



**"CAROL DAVILA" UNIVERSITY OF MEDICINE AND  
PHARMACY BUCHAREST**



**"CAROL DAVILA" UNIVERSITY OF MEDICINE AND  
PHARMACY, BUCHAREST**

**DOCTORAL SCHOOL**

**MEDICINE**

*Novel dermatologic diagnostic techniques in acne*

**PHD THESIS SUMMARY**

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**Content of thesis**

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## Publication list

### 1. Scientific articles, from the thesis research theme, published *in extenso*, in ISI scientific journals:

1. **Cretu S**, Dascalu M, Georgescu SR, CM. S. Personal protective equipment use and face acne in health care providers during the COVID-19 pandemic in Romania: A new occupational acne type? *J Eur Acad Dermatol Venereol.* 2022;36(1):e18-e20. **(FI=9.2)** <https://doi.org/10.1111/jdv.17679> ( Subchapter 5-pages 47-61)
2. **Cretu S**, Dascalu M, Salavastru CM. Acne care in health care providers during the COVID-19 pandemic: A national survey. *Dermatol Ther.* 2022;35(10):e15753. **(FI=3.8)** <https://doi.org/10.1111/dth.15753> (Subchapter 5- pages 47-61)
3. **Cretu S**, Dascalu M, Salavastru CM. Validation of the Cardiff acne disability index and the impact of educational materials in a Romanian cultural adaptation context. *J Cosmet Dermatol.* 2023;22(5):1595-601. **(FI=2.3)** <https://doi.org/10.1111/jocd.15609> (Subchapter 6- pages 62-77)
4. **Cretu S**, Papachatzopoulou E, Dascalu M, Salavastru CM. The role of in vivo reflectance confocal microscopy for the management of acne: A systematic review. *J Eur Acad Dermatol Venereol.* 2023;37(12):2428-39. **(FI=9.2)** <https://doi.org/10.1111/jdv.19327> (Subchapter 8-pages 84-99)

### 2. Posters and presentations, from the thesis research theme, presented at scientific events, organized by national and international societies.

1. **Cretu S**, Salavastru C. Cultural and linguistic adaptation for the Romanian language version of the Cardiff acne disability index: a pilot study on the web-based experience of cognitive debriefing. *Iproceedings*; 2021.
2. **Cretu S**, Salavastru C M. Cognitive debriefing for the cultural and linguistic adaptation for the Romanian language of the Family Reported Outcome Measure, an online pilot study. 31st European Academy of Dermatology and Venereology Congress; 7-10.09; Milan, Italy2022.

3. **Cretu S**, M Dascalu, Salavastru CM. Acne awareness among healthcare providers and medical students-a post-hoc analysis. 32nd Annual Meeting of the European Academy of Dermatology and Venerology; Berlin2023.
4. **Cretu S**, Salavastru C M. Partial responder characteristics from a web-based validation of educational materials for acne patients and of the Romanian cultural adaptation of the Cardiff Acne Disability Index(CADI). 11<sup>th</sup> edition of the Congress of the "Carol Davila" University of Medicine and Pharmacy; 26-28.10.; Bucharest 2023.
5. **Cretu S**, Salavastru C M. Comparative analysis of ultraviolet-induced red fluorescence of facial follicles in healthcare providers in the context of medical face mask usage during the COVID-19 pandemic in Romania-a cross-sectional study. 11<sup>th</sup> edition of the Congress of the "Carol Davila" University of Medicine and Pharmacy; 26-28.10.; Bucharest 2023.

## Introduction

One of the most common skin conditions, with a chronic course of evolution involving individuals from various age groups, acne[1–4] presents with lesions located in visible, cosmetic sensitive areas [2,3]. Because the involvement is easily observable acne patients may present with decreased quality-of-life and this aspect needs in-depth evaluation [5–7]. Consensus is lacking regarding minimal diagnostic criteria for acne. [4]This is one of the reasons why self-stated quality-of-life impairment due to this condition is part of the therapeutic decision making [5,8]. Although effective treatment exists, the clinical signs of improvement take time to show [5,9]. As a chronic condition, medication is usually needed for an extended period, often involving long-term maintenance therapy or several courses of treatment for relapses [3,10]. Treatment adherence is an essential part of successful management and accurate knowledge regarding acne treatment may have a positive impact on adherence, whereas decreased awareness or inaccurate knowledge may have an adverse influence [9]. It may sometimes be the case that acne patients lack the correct information regarding correct treatment of their disease. They may choose to refer to social media or Internet articles of unclear quality, presenting, often inaccurate treatment-related advice [11,12].

I chose to study novel diagnostic techniques in acne, even while going through one of the greatest pandemics of modern history, namely the COVID-19 pandemic, because acne still has many unanswered questions and challenges.

The goal of this thesis was to increase the efficacy of acne treatment. To achieve this, I identified the need for some tools, which were lacking and I also found opportunities to improve already existing instruments, because therapeutic success in acne is dependent on many factors. [5,13–16] Acne diagnostic, severity staging, and evaluation of therapeutic success are currently based on clinical examination and quality of life assessment. [5,13–16] For the latter, disease-specific quality of life measurement tools are preferred.[3,7,17]Because clinic examination offers limited and sometimes biased information, both concerning the acne patient's state at a given time point and treatment effects, attempts have been made to add more details to the current evaluation instruments by using other, more objective techniques [18], such as multispectral analysis, [19,20] dermatoscopy[21] or *in vivo* reflectance confocal microscopy (RCM). [21–25] Moreover, the COVID-19 pandemic brought new challenges, including in how acne is managed, [26–30], and telemedicine has offered a way to maintain contact with patients in order to assess them [31,32]and to monitor their treatment, when in person visits were limited. [28]

## **1.2. Working hypothesis**

By using the above-mentioned novel diagnostic techniques, the care offered to acne patients could be optimised and therapeutic success chances could be increased. The stages of this research centred on multiple aspects of this complex issue, namely increasing therapeutic success in acne patients.

## **1.3.Objectives:**

The objectives of this research are presented below.

**Objective no.1:**The evaluation acne characteristics, in general and those of acne mechanica, in particular, as well as the therapeutic approach employed, in a population consisting of doctors and other health care workers, in Romania, during the initial stages of the COVID-19 pandemic.

**Objective no.2:** Acne awareness assessment within two populations with increased medical literacy, namely medical doctors and medical students. Our starting point was the assumption that the members of these populations could be considered a reference standard for elevated acne awareness, and that the general population, likely, less educated in the matter, would seek answers to their concerns among the members of the studied populations.

**Objective no.3:** Adaptation for the Romanian language and culture of an already validated, acne-specific quality of life measurement instrument for acne patients and of a validated quality of life measurement instrument for family members and close relatives of acne patients, based on reported outcomes.

**Objective no.4:** Development of a guideline-aligned educational material for acne patients.

## **1.4. General research method:**

This thesis comprises a series of prospective, cross-sectional studies and one systematic review. All studies conducted and involving human subjects, are compliant with the principles of the Declaration of Helsinki [33], have received favourable approval from the Research Ethical Committee of “Carol Davila” University of Medicine and Pharmacy, Bucharest, and have been conducted according to the study protocol. Data collection was performed using Microsoft Excel, 2021 edition, automatically for questionnaire studies and manually for studies involving in-person visits. Data statistical analysis was performed using SPSS and R4.3.0, RStudio2023.03.1(Posit Software, PBC, USA). For the statistical analysis I used descriptive statistics, such as frequencies and percentages, together with inferential statistics, such as parametric and non-parametric, within- and between- subjects tests. For continuous variables, with normal distribution, means and standard deviations were calculated, for non-normally distributed continuous variables median and interquartile ranges were determined.

## **2. Personal contributions regarding acne features in health care workers during active epidemiologic restrictions amidst the COVID-19 pandemic**

An immediate effect of declaring the global pandemic status was that health care providers (HCP) needed to use permanently medical face masks [34–36] Dermatological complaints involving the face are common due to medical face mask usage.[32,37–43]

Acne is a common and chronic condition which can be both induced and aggravated by mechanical factors ( e.g. such as medical face mask use). [3,44,45]

The aim of the current study is to assess the impact of personal protective equipment (PPE) on acne for HCP during the COVID-19 pandemic in Romania. This cross-sectional study consisted of a self-administered 41-question questionnaire and considered both the short-term impact in the two months in which the state of emergency was declared, as well as mid-term effects during the pandemic in the 7 to 9 months interval which followed.

The data from this chapter have been published as research articles as follows: „ Personal protective equipment use and face acne in health care providers during the COVID-19 pandemic in Romania: a new occupational acne type?” [46],„Acne care in health care providers during the COVID-19 pandemic: A national survey.” [47] and have been presented as a poster at the 32<sup>nd</sup> Annual Meeting of the European Academy of Dermatology and Venereology, in Berlin, Germany, in the research entitled: „Acne awareness among healthcare providers and medical students-a post-hoc analysis”. [48]

The online survey was applied to HCP in Romania from December 17<sup>th</sup> 2020 until February 17<sup>th</sup> 2021 and was developed using Google Forms. A total of 134 answers were recorded, 116 (86.6%) coming from women. Most of the participants, 107 (79.9%) were doctors. Median age of our cohort was 29 years old.

We found that acne lesions were common amongst the study population from the beginning of the pandemic and 65.7% reported acne worsening when considering the 7-9 months that followed the state of emergency, up until questionnaire response. The most frequently encountered individual problems with people exhibiting acne were: black dots 80 (59.7%), red papules 73 (54.5%), rash 60 (44.8%), erythema 56 (41.8%), nodules 39 (29.1%),

itch 37 (27.6%), and scales 36 (26.9%). While considering affected areas for people exhibiting acne, the chin was most often involved (70.1%), followed by the cheeks (41.8%), nose (34.3%), neck (34.3%), perioral (19.4%) and retro auricular (13.4%).

Cross tabulation for acne onset and current acne treatment were performed. From the entire study population 67 (50%) participants reported currently having acne lesions. In 38 of them (56.7%) treatment was reported. A cross-tabulation found that the people with higher emotional impact of the acne lesions were more likely to report current acne treatment use.

Chi-square tests were run to determine the association between various emotional impacts (i.e., caused by the pandemic itself, of PPE usage, and of lesions) and acne, both during and after the lockdown. As expected, strong associations were observed between the emotional impact of lesions and the actual acne condition, and this effect became stronger after the lockdown. As the pandemic continued after the lockdown, emotional impact of the pandemic itself and of PPE usage was less associated to the acne condition, suggesting that individuals became more accustomed to the pandemic context, while remaining highly impacted if lesions were present. While analysing the correlation between the emotional impact of acne lesions and the current usage of anti-acne medication, cross-tabulations highlighted that the people with a higher emotional impact of the acne lesions were more likely to report acne treatment use ( $\chi(4) = 21.876, p < .001$ ).

Anti-acne topical products used were based on salicylic acid, 61.36% ( $n = 27$ ) participants, topical retinoids (40.91%;  $n = 18$ ), benzoyl peroxide (20.45%;  $n=9$ ), azelaic acid (18.18%;  $n = 8$ ), and topical antibiotics (9.09%;  $n = 4$ ). We identified several common associations between these substances: retinoids and salicylic acid reported by 18.18% ( $n = 8$ ) participants, retinoids and benzoyl peroxide by 13.64% ( $n = 6$ ), salicylic acid and benzoyl peroxide by 13.64% ( $n = 6$ ), as well as azelaic acid and salicylic acid by 9.09% ( $n = 4$ ).

Regarding the openness towards telemedicine, participants in our cohort exhibited a low degree of acceptability; 19 (14.2%) reported previously using telemedicine, and 11 (10.9%) reported its usage for acne treatment.

### **3. Personal contributions regarding the cultural and linguistic adaptation for the Romanian language and validation of the quality-of-life measurement tool, the “Cardiff Acne Disability Index (CADI)” and validation of an educational material for acne patients**

#### **3.1. Validation of the Romanian cultural and linguistic adaptation of the CADI**

As per the standardized translation guideline of the Cardiff University, from the United Kingdom, we performed a cognitive debriefing study, as part the cultural and linguistic adaptation of the CADI for Romanian. Following the successful adaptation of the CADI for Romanian, we conducted a 12-week cross-sectional validation study, which also added the validation of an educational material for acne patients. The validation study involved a self-administered questionnaire, developed using the Qualtrics platform and centred on medical students in their 3<sup>rd</sup> to 5<sup>th</sup> year of study.

The results of the cognitive debriefing study process for the Romanian adaptation of the CADI were presented as a poster, entitled:” *Cultural and Linguistic Adaptation for the Romanian Language Version of the Cardiff Acne Disability Index: A Pilot Study on the Web-Based Experience of Cognitive Debriefing*” [49] , presented at the 9<sup>th</sup> World Congress of Tele dermatology, Imaging and AI for Skin Diseases, held online. The results of the validation process of the Romanian CADI adaptation together with the validation of the educational material for acne patients were published in the research article entitled: “*Validation of the Cardiff acne disability index and the impact of educational materials in a Romanian cultural adaptation context*”. [50]

Guided by the team at the Cardiff University, we underwent the stages of the standardized translation process: forward translation, reconciliation of translated versions, back translation and cognitive debriefing. The latter stage, involved applying the CADI to a group of patients, followed by individual interviews in which each question was discussed. We administered this measure as an online form, developed using the Qualtrics platform. The completion time for each individual question, as well as for the entire survey were automatically recorded. The interviews assessing the comprehensibility and suitability for Romanian language and culture were also held as live online meetings.

The validation study was based on a two-part survey, one with the CADI, administered in two distinct time points, for which the completion time was recorded automatically for each individual question, in both applications. First, the participants answered the questions in the Romanian CADI, then questions from the acne awareness questionnaire. Following, they

received the educational material and afterwards both questionnaires were readministered.

For the cognitive debriefing study, we considered 7 patients, 4 females and 3 males, aged between 19 and 34 years. All subjects were native Romanian speakers. They had mild or moderate acne. Mean completion time for the survey was 3.28 minutes. The mean value for the CADI was 5.4286. All participants agreed that the language used in this quality-of-life measurement instrument was simple, clear, and adequately used in their native language.

For the validation of the Romanian CADI, as well as for the validation of the educational material, at the end of the study period, 153 answers were recorded. After filtering incomplete answers, 95 complete answers were analysed. The mean age of participants was 22.12 ( $\pm 1.147$ ) years, 88(92.6%) were female and lived in urban areas. Acne presence was reported by 71 (74.7%) participants, out of which 52 (73.23%) stated to have visited a physician for this condition. In all cases, the physician's specialization was Dermatology. With a Cronbach's Alpha of 0.807 in the first application of the CADI and a Cronbach's Alpha of 0.839 in the second application, the Romanian adaptation of CADI showed good internal consistency. It also demonstrating high test-retest reliability, with an interclass correlation coefficient (ICC) of 0.987, and the Spearman's rank correlation coefficient ( $r_s$ ) of 0.970 for the overall CADI scores between the two applications. These values are comparable with other cultural and linguistic adaptations, as well as the original English measurement tool. [7,17,51,52] The median overall CADI score was 4 for both questionnaire administrations, and the mean completion time was 49.682 ( $\pm 28.423$ ) seconds for the first application and 25.551 ( $\pm 6.839$ ) seconds for the second one. Acne-affected subjects had higher values of CADI (Mann-Whitney U = 264.5,  $p < .001$  for the 1<sup>st</sup> administration; Mann-Whitney U = 283.5,  $p < .001$  for the 2<sup>nd</sup> administration). The Romanian CADI showed good specificity for acne, as there were significant differences in subjects' responses affected or not by acne, consistent with other literature reports from other languages. [7,17,51,52]

### **3.2. Validation and impact of an educational material for acne patients**

The mean baseline score of our cohort was 15.52 points ( $\pm 1.556$ ) in the knowledge level evaluation questionnaire regarding acne pathophysiology and treatment, from the maximum 21 points. There was an overall increase in the mean score of 18.03 ( $\pm 1.865$ ) after exposure to the educational material. No statistically significant differences were noted between answers from subjects affected or not by acne. The educational material for acne patients was considered useful or very useful by 78(82.8%) participants. Our studies found that the Romanian version of the CADI is a reliable and disease-specific instrument for quality-of-life

measurement in acne patients. It can also be successfully delivered electronically. Additionally, our results suggest a constant and perhaps unmet need for patient education regarding acne, even in highly educated populations. Romanian acne patients can now benefit from an acne specific quality of life measurement tool and an educational material in their language.

#### **4. Personal contributions regarding the cultural and linguistic adaptation for the Romanian language of the Family Reported Outcome Measure (FROM-16)**

The Family Reported Outcome Measure (FROM-16) is a validated tool for quality-of-life evaluation in close family members of patients suffering from chronic dermatologic conditions.[53]The original English version was translated to other languages. For the Romanian language an adaptation had yet to be developed. [54]

The main objective of this study was to evaluate the comprehensibility of the adaptation for the Romanian language of the FROM-16, in a small sample of close family members of patients affected by cutaneous disorders.

Similar to the CADI adaptation, with the support of the team from Cardiff University, we underwent the stages of the standardized translation process: forward translation, reconciliation of translated versions, back translation and cognitive debriefing. The latter stage, involved applying the FROM-16 to a small sample of patients, followed by individual interviews, discussing the questionnaire globally and each individual question. We administered this measure as an online form. The completion time for each individual question and for the entire survey were automatically recorded. The interviews assessing the comprehensibility and suitability for the Romanian language and culture were held as live online meetings.

The results of this study were presented as a poster presentation at the 31<sup>st</sup> Annual Meeting of the European Academy of Dermatology and Venereology, held in Milan, September 2022. The work was entitled : “Cognitive debriefing for the cultural and linguistic adaptation for the Romanian language of the Family Reported Outcome Measure, an online pilot study”.[55]

Six family members of patients with dermatologic conditions were included, 4 females and 2 males, aged between 24 and 43 years, median age 33.5 years old. All subjects were native speakers of Romanian language. Most patients had a single dermatologic condition (n=5, 83.3%). Patients included suffered from atopic dermatitis (n=4, 66.7%) and acne (n=3, 50%), one patient associated both conditions (n=1, 16.7%). Mean completion time for the

survey was 2.68 minutes. The mean score for the FROM-16 was 10.17. All participants agreed that the language used in this quality-of-life measurement instrument was clear and adequately used in their native language. They particularly appreciated that the questions were simple and direct. The original, English FROM-16 has a short completion time, of approximately 2 minutes. [53,54,56] The Romanian adaptation achieved a similar to the completion time. The Romanian lacked a measurement instrument based on reported outcomes to evaluate the quality-of-life impairment in close family members of patients affected by cutaneous disorders. The cultural adaptation for the Romanian language of the FROM-16 has successfully been achieved with the help of teledermatology. During the COVID-19 pandemic, physical visits were limited, and this technique provided the framework to efficiently carry through the stages of the standardized translation process.

## **5. Personal contributions regarding the use of in vivo reflectance confocal microscopy in acne-a systematic review**

For a higher understanding regarding the use of RCM in acne we performed a systematic literature review to assess the usefulness of this technique in acne. The results of this study have been published in the research article entitled: "The role of in vivo reflectance confocal microscopy for the management of acne: A systematic review", published in the Journal of the European Academy of Dermatology and Venereology. [57]

We created a research protocol registered in the International Prospective Register of Systematic Reviews " (PROSPERO) under the record CRD42021266547. The results of our review were reported according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) recommendations. [58,59] Two independent researchers (Ștefana Crețu and Evdokia Papachatzopoulou) performed the literature search in the same day. Data bases investigated were: Google Scholar, PubMed and Clarivate, from their creation to the day of the search. Each of the two researchers performed two separate searches using the following keywords: "acne and confocal microscopy" and "reflectance confocal microscopy and acne." Articles identified contained these key words either in their title or abstract and all the articles selected for inclusion in the review were peer-reviewed.

The risk of bias for the selected articles was assessed using the QUADAS-2 tool [60–63]. Clinical examination was set as the reference standard and RCM was selected as the index test. The 3 data bases searched retrieved a total of 2.184 records. After filtering duplicates, 1608

publications were screened for inclusion based on title and abstract. Finally, we considered the full-text screening of 35 articles and 14 publications were included in the final review.

Overall, the studies investigated considered 291 participants, 216 acne patients and 60 healthy volunteers. Hair follicles examined across all studies were 456 from healthy participants, 1445 from uninvolved skin in acne patients and 1472 acne lesions ( comprising closed and open comedones, papules and pustules). We found 5(36%) articles reporting means and standard deviations [23,24,64–66] and 9(64%) lacking these details. [67–75] Treatment follow-up was the main outcome in 6(43%) studies. [23,67,68,70,71,75] Acne severity was not specified in 4(28.57%) publications. [68,72–74] Most studies, namely 7(50%) included patients with mild-to-moderate acne [23,24,64–66,70,71], whereas the remaining 3(21.43%) considered patients with only mild acne. [67,69,75] The mean confocal parameters reported was 7(+2.45), for studies reporting means and standard deviations. [23,24,64–66] Although with high heterogeneity with regards to how the findings were reported, we found a set of relevant features consistently investigated throughout the articles included: follicular infundibulum size, border type and inflammation. [23,24,64–66] Considering the studies lacking mean values and standard deviations, the mean number of confocal findings reported was 3 (+1.25), lower compared to the former category of publications. [67–75] The overall risk of bias across all studies considered was high or unclear, particularly regarding the patient selection. Using the QUADAS-2 instrument we found that RCM has high applicability in acne.

## **6. Personal contributions regarding the use of *in vivo* reflectance confocal microscopy for assessing the facial skin of medical workers in the context of exposure to medical face masks**

The study conducted aimed to identify aspects related to acne mechanica pathogenesis in the context of medical face mask (MFM) usage in a population consisting of medical doctors and 6<sup>th</sup> year medical students. We evaluated the differences between the unexposed glabella and the chick-chin junction (CCJ), exposed to the action of the MFM, using a multimodal approach based on clinical examination, fluorescent photography (FP), and *in vivo* reflectance confocal microscopy (RCM).

Acne severity staging was performed qualitatively using the Investigator's Global Assessment [5] during in person visits and quantitatively using the methodology of Lucky et al, using clinical photographs and manual counting. [76]

Clinical photographs were obtained using the VISIA-CR device. Frontal and 45-degree side images acquired using cross polarized and parallel polarized light were analysed. Also, we recorded fluorescence photographs, using ultraviolet A radiation (UVA), acquired using the same device, on which we evaluated the porphyrin load by studying the ultraviolet red fluorescing spots (UVRFS), separately, for each of the two facial areas under consideration. [19,20]

From the entire face images, we selected, using the Image J software, two standardized 8x8 mm sections, one from the glabella, the other, from the cheek-chin junction. On these sections we manually counted ultraviolet red fluorescing spots (UVRFS). We recorded the total spot number, measured the area they occupied and calculated the percentage of area covered by UVRFS, separately, for each of the two facial regions. Ultraviolet red follicular fluorescence has been shown to be the result of sebum secretion and microbial activity within the sebaceous follicle. [77–79]

After acquiring a standard 10x10mm dermatoscopic image using the VivaCam (Caliber ID Inc., USA) from each of the two face areas considered. We selected an 8x8mm section to enhance comparison with the fluorescent and RCM images. We manually counted yellow and brown plugs, corresponding to comedones [21,25,80,81], measured their surface and calculated the percentage of covered area.

Based on facial anatomy, we sampled using RCM similar areas of interest in all subjects, regardless of acne history, clinical presence, or absence of acne lesions. We used the commercially available Vivascope 1500 (Caliber ID Inc., USA), at 785nm laser power, to acquire 8x8mm VivaBloc mosaics from each of the two considered regions (i.e., glabella and cheek-chin junction). Three image mosaics set 30µm apart in depth were obtained. For each subject, a minimum of 2 Viva stacks were acquired for non-inflammatory acne lesions in each of the 2 areas investigated. Also using the Image J software, we manually counted and measured hair follicles (HF) from the two facial area evaluated, on 8x8 mm mosaics. We also recorded follicles with bright borders, corresponding to hyperkeratinisation, [23,24,64] content filled follicles [23,24,64–66,70], follicles showing intra or perifollicular inflammatory signs [23,24,64–66,70] and follicles containing *Demodex folliculorum* [65]. Follicles showing grey amorphous material or granular grey debris [66] were considered content-filled. Signs of inflammation were considered the presence of at least one of the following confocal features: small bright dots inside or around HF, white round-to-polygonal cells in the epidermis, exocytosis, and dermal inflammation. [23,24,64–66] Consistent with the methodology

described by Manfredini *et al.* [23,24,64], we evaluated regular (infundibular diameter <90µm) and irregular follicles (infundibular diameter 90-200µm and 200µm) [24] to assess the follicular density and compared it between glabella and cheek-chin junction for each subject.

Stratum corneum (SC) thickness was assessed in the perifollicular area, not the interfollicular epithelium, because we aimed to evaluate HF features throughout all cutaneous layers.

During the 3-month enrolment period, 14 consecutive subjects chose to participate. The mean age was 29.93(±4.53) years old, 11 were female, and the phototypes included were II-IV. There were 11 medical doctors and 3 medical students. Total acne lesion count ranged from 59 to 327 lesions, with a mean of 156(±69).

The mean of the number of inflammatory lesions was 12.43(±11.41) in the glabella region and 21.78(± 11.63) in the cheek-chin region. The difference between the two was statistically significant ( $t=2.871$ ,  $p=.013$ ).

On the 8x8 mm sections selected, we analysed 212 UVRFS from the glabella and 136 UVRFS from the cheek-chin junction region. There were significantly more UVRFS in the glabella region ( $t=4.218$ ,  $p=.001$ ). The overall average percentage of area covered by the UVRFS was 12% for the glabella sections and 7% for the cheek-chin sections. The average area covered by UVRFS was significantly larger in the glabella sections than in the cheek-chin section ( $t=3.853$ ,  $p=.002$ ).

Regarding dermatoscopic examination in acne, the differences regarding the mean number of comedones, the mean area and the mean percentage of area covered were not statistically significant between the two regions.

After processing RCM images, we considered a total number of 9,546 follicles, with a total of 4,966 follicles from the glabella region and 4581 from the cheek-chin junction region. At the epidermal level, there were 77.83% hyperkeratotic follicles in the glabella and 88.04% in the cheek-chin region. A statistically significant difference could be observed between the glabella and the cheek-chin regions as regards the proportion of bight border follicles only at the epidermal level ( $t=2.466$ ,  $p=.003$ ). At the dermal-epidermal junction level, the percentage of follicles showing intra-or peri-follicular signs of inflammation was also higher at this measurement depth in the cheek-chin region than in the glabella, 65.70% versus 54.56%. The difference was statistically significant ( $t=3.465$ ,  $p=.004$ ).

We found that the median SC thickness was 23µm(IQR=6.13) in the glabella, compared with 19.125µm(IQR=4.50) in the cheek-chin region. The differences were statistically significant ( $Z=2.484$ ,  $I=.012$ ). No spongiosis was noted.

## 7. Conclusions and personal contributions

This PhD thesis analysed the current state of knowledge regarding acne in general, in a global context, as well as in the Romanian setting. I analysed the currently optimum treatment choice and follow-up modalities, based on accurate diagnosis of the condition, severity staging, insurance of therapeutic adherence, aiming to diminish treatment failure.

I investigated literature evidence for both acne vulgaris and acne mechanica. In the light of the COVID-19 events, new challenges, and opportunities arose such as the acne mechanica subtype occurring in association to medical face mask usage.

**Objective no.1** entitled “The evaluation acne characteristics, in general and those of acne mechanica, in particular, as well as the therapeutic approach employed, in a population consisting of doctors and other health care workers, in Romania, during the initial stages of the COVID-19 pandemic” was achieved and conclusions are presented below.

In the first study conducted to achieve this objective the findings showed that the Romanian health care workers developed more commonly acne lesions in the facial area covered by the medical face mask, during the COVID-19 pandemic, when epidemiologic restrictions were issued, that the presence of these lesions had a profound and persistent emotional impact, and that lesion management was suboptimal.

Also, as part of achieving this objective, the literature review centred on the use of RCM in acne concluded that there is an increased need of standardisation in this research field considering the research method and data reporting, that the applicability of the technique in acne research is high, with regards to diagnosis, severity assessment and treatment monitoring; the constant and early presence of the bright border follicles in acne patients both in the area covered by lesions, as well as the clinically uninvolved skin of acne patients, together with the ratio between normal and dysmorphic hair follicles in diagnosis and treatment follow-up. Therapeutic monitoring using RCM could offer palpable and early feed-back to patients throughout the course of their therapy and might increase treatment adherence. However, confirming these hypotheses constitutes a path for future research. Other possible directions for future studies are the use of RCM in evaluating truncal acne, or in people with type IV-VI phototypes.

Additionally, for achieving the above-mentioned objective, the conduction of the study centred on the multimodal approach to acne mechanica, developed in association to MFM was necessary. We chose a population of consistent and reliable MFM users made up of medical doctors and 6<sup>th</sup> year medical students, in which we compared two areas of the face, one exposed

to the MFM, namely the cheek-chin junction, and one unexposed, the glabella. The study found through RCM follicular hyperkeratinisation and inflammation, in the area covered by the MFM. We found a thinner stratum corneum in this region, supporting previous findings that skin barrier integrity may be affected by this piece of personal protective equipment. No spongiosis was noted in our study, making the hypothesis of allergy to MFM components less likely. Also, using fluorescent photography, we found that the distribution of UVRFS, known to be an indirect marker of seborrhoea and microbial colonisation, was both in terms of UVRFS number, as well as average area covered, higher in the glabella compared to the cheek-chin junction. This finding is aligned with pre-pandemic studies[82,83], suggesting that microbiota alterations or seborrhoea due to MFM usage may not be the main factors responsible for the lesion's presence. Our observations may guide treatment for acne mechanica and educate patients, especially in avoiding further irritation from unsuitable treatments and unnecessary exposure to antibiotics. This study adds to the existing literature that follicular hyperkeratinisation, inflammation, and irritation may play central roles in the pathogenesis of acne mechanica associated to MFM usage, outweighing other possible alterations. The study was conducted in a setting close to the real-life conditions of the populations evaluated. One of the strengths of our study is that we standardised the surface area examined, in a multimodal approach, considering several distinct techniques, for a comprehensive examination.

Also, having analysed through RCM all the follicular features described on 8x8 mm mosaics, at distinct depths, offers increased consistency to our data regarding the characteristics of the hair follicles in the areas considered. One unresolved issue that remains is causality. This was an observational study identifying association. Future studies are needed to establish causality.

**Objective no.2**, entitled “Acne awareness assessment within two populations with increased medical literacy, namely medical doctors, and medical students” was achieved. The conclusions of the studies conducted for achieving this objective are presented below.

First, in the studies conducted, we found that medical doctors and medical students have relatively high awareness regarding acne, as expected. However, some information lacked or was inaccurate. Knowledge regarding specific aspects such as the importance of an underlying keratinisation disorder in acne, was, more often low, with a tendency of both groups to accentuate the role of sebum secretion and microbial colonisation in acne. An unresolved issue is the assessment of acne awareness within the members of the general population, to precisely establish their knowledge in this matter, and to identify the areas needing improvement.

**Objective no.3**, entitled “Adaptation for the Romanian language and culture of an already validated, acne-specific quality of life measurement instrument for acne patients and of a validated quality of life measurement instrument for family members and close relatives of acne patients, based on reported outcomes” was achieved.

The studies conducted to achieve this objective concluded that the Romanian version of the CADI preserves the characteristics of the original version, with a high degree of specificity for acne, good internal consistency, and high test-retest reliability.

Additionally, the Romanian version of the CADI was evaluated as clear and adequately used in their native language, by the Romanian speakers interviewed. Moreover, we successfully achieved this cultural adaptation through electronic means of communication.

Another study conducted for achieving this objective was the cognitive debriefing study for the Romanian cultural and linguistic adaptation of the FROM-16 quality-of-life measurement instrument. The study concluded that this adaptation was clear and adequately used in their native language by native Romanian speakers. Also, the adaptation was successfully achieved through electronic means of communication, with the help of tele dermatology.

**Objective no.4** entitled “Development of a guideline-aligned educational material for acne patients” was achieved. The study we performed for this objective concluded that there is an ongoing need for patient education regarding acne, even in highly educated populations. The educational material for acne patients was considered useful or very useful by most of the participants. Nevertheless, the evaluation of this educational material was not performed in the context of everyday clinical care, with common acne patients. The impact of this educational material on the treatment outcomes in the real-life clinical setting represents a direction for future research.

### **Personal contributions**

The studies conducted and presented in this PhD thesis brought the following contributions, presented below, with subchapter and page specified.

1. Healthcare providers in Romania are at risk to develop dermatologic conditions in association to medical face mask usage, particularly acne. (subchapter 5, page 50)

2. Acne associated to medical face mask use became more common as the period required to wear masks extended. (subchapter 5, page 50)

3. Areas involved by lesions were in proximity to each other and were suggestive of

acne associated to medical face mask use. In 15.7% of cases the cheeks, nose and chin were simultaneously involved, in the same person and in 14.2% the cheeks, chin and cervical region were affected at the same time, in the same person. (subchapter 5, page 55)

4. The study conducted found that when the affected individuals felt that their acne required treatment, they chose to self-medicate. However, the treatment approaches, substances used and their associations, were rarely guideline-aligned. (subchapter 5, page 53)

5. Participants from this study reported an elevated emotional impact generated by the presence of their acne lesions, higher than that induced by wearing protective equipment or by the pandemic itself. Those who were most affected also showed the highest tendency to seek help. (subchapter 5, page 54)

6. Healthcare workers questioned were reluctant in using telemedicine as a patient. (subchapter 5, page 55)

7. The cognitive debriefing study conducted for the cultural adaptation for the Romanian language of the Cardiff Acne Disability Index (CADI) resulted in the successful adaptation of this quality-of-life instrument for the desired language. Additionally, it demonstrated that following the stages of this process electronically and through telemedicine is possible, with results similar to those obtained in the traditional paper-and-pen, face-to-face approaches. (subchapter 6, page 63)

8. After successful cultural adaptation of the CADI, the tool was also validated for the Romanian language, also in electronic format. (subchapter 6, page 67)

9. The validation study also assessed theoretical knowledge regarding acne pathophysiology and treatment in a population consisting of 3<sup>rd</sup> to 5<sup>th</sup> year medical students, who demonstrated a relatively high accuracy to our acne awareness questionnaire. Despite this, some acne-specific information lacked, and they were achieved after exposure to the educational material under validation. (subchapter 6, page 70)

10. The majority subjects participating in this validation study, specifically 82.8%, appreciated the educational material as useful or very useful. (subchapter 6, page 70)

11. The cultural adaptation for the Romanian language of the FROM-16 has successfully been achieved with the help of teledermatology. (subchapter 7, page 80)

12. Applying the QUADAS-2 tool we found a high level of applicability of RCM in the management of acne. (subchapter 8, page 93)

13. The same study identified a set of RCM features systematically described, with increased relevance for acne evaluation, including, but not limited to, treatment follow-up.

These follicular features are as follows:1) presence of a bright, thick, hyper-refractile follicular border, in the case of acne affected patients, both in clinically visible regions, as well as in clinically uninvolved areas; 2)existence of a ratio between normal and dysmorphic follicles in acne patients, with dynamic evolution under treatment;3)presence of perifollicular inflammation, consequent to infundibular hyperkeratinisation, the latter being the most precocious change noted in the follicles of acne patients. (subchapter 8, page 89-93)

14.Our systematic review identified opportunities to improve for future studies. The overall risk of bias for current studies was unclear or high, particularly because various methodologies, with important differences and various terms referring to the same notion, were used and especially in the patient selection process. Other unclear reports were concerning the order in which the investigations were performed and who made the evaluation for the clinical exam, as well as for the RCM. (subchapter 8, page 93)

15. Our study involving the multimodal analysis of skin findings in the area covered by the MFM, confirmed, through clinical assessment of the subjects, by identifying the presence of open and closed comedones in the area covered by the MFM, that participants were affected by acne. (subchapter 9, page 107)

16.In the same study, we identified significantly more inflammatory lesions in the area covered by the MFM. (subchapter 9, page 107)

17. This study investigated the abundance of UVRFs through fluorescent photography and found that the number of UVRFs and the average area they covered were higher in the glabella compared to the cheek-chin junction. (subchapter 9, page 108)

18.In our study, dermatoscopy in acne failed to identify parameters that may be correlated to confocal ones or other statistically significant differences between the two regions examined, suggesting that the detailed examination RCM provides is challenging to replace. (subchapter 9, page 109)

19. Using RCM we found that bright border follicles are more numerous in the cheek-chin region compared to the glabella in the epidermal layer. These observations indicate that that hyperkeratinisation may be an important event in acne mechanica. (subchapter 9, page 110)

20.RCM allowed the observation of inflammatory findings associated to sebaceous follicles and revealed that the percentage of follicles with peri-or intrafollicular signs of inflammation in the cheek-chin region was higher compared to the glabella. (subchapter 9, page 110)

22. Additionally RCM exam discovered a thinner SC in the cheek-chin area compared to the glabella, supporting the skin barrier damage the MFM may induce. (subchapter 9, page 112)

23. In the study conducted, using RCM no spongiosis was noted, indicating that there is a low possibility that the inflammation be associated to an allergic reaction to the components of the MFM, but more like the consequence of direct irritation. (subchapter 9, page 112)

Directions for future research are multiple and include evolution of the impact on treatment outcomes of the educational material in acne patients; facial skin analysis using RCM, on multiple samples taken from the patient's skin, together with using the technique to identify early adverse reactions of treatment. Also, another potential direction for future research could be the evaluation using RCM of acne patients below the age of 13 years old, which is currently the lower age limit for current studies. What is more, another future direction for research is the study of the quality of life in affected patients and the evaluation of the impact the newly adapted instruments for Romanian language might have in day-to-day practice. Lastly, in the future, patient examination may combine RCM and optical coherence tomography, spectrophotometric and hyperspectral analysis, all of these on portable devices and enhanced optimized with artificial intelligence algorithms.

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