>Week 24</th>
</tr>
</thead>
<tbody>
<tr>
<td>PASI 50</td>
<td>12</td>
<td></td>
<td>↑</td>
</tr>
<tr>
<td>PASI 75</td>
<td>10</td>
<td></td>
<td>↑</td>
</tr>
</tbody>
</table>

### Tabel 1.6. Leptin values in PASI score in group 3

<table>
<thead>
<tr>
<th>Group 3</th>
<th>LEPTIN reported to BMI</th>
<th>Week 0</th>
<th>Week 24</th>
</tr>
</thead>
<tbody>
<tr>
<td>PASI 50</td>
<td>8</td>
<td></td>
<td>↑</td>
</tr>
<tr>
<td>PASI 75</td>
<td>22</td>
<td></td>
<td>↑</td>
</tr>
</tbody>
</table>

### 1.3.4. DISCUSSIONS

It is known that plasma levels of leptin are directly correlated with BMI, fat tissue being a major source of leptin and serum indirectly reflects its fat deposits. Individuals who have mutations in the coding gene for leptin, the Ob gene located on chromosome 7 show morbid obesity and require therapy with leptin. In 1997 there were described for the first time six types of mutations in leptin, which occurred in patients in Eastern Europe, their level of leptin being undetectable by the standard dosage immunoreactive techniques (Montague, Farooqi et al., 1997). The most recent case was reported in 2015 in a child who although had a high level of leptin, he had a functional deficiency of leptin, because he had mutation of leptin receptor, which led to obesity and hyperphagia (Wabitsch, Funcke et al., 2015).

Our study aimed to evaluate plasma levels of leptin in patients with plaque psoriasis and metabolic syndrome and oscillations values in accordance with received treatment and the relationship between this marker of comorbidity and the level of inflammatory activity of disease assessed clinically by PASI score. It was found statistically significant reduction in leptin levels in patients whose disease activity is low, e.g. those who achieved PASI 75 at 24 weeks. In groups 2 and 3, both receiving systemic therapy were achieved these decreases, more important at patients who received biologic therapy. Although in the first group were
patients who had reached PASI50 at 24 weeks of treatment, which corresponds to the reduction of clinically evaluated inflammatory activity, seric leptin levels remained high. This demonstrates the persistence of systemic inflammation correlated with the amount of leptin in patients topically treated. Although local therapy reduces skin inflammation, in the body remains a degree of systemic inflammation, proven by maintaining high values of leptin. In systemic treatment (nonbiologic), was obtained significant reduction in seric leptin levels, different among patients with PASI50 and PASI75. Those who have reached PASI75 have much lower values compared to baseline, low levels of leptin being the marker of reduction of systemic inflammation and disease activity. Patients who achieved PASI50 also show a decrease in leptin level, but this reduction is not so high compared with baseline, which would correspond to a still active disease.

With regards to biological systemic therapy, the third group showed the most important decrease values in the leptin level for patients with metabolic syndrome. Reduction of serum levels is significant even in patients with PASI50, which would correspond to an important reduction of systemic inflammatory phenomena, even if the clinical expression is not as suggestive. Leptin normalisation in some patients with PASI75 and metabolic syndrome who received biologic therapy, supports the anti-inflammatory effectiveness of biological treatments that enable optimal control of systemic inflammation.

These results are consistent with the literature supporting the biological effect of the therapy in reducing inflammation in patients with metabolic syndrome and psoriasis (Puig, 2011). Being a chronic inflammatory disease with both cutaneous and systemic affections, it becomes absolutely necessary to control the activity which results in reducing complications of cardiovascular and metabolic order (Boehncke et al., 2011).

Therapies that allow a decrease of disease activity markers are the systemic ones, even if they are biological therapies, which combine a significant reduction of inflammatory markers such as leptin, or conventional systemic therapies. Essential is the control of the disease as quickly as possible to reduce the inflammatory phenomena that has repercussions both cutaneous and systemic, the most severe being those with cardiovascular risk.

By evaluating leptin plasma levels variations related to treatment in patients with psoriasis and metabolic syndrome we have managed to demonstrate its importance as a marker of inflammation and thus the therapeutic efficacy, but also as a predictor for the occurrence of cardiovascular complications and metabolic disorders commonly associated with prolonged systemic inflammation.

### 1.3.5. CONCLUSIONS

This study shows a correlation between leptin plasma levels in patients with psoriasis and metabolic syndrome and systemic inflammation level, clinical correlation objectified by reducing PASI score. Therapies that significantly lower systemic inflammation, the first being the biological one, followed by systemic nonbiological therapies, induce also reductions in blood leptin levels and indirectly reduce complications associated with metabolic syndrome.
The obtained results emphasize the importance of studying the comorbidities associated with this disease and the importance of using modern therapies to reduce the incidence of various complications. All these have as a main goal improving the quality of life of the patient diagnosed with different forms of psoriasis.

1.4. NEW THERAPEUTIC CONCEPTS IN PSORIASIS

1.4.1. INTRODUCTION

Etiological therapies remain each practitioner's dream, because without if we want, yet we treat the effect of disease and not the cause in most conditions. The use of biological therapies in treating patients with psoriasis has become a common practice in countries with developed economies. Less developed countries, including Romania, are forced to impose strict, centralized measures in order to become available for at least a minimum number of patients with severe forms of disease.

There is evidence that treatment of psoriasis during the first year is conservative and most often based on topical agents, which rarely lead to complete regression of the lesions. Biological therapy is initiated only when local agents, phototherapy and systemic conventional treatments are not effective in patients with moderate or severe forms of the disease (Maza et al., 2012).

Under effective therapy, psoriatic lesions resolve without scarring, which is remarkable, given the number of neutrophils in psoriatic lesions and scarring seen in other disorders mediated by neutrophils, including pyoderma gangrenosum. Effective treatment causes a thinning of the epidermis, reducing inflammatory cells and skin return to normal clinical appearance. Since treatment is discontinued, skin lesions reoccur, usually in the same places where they previously started. Understanding and remission of residual lesions in psoriasis differentiate between treatment and cure of this disease.

There is evidence in other inflammatory disorders (e.g. rheumatoid arthritis, Crohn's disease) that early initiation of systemic therapy, including biologic can improve short and long term results. Both results in the short and long term, have been demonstrated in rheumatoid arthritis (Quinn et al., 2012; van der Kooj et al., 2009). According to the best study, 48% of patients with initial intensive treatment with TNF alpha inhibitors and methotrexate could stop biological treatment and remain in remission four years after study.

A therapeutic approach for a patient can ameliorate cutaneous symptoms of psoriasis and modify the course of disease and thus the burden associated. There is also evidence that despite the disappearance of psoriatic lesions, subclinical inflammation may continue in the skin and may become clinical after stopping treatment, for example during or after a “medication holiday “(Klaus, Goldsmith et al., 2007) . A possible explanation of this phenomenon may be the identification of a subset of T cells called memory cells (Trm), which may contribute to immunological memory of viral infections of the skin (e.g.infections...
with herpes virus). This subtype of T cells play a key role in chronic inflammation, immune-mediated or autoimmune disorder within, as in psoriasis (Clark, 2015).

Modern therapy brings remarkable improvements in psoriasis but for a variable period of time depending on many factors, most likely genetic, environmental, lifestyle etc. Of these, genetic factors likely play a key role in therapeutic response not only in the production of disease. Despite ethnic barriers antigens HLA-DR7 and Cw6 are commonly associated with psoriasis vulgaris, and the therapeutic response is variable.

Biological therapy of eligible candidate patient should fulfill criteria such as safety, tolerability, nonresponse to standard therapy for economic and practical reasons.

**Our concerns regarding the new modern therapies available for dermatologists in treating patients with psoriasis have resulted in the following studies described in detail in habilitation thesis:**

|---|

**1.4.2. ORIGINAL CLINICAL EXPERIENCE AND APPROACH TO TREATMENT STUDY WITH INTERLEUKINE 12/23 INHIBITOR IN MODERATE-TO-SEVERE PSORIASIS PATIENTS**

**1.4.2.1. INTRODUCTION**

There is a large amount of information regarding the functional and immunological significance of the cytokine repertoire involved in psoriasis, including IL17, IL21, IL22 or IL4 (Mease, 2015). Patients with moderate-to-severe psoriasis unresponsive to local or conventional therapy may now benefit from the treatment with new biological agents like anti-tumour necrosis factor alpha (anti-TNF alpha), anti IL12/IL23 and others (Toussirot et al., 2013). Ustekinumab is a monoclonal antibody targeting the p40 subunit common to IL12 and IL23, thus preventing the interactions of those two cytokines with their receptors and blocking Th1 and Th17 inflammatory pathways that lead to psoriasis (Famenini, Wu, 2013).
1.4.2.2. MATERIALS AND METHODS

This was a prospective, observational study performed in order to evaluate the efficacy of Ustekinumab. Eligible patients had a clinical diagnosis of moderate-to-severe chronic psoriasis, defined by a Psoriasis Area and Severity Index (PASI) and Dermatological Life Quality Index (DLQI) score of 10. All considered patients are qualified as candidates for systemic therapy, using usual protocols for all biologic therapy in moderate to severe psoriasis. Both naive and previously treated with other regimens patients were included. All the patients included in this programme for a 12 months therapy had signed an inform consent before enrolment. The clinical study was approved by the Ethical Committees of the two medical institutions.

Dosing was as follows: 45 mg x 5 = 225 mg Ustekinumab in 12 months (for patients under 100 kg) b.w. and 90 mg x 5 = 450 mg/12 months (for patients over 100 kg) b.w. The treatment regimen comprised doses at week 0, week 4 and then every 12 weeks. Gender, age, the previous biological treatment and the presence of arthritis were noted, at the first visit. Each patient was further evaluated at 1, 3, 6, 9 and 12 months, and PASI and DLQI were calculated. The main outcome was the achievement of PASI75. Other endpoints included PASI50, PASI90 and reduction in DLQI.

Continuous variables were presented as mean (±standard deviation). Proportions were compared with the Fisher exact test, baseline continuous variable distributions using the Student t-Test or the Mann-Whitney U test, as required. Overall changes in PASI and DLQI scores over time were evaluated with the repeated-measures ANOVA test, using the Bonferroni adjustment for multiple testing in post-hoc between-groups testing. Time-to-event analysis was performed using the Kaplan Meier method. Statistical analyses were done with STATA/IC 11.2 (StataCorp LP, College Station, TX).

1.4.2.3. RESULTS

A total of 15 patients were included, with a mean age of 50.6 (12.8) years and a male/female ratio of 2:1. Five patients had previous therapy as follows: 2 had Adalimumab, 1 had Etanercept, 1 had Infliximab in a single therapy and the last had Infliximab and Adalimumab. A third (5/15) of patients had arthritis. Patients with arthritis were significantly older than those without arthritis (mean 59.6 vs. 46.1 years, p = 0.02, one side test). No differences in age were seen by gender or previous treatment. There was a significant improvement of both PASI and DLQI scores over the 12 months study period. In terms of the PASI score, there were significant decreases from week 1 to week 4 (mean of 24.1 vs. 9, p < 0.001) and also from week 4 to week 16 (mean of 9 vs. mean of 1.8, p < 0.001), with only minor differences in the following intervals. The same pattern followed the DLQI scores, with significant decreases from week 1 to week 4 (mean of 18.7 vs. 9.1, p < 0.001) and also from week 4 to week 16 (mean of 9.1 vs. mean of 1.5, p < 0.001), and minor decreases after week 16. Both scores exhibited an abrupt
decrease by week 16 and maintained a plateau up to week 52 (Fig. 1.5., Fig.1.6.).

![Fig.1.5. PASI progression over 52 weeks](image)

![Fig.1.6. DLQI progression over 52 weeks](image)

The proportion of patients achieving PASI75 and PASI90 at measured time intervals is shown in Fig.1.7. All patients achieved PASI75 by 12 months, with one patient failing to reach PASI90 at 12 months. This single patient had the lowest baseline PASI score (13) but failed to reach the decrease endpoint. By month 3 the majority of patients had achieved PASI90. The mean time to PASI75 was 3.2 (2.6) months, while mean time to PASI90 was 4.3 (2.7) months. No differences by gender, previous treatment or arthritis were seen, although the group was not sufficiently large to provide enough statistical power for proper subgroup analysis. There were no observed adverse reactions.
in our group, no patient discontinued or was lost to follow-up.

Fig. 1.7. Progression to PASI75 and PASI90 endpoints

1.4.2.4. DISCUSSIONS

One study identified memory T cells in skin lesions in psoriasis patients, who remain in the skin after resolution of lesions during treatment with biological agents, illustrating the importance of this subset of cells in psoriasis (Cheuk et al., 2014). The presence of these cells in skin can explain the disease chronicity and recurrence of lesions in the same anatomical regions.

Cheuk et al (2014) also showed that cells Trm in psoriatic plaques occur after activation, interleukin IL-17A and IL 22, supporting the notion that memory cells in psoriasis are similar Th17 cells with IL-17A as cytokine effector key. In addition to the pathogenic effects of direct IL-17 from T-cells of the keratinocytes, IL-17A is also released by granulocytes and mast cells and plays an important role in the early recruitment of multiple-cell immunity to the target organ. Based on our knowledge of the role of memory cells (TRM) in psoriasis, early inhibition after disease onset, the IL-17A may be a new therapeutic approach, which interferes with the immune system before enlargement and chronic inflammation. This effect is achieved on the one hand by blocking the early inflammatory cells, including Th17, and on the other hand functions by blocking key effectors of memory cells (Trm). TNF inhibitors have been approved for clinical use for over a decade and they have dramatically changed the therapeutic landscape and used for rheumatoid arthritis (RA), psoriatic arthritis (PsA), ankylosing spondylitis (AS) and psoriasis (PSO). Currently, there are five available TNF inhibitors: infliximab, adalimumab, etanercept, golimumab and certolizumab pegol.
Infliximab is an anti-TNF molecule which binds to soluble TNF and membranar TNF. Infliximab may cause lysis of the cells expressing TNF on their surface (as opposed to Etanercept). Recent studies have shown that some patients may benefit receiving higher doses than more frequent administration. Clinically tested on about 1,500 cases Infliximab now reached nearly 400,000 treated patients. Data from the literature suggest that this drug may be useful in the treatment of uveitis, vasculitis, sarcoidosis and chronic sciatica. Beyond the evidence of effectiveness, treatment duration remains to be a problem, so that at 12-20 weeks after stopping treatment the patient may experience disease flare (Reich et al., 2005; Klaus, Goldsmith et al., 2007).

Etanercept is a fusion protein formed by the recombination of two identical chains of the receptors of TNF alpha with subcutaneous administration. Double dose showed no increased effectiveness; moreover postmarketing studies have shown that patients tend to reduce the number of doses without altering the quality of treatment. The product has proven efficiency and rapid onset of activity (Klaus, Goldsmith et al., 2007). Some patients may experience a flare phase of disease for 6-12 months of effective treatment with Etanercept and the causes could be related to the reduction of concomitant therapy too aggressive or the involvement of other immune mechanisms unknown (Papp et al., 2005).

Secukinumab is the first inhibitor of IL-17A approved for the treatment of plaque psoriasis, the moderate to severe forms. It is assumed that early treatment of psoriasis plaques with Secukinumab can block the recruitment of inflammatory cells and antagonize the effects of key cytokines produced by the T cell subset (Klaus, Goldsmith et al., 2007).

The benefits of biologic therapies are huge, however, side effects should also be mentioned because often they can not be neglected. Doctor bears the courage to properly select patients suitable for establishment a biological therapy, because in addition to spectacular efficacy, it presents two major inconveniences categories: side effects caused by medication and the related cost. Potentially all bio-products can induce an undesired immune response. The immunogenicity is associated with reduced levels of serum drug, a diminished response to treatment in many conditions and for more bio-products. The incidence of antimedicine antibody (ADA) varies widely in reported studies. This depends on the therapeutic dosing schedule / route of administration, and other factors, such as the population of study, the use of concomitant medications, and perhaps even more important, the analytical techniques used to measure these antibodies. Bioproducts have a large variation in pharmacokinetics, and therefore the measurement of drugs seems appropriate. The measurement of drug levels is simple, and costs are low. ADA tests facing confounding factors, and the results differ between the types of tests that could lead to confusion or misinterpretation when the user is aware of these things.

The presence or absence of ADA has implications for the response to a second TNF inhibitor. In patients with ADA to their first TNF inhibitor, a second TNF inhibitor may be less effective. These patients may benefit from bio-products with a different mode of action. In contrast, patients who developed ADA for the first TNF inhibitor had a second response to TNF inhibitor. However, they are more prone to develop ADA for the second TNF inhibitor.
Minimum levels can indicate whether the patient is a primary unresponsive to treatment (no response despite adequate levels of medication) or secondary (no response due to insufficient drug levels). In a small percentage of patients ADA can be detected with a test antigen binding (ABT) within a few weeks after induction therapy. Within 6 months of treatment, the ADA are detectable in approximately 2/3 of patients who eventually develop ADA (detectable by ABT). Because low or absent drug levels in serum are associated with lack or loss of clinical response, therapeutic drug monitoring (TDM) could be an important tool in clinical decision making in patients with inflammatory diseases. Regarding the immunogenicity factors, it seems that there is a favorable effect of co-therapy with methotrexate for biological immunogenicity. It is not known if this effect is synergistic or related to the suppression of immunogenicity. Patients with high disease activity had higher levels of ADA after three months of treatment with Infliximab comparing with patients with a lower disease activity. Patients treated with Adalimumab who developed ADA during the follow-up had initially illnesses more lasting and serious indicated by a higher score of disease activity and also by elevated values of CRP and of rate of erythrocyte sedimentation (ESR). This could indicate that the immune status, which is an active inflammatory condition, is affecting the immune response against therapeutic antibodies. Moreover, it has been shown that, for example, certain allotypes IgG or IL-10 polymorphisms have been associated with the formation of ADA.

In addition to the immunogenicity factors influencing disease activity or reduced remission, demographic parameters related to illness may influence treatment outcome. There might be the need for prescribing TNF inhibitors by gender and men tend to get remission more often than women. Other factors such as age, duration of disease or rheumatoid factor status / anti-CCP can potentially influence treatment outcome. Biological products produce depression for immune defense capacity that act at the level of gene linked to immunity, and therefore patient selection must be made extremely careful. Are excluded patients with tuberculosis, viral diseases, infection with hepatitis B, C, malignancies which may all be re-activated with this combination therapy. Lymphomas are also to be avoided in biological treatments. Injection reactions are important and they can go up to anaphylactic shock, arrhythmias, hypertension. But making a careful choice and following rigorously management protocols beneficial effects are spectacular. The second group of undesirable effects is related to the cost price, plus analysis and medical consultation. The cost of biological is ten higher than that of classic therapies, yet pressures to prescribe new therapies are increasingly higher, being important to identify criteria of cost-effectiveness to argue the use of biological therapies. Unfortunately, ordinary public response to psoriasis and issues related to it is not one of recognition, of understanding or willingness to provide assistance. Generally it is difficult to indicate a definite proof of systematic rejection, although exist well documented discrimination and harm. These require further investigation and study. Most commonly it is a matter of carelessness. There is not only carried a disease management or a "case", but also the management of a patient. However, paradoxically, even sophisticated therapies can mark the patient as different, thereby impairing their social work, psychological and physical
activity. The content, form and treatments meaning of interpretation have a potential impact on self-esteem and social reputation, as important as the initial disease. These things can handcuff physically and psychologically the patient, by their nature, emphasizing and exaggerating the effects of the problem that should resolve. It is already known (Raychaudhuri, Gross, 2000) that a third of patients have disease onset before or around the age of 16 and this, viewed from the perspective of law in Romania, involves parents decision concerning the management of the case. Etanercept is indicated from the age of 4 years for patients diagnosed with juvenile rheumatoid arthritis. The studies which had as subjects patients children with psoriasis have demonstrated efficacy of Etanercept in reducing symptoms and increasing quality of life, these results being also statistically sustained (Raychaudhuri, Gross, 2000). One of the research directions that I will follow will have as subject the use of biologic therapies in psoriasis in pediatric patients. I believe that this subject is deficient in dermatological research and in Dermatological Clinic where I work I met sufficient "family cases" of this disease so that we can initiate such a study. The clinical efficacy of Ustekinumab was observed within the first month of treatment, a plateau being observed after week 16. Ustekinumab was well tolerated and there were no drug related adverse reactions in our study group, similar to previously reported results (Ross, 2016).

Between 1st and 3rd month of follow up, consistent PASI75 and 90 responses were observed in Ustekinumab-treated patients and by month 3, the majority of subjects achieved PASI75 response (93.3%) and PASI90 response (73.3%). Only one patient failed to achieve PASI90 during the study period. The impact of this treatment over the patients was evaluated through DLQI. DLQI had an improvement pattern similar to the PASI score, with a decrease by week 16 and a plateau up to week 52. Patient compliance with the treatment regimen was 100%. The results of this study suggest that Ustekinumab is generally safe and efficacious with a rapid response rate in our centre’s experience. Efficacy was well maintained in this study, in which patients continuously received the same dose of Ustekinumab for one full year without interruption or dose adjustment and these data are generally comparable with those reported in the literature (Papp et al., 2013). We can highlight the finding that there were no differences regarding obesity and PASI behaviour (Owczarczyk-Saczonek et al., 2014). The limitations of this small multicentre study were the small sample size, so it did not permit any meaningful subgroup analyses (Leucuţa et al., 2015). The relatively short follow-up might not permit the identification of adverse reactions or immunogenicity of the drug.

1.4.2.5. CONCLUSIONS

The data presented in this study adds to the augmentation of the published work regarding the use of biologics in patients and support the use of Ustekinumab as a new, highly effective option in patients with moderate-to-severe psoriasis. The benefit-risk profile in the
studied group was favourable, consistent with the global studies of Ustekinumab (Kimball et al., 2013; Lebowl et al., 2012; Papp et al., 2008).

Overall, our study suggests that Ustekinumab can be an effective alternative therapy for moderate to severe psoriasis, but further additional studies are needed to evaluate long-term administration and the safety profile.

1.4.3. TREATMENT OF PLAQUE-PSORIASIS WITH ORAL CF101

1.4.3.1. INTRODUCTION

Despite the beneficial effect of the current agents approved for moderate to severe psoriasis, many patients discontinue treatment with biologics or traditional systemic medications because of loss or lack of efficacy, monitoring and safety issues, and lack of tolerance (Torres, Filipe, 2015).

The Gi protein-associated A3 adenosine receptor (A3AR) has been found to be overexpressed in inflammatory cells, whereas low expression of the receptor is found in normal cells. The high receptor expression is also reflected in the peripheral blood mononuclear cells of psoriasis patients (Ochaion et al., 2006).

CF101 is an orally bioavailable A3AR agonist inducing anti-inflammatory effect via deregulation of the Wnt and the nuclear factor kappa-B (NF-κB) signal transduction pathways, leading to the inhibition of tumor necrosis factor-α (TNF-α), interleukin-6 and IL-12, macrophage inflammatory proteins, and receptor activator of NF-κB ligand (RANKL). CF101’s anti-inflammatory effect has been demonstrated in Phase 2 studies in patients with rheumatoid arthritis and with psoriasis, showing efficacy and an excellent safety profile. In an earlier Phase 2 placebo controlled study in patients with moderate-to-severe plaque psoriasis, CF101 at a dose of 2 mg BID demonstrated a statistically significant improvement compared to control as evidenced a 35.3% rate of PASI ≥50 response and a 23.5% rate of achieving a PGA score of 0 or 1 at week 12. CF101 was safe and well tolerated at doses as high as 4 mg BID for 12 weeks (David, Akerman et al., 2012). In the current Phase 2/3 study, CF101 treatment of patients with moderate-to-severe plaque psoriasis has been further investigated, with the demonstration of favorable safety and efficacy through 32 weeks of treatment.

1.4.3.2. MATERIALS AND METHODS

Male and female subjects, aged 18-80, diagnosed with moderate-to-severe plaque psoriasis for at least 6 months’ duration with Physician’s Global Assessment (PGA) ≥3, Psoriasis Area Severity Index (PASI) score ≥10, body surface area involvement ≥10%, who were candidates for systemic treatment or phototherapy, were enrolled into the study. Main exclusion criteria were: other clinical forms of psoriasis, treatment with systemic retinoids, corticosteroids, or immunosuppressants within 6 weeks of the
baseline visit; treatment with moderate-high potency topical corticosteroids (Class I–III), treatment with phototherapy or Dead Sea clima-therapy within 4 weeks of the baseline visit; treatment with a biological agent within a period of time equal to five times its circulating half-life or 30 day, pregnancy; and severe infections or other conditions that would confound the study evaluations or endanger patient safety.

The conduct of this trial was approved by all local Ethics Committees in accordance with the Declaration of Helsinki. Written informed consent was obtained from the patients before inclusion in the study (NCT00428974).

The primary efficacy endpoint during Segment 2 of the trial was the proportion of subjects achieving PASI 75 at week 12, and the secondary efficacy endpoints were the proportion of subjects achieving PASI 75 at week 16 and the proportions of subjects achieving PGA of 0 or 1 at weeks 12 and 16. Efficacy for the OLE period was determined by the change in the proportion of subjects who achieved PASI 75 over time within each group Safety assessments for both segments, including BPCP and OLE, included treatment-emergent adverse events (TEAEs) and changes in vital signs, physical examination, clinical laboratory tests (liver, kidney, hematology, chemistry, urinalysis, and pregnancy test for females), and ECG findings.

An interim analysis was performed using data from the first 103 subjects enrolled in between-treatment comparisons of CF101 to placebo with respect to the proportion of subjects achieving PASI 75 at week 12 using the Cochran-Mantel Haenszel (CMH) test. To adjust for the interim analysis, the primary analysis of PASI 75 at week 12 was performed at the 0.035 significance level. The between-treatment comparisons with respect to the proportion of subjects achieving PASI 75 for visits other than week 12 using non-responder imputation and for week 12 using multiple imputations were considered ancillary efficacy analyses. Analyses of the proportion of subjects achieving PASI 75 at each visit were also performed using observed data, e.g., without data imputation, as ancillary analyses. All TEAEs were summarized by treatment group for each observed system organ class (SOC) and preferred term.

1.4.3.3. RESULTS

A total of 293 subjects with moderate to severe plaque psoriasis met the inclusion criteria and randomized into placebo (n=148) and CF101 2 mg (n=145) treated groups. Most of the patients completed the study (n=260, 88.7%), 125 patients (86.2%) in the CF101 2 mg group and 135 patients (91.2%) in the placebo control group. Reasons for withdrawal from the study included patient request, lack of efficacy, unacceptable concomitant medication or therapy, investigator decision in the patient’s best interest, non-compliance, lost to follow up, or other. 223 subjects completed the open label extension period (n=112 CF101 and n = 111 placebo/CF1010).

For the primary endpoint, the difference between treatment groups was not statistically significant at week 12: 12 of 141 subjects in the CF101 2 mg group
(8.5%), and 10 of 144 subjects in the placebo group (6.9%) achieved PASI 75 at week 12 ($P=0.621$). For a secondary efficacy endpoint, the proportion of subjects who achieved PGA of 0 or 1, the difference between treatment groups was not statistically significant at week 12: 9 of 141 subjects in the CF101 2 mg group (6.4%), and 5 of 144 subjects in the placebo group (3.5%) at week 12 ($P=0.256$). However, positive data were demonstrated at weeks 20 to 32 in the CF101 treatment group, showing linear improvement in PASI 50 (63.5% of patients at week 32), PASI 75 (35.5%), PASI 90 (24.7%), and PASI 100 (10.6%). PASI mean percent improvement was 57% ($P<0.001$) at week 32 (Fig.1.8).

In addition, results regarding efficacy of CF101 were compared to published results from a global Phase 3. CF101 efficacy rates continue to increase past 16 weeks of treatment, whereas those for apremilast appear to level off. Statistically significant improvement over time was also observed in proportion of subjects achieving PGA of 0 or 1 at weeks 24, 28, and 32 for both groups ($P<0.01$ for each time point compared to the rate at 12 or 16 weeks, first cohort and second cohort, respectively). Although not a specific efficacy objective of the trial, cumulative improvement was also observed through week 32 in those subjects who crossed over from placebo to CF101 2 mg after week 16 (Fig. 1.9).

![Fig. 1.8. PASI scores through 32 weeks of CF101 treatment](image)

Although not a specific efficacy objective of the trial, cumulative improvement was also observed through week 32 in those subjects who crossed over from placebo to CF101 2 mg after week 16. CF101 was found to be safe and well tolerated. There were no differences in vital signs, ECG, and clinical laboratory between the CF101 and the placebo. During both the BCP and OLE, compliance with dosing was high in both treatment groups. Thirty-seven of 145 subjects who received CF101 2mg (25.5%) and 29 of 148 subjects who received placebo (19.6%) experienced at least one TEAE. TEAEs tended to be distributed evenly between treatment groups. Infections and infestations (10 of 145 CF101 2 mg subjects, 6.9%, and 13 of 148 placebo subjects, 8.8%) were the
most frequently reported TEAEs, followed by gastrointestinal disorders (8 CF101 2 mg subjects, 5.5%, and 3 placebo subjects, 2%) (Table 1.7.).

The gastrointestinal events reported in the CF101 group were abdominal pain, diarrhea, dry mouth, and nausea. The majority of TEAEs were not considered to be related to study medication (18.6% of CF101 2 mg subjects and 14.2% of placebo subjects). There were no TEAEs considered to be definitely related to CF101 2 mg. The majority of TEAEs were mild (25 in CF101 2 mg subjects, 17.2%, and 20 in placebo subjects, 13.5%). Five subjects experienced severe TEAEs: 4 in the CF101 2 mg group and 1 in the placebo group, none of which were related to study drug. One death occurred during the study in the CF101 group after the visit at week 8. The primary cause of death was unknown and the death was considered by the investigator as not related to the blinded study drug. During the OLE period of Segment 2, all subjects received CF101 2 mg. Infections and infestations were the most frequently occurring TEAEs (23 of 275 subjects, 8.4%). The majority of TEAEs were not considered to be related to study drug (41 subjects, 14.9%, vs 5 subjects with possibly related TEAEs, 1.8%). The majority of TEAEs were mild (31 subjects, 11.3%). Three subjects experienced a severe TEAE, none of which were related to study drug. No change in safety profile and no evidence of cumulative toxicity were seen through 32 weeks.

1.4.3.4. DISCUSSIONS

The present study did not achieve the primary efficacy endpoint at week 12. However, at week 32 PASI mean percent improvement was 57% ($P<0.001$) with cumulative improvement from 16 to 32 weeks. PASI 50, 75, 90, and 100 rates were 63.5%, 35.5%, 24.7%, and 10.4%, respectively, in patients with 32 weeks of continuous treatment with CF101 2 mg BID. Historical placebo responses are very rare at PASI 90 and PASI 100, supporting the notion that the effect was CF101-mediated.
Tabel 1.7. TEAEs during BPCP With Incidence ≥ 3% by SOC Safety Population:

<table>
<thead>
<tr>
<th>Organ Class</th>
<th>CF101 2 mg (N=145)</th>
<th>Placebo (N=148)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any</td>
<td>37 (25.5)</td>
<td>29 (19.6)</td>
</tr>
<tr>
<td>Gastrointestinal disorders</td>
<td>8 (5.5)</td>
<td>3 (2.0)</td>
</tr>
<tr>
<td>Infections and infestations</td>
<td>10 (6.9)</td>
<td>13 (8.8)</td>
</tr>
<tr>
<td>Musculoskeletal and connective tissue disorders</td>
<td>5 (3.4)</td>
<td>2 (1.4)</td>
</tr>
<tr>
<td>Nervous system disorders</td>
<td>6 (4.1)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Renal and urinary disorders</td>
<td>5 (3.4)</td>
<td>4 (2.7)</td>
</tr>
</tbody>
</table>

Moreover, the proportion of subjects achieving PGA of 0 or 1 with CF101 2 mg was significantly increased over time between weeks 24 and 32 (P<0.01 for each time point). Positive response to CF101 was also described in a recently concluded Phase 2b study in rheumatoid arthritis patients treated with CF101 for 12 weeks (Rumen, 2014). It has been reported that A3AR levels are highly expressed in inflammatory tissues and PBMCs of patients with rheumatoid arthritis and psoriasis compared to healthy subjects. Receptor overexpression is attributed to up-regulation of some transcription factors such as NF-kB and CREB, known to be up-regulated in inflammatory conditions.

Ochaion et al. studied the effect of Methotrexate on the expression of A3AR and efficacy of combination therapy in mice with induced arthritis CF101 (AIA). These mice were treated with Methotrexate, CF101 or both agents. Also, the A3AR was determined in peripheral mononuclear cells in healthy subjects and in patients treated with Methotrexate for rheumatoid arthritis. The combination therapy had an additive effect in mice with induced arthritis inflammation and A3AR expression level was higher in patients with rheumatoid arthritis treated with Methotrexate compared with healthy subjects. These data suggest that Methotrexate induces increased expression of A3AR, thus enhancing the effect of CF101, being thus encouraged the use of combined therapy (Ochaion et al., 2006).
The safety profile in the current study is considered excellent, safety being reflected in the Phase 2 study of CF101 including 1000 patients with multiple inflammatory disorders (Avni et al., 2010).

During the last 2 decades, biological drugs including mainly anti-TNFs, anti-IL17, and anti-IL-23 have been used for the treatment of psoriasis, yielding a marked improvement in disease state. The biological agents are very costly, require parenteral administration, and have been associated with both acute adverse reactions and longer term sequelae, including serious infectious complications. On average, the median survival for the biologics in psoriasis was 47 months. Indeed, the real life observational data suggest that loss of biologics efficacy occurs in 10-20% of patients annually. 67% of all discontinuations were attributed to loss of efficacy (Gniadecki, 2015).

1.4.3.5. CONCLUSIONS

Thus, despite major recent advances in the treatment of psoriasis, there is still need for convenient, safe, and effective therapies for many patients. The small molecule drug Otezla (Apremilast), a PDE-4 inhibitor, has been approved for the treatment of moderate to severe psoriasis. Although Otezla outperforms CF101 at week 16 on both PASI 50 and PASI 75, its efficacy appears, at best, to plateau beyond that time point (Papp, 2015), while our results indicate that CF101 efficacy continues to improve through 32 weeks. It therefore seems that although CF101 has a slower efficacy onset, its linear antipsoriatic effect along the 32 weeks of treatment and good safety profile make it a promising drug candidate.
CHAPTER 2
HISTOPATHOLOGICAL ASPECTS IN DERMATOLOGICAL PATHOLOGY

2.1. INTRODUCTION

Skin anatomy analyzed using histological techniques reveals the complexity of this branch of medicine, due to the particular characteristics of the tissue structure and cells. The difficulty and at the same time the beauty of dermatopathology lies in the fact that no other medical speciality has so many names to describe an organ, and, in many circumstances, each term has a histological correlation. On the other hand, the largest organ of the human body may react in so many ways that the histopathological patterns, for instance “perivascular lymphocytic infiltrate with eosinophils”, have multiple correlations with different diagnoses, ranging from post-medication viral exanthema to bullous pemphigoid. Histopathological correlations are the key issue especially when diagnosing inflammatory diseases (Kempf et al., 2008).

Skin biopsy is recommended in all dermatoses, both to confirm the positive diagnosis and to set a differential diagnosis. Skin biopsies should be sampled from recent and unchanged lesions and they are a must in conditions like: bullous dermatoses, skin tumours, tuberculosis, leprosy, sarcoidosis. In general, the histopathological description of a skin biopsy should include two aspects: the tissue reaction picture and the inflammatory reaction picture. Tissue reactions may be classified in major and minor pictures. Major tissue reaction pictures are represented by tissue reactions of the lichenoid, psoriasiform, spongiotic, vesicular-bullous and granulomatous type, as well as of the vasculopathic type. Depending on these aspects, inflammatory dermatoses may be classified in six categories. Moreover, there are minor tissue reaction pictures which complement the major ones and help diagnosis setting. Thenceforth, inflammatory reaction needs to be characterized from the viewpoint of inflammatory infiltrate distribution in the dermis and hypodermis.
Immunohistochemistry (IHC) techniques are considered modern advanced histopathology techniques. Although they are not used as routine methods, they are really helpful in circumstances when common histopathological examination is unable to provide accurate data on diagnosis suspected by the physician. They use primary antibodies to mark certain proteins, and a second type of antibodies which bind to the primary ones. In immunoperoxidase stain, an antibody binds to an enzyme, namely peroxidase, which catalyzes the reactions in which proteins have a specific brown stain. Also, specific patterns are identified on microscopic examination by means of fluorescent marked antibodies. These patterns are considered to be of the diagnostic type and are therefore used in cases when the presence or absence of certain specific proteins may set the diagnosis in skin conditions.

Phenotypic variability of precancerous skin lesions is induced by a variety of cellular and molecular interactions which are normally responsible for the development and maintenance of tissue functions and architecture. Premalignant lesions, a term suggested by Victor Babeș in 1875, are lesions which, in the absence of any treatment, have a high risk of malignant transformation. In 2005, the WHO classifies premalignant lesions into: simple, moderate, severe and “in situ” (Cameleio et al., 2011; Massadi, 2013).

Premalignant skin lesions, the prevalence of which ranges between 0.76-5% in Asia and 13.4% in America, have cellular and molecular characteristics suggestive of the identification of mechanisms specific to the epidermal origin of cancer. The malignant transformation risk of these lesions was reported to range between 6.6 and 36.4%, although a recent meta-analysis study conducted in 2011-2012 showed the risk to be 12.1%.

Early diagnosis and adequate treatment of premalignant lesions may prevent the occurrence of skin cancers (Kivisaari, Kahari, 2013). Although in 1988 Bonquard argued that about 80% of the cancer cases occur as a consequence of malignant lesion transformation, it seems that premalignant lesion diagnosis with or without dysplasia requires reassessment. For instance, until recently, squamous cell carcinoma has been thought to be specific to aged individuals, but its incidence is also high in young individuals with chronic sun exposure. Although this diagnosis is set easily by dermatologists or anatomical pathologists, the prognosis and quality of life of these patients have not improved over the last 30 years. These aspects are due, on the one hand, to phenotypic and molecular variability and, on the other hand, to neglecting the sequential process of this development (Sun, 2013).

Worldwide, over 50% of neoplasms occur at skin level. Nonmelanocytic skin neoplasms, including basal cell carcinoma and squamous cell carcinoma, are the most common type of cancer diagnosed over the last decades in the Caucasian population, with a high incidence of 3-8% per year, starting with 1960 (Sun, 2013).

They are 18-20 times more common than malignant melanoma, with over 1 million cases estimated in 2005. In Europe, USA and Australia they are one of the most common pathologies. An increase in the incidence of squamous cell carcinoma cases was noted not only in hot climate areas, around the Equator, but also in areas with low sun exposure like Finland, where the incidence rate was 4% per year during the last decades. In 2014, these forms of skin neoplasm made up 20% of all skin cancers and were the most frequent forms of
neoplasm to cause metastases. In Egypt, the incidence of squamous cell carcinoma was estimated at 37.02% of the total number of skin cancers (Shevchuk, 2014).

At skin level, the squamous cell carcinoma occurrence risk is influenced by the existence of premalignant lesions like actinic keratosis and chronic sun exposure, as 80% of them are located in sun exposed body areas. American Academy of Dermatology claim that 60% of the individuals aged 40 and older have actinic keratoses (Einspahr et al., 2006).

At oral mucosa level, squamous cell carcinoma development requires the existence of an initial premalignant lesion. A ratio between de novo squamous cell carcinoma and squamous cell carcinoma occurring on premalignant lesions has not been established yet. Leukoplakia, erythroplakia/erythroleukoplakia, submucosal oral fibrosis and lichen planus are considered to be the most common premalignant oral lesions. Keratoses lesions in smokers, discoid lupus erythematosus and epidermolysis bullosa are less common.

As far as these lesions are concerned, a 15% rate was identified in what concerns malignant progression and transformation during the first 7 years since diagnosis setting. The highest percentage of malignant transformation is reported in case of heterogeneous erythroplakia and erythroleukoplakia. Some studies showed that 16.62% of the leukoplakia lesions exhibit modifications suggestive of their malignant transformation into squamous cell carcinoma. The research that focuses on premalignant lesion prophylaxis aims at identifying particular biomarkers involved in the carcinogenesis mechanisms.

As for the cadherin/catenin complex in oral premalignant lesions, the β and γ catenin expression decrease is an important marker of malignant transformation. Desmoplakin and plakophilin-1 marker expression, more particular desmoplakin, may identify tissues with a high risk of malignant transformation in oral dysplasia lesions. Also, the ezrin molecular marker identification is correlated with the Ki-64 index, a tumour severity and proliferation marker (Garcia et al., 2014).

A K1 and K10 expression decrease and a K13 expression increase, as well as cytokeratin K8 modifications were noted in the early stages of carcinogenesis (Presland, Jurevic, 2002). The presence of the maspin marker is an important predictor of premalignant lesion transformation in squamous cell carcinoma in oral mucosa.

Also, the MMP-1, MMP-2 and MMP-9 metalloproteinase expression is associated with a high metastases risk and guarded prognosis. MMP-2 expression, absent in epithelial mucosa cells, is directly proportional to the extent of dysplasia and the protective role of MMP-8 was identified in tongue squamous cell carcinoma (Berardi et al., 2014).

Similarly, defensin expression is correlated with malignant transformation of premalignant lesions such as leukoplasic lesions. Thus, as compared to premalignant lesions, squamous cell carcinoma is characterized by low hBD1 expression and by high hBD3 expression (Brasanac, 2005).

Therefore, the description of the molecular mosaic of the pathology of these lesions facilitates, on the one hand, the setting of an accurate and complete diagnosis in the early stages of malignant transformation, and, on the other hand, allows the use of a specific and minimally invasive therapy.
Our preoccupations related to the positive histopathological diagnosis of skin manifestations materialized in the following works which are described in detail in the habilitation thesis:


### 2.2. LYMPHOMATOID PAPULOSIS

Lymphomatoid papulosis is a chronic, recurrent and self-limiting skin condition characterized by skin lesions of the papule and necrotic nodules type, with papulopustular histological characteristics suggestive of malignant lymphoma (CD30 +) (Bolognia, Jorizzo, Schaffer, 2012). Lymphomatoid papulosis is one of the primary lymphoproliferative CD30 + skin diseases, which, together with primary cutaneous anaplastic large cell lymphomas (C-ALCL), make up the second largest group of T cell skin lymphomas (25% of the cases) (Kadin, 2009).

The first reference to this clinical entity dates back to 1968 and is due to Macaulay (Macaulay, 1968). Specialists have relentlessly argued ever since whether this disease was benign/ premalignant or malignant. Lymphomatoid papulosis makes up about 15% of the cutaneous T-cell lymphoma (CTCL) cases. It is currently considered to be a cutaneous T-cell lymphoma with reduced malignity, having common clinical and histological characteristics with the primary cutaneous anaplastic large cell lymphoma, including the presence of T cells with phenotype and aberrant clone cells with aberrant TCR gene in 60 - 70% of the patients (Bolognia, Jorizzo, Schaffer, 2012).

Our research was aimed at assessing the importance of histopathological data in a case of lymphomatoid papulosis and pointing out the importance of early diagnosis setting and therapy plan determination, as well as the at presenting our diagnosis algorithm option designed to ensure a productive collaboration between the two specialities, namely dermatology and pathological anatomy. A 26-year-old patient, without any significant
medical history, came to the Dermatology Clinic to get examined for an asymptomatic necrotic papulopustular lesion located in the temporal region, with fusion tendency, with its onset 2 months before the patient’s hospitalization. The history of the patient suggests a stage diagnosis of deep folliculitis. The bacteriological exam of the purulent discharge revealed the presence of *Pseudomonas Aeruginosa*. Based on these data, a systemic antibiotic therapy was chosen according to the antibiotic susceptibility testing, and a topical treatment was recommended using antibiotic ointments. The patient’s evolution was positive, as the lesions healed leaving behind hairless scars. Papular lesions reoccurred 4-5 weeks later at the periphery of the scars and the patient was hospitalized again for further tests and therapy plan determination.

The physical objective examination conducted on hospitalization revealed the presence of atrophic scars accompanied by papular lesions and necrosis, as well as ulcerating lesions, located in the temporal and zygomatic areas; the patient also complained of hairless areas and discrete symptoms represented by pain and burns (Fig.2.1). The laboratory tests were within normal limits, except for a methicillin-resistant (MR) *Staphylococcus aureus* isolated there, which was still sensitive to Linezolid, Vancomycin and Teicoplanin. The imaging investigations revealed no pathological changes. Skin biopsy was carried out on specimens sampled from the nodular lesions with central necrosis in the right temporal area. The histopathological exam revealed a skin area with large ulceration covered by fibrinous leukocytic exudate. A polymorphic infiltrate with small lymphocytes, eosinophils and large lymphocytes with nuclei containing eosinophilic nucleoli were detected in the dermis, at the bottom of the ulceration, whereas the cells exhibited a focal sternbergoid appearance, with reduced mitotic activity (Fig 2.2.).

![Fig. 2.1. Papular-necrotic lesions and ulcerations accompanied by hairless patches in the temporal and zygomatic areas](image1)

![Fig. 2.2. Epidermis with ulceration and polymorphic inflammatory infiltrate. HE stain, x40.](image2)

The immunohistochemistry studies indicate the presence of large cells CD30 positive, with nucleoli, CD3 and CD5 positive in most cells in the dermal infiltrate, rare lymphocytes CD 20 positive, rare small lymphocytes CD20 positive, rare small lymphocytes CD 8 positive,
cells CD15 negative in the tumour cells and positive in granulocytes (Fig. 2.3., Fig.2.4., Fig.2.5., Fig.2.6).

The morphological and immunohistochemical aspects together with the clinical findings and disease progression concurred to the diagnosis of type A lymphomatoid papulosis. The patient was examined in the haematology-oncology department in order to decide on the further therapeutic approaches, which consisted of corticotherapy, i.e. Prednison 60mg/day, for 10 days, with progressive dose decreased after 10 days, with haematological-oncological reassessment. His evolution was positive, as the lesions were still remitted at the 1, 3 and 6 month follow-up. Our case deserves to be discussed due to its features: onset at the age of 26 years, i.e. at a younger age than the average age recorded in clinical trials reported in literature, particular lesion location (scalp, beard), association with methicillin-resistant (MR) Staphylococcus aureus. All these aspects delayed the setting of a positive diagnosis.

The lymphomatoid papulosis incidence peak occurs in the fifth decade of life and it rarely affects children and young individuals. The youngest patient reported in literature was 8, and the oldest 84, which means that the average age is 35-45, with a M/F sex ratio of 15.5/1.
Despite the severe clinical picture, the vital prognosis is good in most cases, as 20% of the patients run the risk of developing a neoplasm which may or may not be of the lymphoma type (Kagaya, Kondo et al., 2002).

The etiology of this disease, as well as that of cutaneous lymphomas has not been completely elucidated yet, and among the triggering factors we may list viral infections. There are studies with inconclusive findings as concerns HTLV1, EBV, HSV 1, 2, 6 (Saggini, Gulia et al., 2010).

The most common clinical sign of this condition is represented by hyperpigmentated papule or nodules with necrotic deposits in the centre which are resolved in 3-8 weeks. One may usually note the presence of several to hundreds of lesions in several progression stages. The lesions may be grouped together or scattered across the torso and the limbs, and they may regress spontaneously, leaving behind atrophic hypo-hyperpigmentary scars with varioliform appearance. Cases of restitutio ad integrum have also been reported. Skin lesions are usually asymptomatic (Bolognia, Jorizzo, Schaffer, 2012; Saggini, Gulia et al., 2010). Skin lesion progression may last several months or even tens of years. Lymphomatoid papulosis may be preceded or followed by other cutaneous or systemic lymphomas, such as mycosis fungoides (MF), primary cutaneous anaplastic large cell lymphomas (C-ALCL) or Hodgkin’s lymphoma, in 20% of the patients with skin rashes (Saggini, Gulia, et al., 2010). Most cases have very good prognosis and may be checked and followed up by the dermatologist. The possible spontaneous regression of skin lesions specific to lymphomatoid papulosis is well known. The mechanism of this regression is unknown; the suggested interaction between CD30 and its ligand (CD30L) may contribute to neoplastic T cell apoptosis and skin lesion regression (Mori, Manuelli et al., 1999; Schieman, et al., 1999).

The diagnosis algorithm applied to cutaneous T cell lymphomas (CTCL), including to lymphomatoid papulosis, requires corroborating the clinical, pathological anatomy and phenotypic criteria (Bolognia, Jorizzo, Schaffer, 2012; Benner et al., 2009).

The first stage includes the distinction based on clinical criteria between MF, Sezary syndrome MF and other CTCL, by the dermatologist who knows their clinical signs best. This stage allows the identification of about 65% of the CTCL cases, represented by MF and its variants. This stage may also include the clear cases of lymphomatoid papulosis (Bolognia, Jorizzo, Schaffer, 2012). Knowing the clinical criteria applied to the diagnosis of skin manifestations specific to CTCL allows the identification of about 90% of the CTCL cases in these first two stages. The remaining 10% are represented by rare cases of T cell lymphoma (subcutaneous panniculitis-like peripheral lymphoma, epidermotropic aggressive cytotoxic T CD8 + cell lymphoma, etc.). The cases of lymphomatoid papulosis should be distinguished from skin involvement specific to systemic ALCL, MF CD30 + or other T CD30+ cell lymphomas, as well as from pseudolymphomas, insect bites, viral infections, scabies, atopic dermatitis and others (Bolognia, Jorizzo, Schaffer, 2012; Saggini, Gulia et al., 2010). The second stage consists of skin biopsy examination and determination of the presence of CD30. The group conditions that may be identified include lymphomatoid papulosis, anaplastic large cell lymphoma and they make up about 25% of the CTCL cases.
From the histopathological point of view, one may distinguish between four histological types of lymphomatoid papulosis (Bekkenk et al., 2000):

- **Type A**: with non-epidermotropic lymphocytic infiltrate with flame figures, with Lyt CD30 +, with small multinucleate cells of the Reed-Sternberg type, atypical cells, grouped or scattered, of the neutrophil or eosinophil type;
  - Large atypical cells, with similar morphology to ALCL, are rarely detected in onset and progressing lesions.
  - The presence of isolated neutrophils in the epidermis with moderate acanthosis and parakeratosis is a specific feature.

- **Type B**: rare (<10 %) – characterized by the presence of a band-like lymphocytic infiltrate, with early basal and parabasal epidermotropic invasion.
  - Occasionally, the infiltrate may also occur in the deep epidermis.
  - Atypical small or medium CD3 +, CD4 +, CD30- or CD30+ cells with cerebriform nuclei similar to MF.

- **Type C**: CD30 + monomorphic inflammatory infiltrate, with scattered or grouped T lymphocytes – with histopathological appearance similar to ALCL.

- **Type D**: with T CD8+ epidermotropic cells like in the aggressive lymphoma
  - Intraepidermal cells with CD8 and CD30 expression, with absent T cell antigens.

In most cases, the treatment of lymphomatoid papulosis is unsatisfactory, with relapses when the therapy is stopped. The therapeutic management of the disease requires the careful consideration of the pros and cons of short-term therapy, as well as of its adverse effects (Vonderheid, Sajjadian et al., 1996). The patients whose clinical picture comprises few skin lesions do not need treatment. In these cases, cosmetically favourable results may be achieved by administering small Methotrexate doses (5-20mg/week), by PUVA therapy, by topical mechloretamine or carmustine therapy, or by small etoposide doses. Extensive skin lesions may spontaneously enter a remission process after 4-12 weeks, or they may be treated by surgical procedures or radiotherapy (Bolognia, Jorizzo, Schaffer, 2012; Saggini, Gulia et al., 2010). In our case, the skin lesions underwent spontaneous resolution, with no connection with the antibiotic therapy, which led to confusions in therapy management, since the initial positive response of the lesions was ascribed to the administered agents. The relapse occurred shortly after and we began to question the accuracy of the positive diagnosis. The histopathological examination signed the final diagnosis and enabled us to set an adequate treatment plan. Due to its specific relapses, lymphomatoid papulosis requires long-term medical follow-up (Bekkenk et al., 2003). Lymphomatoid papulosis is a chronic recurrent skin condition, which is rarely dealt with in dermatological practice. The diagnosis of this pathology needs to be set by a team of dermatologists and pathological anatomy specialists, yet the dermatologist plays an essential role in diagnosis setting, as he/she recommends histopathological examination based on particular clinical criteria, when a lymphoma is suspected. The histopathological examination in the second stage of the diagnosis algorithm was also the most important step in setting the right diagnosis in our case.
2.3. AUTOIMMUNITY AND CUTANEOUS IMPLICATIONS OF SYMPTOMS ASSOCIATED WITH HEPATITIS C VIRUS INFECTION

The skin reflects an individual’s health status and many systemic conditions are associated with skin manifestations. Virus C hepatitis is a common condition worldwide; it is therefore recommended that hepatitis C patients pay attention to autoimmune manifestations and vice versa. Autoimmune diseases, including lupus erythematosus, scleroderma, dermatomyositis and Sjogren’s syndrome, are relatively common conditions, but there are many conditions the etiology of which is unknown and for which the theory of autoimmunity is considered. Autoimmune skin manifestations include a wide range of clinical aspects, which may include: livedo reticularis, psoriasiform lesions, lichenoid lesions, lichen sclerosus, vitiligo, alopecia areata or paraneoplastic manifestations. Autoimmunity is characterized by a breach in the immune system tolerance to self antigens. The mechanism by which this breach occurs is multifactor, including genetic and environmental factors. Among the environmental factors, the involvement of infections and medicines is most frequently talked about (Kang, Craft, 2008).

Our study was aimed at analyzing the case of a 65-year-old female patient living in an urban area, who came to the Dermatology Clinic in May 2013 complaining of a rash of erythematous violaceous pruriginous disseminated papules, which had been progressing for about 18 months. The patient’s personal medical history included cholecystectomy, uterine fibroids for which she underwent surgery (total hysterectomy with bilateral ovariectomy), nodular goiter with autoimmune thyroiditis for which she underwent surgery and chronic virus C hepatitis diagnosed when the thyroidsctomy was performed in 2000, for which she underwent Interferon alpha and Ribavirin therapy for 2 years. The patient was diagnosed with insulin-dependent diabetes mellitus in 2002, with generalized vitiligo in 2006, with lichen planus in 2012 and atrial fibrillation, for which she undergoes Metoprolol and Digoxin therapy. The family history revealed the presence of two sisters with insulin-dependent diabetes mellitus. The general physical examination reveals slight muscle weakness, shoulder myalgia, painful abdomen on palpation in the right hypochondrium and hypogastrium. The physical examination revealed lesions represented by flat-topped polygonal erythematous 5 mm papules, bright in some light incidences, isolated or grouped in a ring shape, covered by fine skin flakes, with a whitish network on their surface, scattered across the torso and the limbs, accompanied by a slight itchiness, well-delimited achromatic patches with irregular outline, scattered across the torso, limbs and head (Fig.2.7, Fig.2.8.). We also noted the presence of lesions shaped like a whitish network on the oral mucosa next to the premolars and molars, kraurosis vulvae lesions, with vulvar mucosa surface erosions and whitish reticular lesions on the vaginal mucosa (Fig.2.9.), accompanied by small amounts of brownish vaginal discharge. The physical examination also revealed the presence of punctiform depressions and transverse striations on the finger nails of both hands, xerosis cutis and venous ectasia on both legs.
Fig. 2.7. Lichen planus skin lesions represented by flat-topped erythematoviolaceous papules, isolated or grouped in ring shapes, scattered across the torso and limbs accompanied by vitiligo lesions.

Fig. 2.8. Vitiligo lichen planus represented by well-delimited achromatic patches with irregular outline, scattered across the torso, limbs and head.
The laboratory tests conducted dynamically during the two hospitalizations in the Dermatology Clinic revealed thrombocytopenia (29/05/2013: 81,000 / mm3, 21/08/2013: 94,000 / mm3), hyperglycemia (29/05/2013: 212 mg / dl, 21/08/2013: 380 mg / dl), GGT (29/05/2013: 78 UI / ml, 21/08/2013: 76 UI / ml), high ESR (28 mm /1 hour), immune-electrophoresis proteins (90.2 IgE UI, 445 UI IgA) and anti HCV antibodies in 64.03 UI titre. The findings of the autoimmunity tests (ANA, antiADNdc, antiSSa, anti SSB, anti SCL70) were within normal limits. Skin biopsy specimens were sampled in the papular lesions located on the abdomen, in the vitiligo lesions and sclerous lesions on the genital mucosa. The findings of the tests performed on the papular lesions showed uneven epidermis, epidermal thickening, liquefaction of the basal layer, accompanied by abundant willing band lymphomonocytic inflammatory infiltrate, with epithelial tropism and separated collagen fibres, which support the lichen planus diagnosis. The genital lesion findings revealed changes suggestive of lichen sclerosus: epidermis atrophy, perivascular inflammation, dermis with hyalinization. The vitiligo lesions had normal appearance on the histopathological examination with haematoxylin and eosin staining, except for the absence of melanocytes.

The endocrinological examination set a type II polyendocrinopathy diagnosis, with high TSH values (100 UI), which required Eutirox dose adjustment. The physical examination and the laboratory tests concurred towards the following diagnosis: lichen planus, erosive oral and genital lichen, generalized vitiligo, insulin-dependent diabetes mellitus, chronic virus C hepatitis, class II venous failure according to the CEAP classification, autoimmune nodular
goiter for which she underwent surgery and atrial fibrillation. We noted in this patient an association of autoimmune conditions (diabetes mellitus, vitiligo, lichen planus, autoimmune nodular goiter), against a background of chronic virus C hepatitis, previously treated with Interferon alpha and Ribavirin; this association of conditions may be included in type II polyendocrinopathy (Addison disease, hypoparathyroidism, mucocutaneous candidosis, alopecia areata, type I diabetes mellitus). The relevance of the case within the framework of autoimmune diseases supports the following discussions:

**Autoimmune Infections and Diseases**

A possible infection involvement in autoimmunity has been suggested and the involved mechanism would consist of: immune response modulation by the inflammatory cytokines released (e.g. IFN-alpha) by the antigen presenting cells (APCs), molecular mimicry, cross reactions due to antibody production, or to T cells reacting with both autoantigens and antigens, or to the presence of superantigens which induce a polyclonal activation of the autoreactive T cells (Kang, Craft, 2008). This mechanism has not been fully proven yet, but the association between viral infections such as hepatitis C and Epstein-Barr virus infections, and autoimmune manifestations is very frequent in clinical practice. The pathogenic connection between the HCV and autoimmunity induction has not been fully understood. The mechanisms involved in this immune disorder are still unclear, but they may be connected to the lymphotropic nature of the virus (Böckle, 2012). The HCV may be found in various extrahepatic tissues (salivary glands, kidneys, skin, etc.), thus contributing to infection persistence and reactivation, and at the same time playing an important role in immune system stimulation, including in autoimmune mechanism stimulation.

**Drug-Induced Autoimmunity**

Another discussion concerns drug administration. Certain agents may induce autoimmune diseases or manifestations (e.g. Procainamide, hydralazine, ribavirin, etc.). Ironically, immunomodulatory drugs, such as IFN-alpha, may also induce autoimmunity. Associations have been reported between IFN-alpha therapy of virus C hepatitis of neoplasia and drug-induced lupus associated with nephritis, vasculitis with neutrophilic anti-cytoplasm antibodies, whereas IFN-alpha involvement in lupus erythematosus pathogenicity was suggested as early as the beginning of the 1970’s (Böckle, 2012). Autoimmune disease research has shown a high association rate between chronic virus C hepatitis infection and various autoimmune diseases with clinical and histopathological manifestations located at different levels. Some skin manifestations of autoimmune mechanisms may be worsened or induced by IFN therapy (lichen planus, vitiligo and others).

**Autoimmune Diseases, Skin Manifestations and Chronic Virus C Hepatitis**

The presence of autoimmune diseases in a patient diagnosed with chronic virus C hepatitis (VCH) requires therapeutic management reassessment, in order to consider the possible pros and cons. The association of lichen planus with hepatitis C and the antiviral treatment of hepatitis C and vitiligo are described in literature (Tsuboi et al., 2006). Hepatitis C virus infection, which is a member of the Flaviviridae family, is one of the conditions associated with autoimmune skin diseases, for which this association is well documented.
Chronic HCV infection is associated with clonal B cell expansion, which may lead to autoimmune processes. The phenotypic characteristics of these clonal B cell populations have been reported (e.g. CD20+ CD5+, CD5- CD20+ and CD5+ BAFF+). The autoimmunity and extrahepatic manifestations of HCV infection are related to the same impairment of TH1-mediated immune response generation. Clinical trials have proven the presence of autoimmune diseases in 40-74% of the patients with chronic virus C hepatitis, with regional percentage variations. HCV infection may have extrahepatic location, being detected in the kidneys, blood vessels, blood cells, skin and salivary glands. High anti-HCV antibody production is one of the processes which autoimmune mechanism stimulation relies on; these patients may exhibit other autoimmunity suggestive traits, such as the presence of antinuclear antibodies (41%), rheumatoid factor (38%), anti-cardiolipin antibodies (27%) or anti-thyroglobulin antibodies (13%) (Jadali, Alavian, 2010; Ai, Leonhardt et al., 2003). As regards the haematological manifestations, autoimmune thrombocytopenic purpura is common, the discussed mechanisms having to do with cirrhosis and hypersplenism associated with hypertension, production of antiplatelet antibodies and megakaryocytes, molecular mimicry between HCV and platelet glycoproteins. Autoimmune rheumatologic diseases associated with chronic HCV infection may be represented by Sjogren's syndrome, arthritis and fibromyalgia (Tsuboi, Yonemoto, Katsuoka, 2006), as over 60% of the patients with HCV associated arthropathy exhibit a positive rheumatoid factor. The distinction between rheumatoid arthritis and rheumatoid-like arthritis associated with HCV is complex and difficult to achieve. HCV associated arthritis is symmetrical, impairs large joints and does not generate bone deformation, subcutaneous nodules or inflammatory syndrome, anti-keratin or anti-citrullinated peptide antibodies. Sjogren’s syndrome is one of the most common associations (more than 250 reported cases) with chronic C virus hepatitis, as the existence of viral copies in the epithelial cells of the salivary glands has been proven (there are genetic factors able to promote glandular destruction). Therefore, chronic virus C hepatitis may mimic the clinical, histological and immunological aspects of Sjogren’s syndrome. Autoantibody overproduction is the result of the hyperactivity and expansion of lymphocytes B which generate polyreactive antibodies and rheumatoid factor. Various mechanisms are considered as concerns the pathogenesis of HCV-associated Sjogren’s syndrome, including the molecular mimicry between the salivary glands and HCV and the formation of HCV-associated immune complexes (Jadali, Alavian, 2010).

Systemic lupus erythematosus is also associated with chronic C virus hepatitis, as the two conditions have in common immunological characteristics like autoantibodies (ANA, anticardiolipin) and hypocomplementemia. At the same time, the immune complexes formed due to HCV infection may contribute to lupus nephritis development. Kidney impairment associated with autoimmunity and HCV infection may also be represented by glomerulonephritis, membranous nephropathy, IgA nephropathy (Kamar, 2008). Neurological involvement is considered and represented by peripheral sensory neuropathy, which is by far the most common neurological manifestation, being accounted for by viral RNA identification in peripheral nerve fibres, muscles, brain tissue. Other impairments were:
encephalomyelitis, myelitis, Guillain-Barré syndrome by direct invasion or antibodies production (Morgello, 2005).

Autoimmune hepatitis, characterized by high gamma globulin production and good immunosuppressant antibodies, is common. Two types of autoimmune hepatitis have been described: type I, characterized by the presence of ANA and anti Sm antibodies, and type II, characterized by anti LKM1 antibodies. In this context, hepatitis C is a triggering factor and the disease sets on against a genetic predisposition background. Dermatological manifestation may be the main target of the autoimmune process in chronic C virus hepatitis (Nocente et al., 2003), lichen planus being usually associated, although the trials conducted so far have failed to reach an agreement. Viral RNA was identified on the skin and oral mucosa of lichen planus patients, the combination being supported by in vitro hybridization studies. The immune mechanisms involved in HCV-oral lichen planus association have been suggested by the presence here of viral fragments in the impaired oral mucosa and of lymphocytes T CD4+ and/or CD8+ VHC-specific. The physio-pathological mechanism by which HCV is involved in disease initiation and progression has not been fully understood yet (Pavic et al., 2003).

Vitiligo is another autoimmune skin condition reported in literature, with similar prevalence rates in HCV patients and in the healthy general population (Jadali). Alopecia areata may also be associated, and the role of the liver infection has not been elucidated yet. Nevertheless, alopecia areata, with unknown associations with infections, has been frequently noticed after IFN therapy for hepatitis C. There have been discussions about a possible connection between psoriasis and HCV infection due to studies that reported the existence of anti HCV antibodies in psoriasis patients and RNA-HCV in the lesions of psoriasis and HCV infection patients (Jadali, Mansoury, Jadali, 2006). Autoimmune thyroiditis was the endocrinological condition the most often talked about, being described as one of the most problematic extrahepatic autoimmune diseases associated with hepatitis C; nonetheless, studies have shown contradictory results (Jadali et al., 2005). The pathogenic mechanism is based on two assumptions: direct viral cytopathic effect, secondary autoimmunity induced by molecular mimicry (Piszko, 2006). These patients with thyroid conditions require careful follow-up, especially before and after IFN-alpha therapy.

Insulin-dependent sugar diabetes is indicated in studies as HCV infection susceptibility condition. There are also influences in the opposite direction, since HCV infection affects progression to insulin resistance, which is the best diabetes predictor. This connection has been suggested by recent studies indicating remarkable structural similarities between HCV amino acids and GAD65 (glutamic acid decarboxylase), antigen 2 of Langerhans cell (protein tyrosine phosphatase) and phogrin (Hui et al., 2003; Bogdanos, Rigopoulou, 2007).

The autoimmune manifestations associated with chronic virus C hepatitis may be influenced by interferon and ribavirin therapy. Interferon therapy may be associated with immune-mediated skin lesions and cause the latter’s worsening. Interferon therapy, associated with various ribavirin treatment plans, may exacerbate dermatological manifestations, whereas immunosuppressant therapy of skin conditions may increase the viral load and
worsen liver impairment. IFN-alpha induces autoantibody production in more than half of the patients treated for chronic hepatitis C, the most frequent of which are the antithyroid, antinuclear and antiinsular antibodies (Kolb-Mäurer, Goebeler et al., 2015). The systemic side effects may suggest that the Th17- interleukin axis plays an important role in autoimmune process immunopathogenesis, especially in the case of psoriasiform lesions. The studies conducted on patients under IFN therapy include small groups of patients, and the results are inconclusive, yet lesions were described suggestive of new cases of lupus erythematosus, dermatomyositis (they have histological and genic traits in common, with high IFN-inducible type I gene expression), systemic scleroderma, vitiligo, lichen planus or even new lesions in the absence of hepatitis C. The immunological cascade involved in lichen planus lesion development includes proteins the synthesis of which is controlled by IFN (IF187, IRF1, IFTM1, CXCL 9), proteins which occur in high numbers in the lesions. Thus, IFN favours lymphocyte Th1 accumulation and distinction, followed by keratinocyte migration and adhesion, and stimulates tissue toxicity (Marrack, Kappler et al., 1999). Another association was suggested by vitiligo lesion occurrence, based on antibodies or cytotoxic anti-melanocyte lymphocyte T activation. In the case of this patient, who suffers from a collection of three autoimmune diseases, a genetic predisposition may be taken into account (the patient has two sisters with IDDM), associated with predisposing environmental factors, which contribute to autoimmunity onset. The exact causes of autoimmune diseases remain a mystery, yet it is well known and has been proven by many clinical trials that viruses are one of the most important etiological-triggering factors in this mechanism. Hepatitis C is one of the viruses most frequently associated with autoimmune diseases, autoimmune disease severity exceeding hepatitis severity, which makes it compulsory for doctors to check whether there is a possible association between virus C hepatitis and autoimmunity. The physio-pathological mechanisms have not been fully understood yet, yet we know that molecular mimicry, immune complexes and regulating T cells play an important role in autoimmune reaction associated with HCV. The case described above assesses the connection between autoimmune diseases and hepatitis C virus infection, where the correlation may be interpreted either as a result of genetic predisposing factors associated with environmental factors, or as a result of hepatitis C virus infection (treated with Interferon and Ribavirin) with the onset of vitiligo and lichen planus lesions. The histopathological appearance may help set the diagnosis, this examination being one of the most important in patients with autoimmune manifestations. Skin specimen sampling for biopsy from the lesions may show, in the case of lichen planus, uneven epidermis, thickened epidermis, basal layer liquefaction, under which there is large amount of strip-shaped lymphomonocytic inflammatory infiltrate, with epithelial tropism, which separate the collagen fibres – aspects which suggest a lichen type lesion. The genital lesion findings may reveal changes specific to lichen sclerosus, epidermal atrophy, perivascular inflammation, dense papillary dermis with hyalinization. The biopsy sampled in the vitiligo lesions, with haematoxylin and eosin stain, looked normal, except for the absence of melanocytes.
In conclusion, it is obvious that many histopathological aspects of autoimmunity reveal implications in the skin manifestation panel and it is very important that the pros and cons of immunomodulatory therapy be carefully considered, especially in patients with dermatological conditions and associated autoimmune conditions.

2.4. MULTIPLE CLEAR CELL ACANTHOMA

Clear cell acanthoma (CCA), also known as “Degos Acanthoma” or as “pale acanthoma” is a rare benign lesion, with variable clinical aspect and distinct histopathological characteristics. Described for the first time in 1962 by Degos (Degos et al., 1962) as a rare “epidermal tumour of particular aspect”, clear cell acanthoma has raised special interest since, clinically speaking, it may be easily mistaken for seborrheic keratosis, nummular eczema, pyogenic granuloma, psoriasis, epidermal nevi, cutaneous haemangioma, histiocytoma, poroma, basal cell carcinoma / squamous cell carcinoma (Betti et al., 1995; Monari et al., 2010). From the clinical point of view, CCA looks like a 2-3mm brown-reddish papule or single nodule, sometimes covered by a fine skin flake. Sometimes, it may have a polypoid or pedunculate aspect. CCA is most commonly diagnosed in middle-aged or aged individuals, but it may also be diagnosed in children. Les lesions are usually located on the lower limbs, but there are reports of their occurrence in the inguinal area or on the scrotum, face, scalp, hands, torso, nipples, buttocks, forearms, head, etc. Gender distribution is approximately equal. In general, CCA is a single lesion, but multiple lesions have also been reported (Portal et al., 2013).

Our study was aimed at assessing the histopathological aspects specific to this extremely rare condition. A 71-year-old patient living in the rural environment was hospitalized in the Dermatology Clinic in November 2010 for the presence on the lower 1/3 of the right lower limb of seven pink-reddish 1-2 cm nodular masses. According to the patient, the lesions had a relatively rapid progression of about 2 months. The symptoms were not specific, the patient complaining of mild pain, discomfort and local exudation sensation. The patient had no history of local injuries, insect bites, post-scratching excoriations or other dermatological conditions. The dermatological physical examination revealed that all the lesions had a clear slightly irregular nodular appearance, damp surface covered by skin flakes which tend to form peripheral rings painful when moderate pressure is exerted (Fig.2.10.). The general physical examination revealed the presence of a variety of conditions: chronic venous failure, hypertension, hepatic steatosis associated with hepatomegaly. The paraclinical tests showed moderate leukopenia (3970 white blood cells/mm3), slightly high serum urea (52 mg/dL), cholestasis (GGT- 292UI/L), mild hypercholesterolaemia (216 mg/dL) and lambliasis. The chest X-ray revealed left ventricle hypertrophy and thoracic aorta calcification. The abdominal ultrasonography confirmed the hepatic steatosis and hepatomegaly and revealed two hepatic haemangioma, one 3.4 cm wide in section V and the other 3.2 cm wide in section VII.
Fig. 2.10. Clinical appearance of 1-2 cm in diameter pink-reddish nodule-like skin lesions, with mildly irregular surface, damp or covered by skin flakes, located in the median third section of the lower right limb.

In order to be able to set a positive and differential diagnosis, we have sampled a skin biopsy from a single lesion. After the patient’s informed consent has been obtained, local anaesthesia was performed using Lidocaine 1% and 2 specimens of about 3/4mm were sampled and immediately fixed in 10% formalin solution for 24 hours. Then, the biopsy samples were embedded in paraffin according to the standard histological protocol. Specimen selection was done using a MicromHM350 rotary microtome, equipped with a specimen transfer system on water bath (STS, Microm). Classical Haematoxylin-Eosin (HE), trichromic Goldner-Szekely (GS) and PAS-Haematoxylin stains were used for the histopathological study. For the immunohistochemical study, 4µm thick specimens were sampled on poly-L-lysine covered slides prepared to increase biological material adherence to the slide, after which they were preserved in a thermostat at 37°C for 24h. After paraffin-removal and histological specimen hydration, the biological material was incubated for 30 minutes in a 3% hydrogen peroxide solution. After this process, the specimens were rinsed and boiled in a pH 6 sodium citrate solution, in the microwave oven, for 21 minutes (seven cycles lasting 3 minutes each), for antigenic recovery purposes. For the MNF116 antibody, the antigenic repair was achieved by its boiling in pH 9 EDTA for 21 minutes. After their boiling, they were left to cool for 15 minutes then rinsed in a phosphate-buffered saline (PBS) solution and then in 2% skimmed milk for 30 minutes, in the non-specific site blocking stage. The specimens were hatched with primary antibodies for 18 hours in the refrigerator at 4°C. The second day, secondary biotinylated antibodies (αMs/αRb) were applied on the skin biopsy specimens for 30 minutes, then the specimens were immersed in HRP Streptavidin for 30 minutes. The signal was detected by 3.3’- diaminobenzidine (DAB) (Dako), then a Haematoxylin stain, dehydration of alcohol, xylene clarification and slide fixation in DPX medium (Fluka) were performed. The histological exam revealed epidermis thickening, with
spinous layer thickness increase (acanthosis) associated with kyperkeratosis areas with reduction of the granulous layer thickening. The interpapillary epithelial ridges were long and thickened, and they penetrated the connective tissue of the dermis. The epidermal thickening was irregular and from place to place the epidermis appeared to be thin, made up of only 2-3 rows of keratinocytes, whereas a network of congested blood vessels (arterioles, capillaries, venules) was identified in the surface dermis (Fig.2.11.). This microscopic characteristic may account for the moist appearance of the acanthoma surface and for scale formation.

![Fig.2.11. Overall histological picture, HE, x40.](image)

When classical stains were used (HE, trichromic GS), the keratinocytes in the spinous layer were mildly increased in size and exhibited paler and sometimes heterogeneous cytoplasm. The wide intercellular gaps allowed desmosomes to be revealed. In some areas of the epidermis, several intra-Malpighian leukocyte aggregates (lymphocytes and granulocytes) or diffusely disseminated leukocytes in the epidermis thickness were revealed. A tendency of keratinocytes to acantholysis and necrosis was also noted in the areas where abundant leukocyte infiltrates were identified. The PAS-Haematoxylin stain used to reveal glycogen, showed a heterogeneous layout of glycogen granules in the keratinocytes. The spinous and granular layer cells were the richest in glycogen. The presence of a rich inflammatory infiltrate, made up of heterogeneously disseminated lymphocytes, plasmocytes and granulocytes, was noted in the dermis. Trichromic staining enabled us to notice the presence of large amounts of fibrillar collagen in the surface dermis and local fibroblast activity intensification (Fig.2.12). Three antibodies were used for the immunohistochemical study of keratinocytes. Cytokeratin 7 (CK7) reaction was completely negative in the epidermis (Fig.2.13.), and positive in the epithelium of the excretory canal of the sweat glands. In contrast, cytokeratin 34BE12 reaction was intensively positive in all keratinocytes, except in certain areas where keratinocyte acantholisis and necrosis processes were noted, and areas where many leukocytes were identified, disseminated along the epidermis cells. Keratinocyte reaction to MNF116 (CK MNF116) cytokeratin was intense, except for some of them the cytoplasm of which contained large amounts of glycogen. The immunohistological study of
the inflammatory infiltrate in the dermis showed that it was mainly made up of T lymphocytes. In the papillary dermis, T lymphocytes appear dispersed in the connective matrix of this area, among the angiogenesis vessels. Many T lymphocytes were well represented, sometimes with a nodule-forming tendency. B lymphocytes had a diffuse layout, mainly in the surface dermis and they were less numerous than T lymphocytes. Just like B lymphocytes, macrophages occurred in much smaller numbers than T lymphocytes (Fig.2.14.). CD34 antibodies, which mark angiogenesis (neovascularisation) cells, were used to reveal the vascular structures in the dermis as accurately as possible. This immunohistochemistry technique enabled us to notice that a large number of angiogenesis vessels developed inside the clear cell acanthoma. Small calibre vessels (capillaries, metarterioles, venules) were identified in the papillary dermis (Fig.2.15.) whereas in the deep dermis we noticed, in addition to the capillaries, a large number of arterioles and venules. The multiple clear cell acanthoma diagnosis was set based on clinical and paraclinical data. The treatment included surgical removal of large lesions and dermocoagulation of small lesions. The patient’s evolution was positive, with full recovery on discharge and no relapses. One of the particular aspects of the case was the fast development of nodular skin lesions in about 2 months. According to most studies, CCA usually has a slow progression rate of 2 to 10 years, with minimal or even absent symptoms (Tempark, Shwayder, 2012). We think that the fast development of her acanthoma lesions was due to the other associated diseases, such as chronic venous failure, which causes oedema and stasis in the lower limbs, as well as to her liver impairment. Our patient’s lesions were 1-2 cm in diameter, i.e. they were similar with those of other cases described in literature. Other authors detected larger tumor lesions of over 3-4cm in diameter, on the lower limbs, buttocks or perineum. Our patient’s lesions were located on the lower third of her right calf, they were pale pink and their damp surface was covered by skin flakes. In our opinion, the shade of the lesion was due to the rich dermal vascularization and the damp appearance to the presence of the plasmatic transudate which could also contribute to skin flake formation with epidermis hyperkeratinisation in certain areas. Most CCA cases described so far exhibited skin flakes, sometimes with serous exudation or, occasionally, with serosanguineous exudation (Veiga et al., 2012).
Some authors showed that, due to their rich vascularisation and to the presence of pale epidermal areas, CCA lesions may bleed after minor injuries. The CCA colour may vary from pale pink, like in the case of our patient, to dark brown. The dark pigmented colour may be due to the presence of a large number of melanocytes in the acanthoma; thus, these forms are defined as “melanoacanthomas”, “clear cell melanoacanthomas” or “pigmented clear cell acanthomas”. Bugatti and Filosa described a black hemosiderin CCA, with hyperpigmented macula appearance, with skin flake on its surface, where the histopathological examination revealed a large number of cross blood vessels in the papillary dermis, surrounded by hemosiderin deposits, due to extravasation and erythrocyte destruction (Bugatti, Filosa et al., 2011). Another particular aspect of our case was the simultaneous presence of 7 CCA lesions. The first description of a multiple CCA belongs to Delacretaz (1964). Various authors have diagnosed multiple CCA. Burg et al. found in a 38-year-old patient more than 100 CCAs disseminated on the lower limbs, arms and torso. We found less than 30 cases of multiple CCA in literature.

As concerns the ethiopathogenesis of the condition, all the authors agreed that it is still unknown. Two big theories are currently being discussed: according to the first theory, CCA is an epidermal benign tumor originating in the epidermis, hair follicle or excretory canal of the sweat glands; according to the second theory, supported by most authors, CCA is a reactive inflammatory dermatosis. The histological and immunohistochemical study that we conducted revealed epidermal hyperplasia and a mild keratinocyte size increase, possibly due to the presence of intracytoplasmatic glycogen granules. The glycogen is responsible for the “clear” appearance of these cells in regular stains and in the PAS-positive reaction. Glycogen accumulation in keratinocytes may be due to the absence of phosphorylase, an enzyme involved in glycogen degradation and its metabolization. The presence of glycogen granules in the keratinocyte cytoplasm was also confirmed by electronic microscopy studies. The lack
of CK7 reactivity of keratinocytes and the positive value of the eccrine sweat gland cells made us conclude that CCA does not originate in the eccrine sweat gland epithelium. Moreover, the different keratinocyte reactivity to CKMNF116 proves the metabolic nature of CCA, as the glycogen granules and absent phosphorylase prevent structure cytokeratine synthesis. In our multiple CCA case, the inflammatory alterations were very significant. The inflammatory infiltrate in the dermis was mainly made up of T lymphocytes, B lymphocytes, macrophages, neutrophilic leukocytes and plasmocytes. T lymphocytes and neutrophilic leukocytes were also found in the diffusely disseminated keratinocytes occurring in the basal, spinous and granulous layers. Local fibroblast reactivity, with a connective matrix synthesis increase, especially of collagen, was revealed in the dermis. We also found a network of blood vessels with strongly positive epithelium reaction to the CD34 antibody, a characteristic of the angiogenesis vessels. In the same manner, other authors have shown that intense inflammatory reaction and hypervascularisation support the inflammatory theory of CCA. In our opinion, due to the multiple clinical aspects which are similar to other dermatological conditions, wound biopsy and histopathological examination are vital for positive and differential CCA diagnosis. This is the first multiple CCA case in Romania with a relatively rapid progression of the lesions, located on the lower 1/3 section of the right calf, in a 71-year-old woman. The positive diagnosis was confirmed by the histopathological and immunohistochemical examinations, which revealed CCA-specific microscopic changes in the epidermis, including the presence of glycogen granules in the keratinocyte cytoplasm. The presence of a significant inflammatory reaction in the dermis associated with a dense network of blood vessels and the changes occurring in the connective matrix support the assumption of the inflammatory origin of CCA in our case.

2.5. MICROSCOPIC ASPECTS OF THE PILOSEBACEOUS UNIT AFTER ANTIANDROGENIC TREATMENT OF HIRSUTISM IN WOMEN

Hirsutism in women may be defined as a hyperandrogenism manifestation and/or hair follicle hyper-receptivity to androgens. This means that vellus hairs turn into thick terminal hairs in the androgen-dependent areas (Randall, 2008; Randall, 2007). Vellus hairs are soft, thin, non-pigmented and from a histological point of view it is non-medullary and its diameter may reach 0.03 mm. Terminal hairs have medullaries resembling an inner pocket containing collapsed “proteins” which are not very well known (Azziz et al., 2000). Varied local and systemic factors, as well as cytokines acting directly or indirectly on the dermal papilla and on the external and internal concentric root area known as the hair bulb, are involved in the hair growth regulation process (Deplewski, Rosenfield, 2000). The androgen hormones stimulating pilosity activate the dermal papilla cells by means of androgen receptors (AR) (Rosenfield, 2005). Cyproterone acetate (CPA) functions as an antiandrogenic through its action on the intracellular androgen receptors, and as an antigonadotropic agent by reducing the circulating androgen levels. Its effects on the pilosebaceous structures result from the transformation of the mature hairs into immature vellus hairs and they are directly
proportional to the duration of the antiandrogenic treatment. Just like in other cases, therapy may induce local immune system reactivation. Therefore, antigen-presenting epidermal cells, i.e. the Langerhans cells, are among the first reactive cells.

Paul Langerhans discovered the first dendritic cells in the middle epidermal layer by using the gold salts staining method. It was later proven that the processes in which the Langerhans cells are involved and which present the antigen contain specific granules in the cytoplasm, sending long cytoplasmatic processes between the keratinocytes (Holíková et al., 2001). The Langerhans cell makes up 3-8% of the cell population of the epidermis and some of them are vimentin positive. Immune anti-vimentin reaction has limited specificity. Almost all activated Langerhans cells are assumed to express S100 protein. Despite the fact that S100 has low specificity, it is extremely sensitive. Little data have been published so far about the structure and involutive morphological and immunohistochemical changes of the hair after the systemic antiandrogen therapy of hirsutism. A recent study has revealed that a trophical factor of the nervous system called galanin, which inhibits hair growth by anagen decrease, may be used as a new hirsutism treatment (Holub et al., 2012).

A prospective hirsutism study was conducted between 2000 and 2002 and included 14 women with idiopathic hirsutism, who were diagnosed based on physical, hormonal and ultrasonographic criteria, in order to detect the etiology of their hyperandrogenism. The study was aimed at analyzing the morphological alterations of the pilosebaceous units of hirsutism women before and after 12 months of antiandrogen treatment consisting of CPA 100 mg/day and at confirming these immunohistochemical changes by electronic microscopy. The patients’ age ranged from 16 to 40 years, and their hirsutism on the Ferriman-Galway scale scored between 10+ and -3 points. We administered CPA (Androcur) 100 mg/day associated with oral contraceptives for 12 months, after having obtained the patients’ consent. Biopsy specimens were sampled in the androgen-dependent areas (chin) before and after the treatment. The biopsy specimens were processed by the classical paraffin method. The sections were stained using the haematoxylin and eosin (HE), Van Gieson, Masson, Sirius red and indigo- picrocarmine stains. Additional specimens were immunotreated using S100 protein and vimentin. The immunohistochemical tests were conducted using the LSAB2 system, and the final product was viewed by means of dihydrochloride 3.3 diaminobenzidine. Electronic microscopy tests were performed in 2 cases of Langerhans cell hyperplasia using the Reynolds technique. The HE stained specimens sampled for biopsy before the treatment revealed no significant skin architecture change. An increase in the number of hair follicles and a hyperplasia of the sebaceous glands containing reserve cells (Fig 2.17.) were noted. The deeper part of the epithelial sheath of the hair follicle showed one or more epithelial buds proliferating towards the surrounding connective tissue (Fig. 2.16.). No inflammatory infiltrate or other pathological changes were noted. Hair follicles with well-defined medullaries, with all the layers, sebaceous glands and arrector pili muscles, were noted on the Sirius red and indigo- picrocarmine stained specimens (Fig. 2.18.). Hair follicles with connective tissue cover, Huxley layer, inner root cuticle cover, medullary and cortical components, were noted on the Van Gieson stained specimens. After 12 months of CPA
100mg/day therapy, the following changes were noted in the hair follicles in the androgen-dependent areas, namely on the chin of hirsutism women: pilosebaceous unit atrophy in 9 (64.2%) of the women, inflammatory infiltrate in all the women, multinucleate giant cell granulomas in 1 woman and cells embedded in the basal epidermal layer vacuoles in 4 women. In one of the women the inflammatory infiltrate invaded the epithelial component of the hair follicle. The most common histopathological changes showed hair follicles and sebaceous glands in an atrophic state. Some follicles were in an advanced atrophy state, with only few layers of epithelial cells visible; and they were surrounded by a hyaline condensate without sebaceous glands. The epithelial cells of the cover had clear cytoplasm with hyperchromic nuclei (Fig.2.19.).

From the histopathological point of view, hair follicles became thin in most cases, considering that their medullary was missing. Other follicles had air-filled bubbles in the medullary and started to exhibit atrophic sebaceous glands. Hair follicles with hair bulb and hair matrix in an incipient state, with a rudimentary appearance and partially keratinized cuticle, were among the most frequent changes. Other hair follicles had an axis appearance in the dermis with keratinized medullary cytoplasm or root cover that could not be viewed in all the follicles. Moreover, we found that column-like hair follicles had a mass of cells consisting of incipient changes in matrix and bulb formation, in contrast with mature sebaceous glands. In some sections of the hair follicle we did not notice the dermal papilla in the mesenchymal tissue. In most cases, the medullary had nuclei-less cells or it was totally absent. All the other layers were poorly defined and had a homogeneous appearance between the connective tissue sheath and the cuticle (Fig.2.20.). Another aspect of hair follicles with intense keratinisation processes in the proximity of the epidermis showed that the hair did not penetrate the epidermis, but remained as a rudimentary follicle in its neighbourhood. Moreover, some of these follicles and bulbs seemed to be extensions of the basal epidermal layer (Fig.2.21.), and in other sections of the hair we noted 2 important aspects: the first consisted of an immature sheath structure, whereas the other suffered an ageing process characterized by medullary gaps.

![Fig. 2.16. Hair, Masson stain, x100](image)
A cross section of the hair follicle showed medullary keratinisation and fibrillar keratin structure, as well as a distorted appearance of the hair follicle; trichohyalin was turned into keratin fibrils with intensive cuticle keratinisation (the keratinisation process normally occurs later in this stage). On sections, the medullary was replaced by a series of air bubbles (vesicles or gaps). In this case, an incipient process of concentric intramedullary keratinisation became visible. The connected structures were poorly differentiated. A hair follicle development alteration in the early sheathe and matrix formation stages was visible. Rudimentary hair follicles occurred as horny cysts with parakeratotic structures and degenerated central areas. Other hair follicle involution aspects showed the poorly defined borders of the sheathe layers, with only few atrophic sebaceous glands in their proximity. The hair follicle cortex which became thinner showed degenerated cells, whereas the sheathe layers were not well defined. Some of the cortex cells were replaced by air bubbles (small gaps), defining an ageing process which was visible in aged individuals with disintegrated hair axis. Another characteristic of this process was the absence of the medullary. In other hair follicles we noticed that the medullary and cortex had an almost homogeneous fibrillar structure, or the medullary and sebaceous glands were simply missing. The hair follicle diameter decreased significantly to 30 micrometres (0.03mm).
Moreover, the sebaceous glands were undergoing a disintegration process characterized by blurred outline with adipocyte-like cell appearance; some of the sebaceous
glands had disintegrating cells in the middle, which formed a lipid mass; the content stagnated due to the lack of contraction or arrector pili muscles atrophy. A rudimentary hair follicle in an advanced state of atrophy was revealed in some specimens, whereas the cortex cells had no nuclei, but were in a state of central hyperkeratinisation, with only the concentric fibrillar structures pointing to the initial follicle (Fig.2.22.).

In the Masson stained specimens we found the following pathological changes after the antiandrogenic treatment: inflammatory infiltrate in the epithelial component of the hair follicle in all the women; multinucleate giant cell granuloma in one woman; apoptotic cells of the epithelial cells of the hair follicle in 5 cases; many cells included vacuoles in the basal epidermal layer (intraepithelial lymphocytes). In a single case the inflammatory infiltrate invaded the epithelial component of the hair follicle. The apoptotic cells of the epithelial component of the follicle with acidophilic cytoplasm became detached from other epithelial components and had hyperchromic deformed nuclei. Langerhans cells were found in large numbers in 6 cases after treatment. They were disseminated on all the epidermal levels. Anti S100 proteins were detected mainly in the dendritic cells located in the epidermis, whereas vimentin was positive mainly in the dendritic cells in the basal layer and at the dermal-epidermal junction, as well as in the epithelial component of the hair follicle. The main cytoplasmatic processes extended towards the dermis and, in few cases, thin processes were noted among the keratinocytes. S100-positive dendritic cells were clearly noted in the dermis, especially in the chronic infiltrate areas (Fig. 2.23., Fig.2.24.)

![Fig.2.23. Vimentin-positive cells in the basal epidermal layer and in the upper dermis, x900.](image)

![Fig.2.24. S100-positive cells in the chronic inflammatory infiltrate, x400](image)

2 out of 6 cases with high Langerhans cell counts were chosen for electronic microscopy. A field-specific selection was done on ultrathin specimens. The differentiation criteria of the Langerhans cells from other clear cytoplasm cells were: indented nucleus, presence of nucleolus, lysosomes and multivacuolar bodies in the cytoplasm, and absence of desmosomes. Long-branched cytoplasmatic processes were noted between the keratinocytes and towards the dermis. Finally, we analyzed the specific cytoplasmatic granules described by Birbeck. In both cases, Langerhans cells were found in the middle of the epidermis, and at the
dermal-epidermal junction. Only typical “rode-shape” granules were found in one case. In the other case, both “rode-shaped” granules and tennis-racket-shaped granules were found. Dendritic cells containing Birbeck granules were found in the connective tissue, where the cells were surrounded by lymphocytes.

In hirsutism women in whom usual and special stains were used, we noted a normal appearance of the hair follicle in the androgen-dependent areas before the CPA treatment; a medullary, sebaceous glands and arrector pili muscles were visible.

Hair follicle and hair follicle adnexa involution under CPA 100 mg/day therapy for 12 months consisted of the occurrence of rudimentary immature and non-medullary vellus hairs. We noticed the following changes: hair follicle development stopped in the early stages of sheathe and matrix formation, and the occurrence of air bubbles inside the medullary. In some cases, we noticed the exacerbation of the hair infundibulum occupied by keratosis cysts. Moreover, reserve pilokeratoisis was noted in the deep invaginations of certain basal cells which correspond to rudimentary hair follicles. After treatment, the hair follicle diameter dropped down to 0.03 mm, these findings being similar to other literature data (Whiting, Howsden, 1996). Furthermore, the sebaceous elements were attenuated and the arrector pili muscles were fragmented in a manner which prevented it from contributing to hair verticalization.

Hair keratinisation, which normally occurs differently for each hair, occurred suddenly after treatment administration and skipped directly to the “hard” keratinisation stage. In particular, the Huxley layer was strongly keratinized together with the structures which normally become keratinized among the first elements. A discrepancy was noted between the development of mature sebaceous glands and the development of the hair follicles which were stuck in an early formation stage. Here are the factors regulating the hair growth cycle and the cell division process occurring in the hair matrix: steroid hormones, dermal-epidermal interactions and immune system.

Androgens are the main hormones regulating hair growth (Rosenfield, 2005). Androgens act on the hair follicle directly and/or independently of the serum level, generating a local effect, especially in idiopathic hirsutism (Azziz, 2000). Androgens increase the peripheral activity of 5-alpha-reductase and consequently their effects on the hair follicle. In addition to androgens, hair growth and hair differentiation are also set by local factors and circulating growth factors like TGF, EGF, SCF and VEGF. Factor release occurs in vitro under the influence of androgens (Botchkareva et al., 2001), functioning, at least some of them, by matrix metalloproteinase inducing (MMP). Immunohistochemistry located MMP9 in the lower section of the inner epithelial sheathe of the root, in the Henle layer (Jarrousse et al., 2001). These factors influence 5-alpha-reductase activity in both types. This enzyme may be located in the outer sheathe of the hair follicle root, with lower expression in the dermal papilla. Immunohistochemical studies (Commo et al., 2000) analyzed K19 cytokeratin manifestation, which is a marker of the stem cells of the hair follicle, as long as keratinocytes are similar to multipotent stem cells (Oshima et al., 2001). Similarly, the immunohistochemical studies conducted on our patients after systemic antiandrogenic
treatment revealed inflammatory infiltrate containing both lymphocytes and granulocytes at papillary, perivascular and perifollicular levels. Immunomarking using vimentin and S100 protein revealed the presence of dendritic-like cells in the epithelial tissue of the hair follicle.

Langerhans cells are mobile, considering that they are able to migrate in the dermis, participating in the synthesis of prostaglandin, interferon, lysozyme and certain lymphokines (Romagnoli, 2001; Banchereau, Steinman, 1998). Before antiandrogenic treatment administration, Langerhans cells which were identified with S100 protein and anti-vimentin, were located in the epidermis, demonstrating medium values in what concerns their numbers and distribution. In these cases, we did not note any positive cells in the dermal-epidermal junction or in the papillary dermis. After the antiandrogenic treatment, the Langerhans cells became more numerous. Anti S100 protein managed to identify most Langerhans cells in the epidermis, whereas anti-vimentin revealed dendritic cells in the basal epidermal layer, at the dermal-epidermal junction, in the upper section of the papillary dermis and of the epithelial tissue of the hair follicle. S100-positive dendritic cells were detected in the dermis, predominantly in areas with chronic inflammatory infiltrate.

This study relied on the high number of dendritic cells and on deciding whether the Langerhans cells found in the dermal-epidermal junction are representatives of the migratory cells which moved from the epidermis to the dermis or vice versa. Electronic microscopy revealed long ramified cytoplasmic extensions, either between the keratinocytes, or towards the dermis, and the presence of Birbeck granules. Similarly, we noted dendritic cells containing Birbeck granules in the connective tissue, which are surrounded by lymphocytes. Existing literature data do not describe such aspects. For this reason, the findings achieved by S100 immunomarking should be correlated with the antiandrogenic treatment, since in our opinion hirsutism itself cannot induce such changes. This high number could currently support the assumption according to which reactive mechanisms are induced by local hair follicle involution.

Antiandrogenic treatment led to hair follicle and sebaceous gland atrophy in 64.2 % of the cases. Hair follicle development was influenced in the early stages of matrix and sheathe formation, with the occurrence of vellus hairs and the formation of air bubbles in the medullary and cortex. The morphological and immunohistochemical tests performed on the teguments of 14 patients with idiopathic hirsutism revealed significant changes in the numbers and distribution of Langerhans cells in women undergoing antiandrogen treatment. On the other hand, Langerhans cell activation associated with inflammatory infiltrate in the dermis and hair follicles may be considered a reactive mechanism triggered by the local involution process of hair follicles.
CHAPTER 3
ULTRASONOGRAPHY IN SKIN PATHOLOGY

3.1. INTRODUCTION

Ultrasonography is an imaging assessment method which uses ultrasounds as image formation vector. This imaging technique with cutaneous applicability is a valuable “tool”, which is frequently used in medicine precisely due to features like repeatability, diagnostic value and lack of reported risks for patients, and which has also been employed in dermatology for over 30 years. Significant progress has been made in this field since 1979, when Alexander and Miller conducted an ultrasonography for the first time to measure skin thickness (Jasaitiene et al., 2011). It relies on the transonic wave reflection phenomenon, based on the keratin, collagen and water content of the tissues back to a transducer under the form of an imaging grey scale for interpretation.

This imaging technique has many applications in the diagnosis and therapy of skin diseases, including in melanoma, benign melanocytic lesion and benign tumour pathology, as well as in inflammatory diseases and fat removal, each of these entities being distinctively characterized by ultrasonographic techniques.

Squamous cell carcinoma is the second most common malignant skin tumour after basal cell carcinoma. Although the Gold Diagnosis Standard of this tumour is the histopathological examination, non-invasive diagnosis techniques have been increasingly used lately. In addition to dermatoscopy and confocal microscopy, high frequency ultrasonography is a real help in preoperative tumour assessment (Schmid, Rebecca et al., 2012).

A good correlation between the ultrasonographic technique and histological melanoma-type tumour measurements was achieved by using 20-100MH frequency transducers, which allow a 80-200 μm resolution and a penetrability ranging between 1.5 and 8mm (Warszawik et al., 2015; Jasaitiene et al., 2011). Nevertheless, the value of this technique has been discussed in literature, as all non-melanocytic tumour lesions have a hypoechogenic appearance, which suggests that when used alone this method is not useful in differential diagnosis setting (Desai et al., 2007; Gambichler et al., 2007; Mogensen et al., 2009). Nonetheless, this method should provide precise details about tumour size and thickness. The method should be used complementary in the preoperative stages of tumour lesions (Guitera
et al., 2008). Wortsman assessed in a retrospective study 4338 ultrasound scans and concluded that, when this technique is used to complement the examination protocol, the accuracy of clinical diagnoses increased from 73% to 97% (Wortsman, 2010).

**Our preoccupations related to the skin ultrasonography technique materialized in the following papers described in detail in the habilitation thesis:**

|——|

### 3.2. CUTANEOUS HISTIOCYTOMA

Dermatofibromas, also known as benign fibrous histiocytomas, nodular subepidermal fibrosis or sclerosing hemangiomas, is the second most common skin tumour. From the clinical point of view, it is characterized by a papular prominent lesion, more common in females (4/1), located mainly on the limbs. These tumour lesions are usually painless, although itching and local sensitivity may sometimes occur. The cause of these tumour formations is unknown (Han et al., 2011; Lehmer, Ragsdale, 2011).

The diagnosis of pigmented tumours of the skin may be often difficult (Wortsman, 2010). High frequency ultrasonography and Doppler ultrasonography may characterize these tumours and may help setting the positive and differential diagnosis of pigmented tumours of the skin (Clement et al., 2001; Trojan et al., 2010; Zaitsev, Semenov 2012). Elastography is a non-invasive method which assesses soft tissue elasticity. This method has the ability to increase ultrasonography specificity when assessing nodular cutaneous lesions (Giovagnorio et al., 1999; Harland et al., 1993; Jasaitiene, et al., 2011). This case-control study underlines the importance of the examination of skin tumours using these imaging methods.

Our study was aimed at analyzing the contribution of ultrasound imaging assessment in a particular type of skin lesions, namely histiocytoma. A female patient came to the Dermatology Clinic complaining of an irregular pigmented nodular lesion located on her right thigh. The lesion was initially small, of the fibrous papule type, and it progressed for 6-7 months and reached 2/3 cm in diameter at the time of hospitalization (Fig.3.1.). The patient did not have other tumours, autoimmune conditions or atopic dermatitis and she had not suffered any local injury. The dermatoscopic test revealed pigment at the periphery of the lesion and the presence of leukopigmentary maculae in the middle. Unlike melanocytic tumours, no atypical pigmentary network or vascular arborisations specific to basal cell carcinoma-like lesions was detected (Fig. 3.2.). High frequency ultrasonography (Dermascan C 20 MHz) revealed an 8.3mm thick homogeneous hypoechochogenic well-delimited mass impairing the hypodermis (Fig.3.3.). The grey ultrasound scale revealed a non-homogeneous
hypoechogenic well-delimited skin mass with a 36/32 mm horizontal diameter and a 27 mm thickness index; fluid bubbles were detected inside the mass, which corresponded to cystic areas (Fig. 3.4.).

The Doppler scan revealed the presence of an intratumoral signal, blood vessels located on the borders of the lesion and blood vessels with linear arterial character located inside the tumour (Fig. 3.5.). The vascularisation extent was correlated with the significant vascularisation (dilated vessels) identified inside the tumour on histological specimen examination. Elastography revealed its mixed appearance, as well as the peripheral rigidity and central elasticity of the lesion (Fig. 3.6.). Contrast medium ultrasonography allowed the assessment of intratumour vascularisation, arterial flow and partial wash out time in the concerned area and total contrast medium wash out time in the tumour (Fig. 3.7.).

The imaging diagnosis was benign firm and highly vascularised tumoral mass compatible with fibrous histiocytoma. The histological exam confirmed the benign fibrous histiocytoma diagnosis (Breslow 8mm) (Fig. 3.8.)

Skin tumours have been increasingly numerous and their early diagnosis is a major health issue. Ultrasonographic imaging techniques are an important non-invasive method which provides important data about skin lesions (Claudon, 2008).

In our case, from the clinical point of view, the lesion was large, with exophytic appearance, irregular pigmented surface, with negative retraction sign, non-infiltrative. The clinical diagnosis requires histological confirmation in order to rule out malignant melanoma, pigmented basal cell carcinoma or histiocytoma protuberans. Dermatoscopy only allows ruling out malignant pigmented lesions or pigmented basal cell carcinomas, but not other forms of malignant tumours. Conventional ultrasonography and high frequency ultrasonography provide significant complementary data related to the sizes, structure, elasticity, vascularisation and vascular flow, all these aspects being vital for diagnosis and treatment plan setting. The thickness index values higher than 20MHz, i.e. 8.3 mm, as compared to the histological one (8 mm) may be accounted for by the presence of the inflammatory peritumoral infiltrate. Similar data are also found in literature (Crisan et al., 2013).

Macrocirculation assessed by standard methods, i.e. Doppler scan, and microcirculation assessed by contrast medium ultrasonography reveal typical aspects of tumour benignity: peripheral circulatory pattern and slow contrast medium wash out (Claudon et al., 2008; Seitz et al., 2010; Strobel et al., 2008). Colour code elastography revealed different relations with the histological structure. Previous studies claimed that this technique is a highly sensitive diagnosis method in detecting different malignant lesions, based on the assumption according to which tumour cells are more rigid than normal adjacent tissues (Ishibashi et al., 2012; Hinz et al., 2013; Hinz et al., 2011). The specificity of this case is related, on the one hand, to the histological aspects, more precisely to the presence of xanthomatous cells, to vessel persistence in the fibrous stroma, to the presence of dilated parallel vessels, and, on the other hand, to the ultrasonographic correlations with the morphometry and vascularisation patterns. Combining conventional ultrasonography with
advanced methods (contrast medium ultrasonografia and elastography) has the great potential of improving the future distinction between benign and malignant tumour lesions.

Ultrasonography is a useful imaging method in the “in vivo” study of skin tumours and it provides important information which cannot be gathered by clinical or histological examination. It may also assess the blood flow and skin lesion elasticity.

**Fig. 3.1.** Clinical image of a 2/3cm irregular nodular tumoral mass located on the right thigh

**Fig. 3.2.** Dermatoscopic image of the tumoral mass: with pigment on the edge of the lesion and leukopigmentary maculae in the middle
Fig. 3.3. High frequency ultrasonography image of the tumoral mass

Fig. 3.4. Grey ultrasonographic scale

Fig. 3.5. Doppler scan

Fig. 3.6. Elastography
3.3. INTEGRATIVE ANALYSIS OF SKIN TUMOURS

Basal cell carcinomas (BCC) are the most common malignant skin tumours. Although they used to be considered specific to individuals over 40, their incidence rate has been increasing in the young population, probably due to excessive exposure to ultraviolet radiations (Bolognia et al., 2003). Early diagnosis and minimally invasive therapy which preserves the appearance and functions of the tegument are currently a constant preoccupation (Saurat et al., 2009). The diagnosis based on several criteria (clinical appearance, progress, time, location) was initially complemented by dermatoscopy, which reveals the vascular network inside the tumour, and it has been also supported by conventional and high frequency ultrasonography (US) lately. Ultrasound scanning provides additional information which complements the clinical and pathological diagnosis. It is easily accepted by patients, as it is a non-invasive inexpensive imaging technique, which provides useful information for skin tumour management, progress and prognosis (Crisan et al., 2013). Ultrasound scanning has gained ground in dermatology due to modern technologies and targets, in tumour pathology and also in chronic inflammatory diseases, enhancing the effectiveness of topical and systemic therapies, including of anti-aging therapies. The so-called ultrasonographic techniques provide morphological data in sections (bidimensional or 2D US) and vascular data (quantitative Doppler US- Mod Puls), or qualitative data (colourful flow map). New
techniques, such as elastography, contrast-enhanced ultrasonography (CEUS) (Kleinerman et al., 2012) and high frequency ultrasonography (HFUS) (Korde, 2007) have been introduced in recent years. Each technique provides special information, which may be included in the overall US examinations in order to achieve complex tumour assessment and to guide the doctors towards the best minimally invasive therapy, without any risk of local relapse. The goal of this study was to identify the ultrasonographic, especially morphological and vascular, criteria of skin tumours, in order to develop an integrative and differentiation imaging pattern for benign and malignant skin tumours. Twenty-three patients with large skin tumours were included in the study. The diagnosis procedures were the physical examination, dermatoscopy, high frequency multimodal ultrasonography (US) and conventional US, contact elastography and contrast-enhanced ultrasonography (CEUS). This linear prospective study included 23 patients, 15 men and 8 women, aged between 23 and 83. All these patients had nodular cutaneous tumours in photo-exposed or photo-protected areas. The patients came to the outpatient Dermatology Clinic for ultrasound scans between October 2012 and 10 May 2013. The inclusion criteria were small and medium nodular tumours without marked ulcerations at skin level (face, extremities, back of torso). The patients included in the study followed the same protocol: physical and dermatoscopic examination using a HEINE 20 device in water drops, and high frequency and conventional ultrasonography conducted in compliance with the standard procedures (Badea et al., 2010; Crisan. et al. 2010; Cammarota et al., 1998) and surgical excisions. In all the cases, the diagnosis was confirmed by histopathological examination. The ultrasonographic findings were comparable with the histopathological ones. HFUS with Dermascan 20 MHz device assessed tumour depth in millimetres from the epidermis level. The US tumour index was compared to the Breslow index. The clinical diagnosis together with the dermatoscopic diagnosis revealed elements of malignity (dilated surface vessels, located in the middle and on the edges of the tumour) and benign signs (no visible vessels or a few fine radial vessels on the edges of the tumour, in case of dermatofibromas). Conventional US (2D) was conducted by means of a state-of-the-art iU22, Phillips, The Netherlands device with soft tissue transducer with 7-13 MHz frequency. The echogenicity (in relation to normal dermis), homogeneity and depth of each tumour were assessed. Tumour rigidity was assessed by elastography. Conventionally, blue means rigid tissue, red-elastic tissue and green-intermediate rigidity. Tumour macrocirculation was assessed by colour Doppler flow (CFM) in order to reveal the Dopple pulse and vessels, to distinguish between veins and arteries and to measure velocity. The CFM pattern may be organized (ramified vessels) or disorganized (broken, discontinuous vessels) with central, peripheral or mixed distribution, depending on vessel location inside the tumour. The arterial or venous nature was assessed according to the type of flow (continuous or systolic-diastolic). Velocity was measured by placing the Doppler probe in the middle of the vessel by using the adequate correction angle. CEUS used a standard technique and assessed microcirculation in the tumour bed. The contrast agent (CA) (16 ccm Sono Vue, Braco, Italy) prepared immediately was administered intravenously in the elbow plica vessels, followed by 10ccm of saline solution. A double check was performed in all the cases, and the ultrasound data were
simultaneously displayed on the monitor. The examination continued after the injection and lasted about 2 minutes. CEUS allowed vascular dynamics assessment based on the following parameters: tumour absorption time (seconds after the intravenous injection), CA injection time until reaching the highest intensity (peak time), discharge time (from the peak until CA disappearance) and overall CA duration (until its total disappearance). Intratumoral vessel outlay and contrast agent absorption during the arterial time defined an even loading pattern (full loading of the vascular bed) or an uneven loading pattern. The histopathological examination was done by means of the haematoxylin and eosin stain. The histological diagnosis determined the type of histological differentiation and depth extension (Breslow index), measured from the granular layer to the deepest tumour point.

A comparative data analysis focused only on relevant parameters for tumour description. We performed a correlation analysis (Pearson coefficient), a regression analysis (simple linear regression) and a graphical representation between ultrasounds and histological changes. The correlation between the vascular times of CA dynamics was graphically illustrated (by observation) and by mean calculation. Vascular time interpretation was only graphical due to the small number of benign tumours. Of the 23 tumours included in our study, 18 were malignant and 5 benign (table 3.1.). The ultrasonographic findings are shown in table 3.2. Thus, malignant tumours appear like hypoechoic non-homogeneous masses with many arterial disorganized vessels, occurring both in the middle and on the edges, with a blood flow velocity of >2 cm/sec and faster CA elimination time than in benign tumours. Benign tumours appear as hypoechoic or echogenic masses, with non-homogeneous structures, Doppler signal present only in dermatofibromas, with peripheral blood flow tributary to the viewed vessels, velocity of <2.00 cm/sec. CEUS revealed a poor and uneven contrast agent (CA) load in the vascular bed, the presence of supply vessels in 5 benign tumours in a slow elimination time (Fig 3.9., Fig 3.10.). As regards tumour thickness, there was an important correlation coefficient (i.e. 0.97) between the Breslow index and the HFUS values (Fig 3.11.). Regression analysis indicates the fact that the histological index may be assessed by using the US index. Therefore, the ultrasonographic (US) index may be considered a very important predictive factor from the statistical point of view (p < 0.05), very precise (r =-0.97) for the non-invasive assessment of the histological index. The value of the histological index will be 2.26 mm for a US index of 2.7 mm. Also, a significant difference may be noted between the elimination times of different types of lesions. The CA dynamics analysis revealed a significantly higher value of the elimination time of malignant tumours (38.2s± 15.15) than the elimination time of benign tumours (54.2s ± 8.5) (Fig 3.12.). In our study, elastography revealed the rigidity of all the studied lesions. Although the role of ultrasonography in skin tumours is described in many papers, there are additional aspects, which bring about important information complementing the clinical, dermatoscopic and histological aspects (Vogt et al., 2003; Badea et al., 2013). Skin ultrasonography is a valuable skin tumour analysis method tightly connected to physical and pathological examination. It is an accessible technique, easily accepted by the patient, which provides highly sensitive information, but has low specificity. Different ultrasonographic devices were studied with frequencies ranging between 7
and 100 Mhz, and the pros and cons of ultrasonography in dermatology were listed (Bamber et al., 2013; Lutz, Soldner, 2011).

### Table 3.1. Histological diagnosis of skin tumours

<table>
<thead>
<tr>
<th>Histological diagnosis</th>
<th>Malignant tumours (n=18)</th>
<th>Benign tumours (n=5)</th>
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<td><strong>Malignant tumours</strong></td>
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<td>Keratotic differentiations</td>
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<tr>
<td>Cystic/adenoid differentiation</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Metatypical</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><strong>Benign tumours</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Keratoacanthoma</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Actinic hypertrophic keratosis</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Dermofibroma</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

n = number of cases

### Table 3.2. Comparative findings in malignant and benign skin tumours

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Malignant tumours (n=18)</th>
<th>Benign tumours (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Morphometry</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Echogenicity</td>
<td>Hypoechochogenic (18/18)</td>
<td>Hypoechochogenic (2/5)</td>
</tr>
<tr>
<td>Echostructure</td>
<td>Non-homogeneous (18/18)</td>
<td>Non-homogeneous (5/5)</td>
</tr>
<tr>
<td>Tumour depth</td>
<td>2D US: 1.5 – 6 cm.</td>
<td>2D US: 0.8 – 1.2 cm.</td>
</tr>
<tr>
<td></td>
<td><strong>Dermascan</strong>: 1 – 6.02 mm.</td>
<td><strong>Dermascan</strong>: 1 – 1.05 mm.</td>
</tr>
<tr>
<td>2. Tumour macrocirculation (Doppler US)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Doppler signal</td>
<td>Present 16/18</td>
<td>Absent 3/5</td>
</tr>
<tr>
<td>Proof of vein supply</td>
<td>15/18</td>
<td>2/5</td>
</tr>
<tr>
<td>Circulatory patterns</td>
<td>Disorganized</td>
<td>Relatively organised</td>
</tr>
<tr>
<td>Venous distribution</td>
<td>Central 12/18</td>
<td>Peripheral 5/5</td>
</tr>
<tr>
<td></td>
<td>Mixed 6/18</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt; 2 cm/sec</td>
<td>&lt; 2 cm/sec</td>
</tr>
<tr>
<td>3. Tumour microcirculation (CEUS)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CA absorption pattern</td>
<td>Non-homogeneous 18/18</td>
<td>Poor, non-homogeneous 5/5</td>
</tr>
<tr>
<td>CA absorption</td>
<td>Venous supply 18/18</td>
<td>Venous supply 5/5</td>
</tr>
<tr>
<td>Time until maximum CA signal intensity</td>
<td>9 – 22 seconds</td>
<td>10 – 20 seconds</td>
</tr>
<tr>
<td>Complete wash out (from the peak)</td>
<td>12 – 33 seconds</td>
<td>14 – 26 seconds</td>
</tr>
<tr>
<td>Signal persistence</td>
<td>25 – 90 seconds</td>
<td>27 – 38 seconds</td>
</tr>
<tr>
<td>4. Elastography</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rigidity</td>
<td>Rather rigid 18/18</td>
<td>Rather rigid 5/5</td>
</tr>
</tbody>
</table>
**Fig. 3.9.** Nodular basal cell carcinoma: a) clinical image; b) dermatoscopic image: ramified, dilated tumour vessels; c) histology: groups of basaloïd cells with keratotic and cystic differentiations, with dermis infiltrates; d) HFUS: hypoechogenic masses, with hyperechogenic echoes corresponding to keratotic pearls; e) 2D US: hypoechogenic, non-homogeneous structure, blurred outline, shadow cone, with intratumoral keratinic mass expression; f) Doppler scan: exophytic tumour revealed; the Doppler images reveal the type of arterial blood flow, namely at high velocity and turbulences; g) elastography: relatively rigid mass; h) CEUS dynamics: poorly non-homogeneous load of the vascular bed, a single supply vessel, load limit (24 sec).

**Fig. 3.10.** Actinic keratosis: a) clinical appearance; b) dermatoscopic appearance: surface ulcerations, keratin deposits, without intratumoral vessel view; c) histology: keratin deposits, papillomatosis, inflammatory infiltrate; d) 2D US: hypoechogenic mass, blurred outline, absent Doppler signal, no supply vessel; e) elastography: rigid tumour; f) CEUS dynamics: poor non-homogeneous load, supply vessel, load peak (26 sec).
Our findings are along the same line as those of Wells and his team (Wells, Liang, 2011), and show an important correlation between the ultrasonographical and histological aspects, as concerns tumour thickness. The small differences due to histological over-assessment or US may be accounted for by the perilesional tumour infiltrate, tissue retraction induced by the histological cut and, last but not least, by the examiner’s expertise, as ultrasonography is an examiner-dependent method (Guitera et al., 2008; Dudea et al., 2012). Our study shows the need to cumulate ultrasonographic criteria, based on morphological changes, and hemodynamic criteria, in case of solid skin tumours. An important correlation was noted between the US aspects and the histological ones. Non-homogeneous (hyperechogenic or anechogenic) structures were correlated with rich intratumoral vascularisation, fibrous stroma and type of histological differentiation. Similar aspects were mentioned in literature, without however mentioning the histological differentiation (Wortsman, Jemec, 2013). The blood flow pattern provides information supporting the clinical diagnosis. Thus, the Doppler pattern with central or mixed circulation, with higher velocity in malignant tumours (>2cm/s), as compared to the peripheral pattern with lower velocity in benign tumours (<2cm/s) are elements correlated with clinical and histological criteria. Similar aspects were mentioned in literature (Kohl et al., 2011). When comparing capillary velocity in physiological conditions (1mm/s), we found that the findings of the study were relevant for tumoral lesions. The absence of proof of collateral vessels and vascular bed, in certain cases, illustrates the limited accuracy of Doppler scanning, which has been clearly outrun by CEUS (Badea, 2010). CA analysis suggests that vascular tumour dynamics depends on several factors (vascular resistance, shunts, histological type, location). We found no reports in literature about
vascular CA dynamics in BCC. Studies conducted on a higher number of patients are necessary in order to establish a blood flow pattern for skin tumours. CA tumours consume time and the time elapsed until reaching the maximum signal intensity (peak time) was not relevant in our study. Depending on our results, the elimination time may represent an important differentiation criterion between skin tumours. An analytical study on larger groups should establish the elimination time as an objective skin tumour differentiation criterion. CEUS has been frequently used when assessing tumour pathology of internal organs; yet, as shown here, it may be successfully used in oncodermatology (Seitz et al., 2010; Strobel et al., 2008). Special attention should be paid to tumour rigidity. In our study, elastography revealed rigidity in all the lesions under survey. According to literature, elastography has a > 95% sensitivity and 85% specificity in extra-cutaneous tumour detection and classification (breast, prostate) (Ginat et al., 2008). The role of elastography in skin tumours has been hardly discussed, and some authors pointed out the role of elastography in melanocytic tumours and cutaneous carcinomas, more precisely its high predictive values in patients with clinical skin cancer. (Aoyagy et al., 2009; Schmidt-Wendtner, Dill-Muller, 2008). Our study emphasizes the need to use imaging criteria focused on cumulative morphological and hemodynamic aspects of skin tumours. The complex and multimodal approach, correlated with clinical and histological findings, is an excellent non-invasive skin tumour differentiation method. According to our findings, the malignant imaging criteria in BCC are: non-homogeneous structures, rigidity, abundance, non-homogeneity, arterial vascularisation at velocities of > 2 cm/sec, vessels with central and peripheral location, intense and irregular contrast agent loads, and short elimination time. The limitations of the study are related to the small number of patients, to the selected group and to the operating theatre-dependent investigation, which may be responsible for the errors encountered in the findings. Ultrasonography is a virtual biopsy method. This allows a complex and multimodal approach to skin tumours, which complements the physical and histological examinations, guides therapy management and assesses therapeutic effectiveness and tumour prognosis. It is a modern non-invasive technique used to translate a diagnosis from the clinical to the fundamental research fields. Additional research is necessary in order to turn elastography into an important imaging marker used to differentiate skin tumours.
CHAPTER 4
DERMATOLOGY AND RARE DISEASES

4.1. INTRODUCTION

Genodermatosis is a big group of genetically transmitted rare conditions, the recognition of which is important not only with a view to finding the adequate therapy, but also to identifying abnormalities that may be associated with these frequently multisystemic diseases, including neoplasm (Spitz, 1996). The Bloch Salzberger syndrome (Incontinentia Pigmenti) often has cutaneous impairment as its first sign in all patients suffering from this syndrome. The diagnosis setting criteria were first suggested by Landi and Donnai in 1993, based on the clinical data of 111 patients. These criteria were later reviewed by Minic et al. in 2013 in the light of new information related to the genetic origins of the disease, and new clinical criteria, such as central nervous system abnormalities, ocular abnormalities except for the retina, oral cavity abnormalities, mammary gland abnormalities and histopathological abnormalities were included (Yasmine, Bernard, 2015). Pachydermodactyly was first described in 1973 by Bazex and, according to the references (Sang-Hee Seo, Hyun-Woo Sung, 2011), 88 cases have been reported worldwide. Nevertheless, their total number is undoubtedly higher, as many of them have not been reported yet. The Ekbom syndrome, also known as delusional parasitosis (Freudennmann, Lepping, 2009), was fully clinically described by the Swedish neurologist Karl A. Ekbom in his works of 1938. Before that, it had been known under different names: dermatophobia, delusional infestation or parasitophobic neurodermitis, although this is not a parasite phobia (the patent is not afraid of parasites, but is convinced that his/her body in invaded by them) (Aw et al., 2004).

Our interest in rare skin conditions materialized in the following papers, which will be described in detail in the habilitation thesis:


4.2. BLOCH-SULZBERGER SYNDROME

The Bloch–Sulzberger syndrome is a X-linked genetic condition, with dominant autosomal transmission, due to IKK gamma of NEMO gene mutation, located on q28 (IP2) of the X chromosome. These data supported by genetic studies account for the frequent cause of foetal death in the male gender (Berlin et al., 2002). The number and distribution of cells containing the abnormal chromosome determine the severity of the disease and it is clinically characterized by the occurrence of four stages: vesicular inflammatory, proliferative warty, hyperpigmented and atrophic stages.

A 6-year-old female patient, the only child of parents with no apparent personal pathological history, who was term delivered, with a 2400g weight on delivery, with Apgar score 8, was hospitalized in the Dermatology Clinic. Her mother mentioned the presence, since the birth of the child, of linear hyperpigmented lesions located on her arms, legs and torso, as well as the occurrence, before the child was one year old, of warty lesions located in her right inguinal area, left armpit, front and back of the torso, as well as on her scalp. The patient’s height, weight and intellectual developments were normal. The skin lesions persisted despite various treatments, for which reason she came to the Dermatology Clinic.

The physical tegument and mucosa examination revealed linear hyperpigmented grey-brownish well-delimited lesions on the flexion face of her upper and lower limbs, hyperpigmented lesions looking like bands with winding outline on the anterior face of her left hemithorax and reticular hyperpigmented lesions on the back of her torso and posterior cervical region. Also, warty well-delimited plates were noted in the right inguinal area, and squamous-crust plates, maculae and atrophic hypopigmented plates were detected on the front of her right hemithorax and right armpit area, accompanied by hypohidrosis. Alopecic plaques and depigmented hairs were detected on her scalp. No changes in her skin appendages were detected.

The ophthalmological examination revealed the presence of left congenital palpebral ptosis without anterior pole changes. Nonetheless, the refractometry revealed minor hypermetropia in the right eye, astigmatism and compound hypermetropia in the left eye, as well as minor amblyopia. No dental changes were detected, except for multiple deteriorations, with no skeletal abnormalities.

Laboratory data revealed the presence of moderate leukocytosis, correlated with bacterial scalp infection (the cultures identified the presence of Staphylococcus aureus) and moderate 14 % eosinophilia, with no clinical atopy stigmata.

The histopathological examination of the warty lesions revealed unspecific changes, similar to post-inflammatory residual lesions with the presence of hyperkeratosis, papillomatosis, even hyperpigmentation of the basal layer and the presence of a perivascular and periadnexial lymphocyte and plasma cell infiltrate in the papillary dermis.

The reported case meets the criteria of a classical case of Bloch Salzberger syndrome, supported by the presence of linear hyperpigmented grey-brownish band-like lesions located symmetrically on the Blaschko line paths and hyperpigmented winding lesions on the body,
which may be associated with multisystemic impairment and ocular abnormalities (the patient was diagnosed with congenital orbicular muscle paresis in her left eye and hypohidrosys).

This syndrome is quoted in literature as triggering, in addition to neurological and cutaneous symptoms, ophthalmological impairment and dental dystrophy. It is a rare condition, with only 700 cases reported in literature, being more common in Caucasian individuals. Yet, such cases have also been reported in Asians and Blacks. The first case was reported in 1906, being later defined by Bloch and Sulzberger in 1926 and 1928, as X-linked dominant syndrome. More than 95% of the patients were females. It has been rarely reported in the Klinefelter syndrome, in the male sex (Kenwrick et al., 2001).

The specificity of this case is represented by the absence of the vesicular stage (possibly manifested in utero), by the early onset of hyperpigmented lesions located along the Blaschko line paths, the coexistence of warty lesions and persistence of these lesions on the scalp, in the right inguinal region, left armpit, and the presence of hypopigmented and atrophic plates, associating squamous-crusty or warty lesions on the front of the torso, right armpit and the detection of eosinophilia specific to the inflammatory stage. In this particular case, the lesions may reoccur due to eosinophilia, but the intricate lesions, with hyperpigmented warty predominance with no severe symptoms, did not confirm them.

4.3. PACHYDERMODACTYLY

Included in the category of superficial fibromatoses, pachydermodactyly defines a painless skin condition, characterized by an increase in the volume of the tissues around the proximal interphalangeal joints of the hands, as suggested by its very name (from the Greek pachys= thick, dermatos = skin, dactylos = finger) (Rusu, 2001).

First of all, it is more common in males. Second of all, the hypertrophic changes evolve progressively and symmetrically, being exclusively limited to periarticular skin structures, the tegument involved representing no other semiological aspects (Bucur, Opriş, 2002).

Its incidence in north-eastern Romania has been increasing lately. Thus, we bring into discussion 3 cases of classical, sporadic pachydermodactyly, in three male children, aged 8, 11 and 13, respectively, two from the rural area and one from the urban area, diagnosed with pachydermodactyly during the past year and monitored in the Dermatology Clinic. The children came to the Dermatology Clinic for the diffuse asymptomatic thickening of the skin around the proximal interphalangeal joint of the II, III, IV fingers, in two cases, in bilateral hands (Fig. 4.1.), and in one case, unilaterally.

The anamnesis showed that the changes started several years before, repeated microtraumas competing in their ethiopathogeny, incurred at the agricultural activities in which children from rural areas are actively involved, as well as from the tic of rubbing their hands, especially during emotional times, a behaviour observed by their parents before the occurrence of the “deformations” of their fingers. The global assessment conducted at the Paediatrics Hospital excluded an autoimmune mediated inflammatory pathology, the
biological, biochemical and immunological test results being within normal ranges. Moreover, the hand x-rays objectify the normality of all the joints at this level, the secondary nuclei having an appearance consistent with their age (Fig. 4.2.).

The explorations continued with the comparative assessment of the hands using the ultrasound, which denied the existence of any pathological collection at the level of the radiocarpal, metacarpal-phalangeal, proximal interphalangeal joints, with normal-looking extensor tendons without tenosynovitis. The clinically-obvious deformations were exclusively based on the thickening of the skin. At this level, the skin thickness was 3.4 mm compared with 0.7 mm in the unaffected areas, on the back of the wrist (namely 5 times bigger). The thickness of the skin at the level of the scalp was also measured, however, at this level, it was normal, which ruled out pachydermoperiostosis (Touraine-Solent-Gale syndrome).

Corroborated, the clinical and the imaging appearance establishes the diagnosis of pachydermatous-dactylitis, a diagnosis which was later confirmed by the anatomo-pathological examination, which showed that the epidermis was unevenly thickened, with epidermal papillae slightly elongated, with acanthosis and discreet spongiosis; the corneum layer exceeded the thickness of the epidermis, and the granular one was prominent; the dermis showed marked collagenization (Fig. 4.3.), and the glands and adipose lobules were dissected by thick connective septa (Fig. 4.4.).

![Skin changes with diffuse asymptomatic thickening of the skin around the proximal interphalangeal joints of the II, III, IV fingers bilateral in the patients’ hands](image)

**Fig. 4.1.** Skin changes with diffuse asymptomatic thickening of the skin around the proximal interphalangeal joints of the II, III, IV fingers bilateral in the patients’ hands
In all three cases, the investigations carried out, especially the anatomo-pathological examination allowed to differentiate between the clinically-obvious lesions and those with similar symptoms, such as pachydermoperiostosis, juvenile chronic arthritis, rheumatoid arthritis, gout (gouty tophi), lichen myxedematous, psoriatic acropachyderma, keratoderma, acromegaly, fibrosarcoma, finger arthritis (Heberden nodules), but also joint touch associated with endocrinopathies. Caught in an early stage of the disease, the patients benefited from a favourable response to topical corticosteroid therapy, requiring no other invasive therapeutic methods. The changes described were included in the same degree of severity in terms of touching the soft parts, even if they were present in a greater number of fingers in the children in which bilateral touches were applied.

Potent topical corticosteroids were used in the form of occlusive dressing, maintained for 8-10 hours/day for 3 weeks, after which the frequency of the applications was reduced. Psychological therapy was also recommended, which targeted counselling (both of the child and of the parents), by giving up the action of rubbing hands under the impulse of psycho-emotional stress and learning new relaxation techniques, aimed at correcting/changing this behaviour/tic. The evolution was favourable, achieving improvements after the first weeks of local corticosteroid therapy (Fig. 4.5.). Pachydermodactyly is considered to be a form of localized surface fibromatosis (Weedon David, 2010), which affects young people, the average age being 21.2, according to Sagransky, the male - female ratio being 3:2 (Matthew et al., 2012). These changes often target the dorsal and lateral part of the proximal phalanx of the index and medius, with symmetrical tough, in both hands (Burns et al., 2010). The mechanisms involved in the pathogenesis of the disease are not fully clarified, some authors claiming that it is induced by repeated local micro injuries. In this regard, Sagransky et al. reports 2 cases of pachydermodactyly in workers in the poultry meat processing industry, working at a rapid pace and with significantly large quantities (Diaconu et al., 2002). Other authors recall, as a possible mechanism, the hand rubbing tic which, in time, leads to diffuse skin thickening and hyperpigmentations along the metacarpalphalangeal joints.
The male sex, childhood/adolescence and stress are the main contributory factors in the clinical expression of pachydermatous-dactylitis. The medical literature describes cases of pachydermatous-dactylitis associated with the carpal tunnel syndrome, Dupuytren's contracture, gynecomastia, varioliforme macular atrophy, thyroid disorders, Ehlers-Danlos syndrome, however, the most common association is that with local microtraumas. Starting from these, pachydermatous-dactylitis comprises five clinical forms:

- classical - associated with mechanical strain, covering several fingers;
- localized or mono-pachydermatous-dactylitis;
- familial;
- extended to the metacarpophalangeal joints and the back of the hand;
- asymptomatic - associated with tuberous sclerosis (José et al., 2004).

The clinical elements and especially the history of repetitive trauma are sufficient to establish the diagnosis of pachydermatous-dactylitis, yet the histopathological conclusions are useful to exclude other diseases that manifest similar symptoms (Itin, Beltraminelli, 2009).

Thus, the histopathological examination is not absolutely necessary, given that the diagnosis of pachydermatous-dactylitis is clinical, yet it can be supported by this investigation, which illustrates an increase in the collagen at the level of the dermis, with varying degrees of hyperkeratosis and acanthosis. The dermis is thickened with thick collagen fibres and the
discrete proliferation of fibroblasts, with an increase in their activity and, sometimes, with collagen deposition around the sweat glands.

The association of acanthosis with the thickening of the dermis is not specific to pachydermatous-dactylitis, but it is, nonetheless, one of its characteristics, as well as a useful criterion that helps to differentiate pachydermatous-dactylitis from other similar diseases, such as keratoderma, where the thickening of the dermis does not occur, or fibromatosis, which does not usually dispose of epidermal changes (Itin, Beltraminelli, 2009). The patients discovered in the early stages responded favourably to local corticosteroid therapy, the dermocorticosteroids proving their efficiency through its anti-inflammatory effects and its ability to inhibit cell proliferation as well as collagen synthesis (Diaconu et al., 2002).

The anti-inflammatory effect of corticosteroids is explained through the inhibition of the release of phospholipase A2, the enzyme responsible for the synthesis of prostaglandins, leukotrienes and other derivatives of arachidonic acid. Moreover, corticosteroids also inhibit transcription factors such as activator protein-1 and the nuclear factor kB, involved in the activation of the proinflammatory genes lipocortin and p11CBP (calpactin binding proteins). The immunosuppressive properties lie in suppressing the synthesis and effects of the hormonal factors involved in the inflammatory response, thus inhibiting the migration of leukocytes to the site of the inflammation and interfering with the functions of the endothelial cells, of the granulocytes, mastocytes and fibroblasts. The anti-proliferative role is exercised by inhibiting DNA synthesis and mitosis, providing a partial explanation for the therapeutic action of these agents in the dermatological pathology (Wolff et al., 2008). The effectiveness of topical corticosteroids depends on their potency and on the degree of skin penetration, their activity being closely related to the ability to bind to a specific receptor from cytosol, but also to the vehicle in which it is incorporated (Diaconu et al., 2002).

Apart from local action, the systemic absorption of topical corticosteroids - which depends on the pharmacokinetic properties of the molecule, but also on the integrity of the stratum corneum and the presence/absence of inflammation - is also important (Diaconu et al., 2002). Thus, the topical treatments applied at the level of the skin or mucous membranes benefit from an increased absorption if the skin surface is injured or if occlusive dressings are applied (Buchanan, Courtenay, 2006). The absorption at the level of the skin is done by the diffusion of the dermocorticoid in the stratum corneum. The hydrophilic steroids have a higher rate of penetration through the stratum corneum than through the hydrophobic one; moreover, keratinocyte penetration is faster with lipophilic agents, which explains many of the clinical effects of topical corticosteroids. After the diffusion in keratinocytes, corticosteroids bind to the glucocorticoid receptor from the cytoplasm, inducing the cascade reaction which results in messenger RNA synthesis mediators. The greater the lipid solubility of the dermocorticoid, the greater the binding capacity of the receptor in the cytoplasm.

There are many cells possessing glucocorticoid receptors in the human body, a fact which explains the effects of the glucocorticoids mediated by the receptor. In using local corticoids, a fairly important issue regards the phenomenon of tolerance, which could indicate a discontinuous administration, using a double dose, once every two days.
Withdrawal constitutes another problem which can be avoided, like in using corticosteroids systemically, through the progressive spacing of the applications, or through daily applications, using another topical corticosteroid with a lower potency. The complications which occur mostly in the case of local corticosteroid therapy with powerful agents (class 3 and 4) must also be taken into consideration; these include: skin atrophy, stretch marks, purpura, extending the healing time of wounds, rosacea, pigmentation disorders. Systemically, the most common complications are hypothalamic–pituitary axis suppression and Cushing's syndrome (Diaconu et al., 2002).

The close analysis of clinical elements and the noninvasive minimal investigations can establish the diagnosis of pachydermodactyly and could eliminate unnecessary costs related to sophisticated imaging explorations.

4.4. EKBOM SYNDROME

The Ekbom Syndrome is clinically characterized by pruritus triggered by parasites that crawl under the skin. Patient calls them by various names: insects, larvae, worms, beasts, bugs (Boggild et al., 2010; Fellner, Majeed et al., 2009).

Even thought the syndrome is an illusion of the patient that she/he has parasites, it is always accompanied by tactile hallucinations. The patients feels crawling under the skin, stinging, burning, or pruritus forcing him/her to resort to various methods to remove the parasites, thus causing self-induced skin changes (abrasions, ulcerations) for which she/he then seeks the help of the dermatologist. Moreover, the patient also has visual hallucinations (Boggild et al., 2010), assimilating scaling or waste from clothing or skin. At the beginning, the symptoms can be controlled, the patient being able to have a normal social life. In time, however, she/he isolates from everyone, even from his/her family, in order not to infect others. In their view, parasites also comprise things that surround them, developing an obsessive behaviour for cleanliness and disinfection and sometimes even destroying their goods (Hinkle, 2010).

My personal experience includes 2 cases of 2 patients suffering from Ekbom Syndrome within the dermatology clinic who were directed to specialised psychiatric treatment.

Case 1

The patient C. G., aged 68, from the urban area, without a significant pathological personal history, addressed the clinic in April 2013 due to a papulovesicular skin rash, intensely pruritic, without a typical temporal character of the pruritus, with onset approximately 2 months before, after a bus ride alongside ethnici passengers. Initially, the patient received an etiological antiparasitic treatment (both for scabies and pediculosis), a topical and systemic antipruritic treatment, however, the symptoms persisted, even though, during hospitalization, the skin lesions improved significantly (under supervision, the patient did not cause any more abrasions). Two weeks after he was discharged, the patient returned to the clinic because the symptoms persisted and the skin lesions, intensely excoriated after
scratching, were accentuated. In the patient's account of his medical history, he described pruritus as being triggered by bugs which move under the skin, throughout the body, including the scalp. Moreover, this time, he brought proof, i.e. scaling he collected from the areas of his body which suffered from pruritus, which he claimed to be bugs parasitizing him. Multiple investigations were carried out in order to exclude the organic causes of a pruritus (parasitic, allergic, metabolic, paraneoplastic), and therapy was administered in order to treat the symptoms, but only declarative, without improvement. The patient was redirected to psychiatric examination, suspecting Ekbom Syndrome, but he did not show up for his appointment. Later, for about a year, he made repeated visits to the dermatology clinic with severe pruritic symptoms related to bugs under his skin, the recommended treatments having no results, the patient's skin revealing many intense lesions, some caused by scratching, others by various objects aimed at removing and identifying the parasites. Finally, the patient accepted the psychiatric consultation, which confirmed the Ekbom Syndrome, and followed the recommended psychotropic therapy. After that, the patient stopped coming for dermatological assessment.

**Case 2**

The 72 year old female patient M.A. came to the dermatology clinic with a papulocrustous disseminated rash, with multiple round-oval and linear erosions caused by scratching, covered by hematic crusts, some ulceration-deep, without reason to suspect skin parasites. She declared the onset 2 years before, when she was stung by an insect who laid eggs under her skin. It was then that the pruritus caused by the movement of the larvae started. In time, she followed multiple antiparasitic and symptomatic systemic topical treatments but without relief. The patient admitted to have removed the parasites from under the skin with a nail clipper. The paraclinical explorations excluded any (exogenous or endogenous) cause of pruritus, the patient being directed to the psychiatric clinic during the period of her hospitalization, thus confirming the diagnosis of Ekbom syndrome. Both patients had a particular social status, being isolated from the rest of the family especially due to the presence of these potentially contagious parasites, in their view, the quality of life being, in these cases, much lower.

The Ekbom Syndrome is an affection that mostly affects females, the data available in the literature estimating the sex ratio of 2.8 (Benattar et al., 2004), with onset in older ages, i.e. 64 years old on average (Bourgeois, 2011). The people affected can suffer, before the onset of this syndrome, of social isolation, or can have a paranoid personality (Trabert, 1995). It can be difficult to diagnose a patient who suffers from the Ekbom syndrome, requiring differential diagnosis with hypochondriacal of obsessive-compulsive disorder manifestations. In order to establish the etiology, it is recommended to differentiate between a primary or secondary parasitic delirium. Within this syndrome, delirium is considered to be primary, while hallucinations are limited to skin, the delusions being monothematic. In secondary forms, however, the delusions form a broader picture, having a psychiatric or organic etiology (Lepping et al., 2007). Moreover, it is also recommended to detect the possible etiology of the chronic pruritus in the context of other diseases, such as kidney or liver failure, jaundice,
anaemia, hypovitaminosis or viral infections (HIV, hepatitis) (Dunn et al., 2007). The investigation panel must also target cerebral organic causes, such as dementia, tumors, cerebral infarction or meningoencephalitis, in corroboration with the clinical data.

Even though, at first sight, this syndrome is not extremely severe, as it does not endanger the patient’s life, it is, nonetheless, disabling as regards the patients’ quality of life. There are patients who decide to isolate themselves from their families, who give up their social activity, due to their conviction that they are intensely inhabited by parasites, and they do not want to infest those around them. The diagnosis is confirmed by the psychiatrist, even though the dermatologist is the one who initially suspects it. The difficulties occur when the patient refuses to accept the underlayer of their disease, thus delaying the treatment and the clinical cure of the disease. Patients suffering from Ekbom syndrome seldom seek psychiatric help, due to their difficulty in accepting the fact that their skin lesions do not require dermatological etiological treatment, but psychiatric one (Hinkle, 2011). Psychiatric treatment is, therefore, a challenge, patients showing a resistance called “psychiatrization” in the literature, their frustration leading some to a suicidal behaviour, or to one that endangers those around them. There have been cases successfully treated through electrotherapy or by the administration of antidepressants, but their role is controversial (Bak et al., 2008). The latest studies indicate the therapy with neuroleptics such as Risperidone, Olanzapine and Amisulpride as the first choice therapy (Freudenmann, Lepping, 2008).

Interdisciplinary collaboration is important in order to allow the diagnosis of the Ekbom Syndrome, but especially to choose the correct treatment, for which the psychiatrist is fully responsible (Hinkle, 2011). Many patients do not regard their disease as being a psychiatric one, which is why they delay its diagnosis and treatment by making repeated visits too many dermatologists, trying a variety of symptomatic and etiological antiparasitic therapies which yield no result. All this time, their general condition worsens, the quality of their and their family’s lives is considerable reduced. Establishing a presumptive diagnosis, as early as possible, by the dermatologist increases the patient’s chances to see a psychiatrist as soon as possible and to benefit from the appropriate treatment.
CHAPTER 5
ETHICAL AND LEGAL MATTERS IN DERMATOLOGY

5.1. INTRODUCTION

Ethics (from the Greek ἑθος = custom, habit), also called the science of moral reality, is a branch of philosophy which studies moral principles, norms and values. Medicine has always been accompanied by ethical thought, the medical profession, alongside that of leader and priest, being often seen as a strong profession which requires “strong morals”, based on the Hippocratic oath.

Potter stated in Bioethics (Potter, 1970) that “I chose the root ‘bio-’ in order to represent biological knowledge, the science of the system of beings, and ‘ethics’ to represent the knowledge of the system of human values”. Maurizio Mori claimed that bioethics is the name that would be given to the transition from the traditional ethics of the sacredness of life to the new ethics of the quality of life. Moreover, the International Bioethics Committee (IBC) summarizes bioethics as a field of systematic, pluralistic and interdisciplinary study that approaches moral, theoretical and practical issues applicable to medicine and to life sciences that affect human beings, all of humanity, including the biosphere.

The basic ethical principles are represented by the respect for people, beneficence and justice. The respect for people, mirrored in medicine, in the relationship between physician and patient, targets the ethical conviction that individuals should be treated as autonomous individuals, and that people who have diminished autonomy are entitled to protection. The principle of beneficence lies in the well-known Latin phrase “primum non nocere” as well as in the cumulation of some benefits with the potential to minimize, as much as possible, the negative effects. Claude Bernard was the one who extended the Hippocratic dictum “do no harm” from the therapeutic field to that of research, and noted that people, in this case,
patients under medical supervision, should not be done harm, regardless of the benefits it would bring others.

Deontology comes from Greek ("deontos" - obligation, "logos" - science). Medical deontology constitutes a set of norms regarding the physicians’ conduct, rights and obligations in their relationship with their colleagues and, last but not least, with their patients.

The following are medical documents with deontological content:

- Principles of European medical ethics (approved by the European Economic Community in 1987).
- World Medical Association - 1987 - statement on organ transplants.

The ethical values of the relationship between physician and patient require the compliance with the following principles: mutual trust, patient autonomy, benefit provided to the patient as a result of the medical service performed, compassion, honesty, dignity.

The relationship between physician and patient is under the jurisdiction of the following legal norms:

- Patients’ Rights Law
- Law on Health Reform - Medical Professional Liability
- Order no. 482/2007 of the Ministry of Public Health
- Code of Ethics of the Medical Council

Currently, the normative acts regulating health activities are manifold, starting with Law no. 3/1978 on ensuring the health of the population, as amended, to Law no. 46/2003, on patient rights. The norms provided by the law are related to respecting information confidentiality, obtaining patient’s consent for medical procedures as well as the right to medical information, the right to the highest quality health care. The relationship between physician and patient has many particularities, the patient being entitled to the right to be respected as a human being, without being discriminated against, according to the Code of Professional Ethics and to legal norms, i.e. art. 3 of Law no. 46/2003.

According to the provisions of art. 3 para. 1 of Law no. 74/1995 on exercising the medical profession, the establishment, organization and functioning of the Romanian College of Physicians, the physician having the obligation to prove, in exercising the medical profession, „availability, correctness, devotion and respect for the human being”. Moreover, based on the provisions of the Criminal Code, alongside the other fundamental attributes, the patient’s honour and dignity are protected. The provisions of art. 2 of Law no. 46/2003 attest to the fact that “patients have the right to the highest quality medical care”.

The European Consultation on the Rights of Patients, which authorized the document entitled Principles of the Rights of Patients in Europe, which comprises a common framework of principles for the promotion and implementation of patients’ rights in European member states of the WHO, norms on human rights and values in health care, information, consent, confidentiality and privacy, care and treatment, respectively, took place in March 1994, in Amsterdam.
Our concern for bioethics and deontological ethics materialized through the following papers presented in detail in the habilitation thesis:

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5.2. ETHICAL CONSIDERATIONS IN PSORIASIS

5.2.1. BACKGROUND

Psoriasis is a multifactorial inflammatory reactional predispositional dermatosis, with complex genetic traits, characterized by a major keratinisation disorder. The clinical expression is different, the appearance of lesions being extremely varied, not only from one patient to another, but also in one and the same patient, during different eruptive bouts. Moreover, the extension and severity of the skin rash shows wide individual variations. Recent studies are more and more supportive of the involvement of the neuro-immuno-cutaneous system in the onset and maintenance of the disease (Duits et al., 2002). The effect of the lesions caused by psoriasis is considerable, given the fact that the skin organ is an integral part of every stage of human development, the skin interfering in cultural, interpersonal and personal areas, having a fundamental importance in self-esteem and in forming one’s individual identity. Psoriasis, like any other dermatological problem, is misinterpreted as infectious, which leads to the patient’s rejection and to the “fear of contamination”.

5.2.2. MATERIALS AND METHODS, RESULTS

According to the experts, a giant step forward in treating patients with psoriasis has been made through the introduction of biological therapies. These provide the possibility to intervene in the treatment of psoriasis, even at etiological level, which is extremely important in the context in which, for a long period of time, patients only benefited from symptomatic treatment. The use of biologic therapies in psoriasis has become a common practice in countries with developed economies. However, the less developed countries, like Romania,
are required to impose strict, centralized regulations, in order to make these medications accessible to at least a minimum number of suffering patients.

The legislative norms for the reimbursement of the therapy by the National Health Insurance Company are well-defined and every practicing dermatologist must meet the therapy inclusion criteria of patients suffering from moderately severe forms of psoriasis vulgaris, required in order to be able to draw up a complex assessment file for each patient. Legislative rules for reimbursement of therapy by the National Health Insurance are well defined and each dermatologist practitioner must meet the criteria for inclusion in the therapy of patients with psoriasis vulgaris forms moderately severe disease, is required to make a complex case Assessment for each patient. Consequently, the patients with tuberculosis, viral diseases, infection with hepatitis B, C, neoplasias, which could all be reactivated during this therapy, are eliminated from the start. Moreover, the current legislative norms impose the registration of every patient suffering from psoriasis vulgaris in the National Psoriasis Registry aimed at obtaining a national data base of these patients, which could prove to be useful in monitoring the evolution of the disease. While benefits of the biological therapies are major, the side effects must also be mentioned because, in many cases, they must not be neglected. The physician is responsible to make a correct selection of the patients who are fit for biological therapies due to the fact that, apart from their spectacular efficiency, they are encumbered by two major categories of side effects: the ones caused by medication and the cost-related ones.

The legislative framework in which these therapies must be administered entails that the patient's informed consent on the medical intervention should be granted, according to art. 124 of Law no. 3/1978 (“medical care is provided with the consent of the patient or of the persons who, under the law, are entitled to give their consent”). The patient’s consent should be legally obtained freely and uncorruptedly prior to the provision of the medical service, in accordance with the general norms. The current legality targets the rights and, at the same time, the obligations resulted from the provisions of art. 70-76 of Law no. 3/1978, as well as from some special regulations regarding the patient’s right to medical information, and the obligations of the health care providers. Thus, the law states that the dermatologist must obtain the premise of a validly expressed consent and, in case of biological therapy, s/he must inform the patient with vulgar psoriasis correctly, completely and in terms that are accessible to him/her, in compliance with his/her level of understanding, his/her health condition, the prognosis of the disease and the possible side effects. The literature shows that the general rule of biologic therapy is to inhibit the immune response of the body involved in the pathogenic process. Its action is to block the specific receptors of the cell, of the T lymphocytes, respectively, or to inactivate proinflammatory chemical mediators (cytokines). Given the chronic evolution of psoriasis and its complex impact on the patient’s physical appearance, psyche and social life, the results of the new immuno-biological molecules are spectacular. The clinical trials of biologic therapies showed a significant improvement in the severity of the disease, the latter being quantified according to national and international protocols by determining the following indicators by the dermatologist, and by the patient,
respectively: PASI - Psoriasis Area and Severity Index and DLQI - Dermatological Life Quality Index, which are regularly mentioned in the individual assessment file of the patients suffering from psoriasis vulgaris.

Another problem related to the administration of biological therapies is the high cost of the medication itself, to which the costs of analyses and medical consultation are added. The cost of biological therapies is several tens of times higher than that of classic therapies; at the same time, however, the pressure to prescribe the new therapies is increasingly higher. In this situation, the issue of identifying cost-effective criteria to justify their use is raised. Unfortunately, however, the usual public response to psoriasis and related problems is not one of recognition, understanding and availability to offer assistance. It is usually difficult to indicate a definite proof of systematic rejection, even though there discrimination and prejudice are well documented. These require further investigations, study and, naturally, intervention. In psoriasis, it is not only the management of the disease or of a “case” that is necessary, but also the management of a patient. Nonetheless, paradoxically, even sophisticated therapies can mark the patient as different, thus affecting his/her social, psychological and physical activity. The content, form and interpretation of the treatments have a potential impact on self-esteem and social reputation which is just as important as the initial disease.

5.2.3. DISCUSSIONS

Psoriasis has a significant effect on the patient’s quality of life, leaving a powerful psychological and social mark. The seriousness of its impact on the patient’s life must not be underestimated. The invalidity given by psoriasis is comparable to that of other chronic diseases such as heart disease, diabetes, cancer and depressions (Boniotto et al., 2000). The psycho-sexual impact of psoriasis is manifested at two levels: the effect of the disease on the patient with psoriasis, on the one hand, and the effect of the skin lesions on the partner, on the other hand. The existence of psoriasis-related lesions at genital level is associated with a significant stigmatization for that patient and, eventually, to his/her rejection by a potential sexual partner. The most common feelings associated with psoriasis are depression, anxiety, irritability, nervousness, awkwardness, embarrassment, lack of trust. Stigmatization is especially characterized by anticipating rejection, feelings of weakness, shame and embarrassment, sensitivity to others’ attitude.

In ancient Greece, the term *stigmata* was used to designate a skin sign, a marker of a negative and unusual aspect in a person’s moral status. Such signs warned over the fact that the individual was a blamed person, who should be avoided, especially in public places. Centuries later, in the Christian world, new metaphorical meanings of this word were added. Initially, some skin rashes were considered to be signs of divine grace. However, they eventually began to be seen as surface indicators of underlying psychiatric disorders. Nowadays, the word *stigma* is more often used to indicate shame, dishonour, and less often potential skin manifestations thereof.
A stigmatized person is not merely different in one way or another; this difference is absolutely undesirable. In our view, such a person is not normal, usual, upright. She/he becomes disconsidered, discredited, tainted. The undesirable trait exceeds all the other aspects of his/her personality, reputation, even though, from other points of view, this person can be considered to be positive. The main idea is that this person manifests a destructive, inappropriate potential, and can be a danger for the normal interactions and relationships between people. She/he requires attention and cannot be ignored. Regular people will of course seek justifications and explanations for their reactions and reasoning, constructing real theories to support the strength of their ideas on the potential danger represented by the stigmatized. Paradoxically, apart from numerous supposed imperfections, such theories sometimes include a series of positive, exceptional aspects, as well. Consequently, stigmatization begins by perceiving a difference. Within a complex set of social and psychological processes, the difference is then developed and represented socially and culturally, in different ways. These representations will have severe consequences on the wellbeing and quality of life of the stigmatized person. People with psoriasis are undoubtedly different. The skin affected is not different only at the microscope or at cellular level – it simply feels and looks different in everyday life. Moreover, the clinical aspect of a sick person leaves behind him/her has a visual impact on their environment, which clearly distinguishes him/her from normal-skinned people. These aspects are reunited in a manner that would add new connotations, with a much more important significance than physical and biological terms alone could justify. Indeed, psoriasis seems to have its most notable effects in the social and psychological sphere. Psoriasis can even be traumatic both for the sick, as well as for those who provide care for them, due to the consequences it has on self-esteem and on one’s respect for oneself. Furthermore, it is essential for intimate, interpersonal meetings and contacts with strangers, in which case the threat of an exaggerated, unwanted, attention, inappropriate questions, or rejection, is always anticipated. It was said that psoriasis is like a prison for the people affected, leading to the limitation of their social life and of their occupational options. More importantly, the disease has an impact of long-term relationships and, in this case, the suffering is more intense, more deeply rooted in the identity, in the pattern of life and in the wellbeing of the individual. Thus, the feelings of shame or guilt become a reality. It is often said that skin signs or symptoms are nothing more than mere “cosmetic” problems, without important consequences on the individual affected. However, stigmatization is so pronounced sometimes that this idea is obviously lacking significance. A dermatological disease is not easy to ignore and it is definitely not easy to learn to live with it. On the contrary, it requires the development of complex social and psychological skills and talents which can be learned and practiced not only individually, but also in cooperation with other people who are willing to help and to support the person affected.

Psoriasis is interpreted, understood and labelled (imperfectly, irrationally and falsely, in any case) in various ways which cannot be ignored or avoided. Sociologists and psychosociologists would say that the difference was socially transformed into a deviation. As a result, there is a real meaning in which psoriasis is a socially constructed invalidity, and
those affected are, to some extent, invalid. They discover that they have barriers in the way of their full involvement in the environment, unlike normal, regular people (Jobling, 2001). Invalids are victims of differences, but many people do not believe that their inability and dependence result directly from these differences. All these things can enchain the patient, both physically and psychologically, by their very nature, accentuating and exaggerating the effects of the problem which they have to solve.

5.2.4. CONCLUSIONS

Psoriasis has a significant impact on the quality of life of people who suffer from this disease, leaving a strong psychosocial mark on them. For this reason, its implications for the patient should not be underestimated. The invalidly resulted from psoriasis is comparable to that of other chronic diseases, such as heart disease, diabetes, cancer and depression.

The effect of psoriasis lesions on patients is great, given the fact that the skin organ is an integral part of every stage of human development, i.e. cultural, interpersonal and personal, and has a fundamental importance in self-esteem and in forming one’s individual identity. A huge step forward in providing care for patients with psoriasis was taken by introducing biological therapies which, despite yielding spectacular results, are encumbered by a series of significant side effect, which makes the careful assessment of all medical, social and especially ethical issues to be highly necessary.

5.3. SEXUALLY TRANSMITTED INFECTIONS

STRICTLY CONFIDENTIAL MATTER OR PUBLIC HEALTH ISSUE?

5.3.1. BACKGROUND

Sexually transmitted infections are one of the most common causes of illness among the young population. They have major negative consequences on the health condition of the individuals affected, on their reproductive potential and on that of the communities they belong to. Moreover, sexually transmitted infections (STIs) also have important social and economic implications. The emergence and subsequent spread of HIV and AIDS has had a major impact on STI prevention and combating measures and has complicated the medical services provided to patients suffering from STIs. The world population comprises more young people than ever before, almost half of them being under the age of 25. Every year, millions of them reach the “reproductive age”. Many do this in a safe and controlled way, managing to establish a balance between their goals, education and sexual maturity, as essential elements of human life. Nonetheless, in developing countries, many young people are exposed to increased risks of unprotected sexual practices, such as infection with HIV or with other sexually transmitted diseases. Some people, especially the poor and uneducated, become sexually active without being properly informed and without having access to protection methods (Coleman, Lohan, 2007). There are some cultural and moral justifications
for this lack of information. There is worldwide evidence that informed young people behave more cautiously than the uninformed.

5.3.2. MATERIALS AND METHODS, RESULTS

Sexually transmitted diseases, having an endemic/epidemic potential by definition, raise an issue that is common to all endemic diseases, namely the conflict between the need to protect the population as a whole (the viewpoint of health authorities), and the need to protect the person, as a free individual, holder of certain rights. According to the legal framework in effect, when confronted with an epidemic, such as a cholera or tuberculosis outbreaks, the health authorities usually set regulations on the containment of contagious people in order to avoid the contamination of the entire population. These measures are allowed because the epidemic is known, isolation not being considered discriminatory. Moreover, the person who is isolated knows that she/he is sick and wants, by all means possible, to cure the infection. There are no issues related to human rights violations in this situation because the link between the infection that affects the individual and the epidemic is clear. In the case of STIs, it is much more difficult to prevent contamination by detecting the people infected, and much less accepted, due to the fact that they often appear to be healthy, they still work, and have family and social responsibilities.

From a legal perspective, on the other hand, people with STIs have certain rights that need to be respected in a democratic country, affiliated to the Declaration of Human Rights. Public authorities must avoid the imposition of discriminatory measures which would enhance the natural tendency that each and every one of us have in crisis situation, i.e. that of finding someone “responsible” to lay the blame of the situation, to manifest our frustration and anger. For this reason, in the case of STIs, where there is already a general tendency to reject such a sick person, to consider him/her “impure” among family members, group of friends, colleagues, etc. the public authorities must defend the individual’s rights and avoid discrimination.

The deontological and bioethical medical code mentions individual rights as being included in two categories/types, namely:

- patient rights, established by medical and bioethical deontology, to information, confidentiality and treatment
- human rights (Universal Declaration of Human Rights) – the right to movement, education, free choice, etc.

I studied the literature on the topic and noticed that a frequently debated issue is that of sexually transmitted infections, hence the importance of confidentiality in STIs. Confidentiality has been regarded, for a long time, as a fundamental trait of the relationship between physician and patient. The notion of the confidentiality of the information offered by the patient to the physician is theoretically stipulated in any code of medical practice, from the Hippocratic oath onwards. It is not wrong to say that the medical secret is considered to be important by the patients and that it is implied as being a part of the medical service. The
patients expect the information they disclose to the physician to remain confidential, this being an important part of the medical consultation. Still, why is medical confidentiality so important? The fundamental reason behind the central role that confidentiality has in the field of venereology may be the optimization of the therapeutic relationship between physician and patient. Like priests and lawyers, physicians are capable of keeping secrets. In order to make nursing services more effective, to make patients trust their physician, they have to be convinced that they can talk openly with them.

The literature provides proof of the fact that violating confidentiality affects the level of information offered to patients. Thus, patients will be less honest and open about the information they disclose to health care professionals, which will have a further negative impact on the level of treatment and counselling that patients receive (Holloway, 2004). Maintaining confidentiality in compliance with the deontological and bioethical code is probably the only way to ensure public health. Otherwise, physicians would be discredited as a source of education or treatment. The legal concept of confidentiality is fundamental for pragmatic therapeutic reasons. The consequences of undermining this obligation would not only be damaging to the individual approach of the patient, but also destructive in terms of public health. It is generally accepted that respecting individual autonomy is a fundamental moral principle that allows individuals to have control over their own lives. This is reflected in justice and social policy, as well as in the ethical doctrine. It is precisely the principle of respect for individual autonomy that makes the patient’s choices essential. The patient has the right to decide on which treatment to follow, with emphasis on the importance of informed consent and confidentiality (Lehrer et al., 2007). Maintaining control over one’s own actions can have an important role in the sense of security, freedom of choice and self-esteem.

Another issue related to keeping professional secrecy is the one represented by informing patient contacts, especially within a risk group. The general norms command maintaining secrecy if the patient is behaving in a responsible and appropriate manner. However, there are limits to confidentiality when the patient poses a risk to those around him/her. The principle that ought to be respected in this case is that of the hierarchy of values: the individuals’ life is more important than the right to privacy. If keeping a medical secret endangers the life or health of others, confidentiality is no longer mandatory.

According to the ethical principle of respecting privacy, any human being has the right to confidentiality and to respect for his/her private space. In the relationship between physician and patient, this principle is materialized in “professional secrecy”, which is actually implied in any health care relationship. This principle requires that the physician and professionals in the field of health care do not divulge anything related to the history, health condition or treatments followed by the patient, with the exception of the case in which the patient consents. Any violation of medical secrecy by the physician is susceptible to prosecution and conviction in a court of law (Sankart et al., 2003). Apart for the respect for the patient, professional secrecy finds a second ethical base in the proper exercise of the medical profession. There is no medical practice without a relationship of trust between physician and patient.
The epidemiological norms in effect consider STIs to be a public health issue. Within the National Program for Communicable Diseases and for the Prevention, Supervision and Control of HIV/AIDS, the Romanian law provides free testing through rapid tests and ELISA tests for people at risk in detecting HIV/STI. Syphilis testing and voluntary counselling and testing for HIV is recommended to all patients who seek medical help for STIs. Counselling for STIs (including on HIV-related issues) and presenting ways to prevent such infections are recommended in all cases. The antibiotic resistance of some of the pathogens involved in STIs is increasing, bringing further complications in managing such cases. The laws or regulations in all countries bind physicians to report certain diseases. The health authorities are obligated to carry out epidemiological surveys in order to define the risk of an epidemic and to detect the people infected (sick or just carriers) and their contacts. Their systematic identification is even more important as the spread of STIs is related to the behaviour of those infected. In order to break the transmission chain, these people should be recognized, treated in specialized centres, and educated about sexual behaviour changes. Sexually transmitted infections such as syphilis, HIV/AIDS (mandatory in pregnant women), gonorrhea, chlamydia, hepatitis B and C are diagnosed and treated by the dermatologist/venereologist according to the protocols in the field. Simultaneously, the latter performs notifies these diseases by carrying out an epidemiological survey and issuing it to the Public Health Department. Thus, in case of STIs, the epidemiological investigation starts from the person who requests the consultation and then allows the identification of the source of infection. Both partners are treated and must interrupt sexual activity until healing is confirmed through laboratory samples. Patients sometimes display a certain resistance to participating in epidemiologic investigation regarding the specific manner of transmission of these infections (Woodward, Argent, 2005). The patient’s collaboration entails the recognition of his/her belonging to a risk group or to his/her involvement at one level or another, as a link in the chain of transmission. In fact, groups at risk of acquiring STIs are already subject to discrimination, rejection and social marginalization, especially due to their sexual behaviour. This is the reason why patients often refuse to provide information from their history that would be useful to the epidemiological investigation. The Dermatovenerology Committee and the Ministry of Health and Family stated in the Guide for the Diagnosis and Treatment of Sexually Transmitted Infections, issued in 2001, that providing adequate medical care in STIs remains most important in controlling the spread of these infections, due to the fact that it prevents the occurrence of complications and sequelae and reduces their transmission in the community. Each individual’s choices are private matters, but sexual conduct can have a significant impact on public health.

In practice, prophylaxis employs several different methods in terms of level of efficiency and morality: information, education, behaviour change, condoms. While the condom is a merely a palliative means, simply intended to slow down the transmission of various sexual infections, the real preventive measures aim at changing sexual behaviour through information. However, even in this context of radical prophylaxis, concrete proposals differ.
Information is reduced by some physicians when communicating data, especially about ways of transmission of sexual infections and about protection using the condom, in other words, “safe sex” education. However, changing sexual behaviour aims at reducing the number of sexual partners and their selection. Even though informing partners is sometimes seen as a measure to protect the rights of the society rather than individual rights, its purpose is obviously to help people to respect the individual rights of their partners: the right to be informed, the right to choose, the right to health and, in some cases, the right to life. Public health ethical principles are different from the ones applied in modern bioethics. Bioethical principles were developed at clinical or microethical level, affecting the relationships between individuals, while public health ethics applies at populations or macro ethic level. Solving these principles, e.g. respecting the right to privacy, differs in public health from clinical medicine. Human sexuality issues that are relevant to public health concern reducing morbidity and mortality through STIs, especially in economically and socially disadvantaged regions, by facilitating access to existing means of protection, avoiding infertility caused by STIs, etc. The general ethical issues raised by STIs are related to their prevention, to the recognition and treatment of the people infected and their contacts, to the information and counselling of patients about diagnosis, treatment, evolution, complications, and existing means of prevention (Loewy, 2002). Another important matter is the medical secrecy versus the unconsciousness / lack of responsibility of some patients.

5.3.3. DISCUSSIONS

Patients generally believe that the presence of a sexually transmitted disease has a significant negative impact on their lives, which is a somewhat unexpected idea from the venereologists’ viewpoint, who believe that the majority of these infections are curable. Patients first and foremost assess the impact of such a disease on their personal lives, more so than on their health condition. In addition, a great part of the people infected are not conscious of the fact that some STIs are even easy to diagnose and to treat. Strategies to encourage testing for curable and non-curable STIs are necessary. These must take into account everyone’s attitudes and beliefs regarding the influence of STIs on their sexual relationships, as well as their perception on prevention, diagnosis and treatment methods. We are currently wondering if to tell the patient the truth. We will do it, but under no circumstances must we do it with indifference. Any clinical diagnosis must be immediately followed by a proposal for assistance, support and, of course, treatment. In order to avoid unfounded accusations between partners, it is sometimes recommended to wait for paraclinical confirmation (the results of the laboratory investigations), especially in the case of spouses.

As part of the Christian belief regarding the respect for the individual, medical secrecy and its corollary are very important. However, medical secrecy is not as binding as confessional secrecy, for example, which is absolute and allows no exception. Medical secrecy is binding, but not absolute (Ashcroft et al., 2007). It is delimited, on the one hand, by the patient, and, on the other hand, by the public. If the patient is the carrier of a disease of
epidemic potential, the disease must be reported to the authorities, given that it regards public health. Similarly, if a disease could have dangerous consequences for the health and life of others, this disease must be revealed to the ones concerned. For example, if a physician finds out that a patient with STI is a prostitute, she/he must convince her to give up her work until she is completely cured, even under threat of revealing it to the police, because it concerns the lives of many other people as well. In case of STIs, keeping professional secrecy is more and more important, because their disclosure can lead to destroying a family, to the loss of jobs, to rejection by peers. In addition, if the patient with STI adopts a responsible attitude towards his/her disease and towards those whom she/he could infect, there is no reason to violate professional secrecy, which would be a serious deontological error (Singh, 2005). In a society in which selfishness and indifference to others prevail, keeping professional secrecy is necessary in order to protect the patient suffering from STI. The importance of confidentiality increases in case of sexually transmitted diseases, being essential for the purpose of establishing a relationship based on trust with the patient. Moreover, maintaining medical secrecy favours the patient’s awareness of his/her own health condition, as well as that of his/her partner(s). What is more, confidentiality is a guarantee of the collaboration between physician and patient, of the relationships of trust that will lead to a fair and complete management of the case (diagnosis, treatment, follow-up, counselling). Nonetheless, sexual life and health is an extremely delicate field for us all, which is why some patients behave irresponsibly. The first proof of the patient’s attitude lies in the information which she/he offers to his partner. Someone who discovered that she/he is a carrier of an STI must inform his/her spouse, as well as any other people they have had sexual intercourse with, on the risks to which they have been exposed. Announcing their spouse of their infection is important, but sometimes difficult. There are cases in which the patient wishes to keep the secret from their spouse and family, especially if she/he was involved in extramarital affairs. Patiently, the physician must insist on the importance of the patient’s confession to his/her spouse. The physician’s duty is to convince the patient that she/he is obligated to inform all his/her sexual partners who, in turn, will decide whether or not to seek medical help. In the case in which the patient with STI refuses to inform his/her spouse, the physician is confronted with a dilemma. There is no doubt that the persuasive approach is preferable to violating professional secrecy. The physician must do everything in his/her power to keep in touch with the patient and to convince his/her to inform his/her partner and to do the responsible thing. This awareness could take time. The physician him/herself will not warn a third person on a patient’s diagnosis, unless she/he has explicit permission, or at the latter’s request. This way, public health is better protected, especially as, ultimately, everyone’s responsible behaviour is the surest weapon against the spread of STIs. This solution, which involves deepening the relationship with the patient, maintains the latter’s trust in medical care (Loewy, 2002). The need to ensure as efficient prophylaxis as possible in this field acquires the ethical importance of a social obligation. We must never forget that sexually transmitted diseases, with their seriousness and impact on social life, cannot be placed on the same level with other infectious and contagious diseases, due to the fact that they can be completely prevented. All sexually
active individuals must be correctly informed, adequately counselled and encouraged by the medical and health authorities, and by the media, to give up certain types of sexual behaviour which are known to favour the transmission of infections. Partner notification is the process through which the sexual partners or other people exposed to sexually transmitted diseases are identified, located, assessed, tested, treated and counselled on prevention. The contacts are represented by all sexual partners, by the parents of the infected new-borns, by intravenous drug users who shared the same syringe with needle and by individuals who could be involved in cases of sexual abuse. Informing, treating and counselling partners are recommended in all cases of infection or sexually transmitted syndrome. Not only do these measures have a positive impact on public health (supervision and fight against the disease), but they also have a significant contribution to reducing the risk of patient reinfection. By informing and changing behaviour, decreasing the number of STI cases, as well as educating the young population on the respect for human sexuality is sought. It is, therefore, essential to avoid the joint, public battle against sexually transmitted turning, at a given moment, into the violation of individual patient rights.

5.3.4. CONCLUSIONS

Every individual’s options are private matters, but sexual conduct can sometimes have a significant impact of public health. Public health ethical principles are different from those applied in modern bioethics. The bioethical principles were developed at a clinical or microethical level, affecting the relationships between individuals, the public health ethics applying at a populational or macroethical level. Solving these principles, such as, for example, that of respecting one’s right to privacy, differs in the field of public health than that of clinical medicine.

Prevention occupies a very important place in the ethics of sexually transmitted diseases, which is explained by the absence of the possibility to get vaccinated against such diseases (syphilis, gonorrhoea), on the one hand, and by the absence of a curative therapy in diseases such as hepatitis B and C or HIV, on the other hand. Nonetheless, we know exactly how to prevent these infections.

To conclude, the principle of keeping professional secrecy and confidentiality must be always complied with, with the exception of the case in which it constitutes a danger for another individual or for society.

5.4. SKIN CANCERS

ETHICAL CONSIDERATIONS IN MALIGNANT CUTANEOUS PATHOLOGY

5.4.1. BACKGROUND

Skin cancers are the most common form of human cancer, characterized by a malignant transformation of skin cells. Even through the cell transformation process is
obscure, the explosive development of molecular biology in recent years has led to new
ethiopathogenetic theories which, in turn, have led to changes in the methods of monitoring
the progression of the disease, as well as some features of the physician - patient diagnosed
with cancer (Klaus et al., 2007). In 1787, the Scottish surgeon John Hunter was the first to
perform surgery on a skin lesion which was considered to be malignant. Due to the fact that
the notion of skin neoplasia did not exist at that time, Hunter described the mass as a “mycotic
cancerous excrescence”. 17 years later, in 1804, the French physician Rene Laennec was the
first to identify melanoma as a distinct entity and later on, in 1840, another physician, Samuel
Cooper, reported that therapy was useless in advanced stages of melanoma. After over 200
years, melanoma is still a challenge in terms of clinical diagnosis and treatment (Morton,
2012).

Skin neoplasia, which is diagnosed early, can have a 100% recovery rate. The lack
of information on tumor pathology and the lack of promotion of the notions of prevention by
the media led to the confrontation of physicians with preconceptions and wrong beliefs on
tumoral skin diseases, which are difficult to combat.

5.4.2. MATERIALS AND METHODS, RESULTS

In everyday practice, dermatologists are confronted with the so-called myths about
skin tumor lesions, which sometimes prevent the early, timely presentation of patients in
medical institutions for the purpose of detecting and determining the course of therapy. The
preconception related to the dissemination of the tumor in case of surgery is common among
patients who do not understand that metastases are the final stage of the disease, while
surgeries performed with oncological limits are curative.

Another myth is that of safer artificial tanning, under the pretext of a controlled UV
dosis. Premature skin aging/photoaging is mainly caused by chronic exposure to UVR. Experts claim that about 80% of facial wrinkles are caused by exposure to UVR and, consequently, any form of tan, be it natural or artificial, is aggressive for the skin, through which it tries to repair the damage caused and to prevent future lesions. However, such gene repair can lead to gene alterations, which are triggers of neoplastic processes (Uitto, 1997). Moreover, the idea that, on cloudy days, at the mountain side, or during mountain activities it is not necessary to apply sunscreen is false. The literature states that 80% of UVRs reach the skin and, the higher the altitude at which the patient is located, the more intense the UVRs.

I studied the literature and the data show that Caucasians require maximum 5
minutes of sun exposure to synthesize a sufficient dose of vitamin D. What is more, excessive
sun exposure can induce the transformation of vitamin D into inactive metabolites. On this
basis, studies show that there is no difference in the level of vitamin D between people who
use sunscreen creams/lotions and people who do not (Marks et al., 1997). The myth that
people with dark skin (phototype 5, 6) are less exposed to the risk of skin cancer is also
nourishing among patients. The Environmental Protection Agency presented data according to
which, even though their incidence is lower in case of tumor lesions, their aggressiveness is
Higher. Moreover, global solar protection does not only depend on the high level of the sun protection factor that protects the skin against UVR, and UTVR does not yet have clear standards to provide full protection. Compared to other cases of neoplasia, diagnosing skin forms is easy, taking into account the external location of the target organ. Consequently, the family physician is the first who should notice a suspicious lesion during routine checks, and should ask the patient to undergo a thorough skin examination, the dermatologist being the second physician in line who should fully undress the patient.

Unfortunately, the lack of time or of other concerns most often lead to superficial examination results, limited to the patient’s fields of interest (for example, the face, in the case in which the patient has acne), without having a clear picture of the skin problems and, finally, only one in three dermatologists admits to carrying out a thorough examination of the skin. In most cases, the patient notices the early stage of the lesion and, if she/he was sufficiently informed and addressed a dermatologist right away, therapeutic success is ensured. The dermatologist can recognize the early signs of skin neoplasia, while another specialist can give the same diagnosis only when the disease is more advanced; hence, the question: is it absolutely necessary for the dermatologist to conduct a thorough examination of the patient’s skin, regardless of the latter’s age or condition for presentation?

Unfortunately, this ethical dilemma is not yet regulated and there is no universal guide for skin cancer screening. Approximately 75-80% of non-melanocytic skin cancer affects the cephalic extremity, while the most common form, the melanoma, primarily affects the trunk and then the legs. All these locations are visible to the human eye, easily monitored by the patient and evaluated by the physician.

The norms stipulated in the Guidelines for Diagnosis and Treatment indicate the fact that clinical diagnosis must always be confirmed through a histopathological examination, which can be easily achieved when the health unit is provided with a histopathology laboratory. Thus, the diagnosis is often placed by the general surgeon, by the plastic surgeon, or by another specialist who can perform the therapeutic surgical procedure.

Experts support the existence of the concept of positive and negative patient rights. In the circumstances in which the patient requests the excision of a pigmented lesion, even if benign, the physician does not become a mere executor of the patient’s orders. According to the Minkoff theory, negative rights refer to the fact that the patient is protected against measures that could affect him/her in a negative way, while the positive rights refer to equal access to resources, for the purpose of benefiting from therapeutic care (Minkoff et al., 2010).

The current guidelines for the diagnosis and treatment of skin tumors provide dermatologists with precise indications on the route to be followed in such pathologies. Both the specialty of Dermatology as well as that of Oncology, especially in advanced tumor forms, which require radio therapy or chemotherapy, are the ones in charge of patients with skin neoplasia, with his/her monitoring. However, what happens with the patient, after being diagnosed? Which is the route which must be followed? The current norms entail supervising a patient diagnosed with skin cancer by checking blood parameters, conducting detailed investigations – immunohistology examination or general examinations, such as chest x-ray,
abdominal ultrasound, CT, MRI. Another issue imposed by the current medical guidelines is the prevention of neoplasia by examining the skin of people at risk, with multiple melanocytic lesions. They should be examined regularly by a dermatologist, given the fact that it is well known that the risk of developing melanoma is increased in patients with a high number of melanocytic nevi. There is a theory according to which the genes responsible for a high number of nevi in a person are the same genes that determine the susceptibility to melanoma (Falchi et al., 2009).

The literature reviews show that approximately 80% of NMSC affect the cephalic extremity, the head and the neck; in some cases, postoperative scarring determines major damage to the patient’s physical appearance. Unavoidably, this causes a disfigurement that entails mental changes, such as anxiety, depression or social isolation (Rhee et al., 2005).

5.4.3. DISCUSSIONS

From the moment in which a patient is diagnosed with skin neoplasia, any medical decision brings a challenge for the people involved, requiring the medical staff to act in accordance with the Code of Ethics and Deontology. Communicating this diagnosis is never easy and entails the compliance with ethical, psychological and legal norms in informing the patient, while maintaining confidentiality, to provide correct and adequate information about his/her disease, as well as his/her informed consent in case of treatment. Communicating the diagnosis can raise an ethical problem, especially if it entails discussions with carers or delaying diagnosis in order to be able to communicate a certain diagnosis, transmitting the diagnosis directly, until making the decision of transmitting it by another health care professional. In answer to the question “which of these variants comply with legal and deontological norms?” I believe that the decision should me made based on the age, physical and mental health condition of the patient and on diagnosis circumstances.

5.4.4. CONCLUSIONS

Skin neoplasia is the most common form of human cancer, which often raises ethical problems. The most common principles should, perhaps, be those postulated by Beauchamp și Childress in 2003: the principle of respect of autonomy – respecting the abilities of autonomous people to make decisions, not to do harm; the principle of beneficence – the patient must be the beneficiary of the services provided in the name of the health system, as the patient is the one who supports this system through material contributions; and the principle of justice, in terms of the equity of distributing the risks or advantages to which the patient is subjected in the health system (Beauchamp, 2003). Skin neoplasia still remains a challenge and the ethical principles involved in the therapeutic process will have to combine harmoniously the moral values of the society, the patient’s religious principles, the physician’s professional skills and the therapeutic guidelines in the field.
5. 5. DERMATOETHICS

5.5.1. BACKGROUND

Skin is an integral part of every stage of human development, contributing decisively to self-esteem, to individual identity and to one’s relationship with others. Given the importance of skin in interpersonal relationships, we can attest to the particular character of dermatology, as a profession that addresses skin pathology. Patients with skin problems are often marginalized and stigmatized, which leaves a powerful psychological and social mark of these diseases, which can create real social disabilities. In recent years, considerable progress has been registered in dermatological diagnosis and treatment methods, but also in the manner in which dermatological patients are promoting and defending their rights. These major changes place dermatology in line with the specialties that represent the “changing face” of medicine and, at the same time, in line with the risk specialties (Bercovitch, Long, 2007).

In this context, in continuous change, in a medicine placed in the service of society, the leading place of ethics becomes obvious. In the newly created climate, dermatology must evolve in order to align this specialty with the European and international standards of the 3rd millennia. The dermatological issues that have to be filtered through ethical analysis becomes more and more complex, which “outlines a new subfield of this medical specialty” – dermatoethics. This new subfield shares the problems of all medical specialties, but especially the more and more complex problems specific to dermatology. In the context of the rapid progress of this specialty, dermatoethics is, on the one hand, a factor leading to progress in dermatology and, on the other hand, a prevention factor. Thus, the purposes of dermatoethics are: to enhance specialists’ information on ethical, deontological, forensic and social issues in the dermatological practice, to operationalize norms and ethical principles of the medical practice in algorithms that can be used in the everyday dermatological practice, to encourage dialogue between professionals from different specialties, to update the knowledge in the field of bioethics and to adapt them in dermatology, with the purpose of preventing the violation of the norms specific to the field. In other words, dermatoethics commands that professionals in the field of dermatology be always open to everything that is new, attainable, pertinent and ethically valid.

5.5.2. MATERIALS AND METHODS, RESULTS

Introducing new drugs in skin disease treatments, such as biological therapies, inventing and developing diagnosis and therapeutic techniques (immunohistochemistry, dermoscopy, PUVA, Mohs surgery) dramatically change the possibilities that are available to dermatologists. In the 21st century, these novelties changed the specificity of dermatology from a purely medical specialty into one that is equally medical and interventional. In these conditions, dermatologists are challenged to adapt their knowledge and to exercise their practical skills in order to be able to use the new diagnosis and treatment methods.
A genuine “case study” in this regard is the introduction of biological therapies in psoriasis, an immune-mediated inflammatory skin disease.

The legal framework instaurating such therapies involves a sequence of actions that ought to be taken by the dermatologist, who must draw up an evaluation file, which should include clinical data, appreciated through indices (PASI, DLQI), as well as paraclinical data – which will be forwarded to the Health Insurance Fund. Moreover, in applying revolutionary “back up” treatments, two essential problems are raised: the need to adequately inform the patient on the beneficial effects and risks of the treatment, and the issue of costs, which, most often than not, makes it impossible for all patients to have access to such treatments.

An example of the need for a close collaboration between dermatologists and their patients, aimed at avoiding disastrous side effects that can be associated with certain therapies, is the administration of isotretinoin as a treatment of choice in nodulocystic acne, a disease with a high risk of leaving permanent, disfiguring facial scars. The introduction of isotretinoin in the medical practice, in 1982, was an important step forward in the therapy of this disabling dermatological disease. I studied the literature and the data support that fact that, although this drug demonstrated its effectiveness in the treatment of resistant cases of acne, it also has teratogenic effects, being able to seriously affect the central nervous system and to produce facial congenital malformations in case of in utero exposure. Such foetal malformations are reported with a frequency of about 40% in children born to mothers who followed this treatment during pregnancy. The children who do not suffer from congenital malformations are still at risk of developing cognitive deficiencies. Given the teratogenic potential of isotretinoin, from the moment when it was introduced on the market, different versions of programs intended to prevent pregnancy during the course of treatment were proposed, the final version being introduced in medical practice in March 2006. It refers to iPLEDGE, a program developed by the four companies producing the drug, which requests the registration of all isotretinoin distributors: all physicians who prescribe it, all pharmacists who sell it, as well as all patients who use it, either men or women. The legislative framework entails an extremely rigorous program according to which, before prescribing the drug for the first time to women of reproductive age, two negative pregnancy tests, based on urine or blood, are checked by the prescriber. The female patients of fertile age must also bear the obligation of using two types of contraception during treatment, as well as a month before and a month after its completion.

Moreover, the patients must demonstrate monthly negative pregnancy tests throughout the administration of isotretinoin. This information must be introduced in the electronic iPLEDGE system by the physician who makes the prescription. This program is meant to drastically reduce the number of pregnancies that occur during the treatment with isotretinoin, in order to reduce the possibility of the occurrence of teratogen effects associated with this treatment. Moreover, iPLEDGE is another proof of the fact that only authorized physicians prescribe this drug, 85-90% of them being dermatologists, but also of the fact that this drug is kept on the market, in spite of its teratogenic side effects, and is accessible to patients who suffer from severe acne.
In recent years, discussions on selling cosmetics in dermatological practices broke out. Some commentators claim that such practices question dermatologists’ integrity, while others believe that providing patients with the necessary cosmetics is an extension of the daily dermatological practice. In this regard, the American Academy of Dermatology declared, in 1998, that dermatologists who sell cosmetics in their practices must comply with the standard of acting in the best interests of the patient, making it their most important priority. The main purpose of dermatology is to treat skin diseases and to help patients maintain their skin healthy. This is partly the reason of the influx of cosmetics in the dermatological practice. On the one hand, dermatologists are the specialists who possess the best knowledge in the field of skin care, yet, on the other hand, dermatology specialists, in most cases, also participated in conceiving these products. Lately, the involvement of companies producing or distributing cosmetics and drugs in the field of dermatology has been more and more aggressive. There are many situations in which the unethical collaboration with such companies is able to cause serious harm to this medical specialty. A medical institution is associated with a pharmaceutical company only to then evaluate the products of that respective company. A dermatology clinic establishes collaboration with a cosmetics company, for large amounts of money, only to then have their logo posted in the waiting rooms of the clinic, suggesting the clinic’s support for that cosmetics company to the patients. Dermatologists, like the physicians from other specialties, must carefully assess the potential risks posed by new products and procedures. The physician’s role is to act prudently and to explain to patients the potential side effects of various drugs or cosmetics, especially the long term side effects of the new drugs, warning patients about the incomplete information available about them. All dermatologists must make their potential financial connections with pharmaceutical/cosmetics company public. If dermatologists wish to maintain the patients’ trust and the public’s support, it is imperative for such activities to be condemned as unethical and inconsistent with professional ethics, on the one hand, by the physicians, and, on the other hand, by the professional associations that must develop specific norms in this regard.

5.5.3. DISCUSSIONS

The new directions outlined in recent years in dermatology, focusing on aesthetics, imposed the adherence of dermatology to evidence-based medicine. In aesthetics, assessment is extremely rigorous, and the establishment of a protocol serving to finalize the procedure applied is a must. In recent years, dermatology was “invaded” by cosmetics and other preparations intended to improve the physical appearance of patients.

A major problem in applying biological therapies is the high cost of the drugs themselves and of the medical tests and consultations, which is tens of times higher than that of classical systemic therapies, recommended in the management of psoriasis. If, for example, for a month of treatment with methotrexate (the current standard therapy in psoriasis), the costs are of 100 USD maximum, reaching 2000 USD per months in case of administering an immunobiological treatment (Gheucă, 2008).
At the same time, the pressure to prescribe new therapies is increasingly higher, which makes it necessary to identify cost-effective criteria to justify their use. The problems created by the high costs of this therapy make their presence felt and create the premise for discrimination, both among patients from the same country, as well as among patients from different countries.

The administration of isotretinoin is a clear example of the need to support a close collaboration between dermatologists and patients, starting with explaining the benefits and risks of the treatment, and continuing with careful monitoring throughout treatment. Informing the patient, and the latter’s trust in his/her physician become essential to ensure the patient’s compliance with a backup treatment, with spectacular results, but also with potentially serious side effects. This difficult and demanding procedure of ensuring the safety of the treatment with isotretinoin is, on the one hand, capable of limiting the patient’s freedom, which can lead to his/her giving up the treatment and, on the other hand, it can encumber the dermatologist due to the laborious bureaucratic process it entails, which can make the latter give up considering this substance as a therapeutic option for refractory acne to the conventional treatment.

The activity of securing the administration of isotretinoin is considered to be disproportionate by dermatologists, in the context in which there are other potentially teratogenic therapies for which prescription and supply has not been restricted to a special program. There are patients who, by virtue of their religious beliefs, oppose contraception through any kind of method. Due to the iPLEDGE program, the treatment with isotretinoin is not available to such patients, which is a form of discrimination in terms of medical care.

The security program is completely non-selective and requires all patients to follow the registration procedure, even in the case of male patients, or of female patients who have passed the reproductive age, which is an unnecessary burden on both patients and dermatologist (Weinberg, 2006).

If dermato-cosmethology develops more and more, the issue of including the treatments from this subfield of dermatology into that of methods to improve the human being, with the related ethical problems, arises. By improving physical appearance, dermato-cosmethological treatments are able to offer a social advantage to its beneficiaries, on the one hand, by increasing self-esteem and, on the other hand, by increasing social acceptability. Last but not least, we must not forget that these treatments are expensive and, consequently, are not available to all members of the society, which is likely to create social inequality or to contribute to the deepening of an already existent social inequality (Gheucă, 2008).

The patients’ more and more widespread and easy access to information is a reality. Unfortunately, they sources they use are sometimes misleading, hence some patients’ request for magical elixirs to restore youth. In such situations, dermatologists must remain anchored in reality and within the inherent limits of dermato-cosmethology, without making promises they cannot keep and which, long-term, are able to decrease public confidence in this profession. Professionals who work in the field of aesthetics must ensure a high level of safety, due to the fact that such treatments allow little tolerance for errors or poor results
Due to the selective nature and high costs of dermatocosmetic treatments, the dermatologist’s image runs the risk of changing, from its traditional role as a healer, to that of businessman, meaning that the physician first and foremost thinks about material gains, not about the patient’s best interest, an approach which could undermine the physician’s status and reduce the prestige of this medical specialty.

5.5.4. CONCLUSIONS

The practice of dermatology has undergone a drastic change recently, becoming a high-risk specialty, which entails a careful and honest assessment of the risks and benefits offered to patients, and of its real or potential conflicts of interests (Carroll, 2006).

Dermatoethics can be regarded as a subfield thereof, ensuring the placement of dermatology among the medical specialties in which the respect for the human being, in its entirety, is primordial. The progress of this new subfield of dermatology, aimed at identifying and solving problems that occur both on the background of older practices, as well as motivated by the swift progress in this field, becomes obvious. Based on these considerations, one can argue that selling cosmetics in dermatological practices is acceptable, if not even recommended, if it is based on respecting patients’ interests (Gold, 2006).

Essentially, there should be a clear demarcation between the industry and dermatology, due to the fact that the public’s perception of this medical specialty as being “for sale” endangers patients’ trust in their physicians. Dermatologists must preserve their decisional autonomy, their major interest being the respect for patients’ rights (Franzblau, 2006).
SECTION II

PLANS OF CAREER DEVELOPMENT AND FUTURE EVOLUTION OF RESEARCH

The capitalization on my personal experience in dermatovenereology constitutes the turntable of my future professional and scientific plans. More specifically, my future research will continue in the following major directions:

- conducting studies in the field of psoriasis, with a focus on cardiovascular risk assessment in patients suffering from psoriasis vulgaris, as well as on the role of vitamin D in treating this pathology;
- deepening the analysis of the clinicohistopathological and ultrasound correlations in melanocytic and non melanocytic lesions;
- carrying out a complex assessment of the specific changes related to nail pathology in systemic diseases.

Starting from existing accumulations which define my academic work in terms of education, research and medical care, the projects which I intent to develop entail several essential conditions:

- consolidating the existing logistics base, so as to allow the transition from clinical research studies to approaching pathogenic mechanisms specific to the skin pathology, at tissue and molecular-level;
- enhancing interdisciplinary collaboration;
- rigorous and objective selection of human resources with real potential for research in the field of dermatology, thus ensuring the premise for training within a doctoral program.

Clinicopathological and therapeutic interferences in the evolution of psoriasis vulgaris

Although not extremely common, the possible complications of psoriasis must not be overlooked, especially as they usually occur in severe forms of the disease. These patients have been demonstrated to suffer from increased morbidity and mortality due to cardiovascular diseases, especially associated in cases of a serious skin diseases or drug resistance. Young patients with severe illness, in particular, have an increased risk of developing acute myocardial infarction (Naldi, 2004).

Consecutively, the major desideratum is to optimize diagnosis and treatment protocols in patients suffering from psoriasis vulgaris, by implementing and applying modern clinical
and paraclinical diagnostic evaluation methods, for the purpose of highlighting, as early as possible, the risk of cardiovascular disease. Thus, future research will target the patient suffering from severe psoriasis in association with cardiovascular disease—a priority for the Dermatology Clinic team under my coordination, materialized in a project application, submitted and won at a national competition organized under the aegis of the Romanian Society of Dermatology.

The projects aims at decreasing the cardiovascular risk in patients with psoriasis, undergoing treatment with biological agents, reshaping therapeutic protocols on the administration of monoclonal antibodies, fusion proteins and other molecules existing in the dermatologist’s therapeutic portfolio.

Moreover, my interest in this pathology also targets the analysis of the relationship between serum vitamin D levels and the activity of the disease, in patients benefiting from biological therapy, in various forms of psoriasis.

Given that disease severity is always linked to its impact on the patient’s quality of life, treatment must be complex and customized. Even though the benefits of modern biological treatments are indisputable, the results obtained can be frequently grafted by the occurrence of side effects or, in some cases, by the possibility of therapeutic failure. Recent studies show that 25-hydroxy vitamin D deficiency is very common in patients with psoriasis vulgaris, being potentially involved in non-responsiveness to treatment. In pathogenic terms, 25-hydroxy vitamin D has many implications in psoriasis through: keratinocyte differentiation regulation, immunological modulation by inhibiting IL-2, IL-6 production, interferon gamma transcription blocking, cytotoxic T lymphocytes suppression and natural killer. The topical vitamin D derivatives (calcipotriol, calcitriol) have an immunomodulatory effect on monocytes, macrophages, T lymphocytes and dendritic cells (Monnier, 2016). This study is already initiated within the Dermatology Clinic team, being supported by another project won in a national competition, launched by the Romanian Society of Dermatology.

- **Clinicohistopathological and ultrasound correlations in the melanocytic and non melanocytic pathology**

  The second direction of research which I intend to follow regards the analysis of clinicohistopathological and ultrasonographic correlations in the pathology of melanocytic and non melanocytic lesions. The project will focus on complex, clinical, imagistic and histopathological evaluation of mucocutaneous pigmented lesions, with a major influence on patients’ lives, life-threatening in the short and long run. Starting from the known data on the mechanism of carcinogenesis, the study will be focused mainly on the mixed nevi having the potential to transform to melanoma.

  Firstly, we will clinically identify the predictive factors of melanocytic activity from the structures of nevocellular lesions. Secondly, we will quantify the potentially dysplastic risk from a dermatoscopic perspective, using the imaging videodermatoscopic technique.

  This modern technique offers high resolution dermatoscopic digital images and allows to analyze asymmetry parameters measured on two axes, to calculate two diameters, to assess
margins and surfaces, colour variations, as well as the DANAOS index. This index provides solid criteria to estimate the benignity, dysplastic or malignant type of melanocytic lesions.

The videodermatoscopy technique is also useful in the case of non melanocyte tumor pathology, as a modern method of imaging diagnosis. I

In parallel, the histopathological investigation, doubled by the immunohistochemical examination, will target the assessment of the melanocytic proliferative activity, of the elements involved in neovascularization, and of the related matrix changes, following the validation of potentially morphological prognostic factors.

- The significance of nail pathology in the context of systemic diseases

The third direction of research is represented by the nail pathology, including by deepening the study on the particularities developed in the context of systemic diseases – particularly, scleroderma, SLE and chronic kidney disease.

Nail damage stigmata are obvious and relatively well known in various skin pathologies: psoriasis with various clinical forms (clinically expressed by tip pitting nail changes, “oil stain” discoloration, onycholysis - which are early signs of diagnosis in arthropathic psoriasis), lichen planus, chronic eczema, alopecia areata. The nail pathology comprises three major categories of abnormalities:

1. signs due to developmental abnormalities of the nail matrix represented by Beau’s lines, onychomycosis, pitting, onychorrhexis, trachyonychia, leukonychia, koilonychia.
2. nail bed disorders: onycholysis, apparent leukonychia, splinter hemorrhage.
3. changes due to pigment deposition under the nail blade, such as longitudinal melanonychia, Hutchinson’s sign, green nail syndrome etc.

The motivation behind this project is generated by the relatively modest data available in main stream publications related to the pathogenic mechanisms that lead to the development of nail lesions in systemic diseases. There are many unknown factors entailing the initiation of disturbances in the keratinisation process, in particular, which provide a generous material for clinical and fundamental research.

The clinical examination of the nail – as a complex anatomical structure – will be complemented by the use of imaging techniques aimed at detecting surface and deep nail changes independently, in order to assess the morphological appearance of the nail fold, bed and blade. In parallel, the project aims at carrying out an analysis based on microscopic morphology, complemented by a molecular structural assessment, to allow the identification of pathogenic changes.
SECTION III

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