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ORIGINAL ARTICLE

Relationship Between Psychological Resilience and Attitudes Towards A.I. in Emergency Workers

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Abstract: The impact of artificial intelligence (AI) on our lives can no longer be neglected, and thus, understanding this phenomenon and how humans interact with it is becoming more important than ever. The study emphasizes one of the psychological characteristics that helps us in accepting AI and using it to its fullest, namely the psychological resilience that each of us, and in particular emergency service workers, possesses in different proportions. The analysis of the relationship between the two dimensions (psychological resilience and AI) highlights, among other things, the importance of how organizations can use this interaction to achieve a framework for developing the psychological resources of their employees so that they are increasingly receptive to the use of AI in their work, pursuing the benefits it brings. The study is conducted on 89 emergency service workers and focuses on the positive and highly statistically significant relationship between psychological resilience and attitudes towards AI. The study also particularly emphasizes the relationship between tenacity and self-efficacy, self-confidence, ability to learn from life experiences, rapid recovery from negative events, social and family resources as dimensions of psychological resilience in relation to attitude towards IA of emergency workers.

Keywords: mental resilience, artificial intelligence (A.I.), attitude, emergency workers

INTRODUCTION

People everywhere have suffered from various life events, but these have also provided an open research background from which we can affirm that some psychological characteristics, including psychological resilience, can support a sense of well-being, the ability to quickly restore happiness, post-traumatic growth, and some positive coping strategies after experiencing these events. One of the occupations where increased resilience is needed is that of emergency workers (firefighters, paramedics, police, psychologists, search and rescue teams).

Intervening in emergencies places maximum demands on the cognitive and neurological resources of each person working in such situations. It is thus imperative to understand how the brain processes information, manages stress, and makes quick decisions, because this understanding will lead to optimizing performance and maintaining the long-term mental health of these workers [1, 2, 3, 4]. Also, if we take into account that repeated exposure to traumatic events and high levels of stress can negatively affect the hippocampus, leading to difficulties in concentration, altered prosexic function, memory errors, or even freezes, all this makes it even more important to study the effects of stress on these workers and how they build resilience. One answer to all this may be A.I. through the various ways it offers to stimulate understanding of situations, acceptance of differences in reaction, stimulate coping mechanisms, highlight stress levels (including its various health effects through wearable devices), etc. A.I. can be used in different simulations to understand how they learn, or the different reactions in interventional cases, or afterwards; monitoring with wearable devices of different data and then analyzing them with A.I. (early signs of deterioration of health or well-being can be identified and proactive interventions can be proposed), identification of training needs, alternative learning routes customized on different worker characteristics, personalized training scenarios etc.

AI can be used to create a resilient infrastructure, a resilient organization, and resilient ways of working, and thus contribute to increasing the resilience of the participants in the process. Health is an important component for all people and the systems in which they function [5, 6, 7, 8, 9, 10].

A.I. is very helpful to emergency workers by:

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- rapid data collection and analysis (huge volumes of information can be processed in a short time to provide a quick and accurate overview of the situation);
- predictability and anticipation (A.I. can predict the evolution of an emergency by analyzing existing data, movement patterns of fire, typhoon, etc.);
- optimization of resource allocation (A.I. can help to optimize the distribution of crews, vehicles, and equipment, taking into account factors such as distance, traffic, type of emergency, and availability of resources);
- decision support (through rapid data analysis, scenario generation, AI can provide emergency workers with critical information for decision-making in situations of stress, and uncertainty - e.g., rapid patient resuscitation, prioritizing serious cases, etc.);
- robotics, and automation (robots, drones can penetrate areas that are dangerous for humans, and collect data to streamline intervention);
- effective, and improved communication (A.I. systems can help with real-time translation, answering emergency calls, and relaying information efficiently to teams and the public).

All these provide emergency workers with increased safety, increased efficiency in situations they would not have coped with on their own (e.g. dangerous areas they could not enter), better decisions through access to more complete information or a large volume of information processed quickly, reduced cognitive load (A.I. can take over repetitive tasks or data analysis, allowing staff to focus on human, and operational aspects).

Over the years, researchers have defined resilience in various ways. For example, in some studies [11], psychological resilience is considered to be the ability of individuals or outcomes to adapt successfully to challenging or threatening circumstances. Resilience also reflects a person's ability to maintain stability and equilibrium in the face of difficulties caused by particular life situations, including the epidemics that we are increasingly facing, and people with resilience might also experience positive emotions. It also states that resilience is characterized by positive behaviour, and attitudes in the face of adversity, courage, and perseverance in coping with life's difficulties, possessing a positive attitude even in the face of adversity, enjoying intimate relationships, and expanding social circles.

Other studies [12,13] highlight some key components of resilience, including self-improvement, and particularly positive attitudes maintained during adverse situations, learning from negative and/or positive experiences, novelty seeking, emotional regulation, positive future orientation, and the pursuit of meaningful life goals. Research on mental resilience considers it to be a person's ability to adapt positively to adverse circumstances or to maintain adequate functioning even in the midst of disastrous situations.

Experts are more and more concerned about the effects of life events on the psychological apparatus, especially long-term psychological problems, such as a decrease in subjective well-being, a decrease in adaptability, the onset of psychological trauma, etc., and how to combat them [14]. When the adversity experienced in PTG (Posttraumatic Growth) is high, it can stimulate the person's ability to withstand hostile and traumatic environments, therefore also increases the ability to enhance or accelerate his or her experience of posttraumatic growth [15]. However, research over the last two decades indicates that not all people who face adversity will suffer from mental or emotional health problems. It is known that some people experience positive emotions and psychological growth/development, and this is known as positive mental health, and changes include post-traumatic growth and resilience [16,17]. Scientists' concerns for resilience are all the greater as the world is constantly changing, natural disasters have multiplied, and people are often in stressful situations due to these changes. All the more, emergency workers have more challenges in the current context of climate change and the presence of many natural disasters. Certainly, A.I. facilitates knowledge of data that predicts the occurrence and evolution of traumatic events, and through this, can be useful for building resilient cities, resilient communities, and resilient people in general [18].

Although A.I. can be of real help to emergency workers in building their resilience, there are also some challenges they face in relation to A.I.:

- acceptance of A.I. – the level of acceptance of A.I. is related to the age of people (digital immigrants or digital natives, for example), the degree to which they find it useful, and easy to use;

- dependence on technology – over-reliance on A.I. can lead to problems in the event of technical failures or cyber-attacks;

- data quality – the proper functioning of the A.I. depends on the quality and accuracy of the input data;

- privacy, and ethics – the use of A.I. raises questions about the privacy of personal data, and the ethical aspects of decisions made by algorithms, and this is not yet sufficiently regulated;

- training, and adaptation – emergency workers need to be trained to understand, and use A.I. systems effectively (and this depends on how they are implemented in each institution or system in which they work);

- cost – Implementing and maintaining advanced AI systems can be expensive.

AI is a powerful tool that can revolutionize emergency management by providing essential support to emergency workers and can actively contribute to building their resilience. Resilience has a positive impact on both mental health, and wellbeing, it can help maintain subjective wellbeing, it maintains people's optimism, and belief that things will get better when faced with various

challenges, it includes positive adaptive patterns that are developed over time in response to adversity, and people with high resilience can recover effectively from the impact of everyday stressors, so it becomes all the more important that we can optimize it.

Based on some research in the international literature that has highlighted that positive interaction with technology provides a framework for the development of psychological resources [19, 20], and that artificial intelligence (AI) promotes more effective coping mechanisms by increasing resilience, especially in a professional setting, and during emergencies [21], the objective of the present research was to analyze the relationship between psychological resilience – as a resource that ensures effective adaptation to various situations (new or difficult) –, and the attitude towards AI of Romanian employees.

MATERIALS AND METHODS

The general objective of the research was investigated by means of a questionnaire-based online survey, through a Typeform link containing information about the subjects' informed consent, their socio-demographic data, and the items of the two questionnaires - one analyzing psychological resilience, and the other analyzing attitudes towards AI.

Research hypotheses

The following hypotheses have been formulated to fulfill the general research objective:

Hypothesis 1 - We assume that there are interdependent relationships between psychological resilience and some of its dimensions (tenacity, self-efficacy, self-confidence, ability to learn from life experiences, rapid recovery from negative events, social, and family resources), and the attitude towards IA of emergency workers.

Hypothesis no. 2 - We anticipate that the attitude towards IA can be predicted and explained in terms of the psychological resilience of emergency workers, and its dimensions.

Subjects

The study participants were 89 people, employed in emergency services, from the South, aged between 19 and 43 years (M=29.56, SD=14.26), 51 male, and 38 female. The research sample was a convenience sample, utilizing available subjects, and the only selection criterion was that they were employed. Subjects participated in the research voluntarily, being informed about the research topic, that the results will be confidential, anonymized, and used for research purposes only, and also that they had to fill in two questionnaires (and a set of socio-demographic data) sent via a Typeform link.

Instruments

Psychological resilience was investigated by applying the adapted ARES-i25 scale [22], which contains 25 items distributed on the following scales:

1. tenacity, and self-efficacy – the competence to evaluate the life situation, and the necessary steps to solve the various problematic contexts, and personal resources/limits, as well as the ability to organize resources to solve problems;

2. self-confidence – reflects a positive self-image obtained in objective relation to intuition, and capabilities;

3. ability to learn from life experiences (personal, and/or other people's);

4. rapid recovery from negative life events - tolerance of negative affect, and uncertainty, recovery from failure to adopt resolving means, identification of sources of support, focus on goal achievement, and resistance to disruptive factors;

5. social, and family resources – are the factors external to the person that play a supportive role in coping with various life problems, and in manifesting resilience.

Participants' responses are scored in five steps (1 = strongly disagree, 5 = strongly agree), and the results can be reported both overall and by dimension. ARES-i25 was applied and validated on 423 participants in Bucharest, aged between 19 and 42 years, 260 female, 163 male. Following the factorial analysis, it was found that the scale, at the general level, explains 72.1% of psychological resilience, with coefficients showing that it has a very good internal consistency (Cronbach's $\alpha = 0.83$) at the general level, but also by dimensions ($0.71 < \alpha > 0.89$). ARES-i25 also has content/construct validity, which was also verified by the Delphi method (10 experts). For the present study, the internal consistency of the scale items that were applied to the research subjects (Cronbach's α coefficient = 0.78) demonstrated the fidelity of the ARES-i25 scale in the new context in order to draw valid conclusions.

Attitudes towards artificial intelligence were investigated using the scale constructed by [23], abbreviated ATTARI-12, the authors building on other studies [24, 25]. The 12 items of the scale represent the three facets of attitude (cognitive, affective, and behavioral), with the scale containing an equal number of items for each of these, and reflecting equally positive or negative attitudes towards AI. Participants' responses are scored in five steps (1 = strongly disagree, 5 = strongly agree), and results can be reported both overall and by dimension. The ATTARI-12 was administered and validated on 488 U.S. participants, aged 19-72 years, 211 female, 273 male, and 4 subjects who did not indicate gender. The factor analysis found that the scale, overall, explains 79% of attitudes towards AI, with coefficients showing that it is a unidimensional scale with very good internal consistency (Cronbach's $\alpha = 0.93$), and that it also

has convergent validity, with a statistically significant positive correlation with the scale of attitudes towards specific AI applications (r = 0.93), and that it has convergent validity, with a statistically significant positive correlation with the scale of attitudes towards specific AI applications (r = 0. 60), which proves the psychometric characteristics of the ATTARI-12 scale [26]. For the present study, the scale was translated, and the internal consistency of the items (indicated by Cronbach's α = 0.76) demonstrated the fidelity of the ATTARI-12 scale to draw valid conclusions.

RESULTS

THE IBM SPSS Statistics v23 program was used to analyze and process the data from this research.

For analyzing the first hypothesis, which assumes that between psychological resilience, and some of its dimensions (tenacity, and self-efficacy, self-confidence, ability to learn from life experiences, quick recovery from negative life events, social, and family resources -, and employees' attitude towards AI there are interdependent relationships), and the employees' attitude towards AI, the Pearson correlation statistical procedure was used. The obtained results are presented in Tables 1 and 2.

Table 1. Descriptive statistics for the variables psychological resilience, its dimensions, and attitude towards IA

Research variables	Mean	Standard Deviation
Psychological resilience	64.19	22.35
Tenacity, and self-efficacy	13.22	5.39
Self-confidence	12.97	4.24
The ability to learn from life experiences	16.45	5.13
Quick recovery from negative life events	13.89	3.94
Social, and family resources	17.38	5.36
Employee attitudes towards AI	32.44	11.57

Table 2. Correlations between psychological resilience, its dimensions, and employee attitudes towards AI

Research variables	Employee attitudes towards AI
Psychological resilience	0.91**
Tenacity, and self-efficacy	0.79**
Self-confidence	0.74**
The ability to learn from life experiences	0.83**
Quick recovery from negative life events	0.82**
Social, and family resources	0.75**

*** Correlation is significant at the 0.01 level (2-tailed)*

These results demonstrate a positive and highly statistically significant correlation between psychological resilience and employees' attitudes towards artificial intelligence (AI). Specifically, the more psychologically resilient a person is, the more positive their attitudes towards AI. This strong relationship (r = 0.87) is also supported by the analysis of individual dimensions of resilience. All the dimensions of psychological resilience - including tenacity, self-efficacy, self-confidence, ability to learn from life experiences, quick recovery from negative events, and social and family resources - show strong positive correlations with employees' attitudes towards IA (with correlation coefficient values ranging from 0.74 to 0.91). This suggests that each aspect of resilience contributes to a favorable perception of IA. In conclusion, psychological resilience is a key factor in shaping employee attitudes towards AI. These findings may have important implications for organizations in the context of the ever-accelerating adoption of AI technologies, suggesting that developing employees' psychological resilience could facilitate a smoother and more positive integration of AI in the workplace. There are also statistically significant correlations ($p < 0.05$) between psychological resilience and its dimensions, and between all dimensions of resilience, but these are not within the focus of the research and have not been analyzed or presented in this paper. The effect size of each statistically significant correlation between different variables was calculated using the r2 coefficient; the values of this coefficient ranged between 0.54 and 0.82, thus indicating a high level of association between variables [27], which, beyond the statistical level, adequately reflects reality.

The results of the second hypothesis, predicting that attitudes towards AI can be predicted and explained by the psychological resilience of employees and its dimensions, were obtained by multiple regression. The standard analysis method (Enter) was used, considering 6 predictor variables (psychological resilience, tenacity, and self-efficacy, self-confidence, ability to learn from life experiences, quick recovery from negative life events, social, and family resources) for employees' attitude towards AI (criterion variable).

Examination of the multiple correlation coefficient (with R-value = 0.81) indicates a high correlation between the predictor variables, and the criterion variable,, and its R2 value (0.65) shows that 65% of the variance in employees' attitudes towards AI is determined

by the predictor variables (psychological resilience, tenacity, and self-efficacy, self-confidence, ability to learn from life experiences, quick recovery from negative life events, social, and family resources).

The analysis of variance tested the significance of R, and the value of F (68.22 at p = 0.01) denotes that the predictor variables jointly predict the variance of the criterion variable.

The significance of the individual regression coefficients was assessed using the t-test, and their values (significant at $p < 0.05$) reveal that all predictor variables are important for the estimation of the criterion variable (attitude towards AI). The results are summarized in Table 3.

Table 3. Degree of contribution of predictor variable values to the regression line, and significance of multiple regression

	Unstandardized coefficients		**Standardized coefficients**	**t**	**p**	**R**	**R^2**	**F**	**p**
Model	B	Std. error	Beta						
(Constant)	87.13	11.16		38.45	0.01	0.81	0.65	68.22	0.01
Psychological resilience	42.15	12.43	2.31	10.57					
Tenacity, and self-efficacy	28.52	10.24	1.98	9.38					
Self-confidence	22.14	8.28	0.88	10.22					
The ability to learn from life experiences	21.76	8.33	0.71	11.54					
Quick recovery from negative life events	18.33	5.66	0.56	10.87					
Social, and family resources	14.99	8.47	0.42	11.07					

Based on these data the regression equation can be written: ***Employees' attitude towards*** AI = 87.13 + (42.15) * *psychological resilience* + (28.52) * *tenacity, and self-efficacy* + (22.14) * *self-confidence* + (21.76) * *ability to learn from life experiences* + (18.33) * *quick recovery from negative life events* + (14.99) * *social, and family resources*.

The reliability of the regression equation prediction was verified by calculating the bivariate correlation between the employees' attitude towards AI and its estimation (pre_1), obtaining r = 0.81 (value identical to the multiple correlation coefficient).

The statistical data obtained in the present research indicate that there are 6 predictors of employees' attitude towards artificial intelligence, which both individually and jointly estimate this attitude.

The results show that psychological resilience is the strongest predictor of employees' attitude towards AI, with a regression coefficient of 42.15. This suggests that, independent of its specific dimensions, an overall high level of resilience contributes significantly to a positive attitude towards AI and is a fundamental factor shaping employees' perceptions. The equation shows that attitude towards AI is not only influenced by overall resilience but also by the independent contribution of each dimension. Even though there is a conceptual overlap between overall resilience and its dimensions, the regression equation allows us to see the added impact of each component.

At the same time, these results suggest that organizations wishing to improve employee attitudes towards AI should focus on developing psychological resilience through specific programs, which would lead to easier acceptance of AI and multiple possibilities of its use.

DISCUSSION

The present research started from a number of studies in the literature that have emphasized that positive interaction with technology provides a framework for the development of psychological resources, and that artificial intelligence promotes more effective coping mechanisms by increasing resilience, especially in a professional setting, and during emergencies.

The objective of this research was to analyze the relationship between psychological resilience – as a resource that ensures effective adaptation to various new or difficult situations –, and the attitude of Romanian employees towards artificial intelligence.

The research results (through the significant bivariate correlations identified) proved that people with a high level of psychological resilience have an open attitude towards AI, but also that this attitude is directly proportionally associated with psychological resilience, and its dimensions (tenacity, and self-efficacy, self-confidence, ability to learn from life experiences, quick recovery from negative life events,, and employees' social, and family resources).

Deeper statistical analyses have shown that these variables associated with employees' attitudes towards AI are also predictors of AI.

The novelty of the research is represented by the association between the level of psychological resilience - seen as a resource that ensures adaptation (to uncertainty, difficulties in life) of people from a psychological perspective -, and the employees' reporting to artificial intelligence.

Implementing AI in the workplace often brings with it significant changes: new tasks, the need to learn new skills, redefinition of roles, and sometimes even the fear of replacement. In such a context, psychological resilience acts as a protective shield and an adaptive engine. Resilient employees are, by definition, better able to manage stress and uncertainty, to adapt quickly, to see opportunities rather than threats in AI. In terms of the contribution of specific dimensions of resilience in attitudes towards AI:

- tenacity, and self-efficacy - people who believe in their ability to complete tasks, and overcome obstacles (self-efficacy), and who persist in the face of difficulties (tenacity) will approach AI integration with a proactive mindset, seeing it as a challenge that can be overcome;

- self-confidence - a strong self-esteem helps employees not to feel threatened by AI, but to perceive it as a tool that can enhance their own capabilities, not diminish them;

- the ability to learn from life experiences - this dimension emphasizes the importance of cognitive flexibility and openness to new things. Employees who have demonstrated this ability in the past will be more likely to embrace the new knowledge needed to work with AI.

- rapid recovery from negative events - in the context of AI, this translates to the ability to bounce back quickly from initial frustrations or learning difficulties. They do not give up easily but find ways to move on;

- social, and family resources - a strong support system provides a sense of security and stability, reducing anxiety about change, and allowing employees to better focus on adapting and learning.

This deep underst, anding of the link between resilience, and attitudes towards AI has major practical implications for organizations, namely investing in resilience development programmes, transparent, and empathetic communication (an organizational culture that promotes open dialogue, and underst, anding of employees' concerns can strengthen collective, and individual resilience, facilitating acceptance of AI), .creating a supportive work environment (where difficulties can be discussed, and employees find support in resolving them). In essence, the success of integrating AI into organizational structures depends not only on the technology itself but also, to a large extent, on the psychological preparation of the workforce. By cultivating resilience, organizations not only improve attitudes towards AI but also build a more adaptable, motivated, and prepared workforce for future challenges.

These results cannot be considered as exhaustive conclusions, but in relation to other particularities that could be mediating factors - for example, certain personality traits, openness to novelty, resistance to change, adoption of certain coping strategies, etc. [28, 29, 30].

The results of the present research are in line with the existing literature, with the concept of the importance of socio-technical adaptation of employees,, and the experience, and type of organization play a crucial role in facilitating adaptation to AI; conversely, social, and psychological adaptation of employees can be facilitated with the help of AI-based gadgets, and can reflect on the level of well-being (Giudici, Bento & Falk, 2024). In other words, the differences between organizations in their digital readiness are reflected in the individual's ability to adapt, which supports the socio-technical adaptation framework. Resilience as a central coping mechanism enables individuals to maintain their psychological equilibrium in diverse contexts, including those involving adaptation to new technologies [31, 32, 33, 34, 35, 36, 37].

From the literature review and the data obtained in the present research, it can be considered that psychological resilience as a personal resource helps to adapt to job demands, including those related to AI, helps to understand how AI can be used to increase performance, and well-being at work.

To clarify the influential relationship of psychological resilience on attitudes towards AI research, several directions should be pursued:

- exp, anding the number of subjects (starting from the main limit of this study, consisting of N = 89 subjects);

- to equalize the sample by gender to increase the validity of the conclusions drawn;

- analyzing the role of personality, and other particularities (such as openness to new, resistance to change, stress management, etc.) to establish a psycho-socio-demographic profile of people who would have an open but rational, and cautious attitude towards AI (maintaining a balance between addiction, and fear), respecting ethical principles in the use of AI products;

- exploring the involvement of the organization in developing the psychological resilience of employees to adapt more easily to new technologies, and to ensure an open but rational attitude towards AI.

CONCLUSION

In conclusion, starting from the fact that the research sample contains only 89 subjects, the research has the characteristics of a pilot study,, and the conclusions cannot be extrapolated to the general population of employed people, this being the main limitation of the study. The results of this research are important, however, in that they help to identify trends in the relationship of the influence of psychological resilience on open, rational attitudes towards AI, and to draw directions for further studies to complete the picture of the analysis of this relationship.

Conflicts of interest, and sources of funding

The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript,, and no image was previously published in another journal or is under consideration for publication elsewhere. This research received no external funding.

Authors' contribution

Conceptualization, R.M., A.P., and M.L; methodology, R.M; software, A.C.; validation, R.M., A.P., and M.L; formal analysis, R.M; investigation, M.L, and A.C.C.; resources, A.C.; data curation, A.P., and M.L.; writing—original draft preparation, R.M.; writing—review, and editing, R.M, and A.P.; visualization, M.L..; supervision, R.M; project administration, R.M.; funding acquisition, M.L., and A..C. All authors have read, and agreed to the published version of the manuscript.

Ethics approval, and consent to participate

The study was conducted under the Declaration of Helsinki. The research was conducted under ethical guidelines, and regulations, ensuring compliance with all necessary protocols.

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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ORIGINAL ARTICLE

Positive Experience and Patient Satisfaction – Determinants of Paradigm Shifts in Healthcare

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Abstract: Today's recognition of health as a fundamental value of humanity and the spectacular digital evolution of the environment dominated by permanent and dynamic changes has brought healthcare marketing to the forefront. The trends continuously affecting medical services have generated the need for increased involvement of marketing, with all current developments in healthcare having an indispensable marketing dimension. The successful implementation of a health marketing strategy aimed at enhancing patient satisfaction and fostering a positive care recipient experience, coupled with the cost-effectiveness of the health services offered, has necessitated the development of an adaptive marketing function that harmonizes the set objectives, addresses capability gaps, accommodates cultural realities, and considers individual mentalities and situational circumstances. The direct interaction with patients has made it possible to understand the need for a new paradigm of healthcare services, the demand to comprehend patient satisfaction and positive experience, the call for creating innovative models to address the patient experience, and, above all, the requirement to communicate with the patient continuously and effectively throughout the continuum of healthcare. The challenge for the management of future health systems and organizations is encapsulated in the words of Professor Drucker, who stated that the best way to predict the future is to create it. An exploratory research on the state-of-the-art of the literature was conducted with key references to the most important aspects captured by specialists on positive patient experience and satisfaction, which contributes by highlighting the determining factors that have led to the paradigm shift in healthcare.

Keywords: patient satisfaction, positive experience, new healthcare paradigm, adaptive healthcare marketing, management of healthcare, digital evolution

INTRODUCTION

Given the crucial role of a positive patient experience and satisfaction in enabling individuals who require medical care to make informed treatment choices and prioritize their own needs, the demand for innovative models to address this aspect is evident. These models must reflect the patient-centeredness of healthcare, rather than focusing on the doctor, procedure, or healthcare institution. Also, the models have to consider the medical condition which needs to be seen over the full cycle of care, reflecting the empathy and respect given to patients, the way communication with staff is carried out, the methods by which the information-communication binomial works during the hospitalization period and, of course, confirm the moral values of the hospital unit.

Experience is a core strategy in modern marketing, defined as "the perceptions, emotions and thoughts that consumers have when they come into contact with market services and engage in consumption activities" [1,2]; therefore, the experience is deeply personal and subjective in nature, requires learning and is based on human senses and is persistent over time [3].

Experience is used to influence consumers and is directly related to their level of satisfaction [4]. Four possible strategies are used for its planning:

• designing compelling experiences that stimulate the consumer's interest, attention, and desire to retain information about the service;

• engagement, which is achieved when the consumer feels that they resonate with the service, which they find relevant and necessary;

• emotion induction, which stimulates attention and learning,

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• creating temporary memories, which over time become permanent memories and define new experiences.

The ultimate strategy is to interconnect all these experiences in a holistic, intensive approach, designed to improve the life of the healthcare consumer significantly.

One innovative model for approaching the patient experience is the seven-step model, which includes the following processes: define, research, conceptualize, prototype, choose, implement, and learn. At Stanford University, students took a two-day course offered by the Hasso Plattner Institute of Design, seeking ways to enhance the patient experience in the Stanford Hospital Emergency Department. For the exercise, they role-played patients and their family members to get a sense of what it's actually like to be in the often chaotic atmosphere of an emergency department [5]. The following, among others, were highlighted in this context:

• empathy is a key element of design thinking, a step-by-step approach to problem solving that involves observing and interviewing patients as they go through an experience and then using this information to realize both a prototype and reliable ways to improve the product or process.

• patients wanted a regular flow of information to help them better understand what was happening and how their care providers communicated with each other;

• coordinated and clear communication, in the patients' view, is most helpful in calming anxiety and natural feelings of fear [5].

As is well known, the patient experience, one of the three activities that drive outcomes, along with diagnosis and safe, error-free execution, can be improved by coordinating the patient journey, reducing handoff time, focusing on the audiences of interest, the Disney management approach (every team member is trained to be an effective communicator), and building on traditional health systems (such as the holistic Ayurveda, which emphasizes the unbreakable connections between body, mind and spirit), progressing touchpoints and reforming interactions.

The HealthLeaders Media Intelligence Unit is a division of HealthLeaders Media and has for many years been considered a premier source for health care management research, providing analysis and forecasts through digital platforms, customized reports, customized reports, printed books, conferences, roundtables, peer-to-peer networking technology opportunities (where each computer has equal rights and responsibilities, with network devices sharing resources and communicating directly with each other), and various presentations for senior management [6].

The measurement of patient satisfaction is the application in health care of the concept of customer satisfaction, a concept that comes from both quality management and marketing [6].

As specialists argue, patient satisfaction has an impact on: retaining existing patients and gaining potential ones; the perceived quality of healthcare services by patients as a result of both the interaction with medical staff and the communication through which care recipients are informed about solutions to the medical problems they face, solutions offered by the healthcare facility in comparison to its competitors; compliance with doctors' recommendations; patients seeking a second medical opinion; health outcomes achieved by the health facility in question; satisfaction of the health facility's medical staff; patients not resorting to malpractice lawsuits; patients becoming promoters of the health facility's brand [7].

Several different types of satisfaction questionnaires exist in the medical literature, an established benchmark being, for example, the PSQ-III Patient Satisfaction Questionnaire (a more recent version of the PSQ), a survey of global satisfaction with health care, as well as satisfaction with six aspects of care: technical quality, interpersonal manner, communication, financial aspects of care, time spent with the doctor and accessibility of care [8].

On the other hand, as a short-form version of a hospital patient satisfaction questionnaire, we can consider a combination of a myriad of variants, including the one used by St. Joseph's General Hospital, Elliot Lake [9].

The constant concern about the satisfaction of patients receiving health care services in one of the health care organizations allows resorting to a questionnaire that would allow to objectively assess the degree of patient satisfaction (a significant indicator of health care) treated in a health care facility, and based on the interpretation of the results, to recommend to practitioners solutions to improve this experience, with the effect of optimizing health outcomes and increasing brand awareness in the relevant market.

OBJECTIVES AND METHODS

The main objectives of this study are twofold. First, it aims to examine the key determinants of the emerging patient-centered paradigm in healthcare, with particular emphasis on how positive patient experiences and high levels of patient satisfaction catalyze this paradigm shift. Second, the study evaluates the role of adaptive and innovative marketing strategies in healthcare, including experiential marketing approaches to enhance patient satisfaction and align healthcare delivery with patient needs and expectations. Taken together, these objectives underscore that patient satisfaction and positive patient experiences are central pillars of contemporary healthcare marketing, highlighting the necessity of adopting patient-centered strategies and illustrating how such approaches can drive the transformation of healthcare systems toward more empathetic, responsive, and digitally integrated services.

To accomplish these objectives, an exploratory review of relevant literature was conducted, complemented by a theoretical analysis of key sources. This bibliographic research focused on existing studies addressing the patient-provider service paradigm, experiential marketing in healthcare, and established tools for measuring patient satisfaction, thereby grounding the discussion in well-founded concepts and evidence. Through the synthesis of these sources, the study provides a comprehensive theoretical perspective on the factors driving the patient-centric shift and on the evolution of marketing practices tailored to patient expectations.

RESULTS AND DISCUSSIONS

Paradigm shift in healthcare: patient as healthcare provider

It is well known that paradigms are fundamental frameworks around which disciplines and schools of thought are generated, and most of the paradigms associated with service management have, according to numerous studies, originated from the marketing discipline and perspective. Inevitably, given the generous perspectives that marketing offers, many paradigms associated with services have emerged, with a particular focus on the management sphere.

Given that certain paradigms within service marketing may diverge from traditional operations management principles, leading scholars in both domains have advocated for the development of a renewed and integrative service paradigm.

The review of paradigms in the literature led to the proposal of a customer-supplier service paradigm superior to alternative service paradigms. Thus, the client-provider paradigm (patient-provider of health care services, in the case of health care services) is considered a useful and justified basis for the present and future study of (health care, in our case) services.

The service operations paradigm considered to be prominent is the customer contact model, introduced in the late eighties by R.B. Chase, whose fundamental argument is that the potential efficiency of service operations is limited by the amount of customer contact with the service provider's employees [9].

Furthermore, Chase recommended the famous equation below [10]:

$$\text{Operational potential efficiency} = \int (1 -) \frac{Client\ time\ contact}{Service\ time\ creation}$$

To understand the customer-supplier paradigm, it is necessary to realize that the customer-supplier paradigm does not focus on what customers do but on what they provide as inputs to service processes. This paradigm shift is that customer-provided inputs are the defining characteristic of services, as long as the customer is identified and the process being analyzed is specified. Also, processes rarely occur in isolation; rather, they exist within supply (value) chains, where one process feeds into another process. Thus, the customer-supplier service paradigm clarifies service supply chains, showing how they involve customers both as providers of inputs and as consumers of outputs [10].

For a correct understanding of this paradigm in the sphere of health services, two essential aspects must be taken into account, according to the rich literature [10]:

• Supply Chain Management: the patient-provider paradigm of health care services identifies the complexity of the supply of health care services and shows how to involve patients in their dual roles, those of input providers and output consumers.

• The universal input-output model: the patient-provider paradigm focuses on the patient's needs and wants, which is the defining characteristic of health services.

The primary paradigm in health services has been naturally generated by the interaction between the patient and the healthcare provider and has proven superior to alternative service paradigms, thus providing a viable basis for studying health services.

Examples of adaptive marketing in healthcare

The exponential developments in healthcare necessitate a continuous search for innovative solutions to meet the needs of existing patients, retain them, and attract new ones, while providing high-quality services that take into account the diverse perceptions of all patients. This requires dynamic and creative management strategies, as well as specific patient-centered marketing actions.

The service revolution continues, always presenting new challenges for marketing management, new rules of competition, new methods of organization, new commitments regarding the communication process and interpersonal relationships, as well as new approaches to economy and intelligence [11]. Dynamism, innovation, and technology are now considered essential tools in a scientific approach [11].

Professor Richard K. Thomas argued that "health professionals, especially physicians, fall into a special category, and the fact that they make most of the decisions about patients creates a unique dynamic to health care." [12]

Kotler recommended the development of stronger marketing (i.e., making it more important than production), replacing old marketing with new marketing, the latter of which should be holistic, strategic, technologically oriented, and financially focused [13].

The strong development and affirmation of marketing in the health sector are due to the rapid and diverse evolution of marketing tools, the marketing mix, and especially the various types of marketing that have adapted quickly and effectively in the health sector. One of these is experience marketing, also known as experiential marketing, a specific strategy through which organizations target different categories of consumers with varying cultural values, satisfying their wants and needs by offering emotionally triggering experiences that stimulate their senses and facilitate the formation of personalized bonds [14].

Experiential marketing can include five types of experiences [4]:

1. *Sensory experiences*: occur when human senses are stimulated:

• sight, through attractive colors and shapes, which are part of the brand identity and intended to be memorized by consumers;

• hearing, through music and sounds used as positioning elements;

• sense of touch, to which men are more sensitive than women;

• the sense of smell, which can trigger certain emotions.

Marketing strategies aim to differentiate an organization and its services from other similar organizations and to add value to services through their sensory appeal.

2. *Affective experiences*: These are determined by the inner feelings of the consumer, to whom the use of the service triggers certain emotions, feelings and moods. Marketing strategies are geared towards increasing the intensity of emotions and developing a sense of anticipation of the pleasant feelings that will follow after using the service.

3. *Experiences through thinking*: stimulates the consumer's creativity and challenges them to solve problems, actually encouraging them to get involved in evaluating the service received and the organization. The marketing strategy works in several directions, designed to surprise the consumer, arouse their curiosity, and create controversy by breaking conventional boundaries - actions that address both convergent, rigorous, and systematic problem analysis thinking and divergent, free thinking.

4. *Experiences through action*: involves the consumer in direct interaction with the health care service, towards which he or she takes an attitude and changes some of his or her lifestyle habits. Strategic directions refer to either physical reactions (such as reflex actions or body signals) or changes in mindset and lifestyle.

5. *Relational experiences*: They provide the consumer with the opportunity to learn about entities as a result of the buying and consuming process.

Marketing strategies also have multiple directions:

• public awareness of a particular social category and its advantages;

• identification of the consumer with an established reference group, so that he/she is convinced that he/she has the same needs as the members of the reference group;

• highlighting, whereby membership of the reference group is perceived as beneficial.

The evolution of holistic organizational ecosystems in the context of the broad and ongoing process of digital transformation, as well as the importance of aligning external trends with internal strategic priorities in addressing the new digital transformation model - digital marketing and digital health- are evident [15].

The potential of digital technologies and data to enrich understanding and marketing as a cultural architect is huge. Digital transformation plays a crucial role in advancing healthcare ecosystems, particularly through the strategic use of digital marketing [16]. This has underscored the importance of adopting a strong human-centered approach, effectively managing digital marketing efforts, and integrating artificial intelligence (AI) with the Internet of Things (IoT). Additionally, leveraging AI-driven insights for diagnostics and other applications is becoming increasingly essential.

Nowadays digital technology has become more and more used in everyday activities, and its intensive use and in most areas of activity has brought changes in the structure of society, and even in the psychology of individuals, who are gradually changing their conceptions about life, work, health and education, the ideal way of spending leisure time and the attributes of an optimal work climate. In this general context, it is clear that conceptions of the healthcare process are also changing, and, naturally, they should keep pace with technological progress and incorporate new tools, new treatments, new procedures, and new medicines, in an attempt to simplify and streamline it. Some of the recent spectacular developments in digital marketing worldwide:

• Digital transformation is enabling a closer connection with customers, responding to the new demands of supply chain management (few business functions are as profoundly disrupted by digitization as marketing).

• In the context of an organization's greater interactivity with more empowered customers, amidst facing particular challenges, marketing is now obliged to offer something for each and every customer [17].

• We live, today, in a much more complex world of digitally enabled smart customers, digital products, digital business operations, and digital competitors [18].

• It is important to identify the most useful insights by understanding what is happening at the confluence of people and technology, taking into account the ability of marketing technologies to bridge different technologies [19].

• Marketers need to enhance the customer experience by getting a complete picture of their customers and delivering real-time experiences [20].

• Marketers now have a critical role in the process of continuously identifying digital opportunities along the entire value chain.

• Marketers must first, and best, understand the difference between change and transformation.

• Marketers must integrate people, technology, and best practices, given the accelerated social interaction of social media.

• There are already new digital tools and new ways to drive change [9].

CONCLUSION

The beneficiaries of healthcare services, the patients, are people with fundamentally different lifestyles, varying ages, diverse social backgrounds, differing levels of education, diverse health conditions, and, of course, different expectations and conceptions.

To resonate with these characteristics, the portrait of the ideal doctor has also changed significantly: first and foremost, he must be an excellent professional, a good communicator, approachable and friendly, transparent, enthusiastic, passionate, able to draw realistic conclusions and objectives and provide immediate and effective feedback on health issues, willing to offer professional and emotional support to his patients.

In the midst of continuously identifying digital opportunities along the entire value chain, marketers need to understand the difference between change and transformation better, integrating people, technology and best practices, taking into account the accelerated social interaction of social media, enhancing the patient experience by improving knowledge of patients' needs, wants and attitudes, gaining a complete picture of them and delivering real-time experiences [20].

The transformation of economies into service economies - i.e., economies in which the production and consumption of services predominate - fundamentally changes the structure of society. This economic transformation is being significantly driven by rapid advances in technology, particularly in information and digital technology [21].

This new, highly topical paradigm in healthcare requires not only innovative models of approaching the patient experience and especially patient satisfaction, but also the obligation of permanent, accurate, and clear communication with the patient.

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The authors declare no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration for publication elsewhere. This research received no external funding.

Authors' contribution

Conceptualization MCMS, ITS, ADS, DLDM, AGS, and VLP; methodology MCMS and ITS; software, MCMS and ITS; validation MCMS, ITS, ADS, DLDM, and VLP; formal analysis, MCMS; investigation, MCMS, and ITS; resources, ADS, DLDM, and MCMS; data curation, MCMS, and ITS; writing-original draft preparation, MCMS; writing-review and editing, MCMS; visualization, MCMS; supervision, ADS and DLDM; project administration, MCMS, and ITS. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted under the Declaration of Helsinki. The research was conducted under ethical guidelines and regulations, ensuring compliance with all necessary protocols.

Patient consent for publication

Not applicable.

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REVIEW

Psychotherapeutic Interventions for Combat Soldiers in Complex Clinical Settings

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Abstract: This narrative review examines psychotherapeutic interventions for combat soldiers who deal with post-traumatic distress and face a complex therapeutic setting where the relationships between clients and therapy providers are often conflictual. Despite available emotional aid, gaps between traumatized soldiers' emotional needs and institutional solutions are inevitable and are challenging for clients and therapists alike. These issues are discussed through Israeli soldiers' experiences, a population at high risk for trauma-related distress, due to this country's ongoing security threats. Following a narrative literature review, four key themes emerged: mutual difficulties in admitting mental injury, intergenerational trauma, preserving the soldier identity upon discharge, and choosing among therapeutic opportunities. The study highlights the need and rationale for diverse biopsychosocial interventions for veterans and proposes considerations for enhancing treatment success. Results emphasize the importance of tailored approaches that address both individual and systemic factors affecting veterans' mental health. Guidance for psychosocial interventions is provided, considering the multifaceted nature of combat-related trauma.

Keywords: combat, Israeli soldiers, military, psychotherapy, soldier identity, posttraumatic stress disorder, psychological trauma, veterans

INTRODUCTION

Military service and exposure to war zones affect millions worldwide, putting soldiers and veterans at high risk of physical, emotional, and psychological responses, from resilience to stress-related psychopathologies [1,2]. Posttraumatic stress disorder (PTSD) rates are higher among veterans than in the general population, estimated at 15% of combat veterans [3]. In Israel, military service is mandatory for Jewish and Druze men and Jewish women; many other citizens voluntarily enlist to promote social integration [4].

Active service begins at age 18 for 2–3 years, followed by reserve duty until age 40 [5]. Despite widespread exposure to potentially traumatic combat events, PTSD rates among Israeli Defense Forces (IDF) personnel remain unclear. Recent studies reported a trauma exposure rate of 96% among combat veterans [6], with PTSD estimates ranging from 2% to 16.5% [7–9]. However, the current ongoing conflict has likely increased these rates [10].

Official recognition of mental injuries in Israel, similar to that in other countries, is more complex than physical harm. Providing psychological aid to traumatized soldiers involves dilemmas about the nature, timing, and recipients of interventions, as well as the personal and social consequences [11,12].

The current narrative review [13,14] examines the psychological treatment of military personnel in conflict settings, a perspective that is rarely discussed [15]. It considers the diverse landscape of mental health opportunities offered for Israeli veterans, including official services, the private sector, and non-profit organizations. It aims to contribute to the international discourse on mental health care for military personnel in complex, conflict-prone environments by illuminating considerations for clinicians and policymakers dealing with emotionally injured soldiers or veterans.

This review explores two main research questions:

• What obstacles and dilemmas do professionals face when providing veterans with therapeutic solutions?

• What lessons can be learned from the Israeli experience to help clinicians worldwide better plan therapeutic interventions for military populations?

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MATERIALS AND METHODS

This paper presents a narrative review [13,14] of the literature on psychological interventions for Israeli combat veterans, supplemented by clinical case descriptions. This approach was chosen to provide a comprehensive overview of the topic while allowing for the integration of diverse perspectives and critique, and deepening the understanding of clinical insights through the inclusion of case materials.

A thorough literature search was conducted using PsycINFO and Medline (PubMed), supplemented with manual searches on Google Scholar. The search was restricted to peer-reviewed articles published in English and Hebrew between 1990 and May 2025. Additionally, clinical papers and book chapters that present the treatment of combat soldiers and veterans were also included. Non–peer–reviewed sources were intentionally sought to describe the study's socio-political context, and they included major local and international press. Papers and unofficial media or press publications were excluded if they were not initially published in Hebrew or English. No private or social media forums were included in the current narrative review. Search terms included psychological intervention-related keywords such as Psychotherapy, Group Therapy, Public health system, Private Sector; trauma-related keywords such as Trauma, Post-traumatic stress symptoms, PTSD, Suicidality; and military-related keywords such as Veterans, Soldiers, and Israeli Defense Forces.

The narrative synthesis of the literature focused on identifying key themes and issues relevant to the psychotherapeutic treatment of Israeli combat veterans, in light of global psychotherapeutic trends. These themes were organized into four major categories:

1. Admitting the Injury: Psychological Help for Mentally Injured Israeli Soldiers

2. Intergenerational Traumatic Consequences

3. Preservation of the "Soldier Identity"

4. Making Sense among Therapeutic Opportunities

To illustrate the clinical implications of these themes, case examples were drawn from the author's professional experience as a therapist and clinical supervisor. These case descriptions reflect patterns seen frequently in clinical practice and exemplify complex clinical situations. These composites offer a bridge between research findings and clinical practice, providing concrete examples of how theoretical concepts manifest in real-world therapeutic settings. All presented cases are composites of multiple real cases, with names and identifying details altered to protect client confidentiality.

The integration of literature review findings with clinical case descriptions allows for a rich, nuanced exploration of the psychological and psychosocial interventions that Israeli soldiers receive after developing traumatic distress during exposure to military-related trauma [14]. This approach aligns with the narrative review format, enabling a comprehensive discussion of complex clinical scenarios that may not be fully captured by more structured review methodologies. By combining a broad overview of the literature with detailed clinical examples, this study provides valuable insights for researchers and practitioners working with combat veterans, highlighting the unique challenges and opportunities in this field of psychotherapy. Since the majority of IDF combat soldiers are men, the examples presented describe treatment cases involving male soldiers only.

However, the ideas they illustrate are equally applicable to the experiences of female soldiers. Still, further research is needed to examine the unique challenges faced by female combat soldiers in coping with the emotional post-service impact on their lives, as well as how they utilize therapeutic services.

RESULTS

Admitting the Injury: Psychological Help for Mentally Injured Soldiers

Due to mandatory IDF service, Israel provides various treatment options for military-related distress [16]. IDF mental health services involve about 260 professionals, mostly psychotherapists and psychiatrists [17]. According to the press, these numbers have tripled since October 2023 due to increased demand [18]. Mental health professionals can be accessed across military units, and soldiers can seek help during or after service through their unit's psychologist or the Unit for the Treatment of Combat-Related PTSD. Professionals offer evidence-based psychotherapies in various settings. However, many veterans complain of insufficient or inadequate treatment, according to media reports [19].

The case of Sergeant Y.S., a 26-year-old Israeli veteran who self-immolated in 2021 to protest inadequate mental health support, highlights the challenges that IDF veterans face in seeking help. Unofficial reports indicate that this incident led to increased public awareness and the implementation of the "Nefesh Achat" (One Soul) reform to improve care for traumatized veterans [20]. However, the media keeps raising the voice of many veterans who still report insufficient mental health care [19,21]. Such reports highlight the risk of suicidality and suicide attempts, which have increased among Israeli soldiers in recent years [22], raising questions about the reform's effectiveness. According to these sources, many soldiers and veterans avoid seeking help due to stigma, fear of self-exposure, bureaucracy, or underestimation of their condition [23,24].

These barriers persist despite efforts to improve mental health services.

Intergenerational Traumatic Consequences

In Israel, most soldiers are second- or third-generation veterans, creating a unique context in which combat experience is viewed as a "civilian duty." The country's small size means combat often occurs near where soldiers live. Negative world views among parents can increase trauma-related stress in children through behavioral imitation and cognitive interpretation [25]. Indeed, mothers can play a crucial role in transmitting or buffering intergenerational trauma. These conditions make the effects of military service tangible for other family members and can lead to intergenerational and bidirectional traumatic stress, frequent shared traumatic reality, and secondary stress reactions in families of veterans [26]. Hence, therapists should consider the broader context of trauma, including its connections to a veteran's personal and interpersonal world.

Exemplifying this condition is the case of Josh, 27, who sought therapy 6 years after he finished his mandatory service. Growing up in a village known for its military sacrifices, he served in the armored corps, like his father did 30 years before him. During his service, Josh experienced traumatic events that triggered anxiety, doubt, and PTSD symptoms. Despite this, he described initially feeling undeserving of support and believing physical injuries were necessary to qualify for help. Only years later, when he experienced the horrific sights and fear of the October 7th, 2023, surprising attack by Hamas in the south of the country, he realized how his life followed a reliving of his father's traumatic experiences. As he joined his combat comrades on that day, he understood his emotional reaction was a transgenerational family burden, one he did not choose for himself. Encouraged by his girlfriend, Josh sought treatment for his military-related distress. Although emotionally clear to him, proving the connection between his current condition and past events was complicated, requiring careful navigation of bureaucracy and emotional sensitivities by both Josh and the professionals involved.

Preservation of the "Soldier Identity"

After service, soldiers face stress while readjusting to civilian life due to the stark contrast between military and civilian cultures [5]. Military service emphasizes power, aggression, obedience, and dichotomous thinking, whereas civilian life requires autonomous thinking, contextual understanding, and restraint. Transitioning between these worlds involves a challenging identity shift [27], affecting veterans in terms of personal, interpersonal, familial, and professional aspects of life. Due to their long-term reserve commitments, Israeli combat soldiers must maintain these conflicting identities for years and often never fully relinquish their military ways [28]. Combat experiences can lead to moral injury that exacerbates emotional burdens [29]. Facing longitudinal emotional stress and feeling trapped between ingrained military responses and unfamiliar, emotionally regulated approaches to problem-solving, veterans may resort to substance use [30].

David, aged 34, exemplifies these challenges. As a former infantry soldier, he struggled to adapt his combat instincts to civilian life. His aggressive responses, once valued in the military, became problematic in everyday situations. A turning point occurred when he reacted violently to his 5-year-old son's playful behavior that triggered memories of past military operations. In therapy, David realized how automatic responses to stress that he developed during service were now maladaptive. He grappled with guilt over his current actions as a father and past experiences as a soldier. This led to anger toward the Ministry of Defense, and David questioned the lack of support for veterans transitioning to civilian life and described feeling "used" and "disposable." This example illustrates the complex and long-term impact of military service on personal and family life.

Trauma exposure can interact with established traits among combat veterans, potentially leading to violent behavior [31]. Israeli therapists who are usually veterans may face challenges in helping other veterans process these complex situations, as they often struggle with their own identification or attempts to restore emotional balance in the face of military-related stress. This aspect often becomes a central transferential issue in treatment [32]. Helping veterans process such complicated situations and acknowledging their various emotional origins, as well as their current implications, is a challenging therapeutic task. Clinicians who deal with such conditions often find themselves either identifying with their patients or trying to help them reach a more balanced state, in which they can once again find order in their inner world. These reactions by clinicians are reflected in their hardships related to bearing witness to these violent acts [33]. Press and media reports indicate this situation is especially complicated for minority soldiers, who may face racism during service [33–35]. These individuals can experience both peer criticism and the stigma of seeking help [36]. Providing therapy in such contexts with multiple conflicts requires significant effort to build trust and emotional safety, along with a mature clinical attitude [37].

Choosing among Therapeutic Opportunities

Israeli veterans often struggle with the emotional toll of their service and rely on functional coping methods that can generate emotional avoidance and loneliness [5,38]. Like in other countries, many veterans hope time will heal their wounds or perceive their PTSD symptoms as minor, leading to underutilization of official support systems [40,41]. A recent study found 21% of Israeli combat veterans dealt with subthreshold PTSD, while 14% had clinical levels [39]. To address this increasing need, nongovernmental organizations have developed group intervention programs focusing on raising awareness, adjusting to civilian life, and enhancing emotional resilience [42,43]. These programs combine being in nature and outdoor activities, stress reduction techniques, and group processing of service-related events. They aim to enhance veterans' emotional coping, reduce guilt, and promote social recognition in the army service's psychological impact, helping veterans resolve unfinished business from their service in a supportive environment.

An example of these group interventions is the case of Daniel, 32, who grew up in an orthodox, traditional, low-income family. He described army service as a chance to achieve social mobility and volunteered for an elite commando unit, where he served as a sniper. After his service, he experienced emotional and physical stress, depression, and life dissatisfaction. He didn't connect these issues to his military service until participating in a group intervention program with his unit. There, he realized his depression stemmed from his role in killing others while in the military. The program helped him process a particularly conflictual incident during which he shot a young attacker. This shared experience with comrades helped him feel legit for his distress, relieve the big parts of it which were related to social demands and expectations, and seek further future therapy.

As described, army service often provides the first opportunity for minority youth to integrate into broader Israeli society [44]. However, minority soldiers often cope with both inner stress and social criticism when facing traumatic events and their consequences. Their race and socioeconomic background can impair resilience, increasing emotional vulnerability [37]. In such conditions, acknowledging combat-related emotions in therapy requires confronting difficult feelings, leading many young soldiers and veterans to avoid emotional processing and dwell in emotional numbing [45].

Sharon's case, a 23-year-old soldier who participated in a "friendly fire" incident where an Israeli soldier was killed, demonstrates this state of affairs. Right after this deadly incident, a group session with the unit's psychologist was provided, but all the squad's members remained silent throughout this whole session. In a later individual session, Sharon gave mostly rational answers to the therapist's attempts to reach his emotions, detaching from any emotional connection. Recognizing this as a defense mechanism, the therapist acknowledged the normalcy of stress and emotional detachment in such situations. He validated the ongoing challenges of combat service and encouraged Sharon to seek support, including after service completion. This message of partnership and hope proved crucial for Sharon's present coping and future development.

DISCUSSION

Balancing the Opposite Ends

Israeli society, like many others, often overlooks veterans' needs, focusing primarily on major psychopathologies while missing cases of long-term subthreshold PTSD [40,42,43]. Awareness has grown in recent years that combat soldiers need time to integrate into civilian life after service, with volunteer and "third-sector" organizations supporting this transition [5]. Providing therapy in this context is challenging due to unresolved questions about optimal timing and format [46]. Even though early and tailored interventions that leverage established social cohesion are considered beneficial [47], the effectiveness of psychological aid for young, cynical soldiers or veterans remains uncertain. The multicultural nature of Israeli society makes this tough clinical situation even more complex. Although army units are cultural melting pots, veterans often return to their original communities after service [44]. Balancing soldier and civilian identities is complicated, especially for people from traditional, patriarchal backgrounds in which admitting emotional hardships and seeking help through individual therapy can be intimidating [28,48]. Social workers must recognize both general and culture-specific aspects of these situations to support the integration of veterans into civilian life effectively [38,49]. This requires addressing controversies and the unique cultural needs of diverse populations when providing beneficial psychological aid. According to the press, these challenges have become even more difficult during the past 2 years, because Israeli reservists have been required to undertake numerous extended service rotations amid an atmosphere of extreme social division in Israeli society [49].

Resilience and social support are critical resources that can help combat veterans cope with PTSD and other stress-related symptoms. Although resilience levels among veterans are generally high, their impact often depends on individual coping skills [6]. Prior research and clinical experience have suggested that interventions should encourage authentic emotional expression, strengthen social relationships, and provide stress-management strategies, thereby reducing distress following military service without compromising resilience. Social support can play a particularly important role in mitigating symptoms of depression, like social withdrawal and emotional restriction [50]. Concurrently, identity integration remains a central challenge for many veterans during the transition from military to civilian life, particularly as they reconcile traumatic experiences and navigate societal expectations [27]. These processes differ based on cultural, social, and political contexts, highlighting the need for culturally competent practice. Group-based processing with peers can facilitate the integration of traumatic experiences into veterans' personal and social identities. Prior research [51] and clinical practice provide evidence of veterans who benefit from such interventions across a wide range of ages, depending on their emotional preparedness to utilize psychological help. Hence, practitioners must remain attentive to local cultural and familial attitudes toward military service and norms around emotional expression, which shape how veterans manage post-service adjustment and distress. These issues may be even more complex when dealing with female veterans' post-service emotional distress, which requires further study.

This study has limitations related to its narrative review methodology, which may have introduced author bias in the selection and interpretation of materials. Unlike systematic reviews, this approach does not reflect objectivity, reproducibility, or an exhaustive evidence search. However, it allows for holistic analysis, interpretation, and critique of the focal topic. This review synthesized current knowledge on treatment for Israeli veterans and highlighted knowledge gaps. It emphasizes the need for carefully considered solutions to treating traumatized veterans and encourages therapists to address their service-related emotional wounds, which may surface during therapy. Future research should explore the complex professional and relational aspects of this clinical area.

CONCLUSION

Both professionals and lay people who deal with PTSD symptoms often lack knowledge about the complexity of this condition and its effective treatments. Social workers and psychotherapists should provide traumatized veterans with comprehensive bio-psycho-social solutions. Professional coordination across sectors is crucial for optimal therapeutic outcomes, and public education is needed to help veterans recognize when and where to seek care. Although support for PTSD among veterans is a global need, recent developments in Israel and other countries demonstrate that progress in this field is achievable and should be prioritized by professionals and policymakers.

Conflicts of interest and sources of funding

The author declares no conflict of interest. No artificial intelligence automatically generated text was inserted in this manuscript, and no image was previously published in another journal or is under consideration for publication elsewhere. This research received no external funding.

Authors' contribution

As the only author, the author is responsible for the design, data collection, reviewing, interpreting the results, and revision of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

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REVIEW

The Impact of miRNAs in Diabetes Mellitus

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Abstract: Diabetes mellitus refers to metabolic disorders whose main characteristic is chronic hyperglycaemia. The cause is either disturbed insulin secretion, insulin resistance, or usually both. MicroRNAs represent a subclass of non-coding RNA molecules that are short in length, about 17-26 nucleotides. Since they are circulant and can be tissue-specific, their use as diagnostic biomarkers or screening for different diseases is currently undergoing deeper studies. It was found that 22 miRNAs were associated with the pathophysiology of T1DM, 34 with T2DM, and 16 miRNAs were identified to be common amongst T1DM and T2DM. All of them were reconfirmed in at least two separate studies.

Keywords: microRNA; diabetes mellitus; insulin resistance; pancreatic β cells; gene silencing

INTRODUCTION

Diabetes mellitus (DM) encompasses a group of heterogeneous diseases characterized by hyperglycemia. The most prevalent forms of disease are Type 1 DM (T1DM) and Type 2 DM (T2DM). IDF has also just confirmed type 5 diabetes. It is reportedly characterized by severe insulin deficiency, mainly caused by undernutrition during childhood or adolescent years [1]. T1DM affects approximately 8.75 million individuals globally, whereas T2DM currently impacts about 6.28% of the world's population, accounting for 80% of all diabetes cases. DM represents a significant health issue both in terms of prevalence and the impact of its chronic complications, which can lead to organ failure (e.g., kidneys, retina, heart) [2-4]. Projections indicate that these values are expected to increase significantly in the coming years [5].

T1DM and T2DM are multifactorial diseases. T1DM is typically diagnosed following extensive destruction of insulin-secreting pancreatic β cells (70-80%) by T lymphocytes, resulting in the presence of self-reactive autoantibodies (T1A-DM). Non-immune mechanisms account for 10-30% of T1DM cases (type 1B - T1B-DM subclass), which are classified as idiopathic T1DM [6-8]. Idiopathic T1DM does not have an autoimmune background. Although it can present with insulin deficiency, this subclass of DM does not have a clear trigger. Fulminant T1DM represents another subclass that is also non-immune-mediated. It manifests by fast β-cell death, which may be caused by environmental and genetic backgrounds [9]. Altered insulin secretion, often paired with insulin resistance, represents the main mechanism involved in T2DM [10].

The predisposition for DM remains only partially understood. With some exceptions, a complex network of genetic and epigenetic factors can predispose individuals to T1DM and T2DM [11-15]. Since 2004-2005, exosomal microRNAs (miRNAs) have been included in the list of factors associated with DM and its chronic complications [16-17].

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miRNA biosynthesis

Small RNA molecules (e.g., microRNAs -miRNAs; small interfering RNAs -siRNAs) are synthesized in the nucleus and subsequently transported to the cytoplasm. The origin of the miRNA molecules is a primary transcript that contains hairpin-shaped

structures [18]. The RNase Drosha recognizes and cuts these hairpin structures in RNA, resulting in a miRNA precursor (pre-miRNA) of about 70 nucleotides in length. These molecules exit the nucleus (by means of exportin-5 complex) and in the cytoplasm are recognized and cut at the loop part by the enzyme Dicer [19]. The double-stranded miRNA is then loaded into the Argonaute (Ago) protein complex, and a complete silencing complex is formed, and the miRNA is used as a template for target recognition [18].

These double-stranded RNA molecules are utilized by the RNA-Induced Silencing Complex (RISC) to regulate gene expression [19]. It has been reported that the structural similarity of these miRNA hairpins and anti-Tn-C aptamers indicates miRNA functionality beyond RISC, making them even more sophisticated regulators [20]. The RISC complex acts as a translational repressor or as an mRNA breaker, depending on the number of base pairs that form between base pairs. One miRNA can target multiple genes, and they often return to silence the primary transcript from which they were synthesized.

The silencing process has critical roles in the physiological processes (e.g., cell differentiation, organism development) [21]. The concentration of miRNA was estimated between 1000 copies/ 1000 mm3 cells and around 200 copies/cell (or 166pM). It has been reported in previous studies that the median half-life of miRNA is approximately 5 days, with some exceptions (e.g., miR-155 was reported to have lower stability) [20]. The temporal and spatial deregulation of miRNA expression and the silencing process can result in pathological consequences (e.g., by modulating the expression of pro-inflammatory and pro-apoptotic effectors), which impact disease onset, progression, and severity [18,22]. The possibility of repeated sampling has increased scientists' interest in assessing the potential of miRNAs as biomarkers for malignant (e.g., lung adenocarcinoma, gastric or breast cancers) and non-malignant diseases (e.g., including DM) [23-27]. It should be taken into consideration that the multi-role of these molecules when comprising a panel for clinical usage. Thus, miR-21, miR-326 (e.g., glioblastoma), miR-34a, miR-2a (e.g., hepatocellular carcinoma), miR-155 (e.g., pancreatic ductal adenocarcinoma), miR-23b (e.g., melanoma, prostate cancer), miR-98 (e.g., squamous cell carcinoma; head and neck carcinoma), miR-186 (e.g., Hodgkin's lymphoma), miR-223 (e.g., chronic myeloid leukemia) were associated with different malignant diseases. But some of these miRNAs were also associated with different nonmalignant diseases (e.g., miR-21- cardiomyocyte hypertrophy; immune response in sepsis; miR-326 and miR-186 - multiple sclerosis; miR-590- extrinsic cardiomyopathy, autoimmune myocarditis; miR-223 - hereditary neutrophilia) [28].

STUDY OBJECTIVES

The study aims to perform a bibliometric analysis to evaluate research trends in the field and to identify and characterize miRNAs implicated in DM using curated bioinformatic resources.

MATERIALS AND METHODS

This study was conducted in two main phases: an analysis of the scientific literature and the identification of miRNAs altered in DM based on records in specialized databases.

Bibliometric Analysis. A comprehensive literature search was performed using the Scopus database [29]. The search strategy targeted the Title and Abstract fields with the following keywords: ("hsa-microRNA" OR "hsa-miRNA" OR "microRNA" OR "miRNA") AND ("type 1 diabetes" OR "type 2 diabetes" OR "T1D" OR "T2D") AND ("human" OR "humans" OR "Homo sapiens") NOT ("mouse" OR "mice" OR "rat" OR "rats" OR "murine" OR "animal model" OR "in vivo" OR "non-human" OR "rodent"). The initial search yielded 1730 articles. After excluding irrelevant subject areas (e.g., Engineering, Mathematics, Materials Science, Arts and Humanities, Social Sciences, Physics and Astronomy), 1,707 articles remained. Further filtering by document type (research articles only) reduced the number to 1085. Applying language (English) and publication stage filters resulted in a final dataset of 1051 articles. The literature analysis was conducted using the Bibliometrix R-package (version 4.4.0) and Biblioshiny (version 5.0), which enabled descriptive statistics, thematic mapping, and trend analysis [30]. All analyses were performed in R (version 2025.05.0, Build 496).

MiRBase [31] and TargetScanHuman 8.0 [32] were consulted to check for predicted target transcripts and conservation status of each miRNA. Diseases associated with miRNA were extracted from the Human ncRNA Gene Database [28].

RESULTS

Literature data analysis

The timespan of the analyzed publications was 2008 to 2025. The dataset comprised 1051 documents published across 440 sources, involving a total of 6409 authors and citing 49120 references. The annual growth rate of scientific output was 18.34%. The average age of the documents was 5.34 years, and the mean number of citations per document was 28.12.

Interest in the publication of this data began between 2008 and 2012 (Figure 1). A high increase was noticed from then on until 2015, with a spike from 2016 to 2022.

As for the average number of citations per year, it aligned with the wave of scientific production (Figure 2). The first spike was observed in 2009, followed by a second spike in 2012. A slight rise was noticed again, from 2018 to 2020.

Figure 1: Annual scientific production of data regarding miRNA and DM.

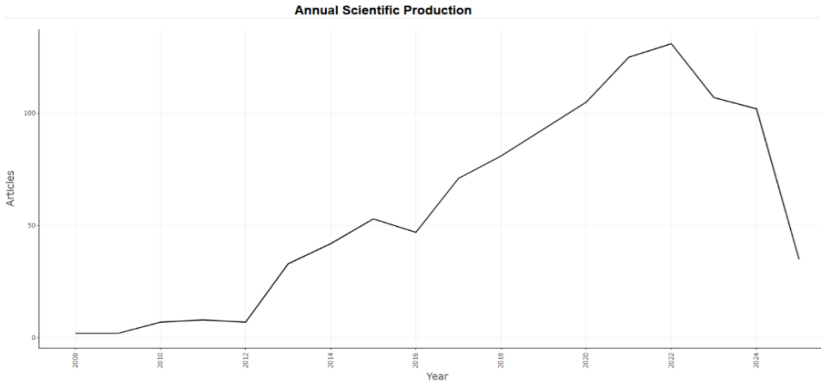
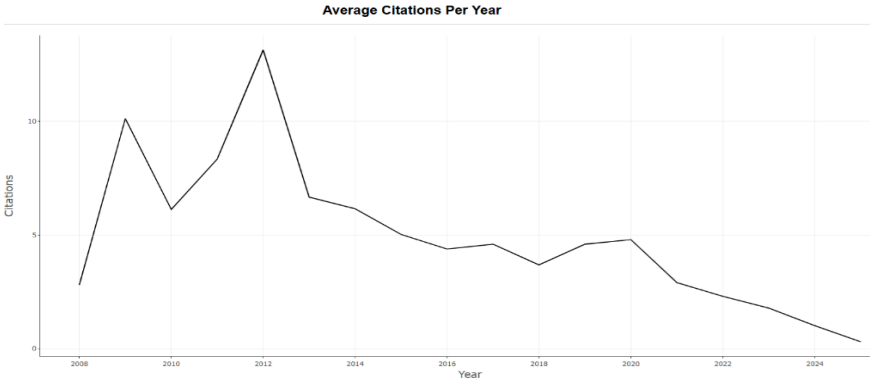


Figure 2: Annual scientific production of data regarding miRNA and DM.



The 1051 articles were published in various sources. The majority of them can be found in PLOS One, Frontiers in Endocrinology, International Journal of Molecular Sciences, and Scientific Reports. Other sources included: Acta Diabetologica, Journal of Clinical Endocrinology and Metabolism, and Molecular Medicine Reports (Figure 3).

Figure 3: Most Relevant Sources containing the included articles.

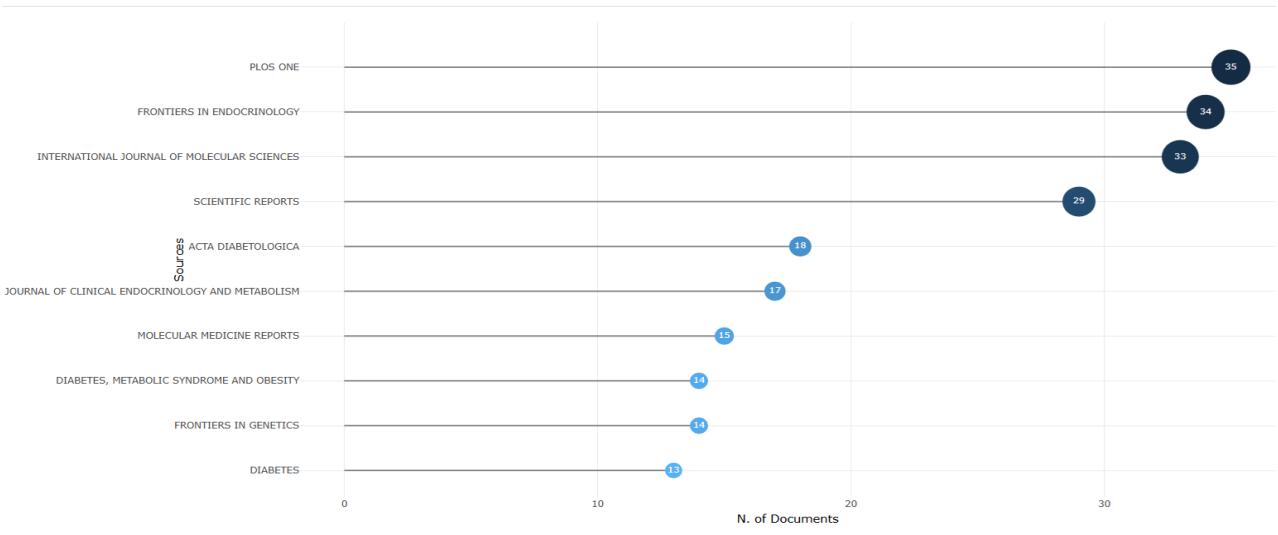


Figure 4: Most relevant authors of articles included in this study.

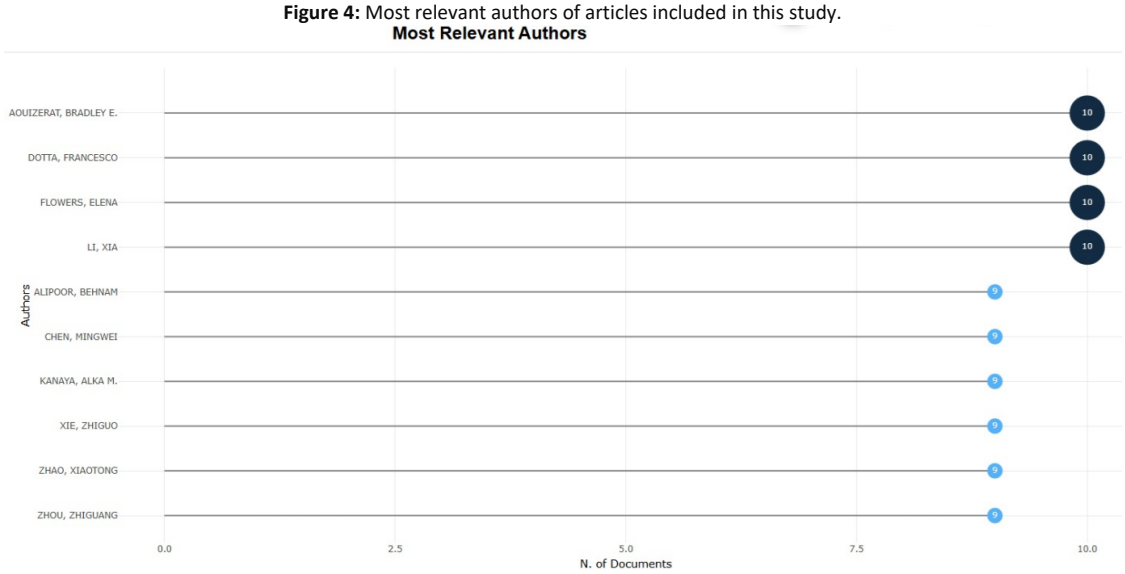
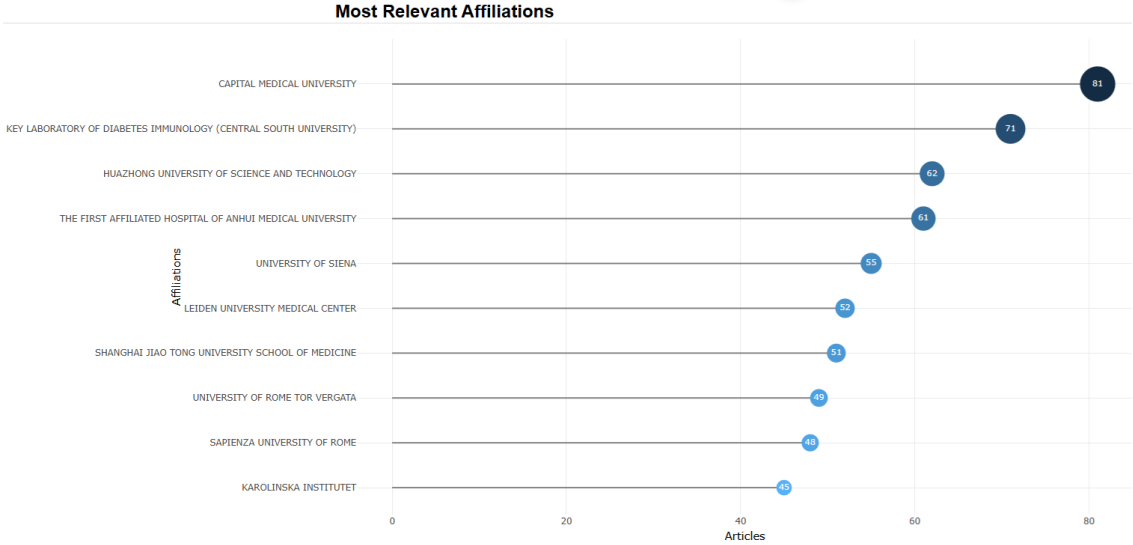


Figure 5: Most relevant affiliations of authors whose papers were cited in this article.



From the 6049 authors who participated in the research included in this paper, some of the most relevant are Dotta F., Flowers E., Li X., Chen M., and Zhao X. (Figure 4). Their affiliations include: Capital Medical University, Key Laboratory of Diabetes Immunology, University of Siena, and Huazhong University of Science and Technology (Figure 5).

Speaking at a global level, the papers with the most citations included in this study were [2,33-36]. The sources in which the works of the aforementioned authors were published include, in order: Oxidative Medicine and Cellular Longevity, Acta Diabetologica, The FASEB Journal, Journal of the American College of Cardiology, and The Journal of Clinical Endocrinology & Metabolism (Figure 6).

The trending topics identified in the reviewed articles highlight the evolving focus areas and emerging themes within the research field over time (Figure 7). Authors' keywords pinpointed in articles that constitute the database change over the years. First time span consisted of keywords such as: “cytokines”, “miR-375”, “single nucleotide polymorphism”. In recent years, keywords such as “diagnosis”, “molecular mechanisms”, “biomarker”, and “type 2 diabetes mellitus” were employed.

The co-occurrence network of authors’ keywords and the microRNA species miR-21, miR-126, and miR-146a highlights the relationship and thematic connections within the literature contained in the database of this paper (Figure 8).

The distribution of corresponding authors by country mirrors the ranking of the most cited countries (Figure 9; Figure 10). Leading the list are countries such as China, the United States, and Italy, which also represent the most active contributors to DM research. In terms of citation impact, Italy is followed by the United Kingdom, Iran, Spain, and Japan.

Figure 6: Most Globally Cited Documents cited in this paper.

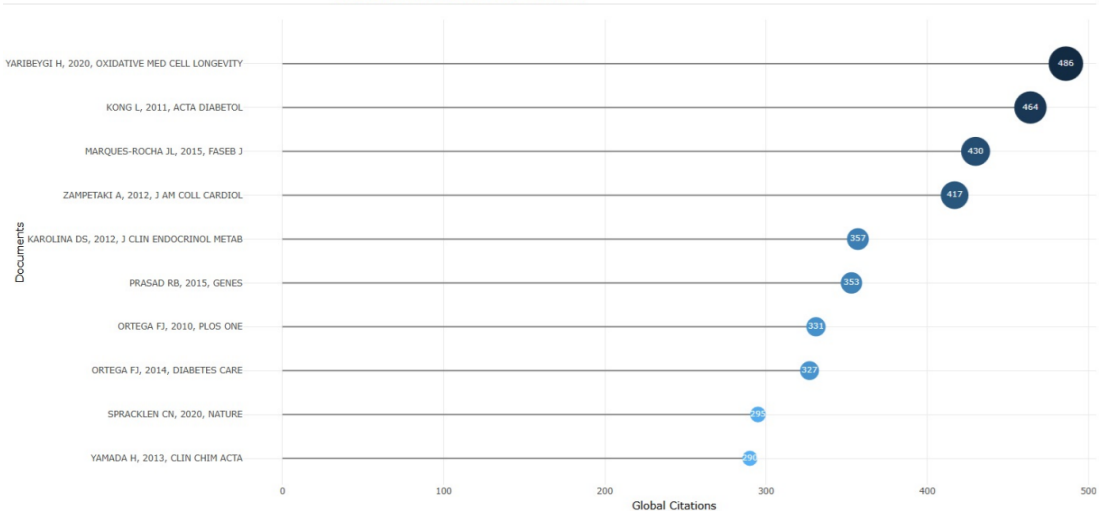


Figure 7: Trend topics identified in articles used in this systematic study.

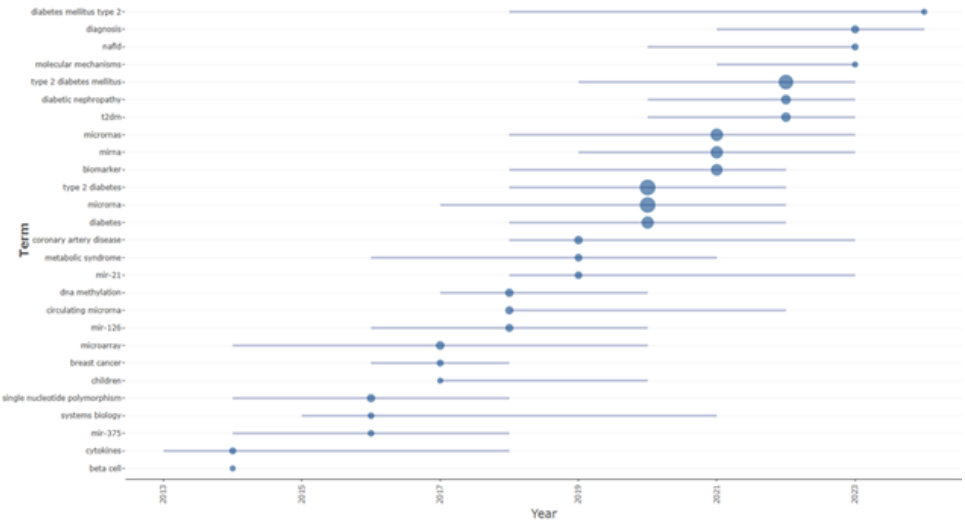


Figure 8: Co-occurrence network of authors' keywords (miRNAs: miR-21, miR-126, and miR-146a are represented in a green triangle).

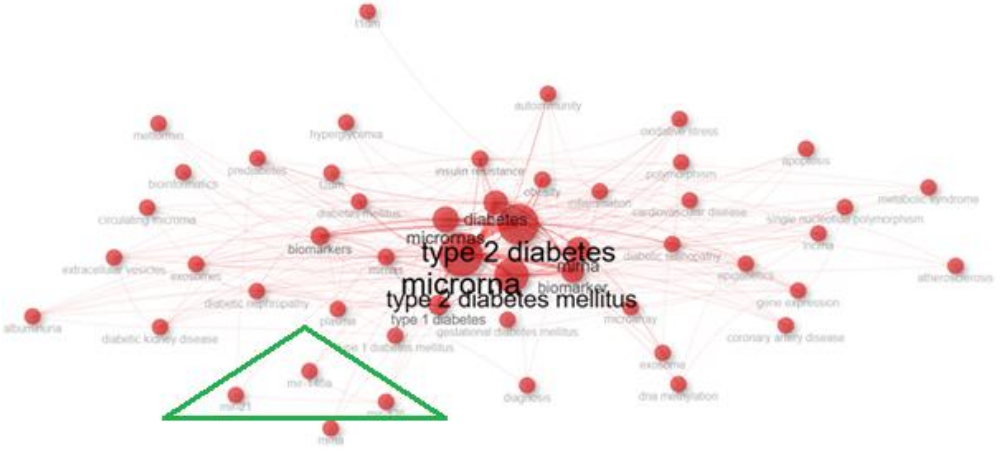


Figure 9: Authors' corresponding countries that were included in this study.

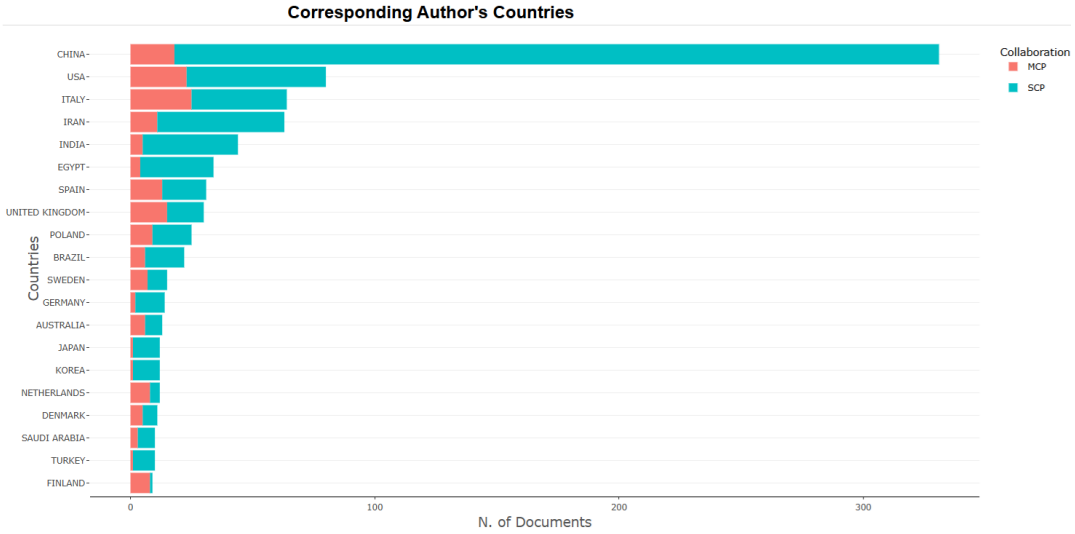


Figure 10: Most cited countries on subjects referring to miRNA and DM.

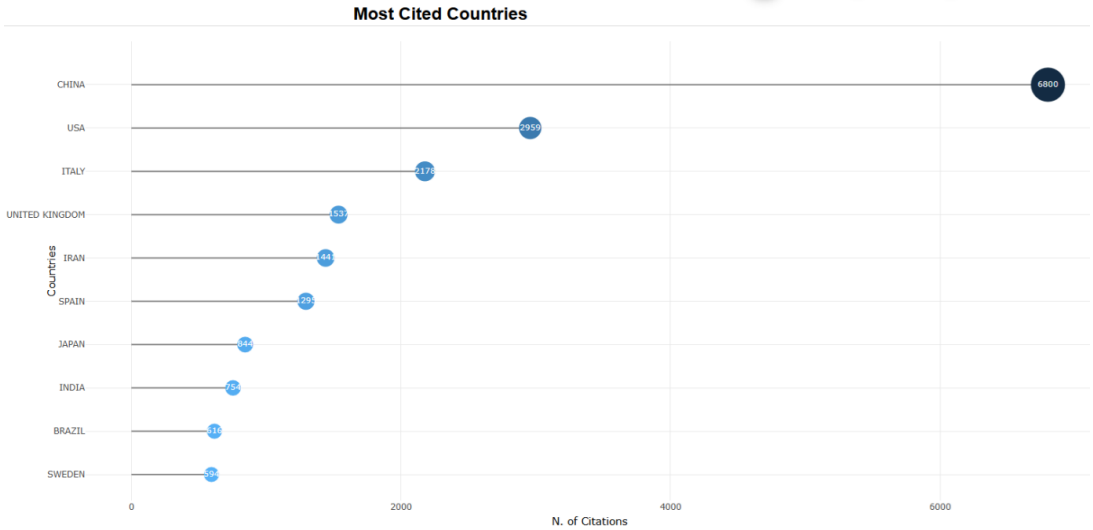


Figure 11: World map of countries collaborating on research on miRNA and DM

Visualization of international research collaborations by country is seen in Figure 11. Highlighting the global networks formed through co-authorship and cross-border scientific partnerships, its scope encompasses every continent.

miRNAs expression and DM

Bioinformatic analysis can be conducted in order to predict possible miRNA targets. Examples of databases constructed for this purpose are miRBase [31], TargetScan [32], and miRecords [37]. For this research, miRbase [31] and TargetScanHuman v8.0 [32] were used for consulting target transcripts and conservation status for each miRNA. Target gene IDs can be found in Tables 1, 2, and 3. MiR ID was normalized according to at least two articles and two miR databases, according to that miR's sequence.

Table 1: microRNAs downregulated (↓) or upregulated (↑) in T1DM analyzed in at least two studies.

miR in T1DM	Target Gene/Mechanism in which they are involved	Referenes
miR-326 ↑	TNFSF14, 15; CEP85	Zheng et. al., 2017
miR-23 ↑; miR-98 ↑; miR-590-5p ↑	Trail, Faslg	
miR-186 ↑	CXCL13; IRF8; STAT4	[38]
miR-223 ↑	HSP90B1; TP53; MCL1	
miR-142-5p ↑	HERPUD1	[39]
miR-155 ↑ / ↓	CASP3	[39]; Katsarou et. al., 2017
miR-100-5p ↓	RAVER2; AP1AR	Ferraz et. al., 2022
miR-181a-5p ↑	ZNF; TNFSRF11B	Liu et. al., 2019
miR-1275 ↑	KCNC3; KIR2DL4	[22]
miR-30b-3p ↑	CCL28, 22; TAF8; NCR3	Nizam et. al., 2024
miR-25-3p ↑	HIPK3; SNAPC1; RBM47	Liu et. al., 2019
miR-10a-5p ↑	CRLF2; HOXA3	Santos et. al., 2022
miR-98 ↑	IGDCC3; IGF2BP2; CCL3	Khan et. al., 2020
miR-22-3p ↓	H3F3C; GADD45A	Kaur et. al., 2015
miR-16-5p ↑	TNFSF13B; BTLA	Gao et. al., 2020
miR-574-3p ↓	NKG7; IL6; ATPIF1; IFNLR1; SRF	Garcia-Contreras et. al., 2017
miR-103a-3p ↑	SPI1 1; AGO4	Assmann et. al., 2018
miR-454-3p ↑	IRF1; IGF	Erener et. al., 2017
miR-450a-2-3p ↑	IFG2; IL6ST; NOX5; IL17A	Takahashi et. al., 2014
miR-100-5p ↓	INSM1; AGO2	Hezova et. al., 2010

TargetScanHuman v8.0 was used to query each miRNA to check predicted target genes.

Some factors involved in the pathophysiology of T1DM (e.g., ILs, tumor necrosis factor) can interfere with the expression of these miRNAs and thereby modulate the efficiency of silencing processes. These observations complicated the relationship between T1DM and miRNAs.

ABERRANT MICRORNA EXPRESSION IN TYPE II DIABETES

Database analysis

MiRNAs can interfere with T2DM physiopathological mechanisms [40] (Table 3). Some of these molecules were associated with both T1DM and T2DM (Table 2).

Although there is significant interest in developing new biomarkers for DM, only a few miRNAs have been consistently associated with T2DM in at least two studies (Table 3).

Table 2: microRNAs downregulated (↓) or upregulated in (↑) T1DM & T2DM analyzed in at least two studies.

miR in T1DM & T2DM	Target Gene/Mechanism in which they are involved	Referenes
miR-21 ↑	IL12A; FASLG; CCL1	[41]
miR-34a ↑	CDIP1; MSR1; PDCD6	
miR-146a ↓	INSR; ZNF; GRIN2B	
miR-29 ↑	NFIA; ATAD2B	Massart et. al., 2017
miR-142-3p ↑	CASP3	[39]; Zhu & Leung, 2015
miR-24-3p ↑	FASLG; BCL2L14	Blanco et. al., 2023; Garavelli et. al., 2020
miR-148a-3p ↑	ATP6AP2; GADD45A	Grieco et. al., 2018; Ghoreishi et. al., 2022

miR-150-5p ↓	MYB; TADA1; AIFM2	Kim et. al., 2019; Qiu et. al., 2021
miR-210-5p ↑	ERP29; GDF11	Patra et. al., 2023
miR-342-3p ↓	OSER1; SPI	Jiang et. al., 2020
miR-375 ↑	INS; HOXA5; ATP1B1	Higuchi et. al., 2015; Marchand et. al., 2016
miR-101 ↑	UBE2D1; APP	Santos et. al., 2019; Higuchi et. al., 2015
miR-199a ↑	PAWR; ACOX1	Wang, et. al., 2018; Yang et. al., 2020
miR-335-5p ↓	CASP; IL17RD	Li & Zhang, 2021; Hezova et. al., 2010
miR-20b-5p ↓	PDCD; IRF9	Katayama et. al., 2019; Hezova et. al., 2010
miR-15b ↑	CYP7A1; CASP	[42]
miR-186	CRP	
miR-155 ↑ / ↓	BCL2	Corral-Fernández et. al., 2013; Katsarou et. al., 2017

TargetScanHuman v8.0 was used to query each miRNA to check predicted target genes.

Table 3: microRNAs downregulated (↓) or upregulated (↑) in T1DM analyzed in at least two studies.

miR in T2DM	Target Gene/Mechanism in which they are involved	Authors & Year
miR-143	IGFBP; FADS6; ITM2B	[43]
miR-142-3p	BCL2	Zhu & Leung, 2015
miR-126	INSR; SCL7A5	Zhu & Leung, 2015
miR-375	INS; VEGFA; HOXA5; ATP1B1	Poy et. al., 2009; Chakraborty et. al., 2014
miR-26a-5p	POLR3G; INS; BAX	Xu et. al., 2020;
miR-146a-5p	IGSF1; IRAK1; PSMA4	Li et. al., 2012
miR-1 ↓	CYP7A1; XCR1; PRPF39	Chakraborty et. al., 2014
miR-16 ↓	RRM2B; MRPS22; C8B; PDX1	Bork-Jensen et. al., 2015
miR-30e ↓	GABRB1; IL1R1APL2; SOCS1	Dieter et. al., 2019
miR-9 ↑	DSCC1; GLT1D1	Al-Muhtaresh et. al., 2018
miR-33a ↑	TEX38; TLR4; PTPLAD1	Saeidi et. al., 2023
miR-320a ↑	ETFA; TRIAP1; RGS9BP	Du et. al., 2021
miR-148b ↑	GADD45A; MMD; TGFA	Liang et. al., 2020
miR-21 ↓	IL12A; FASLG; GPR64	[44]
miR-29a ↑	MANEA; HOXA10; RTP3	Massart et. al., 2017
miR-34a ↑	MSR1; PDCD6; GORAB	Shen et. al., 2017
miR-103	RNF38; TRIAP1; AGO4	Rafiee et. al., 2024
miR-15a ↓	POLR3K; SLC22A9; RAVER2;	Al-Kafaji et. al., 2015
miR-155 ↓	H3F3A; ZNF652; FGF7	Katsarou et. al., 2017
miR-221 ↑	GABRA1; KCNQ3; SOCS5	Liu et. al., 2018
miR-145 ↓	ATP6V0B; ITGB8; CDC37L1	He et. al., 2020
miR-24 ↓	SNN; BCL2L11; KCNK2	Xiang, 2015
miR-182 ↑	TMEM50B; CHIC1; VLDLR	Krause et. al., 2024
miR-192 ↑	insulin-like growth factor 2; SLC44A1	Jaeger et. al., 2018
miR-194 ↑	PRKAR1A; SLC10A7; BNIP2	Jaeger et. al., 2018
miR-223 ↑	FBXW7; IL6ST; HSP90B1	Sánchez-Ceinos et. al., 2021
miR-130a ↑	IGF1; TGFBR2; HIVEP2	Ofori et. al., 2017
miR-19a ↑	QKI;	Yan et. al., 2020
miR-26b ↑	ID2; IGFBP3; SOCS1	Xu et al., 2015
miR-27a	FOXO1, FOXA2, STAT3	[45]
miR-27b ↓	GOLM1; GPAM	Ghoreishi et. al., 2022
miR-28 ↑ / ↓	TRAF3	[45;46]

TargetScanHuman v8.0 was used to query each miRNA to check predicted target genes.

DISCUSSION

Literature data analysis

The utility of miRNAs as biomarkers for screening or diagnosis of T1DM is of high interest. There have been some attempts to propose a panel of miRNAs for elaborating these tests, although normalization of miRNA levels is still necessary [47].

A systematic review published in 2017 mentioned 48 miRNAs that were investigate in tissues relevant for T1DM pathogenesis (e.g. serum, plasma, pancreatic tissue, blood cells); for 11 of them the expression level differ significantly in T1DM patients and control subjects (e.g. miR-21-5p, miR-24-3p, miR-100-5p, miR-146a-5p, miR-148a-3p, miR-150-5p, miR-181a-5p, miR-210-5p, miR-342-3p, miR-375 and miR-1275) [22]. There are certain miRNAs (e.g., miR-375) that directly influence specific pathways leading to diabetogenesis. These molecules target genes that modulate immune T cell activity. Human and mouse T lymphocytes have been shown to release exosomal miRNAs (e.g., miR-142-3p, miR-142-5p, and miR-155), which can predispose or support the apoptosis of pancreatic β cells [39]. Additionally, overexpression of some miRNAs can reduce insulin synthesis, insulin secretion, or their biological effects [48].

Cytotoxic attack in T1DM is mediated by proinflammatory cytokines, such as interleukin-1β (IL-1β) and tumor necrosis factor α (TNF-α). These cytokines have been found to induce the synthesis of miR-21, miR-34a, and miR-146a in vitro, in human and mouse pancreatic cells [41].

miR-21 is predicted to target multiple transcripts (e.g., IL12- natural killer cell stimulatory factor and cytotoxic lymphocyte maturation factor, CXCL1- chemokine ligand 1). Increased levels of IL-12 could be predictors of T1DM. IL-12 plays a part in determining the levels of CD4+T cells and increasing the CD8+ T cells' toxicity in DM [49]. It also has a role in activating the apoptotic pathway of the β cells. Existing literature proved the implication of the CXCR1/2 axis, CCL4-CCR5 axis, and CXCL10-CXCR3 axis in the inflammatory background of DM. These pathways were proven to be engaging more aggressive immune T-cells. This furthermore accentuates the β cell death [50].

A decrease in miR-155 expression has been shown to impair the function of regulatory T cells (Tregs), which exhibit an upregulated expression of suppressor of cytokine signaling 1 (SOCS1) upon maturation. Treg cells have a primary role in alleviating the aggressivity of CD4+ and CD8+ T cells. Therefore, their underdevelopment has been linked with one of the causes of T1DM, implicating the STAT3 mutations. The signal transducer was reported to recognize and enhance the miR-155 promoter. The development of Treg cells is also threatened by mutations in the FoxP3 gene, and its altered transcriptional activity has been linked with the presence of miR-155 [51].

The apoptotic genes (Trail, Faslg) are predicted to be targeted by miR-23, miR-98, and miR-590-5p. These miRNAs are overexpressed in CD8+ T cells of T1DM patients [52]. MiR-326 targets different genes (e.g., the gene coding the vitamin D receptor), which modulate the function of the immune system [44]. Expression levels of miR-326 were found to be increased in lymphocytes of T1DM patients who tested positive for the islet-specific autoantibodies IA-2A and GADA [53].

MiR-34a is known to inhibit macrophage polarization, thus impairing tissue homeostasis and repair. This miRNA is involved in the regulation of insulin signaling and glucose metabolism through the targeting of specific mRNAs, including those of sirtuin 1 (SIRT1) and vesicle-associated membrane protein 2 (VAMP2) [54]. Dysregulated miR-34a in cancer patients has been proven to lead to low survival chances. It targets multiple oncogenes and plays a part in apoptosis mediated by the p53 complex, the tumor suppressor gene [55].

In a cross-sectional study, Januszewski and his coworkers demonstrated increased levels of miR-186 and miR-223 in T1DM patients. MiR-186 is known for its role in apoptosis and chemokine and insulin signaling pathways [42]. MiR-186 is also known for being a tumor suppressor in prostate cancer. There was an increase in granulocytes in model mice with miR-223 deficiency, and its expression was highly decreased in patients with active nephritis. This miR-223, along with others, has been associated with multiple cellular processes: p53 pathway regulation, cell cycle regulation, tumor metabolism, and apoptosis [38].

The cumulative roles of miRNAs in T1DM are various, implicating many cellular and molecular pathways and mechanisms. Extensive research is needed to further validate the expression levels in vivo model organisms [47].

miRNAs expression and DM

Certain miRNAs found in scientific publications or databases are associated with the pathogenesis of diabetes or with particular features of diabetic patients. Subjects with impaired glucose tolerance presented abnormal levels of miR-18b [45]. miR-143 has a role in precursor adipocyte differentiation and in predisposition for T2DM (e.g., by targeting the insulin-AKT pathway) [43]. MiR-21 targets mainly FOXO1, FOXA2, and STAT3 transcripts. Its levels were considered informative for gluconeogenesis [56], insulin resistance [57], and altered glucose tolerance [45]. Markers of low-grade inflammation in T2DM (e.g., IL6, TNFα) are also associated with changes in miRNAs. TNFa enhances the intracellular accumulation of miR-223, which targets genes involved in lipid and glucose metabolism, inflammation, and T2DM (e.g., IGF-IR, Hsp90, FOXO1) [58;59].

A randomized community-based study analyzed a sample of 1000 individuals. It was identified several downregulated miRNAs (e.g. miR-126, miR-15a, miR-29b, miR-223, miR-20b, miR-197) in the plasma samples from T2DM patients [46]. Upregulated (e.g., miR-140, miR-142, miR-222) or downregulated (e.g., miR-125b, miR-126, miR-130, miR-192, miR-195, miR-423-5p, miR-532-5p) miRNAs in T2DM patients were also identified in a cross-sectional, double-blinded study. It was observed that the expression levels of four

miRNAs (e.g., miR-126, miR-140-5p, miR-195, miR-423-5p) demonstrated a strong ability to distinguish subjects with T2DM from those with normal fasting glucose levels [60]. MiR-126 has been reported by a considerable number of research groups to be a suitable biomarker for the assessment of T2DM progression [44, 46, 61]. MiR-186 expression levels were found to be statistically significantly associated with HbA1C levels in T2DM patients and with the risk of diabetic neuropathy [62].

There are numerous factors to consider when evaluating the potential of miRNAs as biomarkers for DM. For instance, the dynamics of miRNA concentration may be correlated with causes of dysregulated expression (e.g., number of cells releasing these small non-coding molecules or with miRNA overexpression in individual cells), miRNA half-life, and factors influencing their stability. Other factors can also significantly impact the significance of results. For example, an increase in adipose tissue volume can increase the levels of some miRNAs; these associations may influence the results, particularly in studies involving obese patients with T2DM [45]. The timing of miRNA testing poses a challenge, especially in presymptomatic stages of diabetes [63]. It has been hypothesized that dysregulated miRNAs may be present only in specific cells or tissues. Comprehensive research involving paired analyses of body fluids and biopsy tissues, along with validation studies, could be beneficial for future investigations [64]. There also remains a critical need for the standardization of miRNA analysis across different research platforms, tissues, and conditions to facilitate the development of clinically useful biomarkers [65].

The size of the sample pool influences the study's power and the reliability of results. Studies with small sample sizes (e.g., fewer than 10 patients and 10 control subjects) may yield biased outcomes [66]. Consequently, findings from such studies may not be corroborated by research involving larger sample sizes. Therefore, the statistical significance and clinical relevance of some miRNA studies may be limited. Reliable statistics are needed against large sample pools and of different categories.

CONCLUSION

MiRNAs can target a large number of transcripts, and their levels in specific tissues change during the natural history of the disease. But these factors also increase the difficulty of identifying the relevant genes for diabetogenesis and validating the clinical use of specific miRNAs. Standardization in profiling techniques and validation of these biomarkers across large, diverse populations remain essential steps toward their clinical application.

This review underscores the emerging role of miRNA as one of the key molecular players in the pathogenesis, diagnosis, and potential treatment of DM. Numerous studies have identified distinct expression patterns of specific miRNAs (e.g., miR-21, miR-126, miR-143, miR-155, miR-223, and miR-375) that correlate with immune dysregulation, β-cell apoptosis, insulin resistance, and metabolic imbalance.

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Authors' contribution

Conceptualization, X.I.P. and DC.; methodology, X.I.P., RN and DC; software, D.C. and X.P; validation, X.I.P., O.A.A.T, I.R, L.B., I.A.E. and S.N.; formal analysis, D.C.; investigation, D.C, X.I.P. and R.N; resources, D.C.; data curation, X.I.P., O.A.A.T, I.A.E, S.N, and R.N.; writing- X.I.P. and D.C; writing-review and editing, O.A.A.T, I.R, L.B., I.A.E, S.N. and R.N.; visualization, D.C.; supervision, D.C.; project administration, L.B.; funding acquisition, L.B. All authors have read and agreed to the published version of the manuscript.

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REVIEW

The Novelty of Contrast-enhanced Ultrasound in the Pathology of Liver Tumors. A Narrative Review of the Literature

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Abstract: Liver pathology represents, nowadays, a major cause of mortality and morbidity worldwide. Since MRI helps in limited cases in the differential diagnosis of various pathologies concerning liver tumors, other modern diagnostic strategies should be prioritized. Besides various tumoral biomarkers, contrast-enhanced ultrasound (CEUS) plays a substantial role in differentiating various liver pathologies due to its availability and specificity. Various contrast-enhanced substances used for CEUS assist mainly in detecting focal liver lesions and differentiating their malignant character. This review focuses on the main indications and limits of this novel investigation, considering that patients presenting with various liver pathologies are more challenging. We highlighted in our review the similarities with the CT/MRI imaging methods in reference to the specificity and sensitivity of the method, in its potential ability to detect various liver tumors. Moreover, the contrast agents used by CEUS are eliminated throughout the lungs, showing no evidence of hepatotoxic, nephrotoxic, or cardiotoxic events. In conclusion, the accessibility and safety of the procedure and the absence of the use of ionizing radiation point out that CEUS could be a stand-alone technique in the diagnosis and evaluation of complex liver pathologies.

Keywords: contrast-enhanced ultrasound; hepatocellular carcinoma; viral hepatitis; liver cirrhosis

INTRODUCTION

The diagnosis of various liver tumors remains, nowadays, challenging even if new diagnostic and prognostic tools are continuously developed [1–5]. In this context, contrast-enhanced ultrasound (CEUS) has been a widely used imaging method in Europe and Asia for over 10 years. CEUS consists of an ultrasound that employs contrast agents for increasing the accuracy of the investigation, typically injected intravenously. It can be compared with other contrast-enhanced imaging methods, such as CT or MRI, and it possesses the advantage of a real-time assessment of different focal liver lesions, improving their detection and characterization [1,2]. CEUS has become more popular in recent years than CT or MRI due to the fact that it has almost no adverse reactions, does not make use of any type of ionizing radiation, and can be employed successfully in patients with impaired renal function [1].

CEUS uses a special type of contrast agent that consists of a microsphere with an albumin, phospholipid, or surfactant-based shell and a gas filling, which subsequently forms a microbubble [2]. These microbubbles do not usually surpass 7 µm in size and have a mean diameter of 2.5 µm. These small sizes allow the microbubbles to freely circulate in the capillaries and impede them from passing through the vascular endothelium into the interstitial space, thus rendering them exclusively intravascular [2,6,7].

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The microbubbles are excreted in two different ways depending on their components – the shell is metabolized in the liver, while the gas filling is eliminated through the lungs in about 10 to 15 minutes, even in patients with chronic obstructive pulmonary disease. This characteristic allows the utilization of these contrast agents in patients with renal failure, thus posing no risk of nephrotoxicity, when other contrast-enhanced methods such as CT or MRI are not indicated [2,6,8,9].

There are many contrast agents available around the world, but the most conventional are the sulfur hexafluoride microbubbles (SonoVue, Bracco), the perflutren lipid microspheres (Definity, Luminity), and the perfluorobutane microbubbles (Sonazoid).

SonoVue is the contrast agent most often used in Western countries and comprises a phospholipid shell that surrounds the sulfur hexafluoride microbubbles, resulting in a diameter of 2.5 μm. The agent is usually injected as a bolus of 2.4 mL in a peripheral vein (the antecubital vein), together with a 5 mL to 10 mL saline solution, using a large enough needle (more than 20-gauge) to ensure that the bubbles do not rupture during the procedure [1,2,6].

Sonazoid is mainly employed in Japan, Norway, and South Korea, and uses perfluorobutane microbubbles. Although it is similar to the other contrast agents used in CEUS, unlike those, Sonazoid can be phagocytized by the Kupffer cells and allows an additional evaluation of the liver in the Kupffer phase or the post-vascular phase. The agent can persist up to several hours in the liver and spleen after its vascular phase [1,6].

INDICATIONS - GENERAL CONTEXT

Even though the most common clinical application of CEUS is the characterization of focal liver lesions and differentiating between malignant and benign masses, there are studies and guidelines that show there are numerous other uses for this technique. As an intravascular contrast-agent imaging method, CEUS can provide information about different vascular diseases that may affect the carotid arteries or the aorta and other abdominal blood vessels, or it can help assess the thrombosis of the portal vein [10,11].

CEUS plays an important role in the imaging of the extravascular structures, mainly cavities, either physiological, such as the peritoneal, pleural cavities, biliary and gastrointestinal tracts, or non-physiological, including cysts, abscesses, or diverticula [12,13]. Thus, CEUS is used in gynecology to evaluate the uterus and the fallopian tubes, in nephrology and urology to help diagnose ureteral reflux and to discern between bladder or renal tumors and cysts or testicular masses. Excluding the liver evaluation, CEUS also has other applications in the assessment of the gastrointestinal tract.

It is successfully used in patients with inflammatory bowel diseases to estimate disease activity or with fistulae and abscesses, and at the same time, it can be administered orally in order to examine the bowel lumen [12–14].

CEUS can be successfully used for assessing different focal liver lesions seen on standard ultrasound and is indicated for the following: hepatic nodules larger than 10 mm (for nodules smaller than 10 mm CEUS can be attempted by an expert examiner); LI-RADS-graded liver lesions as LR-3, LR-4 or LR-M on CT or MRI: monitoring enhancement pattern changes over time for LI_RADS-graded lesions as LR-3 or LR-4; detecting arterial phase hyperenhancement (APHE) when it is absent on CT or MRI, but still suspected; guiding liver biopsies or treatments for difficult to visualize lesions, aiding in choosing the right lesion to be biopsied or reevaluating prior biopsied lesions with inconclusive histopathology; assessing treatment response; making the differential diagnosis between a tumor inside a vein or a simple thrombus [15].

One of the most important indications for CEUS is liver mass characterization and not just detection, with some studies showing an equivalence or even a superiority compared to CT or MRI scans. This ultrasound can also help in differentiating benign from malignant nodules [16].

The most common benign lesions that can be diagnosed using CEUS are hemangiomata, focal nodular hyperplasias (FNH) and hepatic adenomata. Malignant masses which are evaluated by CEUS are hepatocellular carcinomata (HCC) and non-hepatocellular tumors, that include metastases, intrahepatic cholangiocarcinomata (ICC) and lymphomata [16–18].

Liver lesions in CEUS can be described using two features that appear in different phases of the ultrasound: arterial phase hyperenhancement (APHE) and washout [1,16].

If there is no washout present and the enhancement in the arterial phase is sustained, there is a high probability that the mass is benign. In comparison, malignant masses show a decline in the enhancement (washout) in the portal or late phase. Furthermore, the intensity and the timing of the washout can be employed to differentiate between types of liver malignancies.

HCC nodules are most likely to display a late (in over 1 minute) and weak washout, while non-hepatocellular malignancies exhibit early (in less than 1 minute) and intense washout. In order to detect liver metastasis, a sweep of the entire liver in the portal venous phase is essential [1,2,16,17,19]. The benign lesions are usually described by specific patterns of enhancement in the arterial phase. Some examples of these patterns are peripheral discontinuous nodular enhancement (hemangiomata), stellate vessels with centrifugal filling (FNH) and centripetal filling (some adenomata) [16,20].

TECHNICAL EXECUTION

To correctly perform an ultrasound using contrast agents, firstly, it is necessary to have a properly trained examiner who should be able to recognize focal liver lesions and vascular disorders, to assess the effect of a treatment over a lesion and to compare different findings with other imaging methods such as CT, MRI or PET-CT. Secondly, the ultrasound equipment needs to have appropriate hardware, for example, scanners that can cancel soft-tissue enhancement, and software that can display both the standard ultrasound image and the CEUS image simultaneously [15,16]. The patient should be in a supine position with the abduction of the right arm, and before injecting the agent, the examiner should be aware of the medical history of the patient, of the clinical aspects and also of any existing cysts or calcifications that may be wrongly interpreted as malignant [1,12,15,16,21].

For liver imaging, the recommended dose of SonoVue, the most frequently used contrast agent, is 2.4 mL. This value can be decreased by 20-25% if the patient has a low BMI in order to avoid the excessive enhancement of the liver or it can be increased if the lesion is very superficial [15]. The substance is then injected through a large IV line (20-gauge minimum) as a bolus in the span of 2-3 seconds, immediately followed by a saline flush of 5-10 mL [2,7,15].

Interpretation of CEUS is made by evaluating the three vascular phases that follow the injection of the contrast agent. These phases appear as a result of the double vascularization of the liver, namely the hepatic artery and the portal vein [21].

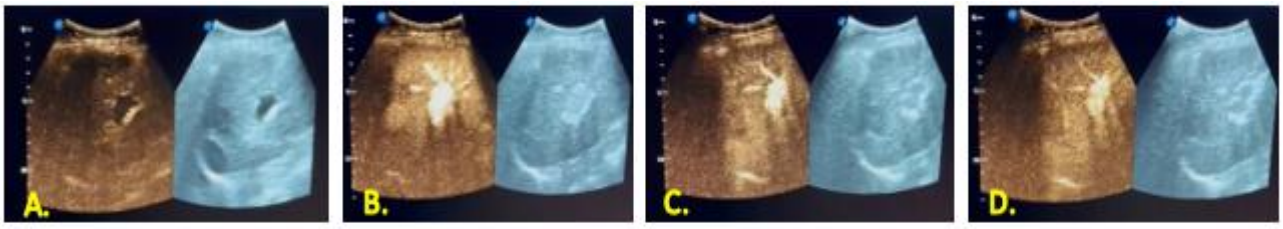
The arterial phase begins at 10 to 20 seconds after injection and ends at 30-45 seconds and appraises the arterial vascular supply of the liver lesion by degree and pattern [1,21]. The portal venous phase begins immediately after the arterial phase at 30-45 seconds and can last up to 120 seconds (2 minutes). This phase marks the entry of the contrast agent in the portal vein and is characterized by diffuse and maximal enhancement of the liver parenchyma [1,21]. The late phase is represented by the amount of time passed from the end of the previous phase until the clearance of the contrast agent from circulation and it can vary from 2 to 8 minutes (120–480 seconds), depending on the type and dose of the agent [1,21]. The post-vascular phase only appears if Sonazoid is injected and it is the result of the contrast loading of the Kupffer or other phagocytic cells. This particular phase usually starts after 8 minutes (480 seconds) following the initial injection and can be seen even after several minutes or hours [1,21] (Table 1).

Table 1: Vascular phases and their duration in contrast-enhanced ultrasound (CEUS)

	START	END
Arterial	10-20 s	30-45 s
Portal venous	30-45 s	120 s
Late	>120 s	Approximately 4-8 min (bubble disappearance)
Post-vascular	>480 s	Approximately 30 min

In the following images, an HCC nodule can be seen during a contrast-enhanced ultrasound. The hyperenhancement in the arterial phase and the late and mild washout in the later phases should be noted (Figure 1).

Figure 1: HCC nodule as seen during CEUS. (a) After 0 seconds (moment of contrast injection), (b) After 30 seconds (arterial phase); (c) After 90 seconds (portal venous phase); (d) After 140 seconds (late phase). Image credits: Teodora Isac.



BENEFITS

The contrast agents used for CEUS have a few major advantages in comparison to those used for CT or MRI. The most important characteristic is the increased safety of administration, with almost no side effects reported in the literature. There is no evidence of either cardiotoxic, nephrotoxic or hepatotoxic events, nor any interference with the thyroid function as they do not contain iodine and no need for any specific laboratory testing or fasting before the procedure [2,15,21]. Therefore, these contrast agents can be harmlessly administered to patients with renal failure and CEUS can be considered the first-line imaging method for these particular cases [2,21]. Some studies show that when adverse reactions do appear, they are: headaches, chest pain or chest discomfort and nausea, all other symptoms being extremely rare [21,23,24].

Compared to CT contrast agents, CEUS agents have a significantly lower incidence of hypersensitivity events and the probability of an anaphylactoid-type reaction is extremely low, with a rate of 0.001% and a rate of 0.0006% of fatalities after SonoVue administration [2,21]. Some studies show that CEUS is also safe for use in pediatric patients or pregnant women [21]. Following these observations

in the population, the 30-minute period of monitoring previously recommended after injection of contrast agents was eliminated [15].

CEUS has the advantage of being a real-time investigation with a higher sensitivity for detecting APHE in focal liver lesions than CT or MRI and is one of the most important imaging methods for identifying HCC nodules [6,21]. CEUS can categorize a rapidly enhancing hemangioma as LR-1, often wrongly diagnosed as LR-3 or LR-4 by CT or MRI, and can also differentiate an arterioportal shunt from a true HCC nodule, which can confuse the previously mentioned types of scanning [6].

CEUS was successfully employed for guiding treatment procedures or for evaluating treatment response. When performing a US-guided thermal ablation of a liver nodule, often an HCC one, CEUS showed a rate of 95.2% of patients with complete tumor ablation after a single procedure compared to 32% of patients after using a standard US. The rate of tumor recurrence was reduced to 16% as compared to 48% and the patients that benefited from CEUS guidance showed better progression-free survival rates [16,25,26]. The sensitivity of CEUS for post-treatment assessment is more than 80% and the specificity nears 100%, in some studies exceeding other imaging modalities, and, if used immediately after the ablation, is able to evaluate hemorrhages or hepatic infarctions [26].

Another treatment procedure after which CEUS can be used to determine residual tumor is transarterial chemoembolization (TACE). When employed after 4 weeks, it can successfully differentiate between a real active tumor and post-procedural inflammation. Some studies show that CEUS can visualize residual HCC nodules after only 1 day following the procedure, with higher rates of detection than CE-CT after 1 month [25]. It also has the advantage of detecting smaller areas of viable tumor that cannot be correctly appreciated by CT or MRI [26].

Though limited, there are studies that attest to the use of CEUS for evaluating patients one week after transarterial radioembolization (TARE) based on its ability to detect changes in tumoral perfusion [26].

Some studies evaluate CEUS as a monitoring tool for systemic therapies and their effects on tumor angiogenesis [25].

LIMITS

Even if CEUS is one of the most efficient imaging methods for liver nodules, there are some limits that need to be taken into consideration.

First of all, being a real-time evaluation, it is not possible to examine more than one lesion at the same time or to visualize the entire liver in the arterial phase; for this instance, a second bolus of contrast agents is needed for the same patient [2,15]. For patients with multiple liver nodules, contrast-enhanced CT or MRI is recommended [22].

Secondly, areas that are difficult to examine on a standard ultrasound are most likely the same when using CEUS and it can be troublesome when the lesion is smaller than 10 mm or is located subdiaphragmatic or very deep [15,22]. Small, simple cysts can be misinterpreted as metastatic lesions because both of them appear hypoechoic on CEUS [2].

For nodules that require complete imaging staging or monitoring of changes in size over time, CT or MRI are more likely to be of use, because CEUS alone is either not sufficient or it is very difficult to capture exactly the same image as the first time on serial US exams [6,22,27].

Even though CEUS is better than standard US, it is still examiner-dependent and the quality of the images may differ with movement artifacts or other interferences [2]. Some limits may also appear in the technique itself, for example, artifacts related to the dosing of the contrast agent, destruction of the microbubbles by the US beam, or inadequate tissue suppression as seen in a fatty liver [16].

CEUS LI-RADS and HCC

In order to create standardized reports for liver nodules, mainly HCC, seen on CEUS examinations, a scoring system has been designed called LI-RADS (Liver Reporting and Data System) [1,28,29]. It helps improve communication between different examiners and reduce confusion and diagnostic errors in patients with high risk for HCC [28]. The nodules observed with CEUS are then classified as: LR-1 (definitely benign), LR-2 (probably benign), LR-3 (intermediate malignancy probability), LR-4 (probably HCC) and LR-5 (definitely HCC) [1,28] (Table 2). Because CEUS is also able to evaluate other types of liver masses, additional categories have been added: LR-M (malignant, but not specific for HCC), LR-TIV (tumor present in the vein) and LR-NC (not categorized due to omission or image degradation) [1,15,30,31]. The sensitivity of CEUS for detecting HCC lesions is dependent on the mass size. For nodules of less than 2 cm, the sensitivity can vary in different studies from 53.6% to 83.3% and for nodules bigger than 2 cm, it ranges from 91.3% to 94.5%, with a mean of approximately 78.3% and a specificity of around 93.1% [32].

If a nodule shows rim-like APHE, which means the enhancement is more intense in the periphery, forming a halo around it, the category according to CEUS LI-RADS should be LR-M and they are usually liver metastasis or other malignant lesions, but not HCC. If the nodule has a peripheral discontinuous globular pattern, it should be included in the LR-1 category as a benign hemangioma [1,33].

Table 2: CEUS LI-RADS diagnosis (modified after [6])

APHE (arterial phase hyperenhancement)	**NO**		**YES** (not rim and not peripheral discontinuous globular)	
Rim APHE or early washout (<60 s) or marked washout – LR-M Peripheral discontinuous globular – LR-1 (hemangioma)				
Nodule size	Nodule size	≥20 mm	<10 mm	≥10 mm
No washout	LR-3	LR-3	LR-3	LR-4
Late and mild washout	LR-3	LR-4	LR-4	LR-5

In a study that took place between January 2010 and December 2018, 382 patients (with a total of 464 liver lesions) with chronic liver disease at high-risk for hepatocellular carcinoma were included [28]. The exclusion criteria were: transarterial chemoembolization, radiofrequency ablation, and systemic treatment for HCC [28]. The 464 liver lesions were divided into: 68 non-HCC non-malignant lesions (hemangiomata, simple cysts, fatty infiltration, regenerative nodules), 359 HCCs and 37 non-HCC malignant lesions (liver metastases, other types of malignancies). By using CEUS LI-RADS, all of the 464 nodules were classified and the percentage of HCC was established for each category. From the total number of lesions examined, 22 of them were part of the LR-1 category and the other 8 lesions were classified as LR-2. Among those, the HCC rate was 0%. 26 lesions were included in the LR-3 class, but 11 (42.3%) of them were HCC. From the 106 nodules that were included in the LR-4 category, 85 (80.2%) were also confirmed to be HCC. 264 nodules from the total of 464 were categorized as LR-5 by means of CEUS and the HCC rate in this category was 258 (97.7%). For the remaining lesions, 11 were classified as LR-TIV and all of them (100%) were confirmed as HCC, and 38 were classified as LR-M; only 5 (13.2%) of those actually were HCC [28].

The sensitivity of the LR-5 category for the diagnosis of hepatocellular carcinoma was 71.9%, with a specificity of 94.3%, a positive predictive value of 97.7%, a negative predictive value of 49.5% and an overall diagnostic accuracy of 76.9% [28].

By combining the LR-4 and LR-5 categories, they managed to improve the diagnostic accuracy to 90.7%. In this case, the sensitivity was 95.5%, the specificity 74.3%, the positive predictive value 92.7% and the negative predictive value 82.9% [28].

This study has once more shown that CEUS is comparable to CE-MRI and CE-CT in the diagnosis of hepatocellular carcinoma and can be used as a first step to describe liver lesions in high-risk patients during the HCC surveillance. Moreover, using CEUS instead of CT/MRI limits the use of unnecessary imaging techniques in the case of non-malignant tumors [28].

Differentiating an intrahepatic cholangiocarcinoma from a hepatocellular carcinoma in patients with liver cirrhosis can be difficult. The CEUS pattern of the intrahepatic cholangiocarcinoma in this study was a heterogeneous and rim-like enhancement in the arterial phase and a portal venous and late vascular phase washout. The key feature of the HCC is represented by the arterial hyperenhancement of the nodule, followed by a late (after one minute) or absent washout [28]. CEUS has shown a good performance in the diagnosis of HCC, with good sensitivity and specificity using the LI-RADS algorithm. However, an HCC nodule lower than 2 cm has shown a lower sensitivity (56.3%) as compared to 78.9%, representing the sensitivity of HCC nodules higher than 2 cm.

In spite of all the limitations of CEUS presented in this study, the CEUS LI-RADS algorithm for HCC diagnosis remains a useful non-invasive clinical practice [28].

In conclusion, for a nodule to be diagnosed as HCC, it needs to show an APHE, not rim-like and not peripheral discontinuous globular patterns and late and mild washout, as compared to other malignant lesions of the liver categorized as LR-M, that reveal a rim APHE and early, marked washout [30,31,33].

Specific HCC-related conditions and CEUS contribution

CEUS could help differentiate AFP-negative hepatocellular carcinoma from other liver malignancies. A total of 99 patients with liver disease and AFP-negative primary liver malignant tumors were included in a study that took place between January 2018 and February 2021. While the inclusion criteria were the following: serum AFP negative for all patients, the CEUS LI-RADS criteria applied to all patients and CEUS performed within 2 weeks before the liver surgery or biopsy, the exclusion criteria were the following: tumor lesion below 1 cm, thrombosis of the vein, multiple tumors, other treatment modalities in advance of the CEUS examination [28]. They performed CEUS using Siemens ACUSON Sequoia and Philips IU22/EPIQ7 and SonoVue as the contrast agent [33].

According to the results of the biopsy, the 99 intrahepatic lesions were divided into 38 cases of other malignancies (3 cases of HCC combined with ICC, 31 cases of ICC, 3 cases of lymphoma, 1 case of spindle cell sarcoma and 1 case of angiosarcoma) and 61 cases of HCC [33]. The study showed that there were no patients in the LR-1, LR-2 or LR-3 categories in both the HCC group and the OM group. In the HCC group, there were 13 patients in the LR-4 category, 38 patients in the LR-5 category and 10 cases in the LR-M category, while in the OM group, there were 3 patients in the LR-5 category and 35 cases in the LR-M category [33].

The table below shows the sensitivity, specificity and both positive and negative predictive values for the diagnosis of hepatocellular carcinoma or other liver malignancies using the CEUS features [33] (Table 3).

Table 3: CEUS characteristics and their efficacy in the diagnosis of both HCC and OM (modified after [33])

HCC characteristics	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Diagnostic compliance rate
APHE (but non-rim)	95.1%	63.2%	80.6%	88.9%	82.8%
Mild washout	88.5%	65.8%	80.6%	78.1%	79.8%
Late washout	75.4%	84.2%	88.5%	68.1%	78.8%
LR-4	21.3%	97.4%	100.0%	44.2%	51.5%
LR-5	62.3%	92.1%	92.7%	60.4%	73.7%

Other primary malignancies (OM)	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Diagnostic compliance rate
Rim APHE	57.9%	96.7%	91.7%	78.7%	81.8%
Marked washout	65.8%	95.1%	89.3%	81.7%	83.8%
Early washout	84.2%	82.0%	74.4%	89.3%	82.8%
LR-M	92.1%	83.6%	77.8%	94.4%	86.9%

Although AFP is a serological marker of great importance in the early screening of HCC, there is a important number of patients who are AFP-negative and present intrahepatic nodules. Therefore, CEUS is a safe, convenient, cost-effective and radiation-free tool that can reveal microvascular vascularization of the tumor. However, it may be difficult to differentiate HCC nodules from ICC nodules in cirrhosis due to the overlap between the enhancement patterns [33].

This study shows that the LR-5 category has a specificity of 92.1% for HCC nodules that are AFP-negative and a specificity of 62.3%. The LR-M category represents an optimal way to differentiate other liver malignant lesions from HCC nodules. The LR-M category in this study revealed a sensitivity of 92.1% and a specificity of 83.6%. [33] Moreover, CEUS LI-RADS has a great application value for the diagnosis of HCC versus OM in patients at risk of HCC. CEUS LI-RADS can be used to determine the presence of focal intrahepatic lesions, even at an early stage when AFP is negative. Nevertheless, CEUS LI-RADS needs continuous improvement in order to diagnose and differentiate other liver malignancies efficiently [33].

Furthermore, CEUS proved to be helpful in the evaluation of the post-treatment response in patients who underwent transarterial chemoembolization (TACE) for HCC nodules. TACE is a therapeutic option widely used for unresectable hepatocellular carcinomas, given the fact that the majority of patients with HCC are usually diagnosed when the disease is at an advanced stage [34]. Patients who underwent TACE as treatment for HCC need to be accurately evaluated to establish the subsequent plan of therapy. CEUS can depict tumor necrosis after TACE, as early as 1-2 months, while the shrinkage of the tumor can take longer to occur [34]. This study wanted to describe the patterns of residual HCC on CEUS and compare the accuracy of CEUS with that of CT in the assessment of HCC response to TACE [34].

This study took place between February 2010 and June 2015 and included patients with HCC who had undergone TACE. They were divided into two groups: those who were assessed for the very first time after the procedure for tumor response and those who were suspected of tumor recurrence due to raised serum AFP.

The inclusion criteria in the study were the following: single mass or multinodular HCC less than 50% of the liver, entire nodule seen on standard US, nodule smaller than 15cm, while the exclusion criteria were represented by the presence of comorbidities (renal and heart failure), severe cirrhosis, HCC involving more than 50% of the liver and breastfeeding women. After applying the exclusion criteria, 50 patients with 70 liver lesions were evaluated by CEUS, but only 57 masses by CEUS, CT and MRI [34].

MRI detected residual disease in 36 masses out of 57; meanwhile, CEUS successfully identified 34 out of 36, resulting in a sensitivity of 94%. The specificity of CEUS compared to MRI was 100% regarding the 21 masses with no recurrent disease. The positive and negative predictive values of CEUS were 100% and 91% [34].

Compared to the multiphase CT, the sensitivity of CEUS was 94% (vs. 50%), while the specificity was 100% for both imaging tools. These results may be explained by the Lipiodol substance used for TACE, which may appear similar to a residual viable tumor. Therefore, the CT scan may be confusing, while CEUS, by not being affected by Lipiodol, can detect even tiny nodules as early as one week after TACE. After 4 weeks post-TACE, CEUS achieved superior sensitivity rates than CT and MRI (100% vs. 50%) [34].

CEUS could be considered an effective alternative technique for identifying early tumor response of HCC after TACE (using Lipiodol substance). However, it is recommended that, in the long term, CEUS be used together with MRI/CT for a better evaluation of the tumor response [34].

CONCLUSION

CEUS is a real-time imaging method used in many areas of interest, including the study of liver masses. It is shown in many studies that the sensitivity and the specificity of CEUS compared to CT/MRI are similar, or even superior, in the diagnosis and surveillance of hepatocellular carcinoma and other malignant liver nodules. The contrast agents used by CEUS are eliminated throughout the lungs,

showing no evidence of hepatotoxic, nephrotoxic, or cardiotoxic events. In order to systematize the assessment of the HCC nodules, the CEUS LI-RADS classification was introduced.

Ultimately, although CEUS has its limitations, there are great perspectives in the appraisal of liver nodules using this imaging technique. The accessibility and safety of the procedure and the absence of the use of ionizing radiation reinforce the idea of using CEUS in the near future as a stand-alone technique in the diagnosis and evaluation of the response to TACE for the hepatocellular carcinoma nodules.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. This research work received no external funding. No AI tools were used to draft or edit the manuscript.

Authors' contribution

All authors have read and agreed to the published version of the manuscript.

Patient consent for publication

Not applicable.

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REVIEW

The Transformative Role of Artificial Intelligence in the Future of Radiology

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Abstract: Recent advances in technology and artificial intelligence have transformed radiology. Artificial intelligence, which uses machine learning, deep learning algorithms, and cellular neural networks, is now applicable to image analysis, enabling the analysis of large volumes of data and the early identification of lesion features, such as tumors. Integrating artificial intelligence into clinical workflows increases the efficiency of medical report generation and enables efficient prioritization of complex or urgent cases. Predictive analytics based on artificial intelligence uses extensive patient data, including demographics, medical history, tests, and imaging, to build more accurate predictive models. Among the areas where artificial intelligence is successfully implemented in radiology is the automated detection of lung nodules. In the field of neurology, artificial intelligence helps track progressive lesions through volumetric analysis of the brain and surveillance of demyelinating diseases. In oncologic radiology, artificial intelligence is used for automated image segmentation, lesion review, and standardized report generation. Therefore, in this ever-evolving landscape, radiologists must embrace emerging technological advances in order to occupy a unique position at the intersection of different diagnostic fields.

Keywords: artificial intelligence; radiology; magnetic resonance imaging; computed tomography

INTRODUCTION

The Current State of Radiology and Its Importance in Modern Medicine

Radiology has been closely linked to the degree of technological evolution and has experienced revolutionary developments in recent years. This review examines the implementation of Artificial Intelligence (AI) in radiology, examining its practical applications, associated challenges, and potential paths for progress.

Radiology is a medical field that, through several imaging technologies, including Magnetic Resonance Imaging (MRI), Computed Tomography (CT), Positron Emission Tomography (PET), ultrasound, and conventional radiology, contributes to the formation of medical diagnoses [1].

Challenges facing radiology today include a high workload that is not adapted to the existing workforce, corporatization, streamlining of workflow, and burnout. All of these contribute to the "perfect storm," creating pressure on radiologists and the institutions in which they work [2].

In this context, AI can contribute to the evolution of radiology, from improving image acquisition to image interpretation with the help of advanced image analysis [1].

Understanding Terminology: AI, ML, and DL

Artificial intelligence has a contribution in radiology through machine learning (ML) and deep learning (DL) algorithms that play a role in image analysis.

Artificial intelligence is the replication of human intelligence in machines programmed to simulate human thought and action, with the aim of creating intelligent systems that operate autonomously and have the ability to solve problems that would normally require human intelligence.

Machine learning algorithms have emerged in recent years and are based on the introduction of decision trees, support vector machines, and neural networks, with these algorithms forming the basis of image analysis.

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Deep learning is a subset of ML that uses multilayer artificial neural networks, which allow DL algorithms to understand complex data, which is successful in visual recognition. The layers are learned from the data, with architecture inspired by the human brain [1, 3].

HOW ARTIFICIAL INTELLIGENCE IS CHANGING THE FUTURE OF RADIOLOGY

AI is crucial to radiology's future in many aspects:

Intelligent image analysis algorithms

Through DL, AI can analyze large volumes of unstructured data, such as images, and learn patterns to automatically recognize features from the raw data. Through these technologies, and particularly through Convolutional Neural Networks (CNNs), AI has revolutionized the early identification of lesion patterns in CT scans [3].

Increased workflow speed

Integrating AI into clinical workflows gives radiologists quicker access to advanced imaging, speeding up results and helping prioritize complex or urgent cases [1]. There are areas where the use of artificial intelligence tools has reduced the workflow of radiologists by about 34%, such as in the case of breast cancer screening [4]. One of the main problems that arises when implementing AI in radiology is the lack of standardization between platforms and the fact that there are multiple AI tools with different user interfaces, which ultimately leads to an inefficient workflow, as radiologists must access different systems to reach the information provided by AI. The existence of a centralized AI platform, which integrates multiple diagnoses, with a single interface, can facilitate the workflow [5].

Predictions based on image analysis

The ability to make an accurate prediction of a patient's prognosis is essential for timely interventions. The emergence of predictive analytics through artificial intelligence allows the analysis of a large volume of patient data, including demographic data, medical history, and previous tests, which, together with imaging, can be used to establish predictive models with greater accuracy [6].

The role of AI in education

The current approach to education emphasizes a series of theoretical notions and a practical case-based experience. However, the method has its limitations, exposing learners to a limited number of cases compared to real-world work situations, as well as limited access to expert radiologists in certain fields. This is an area where AI can have a transformative impact through its ability to analyze large volumes of data and provide real-time feedback to complement the knowledge acquired through traditional education. Also, the existence of work platforms with simulations of real cases, connected to large databases, can increase the quality of training for students and residents [7].

Another transformative element is the inclusion of interdisciplinary education; radiologists must go beyond the traditional focus and have experience in fields such as genomics, pathology, laboratory medicine, oncology, molecular medicine, and immunology. Thus, the radiologist can understand the complex data that modern AI systems introduce into analysis to generate multimodal results. Radiologists occupy a unique position at the intersection of diverse diagnostic fields and play a central role in the diagnosis and care of patients, which is why their expertise in placing imaging data in an integrative clinical context is essential. Interdisciplinary collaboration is the way to ensure that radiologists are active users of artificial intelligence. Another area that can be improved is communication skills, an area in which the existence of structured reports also helps interdisciplinary communication and the transmission of clear conclusions [8].

Radiology training programs should prepare radiologists to understand artificial intelligence and to be able to make decisions under uncertainty without being influenced by cognitive biases, a situation that training in risk assessment and probabilistic reasoning helps to improve. This shift involves more than simply adapting the curriculum; it involves an integrative mindset focused on continuing education and an education that emphasizes interdisciplinary collaboration. These are the values that can help radiologists adapt to the complexity of the modern data they face [8].

AREAS WHERE AI IS SUCCESSFULLY INTEGRATED IN RADIOLOGY

Oncology

Correct interpretation of images, considering tumor heterogeneity and imaging characteristics, is a key factor in cancer diagnosis. By integrating AI into the oncology imaging workflow, multidimensional information about tumors can be extracted, which together can aid in tumor staging and stratification, molecular diagnosis, metastasis prediction and treatment response [9]. The main directions of focus for the inclusion of AI in oncology imaging include automatic image segmentation, lesion review, and the generation of standardized responses based on automated analysis. A significant aspect in oncology is the development of radiomics, but also the contribution of AI in radiotherapy.

Among the areas where artificial intelligence is successfully integrated into radiology is oncology, where techniques for automatic image segmentation, lesion review, generation of standardized responses based on automated analyses, and the development of radiomics have been successfully implemented.

Automated Image Segmentation

DL significantly contributes to image segmentation and classification and therefore increases the speed and accuracy of diagnosis. CNNs contribute significantly to the ability of artificial AI to learn complex patterns, which are found in fields with visual components, such as radiology. This is possible due to the complex, layered architecture of these networks, which have demonstrated increased ability in object detection and segmentation. CNNs have shown a key role in segmenting brain tumors in MRI examinations. Other applications where CNN segmentation has been successfully applied in radiology are the individualization of lung nodules, also distinguishing between benign and malignant tumors in mammography [1, 10, 11].

Review of lesions

Radiology exploits the most useful AI algorithms to automatically identify, locate, and recognize abnormalities found on medical images, whether they are tumors, fractures, or other types of injuries. Among these, DL and reinforcement learning help AI systems increase diagnostic accuracy, helping to halve the radiologist's workload and medical decision-making time. DL models can identify small lesions more easily through resolution enhancement techniques such as interpolation and super-resolution. To increase the performance of AI analysis algorithms in medical object detection, image pre-processing procedures are applied. Among the image preprocessing techniques used in artificial intelligence for object detection in radiology, there are some important ones, such as spatial resampling, intensity normalization, image registration, data augmentation, noise reduction, and segmentation. Data augmentation techniques include rotating, scaling, and flipping images to create larger data sets for use in AI models. Radiological applications of AI object detection apply to both tumors and other types of lesions in a wide range of examinations, including X-ray and CT scans, but one of the most innovative applications is CNN-based segmentation in MRI imaging [12].

Development of standardized reports based on automated analysis

The existence of structured reports in radiology improves workflow efficiency by faster generation of medical reports, especially when there are variable templates for various clinical aspects. The European Society of Radiology (ESR) emphasizes the need to use structured medical reports to streamline workflow and reduce the potential for errors. The use of structured reports that include a standardized list of issues reduces the risk of error by guiding the radiologist through a system of reviewing key points. At the same time, a structured approach ensures better communication between specialties and ultimately contributes to better patient care. With multiple AI tools, there is a risk that radiologists will be overwhelmed, but AI algorithms for rapid image analysis and standardized reporting can create consistency and make these disparate AI tools work together more effectively [5].

Radiomics

Among AI techniques, ML and radiomics involve extracting predefined features from radiological images through a data characterization algorithm. These features encompass various aspects of tumors, such as intensity-based measurements, shape characteristics, volume, heterogeneity, vascularity, and effect on surrounding structures. The entire process aims to increase diagnostic and predictive capabilities [1, 9].

Radiotherapy

Technological progress in diagnostic imaging has improved the efficiency of radiotherapy by introducing artificial intelligence software that optimizes the segmentation of tumors and organs at risk, prognostic assessment, treatment planning and optimization, thus saving considerable time for the radiation oncologist [13].

Lung nodule detection and classification

An advantage and a significant improvement of the workflow in radiology services appeared with the programs for automatic analysis and segmentation of pulmonary nodules [14]. These programs have become routine and are permanently attached to PACS systems, identifying, measuring dimensions, analyzing the structure, density and location of pulmonary nodules with dimensions between 3-30 mm [14].

There are also limitations in the automated analysis of pulmonary nodules, among which the most significant are the false identification of vascular trajectories as nodular hyperdensities, and other limits in identifying lesions in areas with ventilation disorders, with motion artifacts or with fibronodular sequelae. However, automated analysis programs are continuously improving and shortening the times for preparing medical reports, increasing work efficiency, and becoming working tools for the redistribution of highly qualified human resources in other directions where they are still irreplaceable [15].

Another useful contribution is the automated comparative analysis of the evolution of pulmonary nodule sizes, in accordance with the intervals specified by specialist guidelines, such as that of the Fleischner Society, an aspect that contributes to establishing the most appropriate therapeutic and follow-up conduct [16]. Automated analysis generates medical reports that enter patients' electronic records in parallel with the generation of a set of images with automatically marked lesions [15]. A concrete example in practice is that provided by CAD-type analyses in the identification of undetected tuberculous lesions.

Trauma with applications in military medicine

In imaging departments serving emergency rooms in civilian or military field hospitals, cerebral hemorrhagic lesions, or hemorrhages in any part of the body, represent the most common cause of post-traumatic death. Rapid recognition of hemorrhages and the most appropriate therapeutic interventions are of paramount importance. The role of AI systems using ML is to optimize diagnosis in these situations through algorithms that recognize hemorrhagic lesions and assess their risk potential through CT and FAST ultrasound methods [17]. These programs use semiological notions of lesion recognition, such as Language Models (LM) for automatic 3D labelling to recognize hemorrhage subtypes based on brain segmentation, midline deviation, mass effect, cerebral edema, and hyperdensity values. Diagnostic models in neurotraumatology utilize various emergency diagnoses, including hemorrhage, ischemia, cerebral infarction, space-occupying lesions, mass effect, hydrocephalus, and midline deviation. Neurotraumatology lesion recognition models based on imaging data obtained through radiography, computed tomography, and MRI can also be used for the rest of the body, through 3D anatomical segmentation, 2D CNNs software, and Recurrent Neural Network (RNN) [18].

Neurology

By using the Picture Archiving and Communication System (PACS) system, lesions with complex neurological substrates can be effectively detected and characterized through automatic algorithms that have entered routine analysis in some types of cerebral pathologies.

Automatic Cerebral Volumetry consists of an efficient automated analysis based on segmentation using the 3D T1 MPRAGE sequence. For reference, the images and measurement values are compared with those of a comparative database of patients from the same age groups for whom the brain volume is reported. Thus, this program provides cerebral volumetric assessment in case of suspected dementia, by evaluating the frontal, parietal and temporal lobes, and particularly the hippocampus. Brain volume assessment can also be extremely useful in multiple sclerosis and other degenerative diseases, such as Multiple Systemic Atrophy (MSA), Progressive Supranuclear Palsy (PSP), and Cortico-basal Degenerative Disease (CBD).

The program can also analyze the volume of specific brain areas such as the caudate nucleus, putamen, globus pallidus, cerebellar cortex, and midbrain [19].

The *Evaluation of Arterial Intracranial Vascular Lumen* uses a Time-of-flight (TOF) acquisition for analysis before being processed with the Maximum Intensity Projection (MIP) program. The analysis program is attached to the PACS system and generates a medical report that includes measurements and imaging of the identified lesions. In the case of intracranial aneurysms, it is estimated that 10-15% of them are underdiagnosed, and these automated analysis programs can improve the anomaly detection rate with an accuracy of almost 100% [20].

The *Characterization of a Demyelinating Lesion* is based on the analysis of 3D Fluid-Attenuated Inversion Recovery (FLAIR) sequences that highlight the location of the lesions. The analysis includes classification of the lesions using various reference systems, such as the McDonald and Fazekas criteria.

The program can generate a medical report that includes data on the location and size of the lesions, the presence of new lesions compared to previous examinations, and key illustrative images that are included in the report. Thus, this type of analysis can be extremely useful in all demyelinating diseases, including multiple sclerosis [20].

Brain tumor analysis allows for the automated assessment of tissue structures associated with tumor presence in entities with different anatomopathological substrates. Automated tumor lesion analysis is performed after intravenous administration of a contrast agent, which evaluates the behavior and uptake of the contrast. Automated lesion analysis is applied to highlight and differentiate various lesions and tissue components, which can be especially useful in the characterization of glioblastoma-type lesions, metastases and other tumor types, such as meningiomas. Also, complex data analysis allows for the possibility of integration into radiotherapy and neurosurgery programs [21].

Prostate

AI has become a commonly used tool for the diagnosis of prostate cancer, useful for both radiologists and urologists in multiparametric MRI examinations. The speed and efficiency of prostate cancer diagnosis and staging are among the greatest achievements for AI in prostate MRI evaluation [22].

Prostate cancer staging is performed using the PIRADS classification system and is integrated into the PACS system. The AI-generated medical report provides PIRADS classification, prostate volume, and detailed multiparametric analysis of prostate lesions, including nodule identification and categorization. In addition, when available, previous examinations can be evaluated comparatively. Medical reports also include appropriate graphical representations and dimensional assessments, as well as mapping of prostate lesions [22, 23].

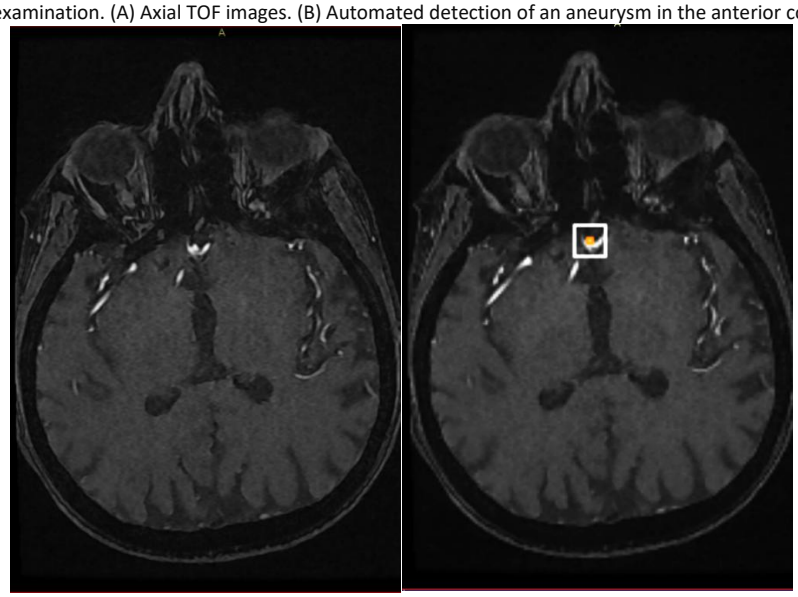
When the patient's PSA analysis is also available, this aspect can be represented in tables and associated with the presentation of prostatic density in the workflow report. This medical report can also be effectively used for image-guided biopsy, depending on the representation and arrangement of the most representative lesions [22, 23].

CLINICAL IMPLEMENTATION OF ARTIFICIAL INTELLIGENCE, CASE STUDIES AND APPLICATIONS

Case 1. Detection and analysis of intracerebral aneurysms using artificial intelligence

By analyzing brain MRI, TOF sequences, AI software can analyze the intracerebral vascular lumen and thus identify the presence of intracerebral arterial aneurysms and estimate their location and size (Figure 1).

Figure 1: Brain MRI examination. (A) Axial TOF images. (B) Automated detection of an aneurysm in the anterior communicating artery.

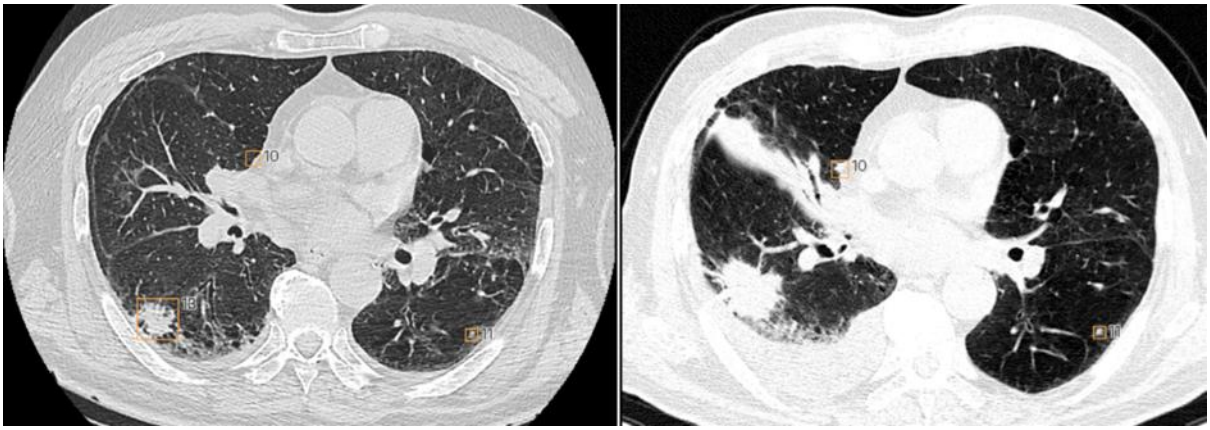


(A) **(B)**

Case 2. Automatic identification and dimensional monitoring of pulmonary nodular lesions

With the help of automated AI programs, in the case of thoracic CT examinations, the lung parenchyma can be analyzed for the presence of lesions, thus nodular lesions are identified, counted and measured automatically, and if there are previous examinations, the dimensional progression of the lesions can also be automatically assessed. Thus, with the help of AI tools, such as Rayscape, clinical impact improvement and shortening of diagnostic times are observed [24] (Figure 2).

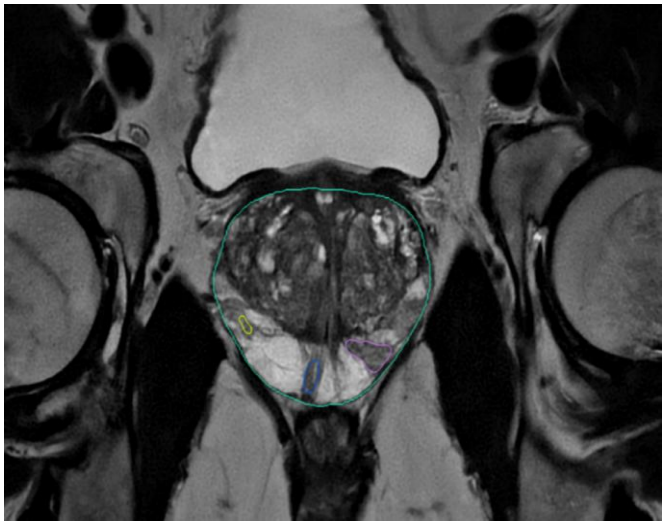
Figure 2. CT examination of axial sections, lung parenchyma window. (A) Automatic identification of pulmonary nodular lesions and dimensional assessment. (B) Automatic comparative analysis of pulmonary lesions with dimensional progression assessment.



Case 3. Automated prostate volume analysis and lesion characterization

Automated prostate analysis on MRI examinations is an area where AI has demonstrated progress and efficiency, so that prostate volume is assessed, lesions are identified, measured and classified according to PI-RADS classification, and a mapping of the most significant lesions is made in the report (Figure 3).

Figure 3. MRI examination, coronal T2 images of the prostate with automated prostate volume analysis and characterization of nodular lesions.



METHODS AND INDICATORS FOR EVALUATING THE EFFECTIVENESS OF AI PROGRAMS

The most useful evaluation method is the one that uses the Area Under the Curve (AUC) indicator in binary detection. The method is derived from the Receiver Operating Characteristic (ROC) curve, which uses sensitivity and specificity as parameters to state whether a lesion is present or not [17]. In the ideal mode, the optimal value of AUC is 1. Between 1 and 0.5 AUC has the appearance of a random model and below 0.5, it cannot be brought into discussion. At the current time, the interpretation in an efficient model has AUC values close to 0.9. The evaluation of the AUC indicator has, in turn, references and standards related to diagnosis established at the level of junior and senior radiologists with expertise. This level of over 0.9 is more easily achieved in lesions defined by few parameters and with clear values, such as hemorrhages, fractures, pneumothorax, lesions from mammographic examinations, while multiparametric defined lesions, such as prostatic or hepatic ones, require a senior radiologist-type standard of expertise. In the radiological evaluation of polytrauma in emergency services, high AUC values, above 0.9, and short processing time are required [25].

CONSIDERATIONS AND CHALLENGES

• Legal and ethical implications

The legal and social implications of artificial intelligence technologies in radiology need to be seriously assessed. One concern is the lack of transparency behind the decision-making process through artificial intelligence. Another key aspect in the implementation of artificial intelligence is the lack of a legislative system that regulates the level of involvement of artificial intelligence. Medical liability in case of malpractice is attributed to the radiologist. AI tools must meet rigorous validation and approval standards to establish their safety. Once approved, they must be continuously monitored to ensure that the results they provide are dependable. The implementation of AI systems requires adherence to established standards, appropriate regulatory approval, and awareness among radiologists and medical practitioners regarding their limitations, ensuring these factors are carefully considered [1, 26].

• Bias and generalization

Despite the development of artificial intelligence, when implemented in real life, its performance varies for certain subgroups. The question arises whether the goal should be fairness to the individual or fairness to the group. It has been observed that artificial intelligence takes shortcuts in the learning process, which can lead to biased results towards certain subgroups, for example, when multiple factors, including age, gender, and race, are taken into account in the diagnosis [27].

• Familiarization, incorporating AI into existing workflow systems

The integration of artificial intelligence into clinical radiology faces several challenges. One of these is the existence of robust hardware capable of managing the substantial amounts of data generated by medical imaging systems, which implies investment in such infrastructure. On the other hand, this new hardware and software must be able to integrate with existing radiology systems. There is also a need for training of radiologists and for them to collaborate with artificial intelligence developers [1].

A LOOK INTO THE FUTURE OF RADIOLOGY

The future transformation of radiology includes the integration of Augmented/Virtual Reality (AR/VR) and AI. AR/VR technologies are expected to play a significant role in radiological practice and radiological education by improving the visualization of radiological images, thereby contributing to diagnosis and treatment planning. Also, through machine learning, AI improves its image analysis

capabilities, thereby contributing to reducing diagnostic errors. A tool based on AI is Computer-Aided-Diagnosis (CAD), which can contribute to the development of an integrative diagnosis by incorporating radiological, anatomopathological and genomic information into the AI-assisted workflow [1].

CONCLUSION

Artificial intelligence is developing rapidly, and understanding its use in medical practice is mandatory. Radiology is transforming, and specialists must keep up with it, adopting these innovative technologies and being integrated into multidisciplinary clinical teams, with collaboration between healthcare providers being paramount to fully capitalize on the benefits of this scientific knowledge.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. This research work received no external funding. No AI tools were used to draft or edit the manuscript.

Authors' contribution

Conceptualization, D.C., and A.S.C.; methodology, D.C., and A.S.C.; software, D.C., and A.S.C..; validation, D.C., and A.S.C.; formal analysis, D.C., and A.S.C.; investigation, D.C., and A.S.C.; resources, D.C., and A.S.C.; data curation, D.C; writing—original draft preparation, D.C., and A.S.C.; writing—review and editing, D.C., and A.S.C.; visualization, D.C.; supervision, D.C. All authors have read and agreed to the published version of the manuscript.

Patient consent for publication

Not applicable.

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ARTICLE

Salivary Investigation of the Complex Relationship between NLRP3 Inflammasome, Leptin and Total Antioxidant Capacity in the Context of Periodontal Disease

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Abstract: Periodontal disease is a chronic pathology, resulting from the action of microbial communities that become dysbiotic, accompanied by immune response impairment in periodontal tissues, leading to an inflammatory process that promotes progressive tissue destruction. Abnormal activation of nucleotide-binding oligomerization domain (NOD-), leucine-rich repeat (LRR-), and pyrin domain (PYD)-containing protein 3 (NLRP3) promotes chronic inflammation. Research shows that leptin and oxidative stress (OS) are involved in the activation of the inflammatory pathways, including the activation of the NLRP3 inflammasome. In this context, the main objective of our study was to determine the levels of the NLRP3, leptin, and total antioxidant capacity (TAC) in the saliva of patients with periodontal disease. The present cross-sectional study included 44 patients with periodontitis, while the control group was represented by 19 clinically and biologically healthy adults. Saliva samples were collected from all participants in the study, and salivary levels of NLRP3, leptin, and TAC were determined using the Enzyme-linked immunosorbent assay (ELISA) and commercial kits. The results show that NLRP3 had statistically significantly increased levels in the study group versus the control group ($p< 0.00001$). Although TAC was also increased in the patients compared to the healthy subjects ($p=0.491$), no statistically significant differences were found. On the other hand, leptin levels were significantly lower in periodontitis patients compared to the control group ($p< 0.00001$). The results obtained in the present study are promising, but further studies are needed to obtain a more comprehensive understanding of the complex molecular mechanisms underlying periodontal disease.

Keywords: periodontal disease; NLRP3 inflammasome; leptin; TAC; saliva

INTRODUCTION

Around 700 species of oral bacteria form the oral microbiome [1], a term used by molecular biologist and Nobel Prize laureate Joshua Lederberg [2, 3] to define the mixture of symbiotic, commensal, and pathogenic microorganisms present in our organism [4]. Periodontal disease is a chronic inflammatory disease [5] with origins in the dental plaque [6], being a result of microbial communities that become dysbiotic, corroborated with immune response impairment in periodontal tissues, leading to an inflammatory process that promotes progressive tissue destruction [7, 8].

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In the beginning, the inflammation induced by anaerobic bacteria [6] leads to gingival bleeding, pain, and swelling, which are clinical traits of gingivitis, which affects almost 90% of the population and is considered to be the mildest form of periodontal disease [9, 10]. The progression of the disease, in the lack of appropriate treatment, leads to irreversible attachment loss of the periodontium and alveolar bone destruction, thus causing periodontitis [9], which, if left untreated, results in tooth loss [11, 12].

Periodontal disease affects not only the quality of life of the patients because of the associated symptoms and dysfunctions, but also has a considerable effect on society, even from an economic point of view [13], becoming a global health burden [14]. Moreover, by favoring the transfer of periodontal pathogens and pro-inflammatory agents to other tissues, periodontal disease has been linked to systemic diseases [15], including diabetes and heart disease [9], respiratory infections [16], Alzheimer's disease [17], neurodegenerative diseases[18], autoimmune diseases, and even malignancy [19].

Recent research has shown that the development of periodontal disease is not promoted only by bacterial, but also by viruses, environmental factors (e.g., smoking) and lifestyle of the host, and it involves genetic mechanisms and immune response [5, 7], new studies focusing on the development of alternative therapies such as vaccines or antioxidant agents to improve the prevention of the disease [20].

The activation of inflammasomes represents one of the core pathways linked to inflammatory diseases [21]. The nucleotide-binding oligomerization domain (NOD-), leucine-rich repeat (LRR-) and pyrin domain (PYD)-containing protein 3 (NLRP3) inflammasome [22], found in the cytosol, is a supramolecular complex that comprises receptor protein NLRP3, adapter protein ASC (apoptosis-associated speck-like protein containing a caspase recruitment domain) and caspase-1 [23]. These three components of the inflammasome have different functions. While receptor protein NLRP3 can detect endogenous danger signals and enlist downstream targets [23, 24], ASC links NLRP3 to caspase-1, which is involved in cytokine release and pyroptosis (inflammatory cell death) [22, 24, 25] via gasdermin D [24]. NLRP3 can be activated by cellular alterations [23], microbial infections, or environmental stimuli [26]. The activation of NLRP3 leads to protease caspase-1 activation, which in turn promotes the production of cytokines IL-1β and IL-18, thus upholding inflammation [26]. Furthermore, abnormal activation of NLRP3 promotes chronic inflammation, hence being involved in the development of numerous diseases [25], such as cardiovascular diseases [27], metabolic diseases [25], autoimmune diseases [28], cancer [25], periodontal disease [29], and, as it was recently discovered, COVID-19 [30].

So, the NLRP3 inflammasome is responsible for the transformation of IL-1β, one of the most important pro-inflammatory cytokines linked to periodontal disease, into its biologically active form [31]. This mediator of inflammation can induce osteoclast differentiation, thus playing a crucial role in alveolar bone resorption, specific to periodontitis. Moreover, NLRP3 activation mediates osteoblasts' apoptosis via IL-1β and IL-18, affects the fibroblasts in the periodontal ligament via IL-1β and IL-6, and is involved in leukocytes' regulation in inflamed periodontal tissue [32].

Research shows that leptin and reactive oxygen species (ROS) can activate inflammatory pathways, including the NLRP3 inflammasome [33]. Leptin, a peptide hormone produced primarily by adipocytes [34], is involved in the regulation of food intake, additionally having an important role in proinflammatory immune responses [35]. It is also produced by the salivary glands, which are responsible for leptin storage and secretion as well [36]. In periodontal disease, leptin has been shown to be able to regulate the ratio between osteoprotegerin and receptor activator of nuclear factor kappa beta (RANKL) in gingival fibroblasts [37], hence influencing bone remodeling [38]. In a recent study conducted on mice, Han et al revealed that leptin promotes macrophage polarization in periodontitis via NLRP3 inflammasome [39]. Furthermore, leptin was found to promote oxidative stress (OS) [40].

OS has already been proven to be one of the key players in periodontal disease development and progression, contributing to tissue destruction and tooth loss [41]. OS is characterized by the loss of balance between oxidants and antioxidants, promoting the formation of ROS and free radicals, which can play an important role in inflammation, despite their reduced lifespan [42]. In periodontal disease, the activation of the innate immune system produces a storm of proinflammatory cytokines and overproduction of ROS [41]. On the other hand, in chronic inflammation, such as the one present in periodontitis, overproduction of ROS, mainly by neutrophils [43], can not only induce OS, and activate proteolytic enzymes but can also activate different signaling pathways [44].

In order to investigate the complex mechanisms involved in the pathogenesis of this condition, the main objective of our study was to determine the levels of the NLRP3, leptin, and TAC (total antioxidant capacity) in the saliva of periodontitis patients.

MATERIALS AND METHODS

Study groups

The present cross-sectional study included 44 patients with periodontitis, diagnosed according to the 2018 AAP/EFP Classification of Periodontal & Peri-implant Diseases [45, 46].

Inclusion criteria for the study group:

• Presence of at least 14 teeth on both dental arches combined;

• Interdental clinical attachment loss (CAL) is detectable in ≥2 non-adjacent teeth or buccal/ oral CAL ≥3 mm with periodontal pocket depth >3 mm is detectable in ≥2 teeth;

• CAL cannot be attributed to non-periodontal causes such as:

1) gingival recession of traumatic origin;

2) dental caries extending into the cervical area of the tooth;

3) presence of CAL on the distal aspect of a second molar and associated with malposition or extraction of a third molar;

4) a combined endo-periodontal lesion;

5) occurrence of a vertical root fracture [46].

Exclusion criteria for the study group:

• chronic alcohol consumption;

• administration of contraceptive medication;

• pregnancy or breastfeeding;

• periodontal treatment less than 6 months before sampling.

Among the 46 patients initially recruited from the Periodontology Department of the Faculty of Dentistry, “Carol Davila" University of Medicine and Pharmacy, and two private practices, 2 were excluded because they had active autoimmune diseases, which could have altered the results, given the fact that inflammation is also at the center of these pathologies.

The control group was represented by 19 clinically and biologically healthy adults with no periodontal disease, other oral disorders, and any known systemic pathology.

All the participants in this study were informed about the aim of the study and their voluntary participation. It was also explained to them that by agreeing to take part in the research, they would have no additional risks to their periodontal treatment, nor any costs or benefits of any kind. Moreover, the subjects included in the study were assured of their anonymity in the processing and publication of the data obtained. Furthermore, they were informed of the right to freely withdraw from this study at any time without any type of repercussions or penalties. After they completely understood the nature and purpose of this research, they signed the informed consent. This study was approved by the Ethics Committee of the Carol Davila University of Medicine and Pharmacy (22976/17.08.2022).

Sample collection and biomarker determination

All subjects included in our study received written information regarding the collection procedure before sampling. Saliva was collected during their next appointment, in the morning, between 8 and 10. They were recommended to avoid food and drink intake, as well as any oral hygiene method, for 12 hours before the collection of the sample. If this was not possible, they had to refrain from eating and drinking for at least 60 minutes before sampling.

Additionally, smoking and chewing gum were also restricted in the last hour before the sample collection, while physical effort was forbidden 30 minutes before the collection. Another requirement was to avoid using lipstick or similar products that morning. In order to remove food scraps and exfoliated cells, each participant in the study rinsed their mouth with distilled water before the collection of the samples.

During the procedure, they were advised to relax, not swallow, and not communicate with others. Three mL of unstimulated total saliva was collected from each participant in the study. Once retrieved in sterile tubes, the saliva was transported to the laboratory on ice in a special transport box for biological samples. Samples were then centrifuged at 3000 rpm for 15 minutes and aliquoted. Until further analysis, they were kept in the freezer, at -80°C.

The three biomarkers investigated in this study were determined using the Enzyme-linked immunosorbent assay (ELISA) and commercial kits (DRG Instruments GmbH, Germany – TAC and leptin; Abbexa LTD, Cambridge, UK for NLRP3). The determination of all parameters was performed according to the instructions of the manufacturer.

Statistical analysis

All the data from the study were analyzed using IBM SPSS Statistics 26 (IBM, USA). Data were expressed as means ± std. Deviation, as well as medians, depend on the distribution's normality. The Shapiro-Wilk test was used to evaluate data distribution. The non-parametric Mann-Whitney U test was then applied to compare quantitative independent variables between the groups. Correlations were assessed using Spearman's rho correlation coefficient. A p-value lower than 0.05 was considered statistically significant.

RESULTS

The patient group included 44 subjects, among whom 21 were males (47%). The mean age was 51.8 ± 9.9 years, with a median of 51.5 years. The average number of remaining teeth in the patient group was 21.8 ± 4.6, with a median of 23. Eleven patients (25%) suffered

from hypertension, diabetes mellitus, hyperthyroidism, or osteoporosis. The control group was represented by 19 healthy subjects, among whom 5 were males (26%). Their mean age was 28.5 ± 13.8 years, with a median of 19 years.

The results obtained show that when all patients with periodontal disease were compared with the control group, NLRP3 had statistically significantly increased levels in the study group (Table 1, Figure 1). Although TAC was also increased in the patients compared to the healthy subjects, no statistically significant differences were found (Figure 2). At the same time, leptin was significantly decreased in patients with periodontal disease compared to the control group (Figure 3).

Table 1: NLRP3, TAC and leptin salivary levels in periodontal patients and the control group

Biomarker	Group	Median	Mean ± Std. Deviation	p-value*
NLRP3	Patient	1.1372 ng/mL	1.117 ± 0.332 ng/mL	**< 0.00001**
	Control	0.7382 ng/mL	0.776 ± 0.208 ng/mL	
TAC	Patient	76.741 µmol/L	114.127 ± 119.398 µmol/L	0.491
	Control	70.838 µmol/L	94.553 ± 101.928 µmol/L	
Leptin	Patient	5.894 ng/mL	6.124 ± 2.064 ng/mL	**< 0.00001**
	Control	8.827 ng/mL	8.618 ± 1.478 ng/mL	

**Mann-Whitney U Test*

Figure 1: Salivary levels of NLRP3 in the patient group *versus* the control group

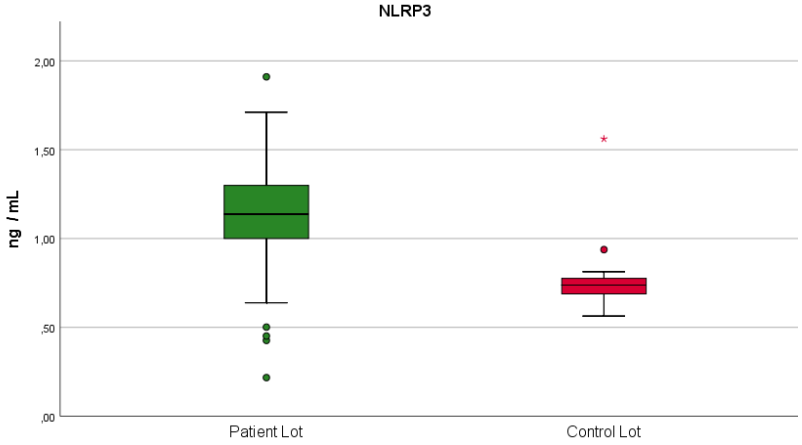


Figure 2: Salivary levels of TAC in the patient group *versus* the control group

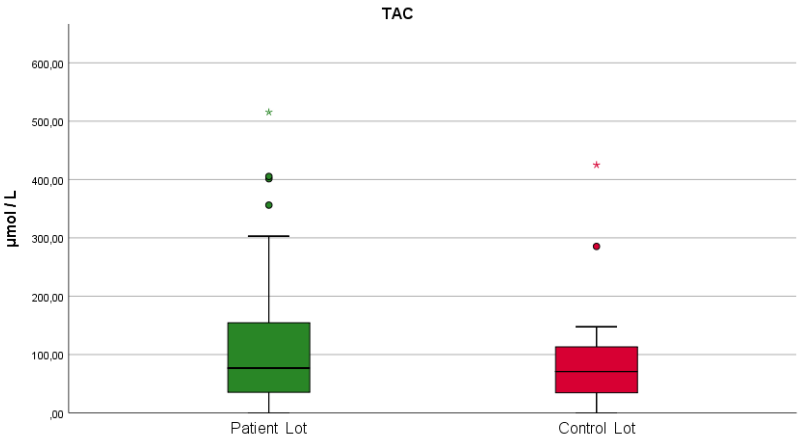
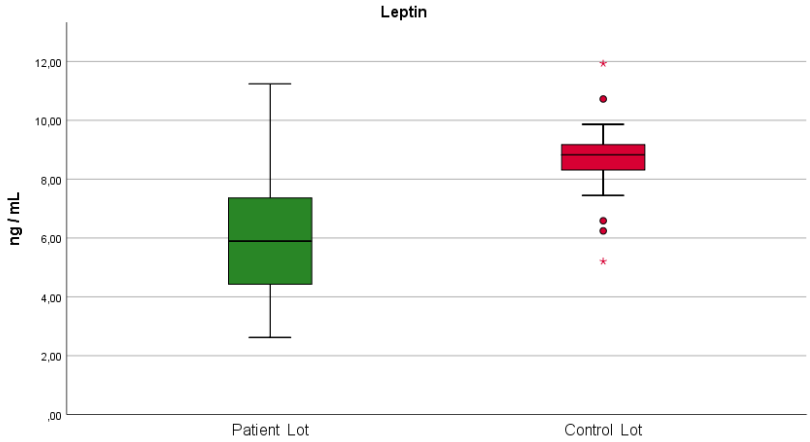


Figure 3: Salivary levels of leptin in the patient group *versus* the control group



None of the three determined parameters displayed significant differences when comparing patients with periodontitis with no associated systemic disease *versus* patients with periodontitis and associated systemic conditions (Table 2, Figures 4, 5, 6).

Spearman's rank correlation coefficient was used to test intragroup possible associations between salivary parameters. No significant correlation was found between salivary levels of leptin and NLRP3 when including all patients or when analyzing just patients with periodontal disease without associated systemic conditions. Also, no significant correlation was found between salivary levels of NLRP3 and TAC or TAC and leptin when including all patients or when analyzing just patients with periodontal disease without associated systemic diseases. No statistically significant correlations were found between the concentrations of the analyzed biomarkers and the number of remaining teeth when taking into consideration all periodontal disease patients, nor when including only the patients without associated systemic diseases (Table 3).

Table 2: Comparison of the biomarkers' salivary levels in periodontitis with no associated systemic disease *versus* patients with periodontitis and associated systemic conditions

Biomarker	Group	Mean ± Std. Deviation	p-value*
NLRP3	No Associated Diseases	1.13 ± 0.369 ng/mL	0.356
	Associated Diseases	1.077 ± 0.189 ng/mL	
TAC	No Associated Diseases	112.041 ± 112.434 µmol/L	0.828
	Associated Diseases	120.387 ± 115.215 µmol/L	
Leptin	No Associated Diseases	6.346 ± 2.011 ng/mL	0.115
	Associated Diseases	5.457 ± 2.174 ng/mL	

**Mann-Whitney U Test*

Figure 4: Salivary levels of NLRP3 in patients with periodontitis with no associated systemic disease *versus* patients with periodontitis and associated systemic conditions

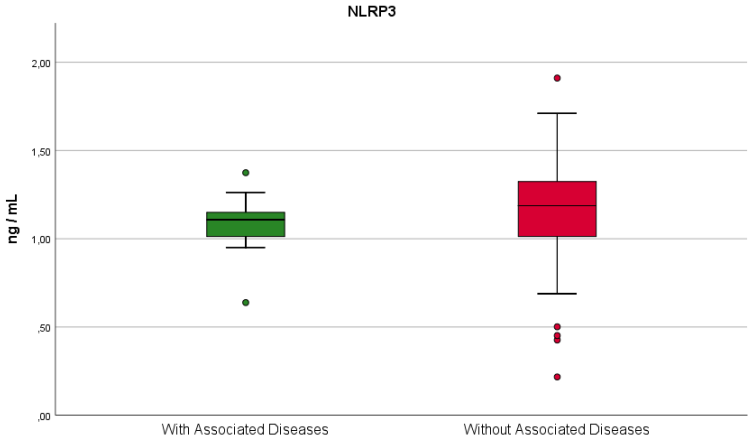


Figure 5: Salivary levels of TAC in patients with periodontitis with no associated systemic disease *versus* patients with periodontitis and associated systemic conditions

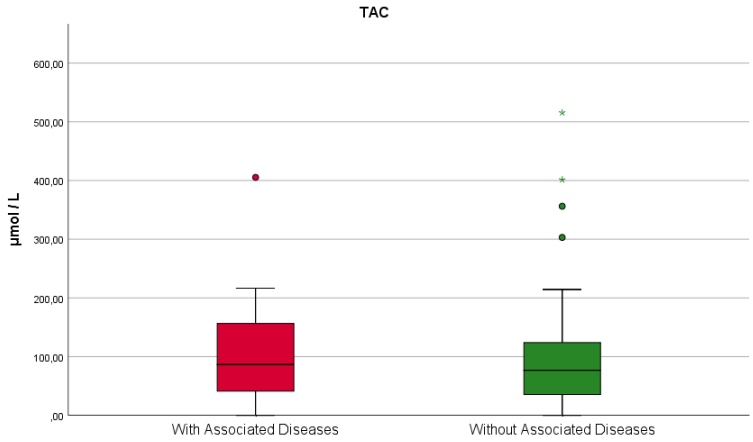


Figure 6: Salivary levels of leptin in patients with periodontitis with no associated systemic disease *versus* patients with periodontitis and associated systemic conditions

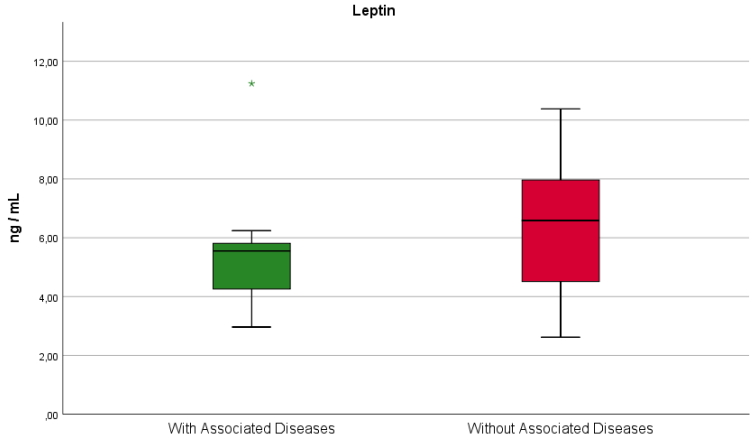


Table 3: Correlations between the determined biomarkers

Correlation	Spearman Rho	p – value
Between the number of remaining teeth and the NLRP3 levels for all patients	0.175	0.255
Between the number of remaining teeth and Leptin levels for all patients	0.095	0.541
Between the number of remaining teeth and the TAC levels for all patients	-0.158	0.305
Between the number of remaining teeth and the NLRP3 levels for patients without associated disease	0.223	0.213
Between the number of remaining teeth and leptin levels without associated disease	-0.063	0.729
Between the number of remaining teeth and leptin levels for patients with associated diseases	0.378	0.252
Between the number of remaining teeth and the TAC levels for patients without associated disease	-0.088	0.626
Between leptin and NLRP3 in all patients	0.147	0.340
Between leptin and NLRP3 in patients with no associated diseases	0.234	0.189
Between TAC and NLRP3 in all patients	-0.171	0.267
Between TAC and NLRP3 in patients with no associated diseases	-0.131	0.467
Between TAC and leptin in all patients	-0.168	0.276
Between TAC and leptin in patients with no associated diseases	-0.059	0.746

DISCUSSION

Saliva has already proven to be a reliable diagnostic, prognostic, and monitoring fluid for both oral and systemic diseases, being very rich in molecules and macromolecules that can be used as biomarkers [48]. Furthermore, the collection procedure is easy, it is more efficient in terms of costs, has a lower risk of cross-infection compared to the blood, and most importantly, the sampling is non-invasive [48, 49]. However, when determining the salivary levels of these biomarkers, we have to keep in mind that their concentrations are frequently decreased compared to the blood. Additionally, there are several factors that can influence their levels, including the moment of the day or the level of stress [49].

In recent years, inflammasomes have represented a major research interest due to their potential to determine an inflammatory response by integrating signals from different pathogens and altered cells [50, 51]. The most studied of the inflammasomes in systemic diseases, NLRP3, has also gained attention regarding its connection with periodontal disease [52]. The dysbiosis and inflammation associated with periodontal disease lead to the activation of the components of the NLRP3 inflammasome. Apart from promoting pyroptosis and neutrophil recruitment, once activated, NLRP3 promotes the overproduction of cytokines and antibodies, as well as the release of prostaglandin E2. In the end, all these events result in the destruction of the periodontium [53].

The results of previous studies performed on animal models were not conclusive. Yamaguchi et al induced periodontal disease in mice by orally injecting them with Porphyromonas gingivalis, showing that this microbial species, involved in the inflammatory process characteristic of chronic periodontitis, activated the NLRP3 inflammasome, leading to a response from the cells of the innate immune system and involvement of the IL-1 family of cytokines [54]. Nevertheless, a recent study by Rocha et al. performed on mice with induced periodontal disease revealed different results, showing that the inflammation associated with periodontitis and the consequent bone resorption was not determined by NLRP3, which played a minor role in the process, but rather by caspase-1 [55]. The results of our study showed statistically significant increased salivary levels of NLRP3 in patients with periodontitis compared to the control group ($p< 0.00001$, Table 1, Figure 1), suggesting a possible association of NLRP3 with the pathogenesis of this oral condition. Furthermore, the data we obtained did not show any correlation between NLRP3 salivary levels and the number of remaining teeth on the dental arches (Table 3).

When comparing the salivary levels of NLRP3 in patients with periodontitis and without systemic diseases to those who have associated systemic diseases, we were unable to find any statistically significant difference (Table 2). These results indicate that NLRP3 levels are mainly due to the presence of periodontal inflammation and not to the coexistence of other pathologies. Isola et al reported that patients with periodontitis and patients with periodontitis and type 2 diabetes had increased NLRP3 levels, both in the serum and saliva, not only compared to healthy controls, but also versus patients who had type 2 diabetes, but did not have periodontal disease [53]. Using samples from the gingival crevicular fluid and gingival biopsies, Garcia-Hernandez et al. showed that patients with periodontitis and uncontrolled type 2 diabetes displayed upregulated genes and proteins associated with NLRP3 activation compared to patients who had only periodontitis [56].

A systematic analysis from 2020 concluded that the levels of leptin both in plasma and saliva reported in the analyzed studies were too different, and although a decrease in salivary levels of leptin was observed in periodontal patients compared to healthy subjects, that data was not sufficient to establish the relationship between the disease and levels of leptin [57].

Similar to other studies, our results showed that leptin had significantly decreased levels in the saliva of patients with periodontal disease compared to those of the control group ($p< 0.00001$, Table 1, Figure 2) [58]. Leptin has already been shown to activate NLRP3 inflammasome in other inflammatory diseases, including systemic lupus erythematosus, and autoimmune disease characterized by the presence of the inflammatory process [59]. Despite both NLRP3 and leptin showing increased levels in periodontitis, we could not find a correlation between the two biomarkers.

TAC was defined by Miller as the "measure of the antioxidant capacity of all antioxidants in a biological sample and not just the antioxidant capacity of a single compound" [60]. Since ROS are naturally counteracted by antioxidants, determining the total antioxidant capacity has become one of the common and important ways to monitor the effect of oxidative stress in periodontal disease. However, the results regarding TAC levels in periodontal disease are controversial, with research showing both significant increases in periodontitis patients compared to healthy controls, as well as no significant differences [61].

On the other hand, Toczewska et al reported lower levels of TAC in both the saliva (which was a mixture of non-stimulated and stimulated saliva) and the gingival crevicular fluid of periodontal patients [62]. A decrease in the antioxidant capacity of patients with periodontal disease was also reported by Diab-Ladki et al [63]. Moreover, similar results were obtained by Brock et al, although their data did not show a statistically significant difference for salivary levels [64]. In our study, the determination of TAC displayed higher salivary levels in periodontitis patients compared to the control group ($p=0.491$, Table 1, Figure 3), but no statistical significance was found. This difference could be explained by the fact that the control group consisted of healthy subjects who had a mean age lower than that of the periodontitis patients. Limberaki et al previously reported that, contrary to expectations, younger people have lower TAC levels in the serum than older people [65].

Previous research revealed that while mitochondrial dysfunction and ROS overproduction can promote NLRP3 activation, their existence is not imperative for this to happen. Additionally, DNA damage caused by ROS can activate the inflammasome, but its release can happen downstream of the activation of the inflammasome [66]. This could explain why our results showed no statistically

significant correlation between the salivary levels of NLRP3 and those of TAC.

Our study has several limitations, including the study groups being relatively small. Additionally, there was a significant mean age difference between the study group and the control group. Moreover, although saliva was collected following the same recommendations for all participants in the study, the salivary flow of each participant could have played a role in the level of the biomarkers [67]. Nevertheless, they reveal that the data obtained by our research group suggests the involvement of NLRP, leptin, and oxidative stress in periodontitis.

CONCLUSION

The results obtained in the present study are promising, but they incite further research that should be more focused on the relationship between these biomarkers and other factors that could influence the outcome, for example, the different types of bacteria involved in periodontal disease. Moreover, analyzing not just the saliva of the patients, but their gingival crevicular fluid could provide better insights. Future perspectives could be offered by determining these biomarkers in dynamic, before and after periodontal treatment, as well as correlating them to clinical parameters. All these represent objectives for our future research, which would offer a more complete picture of the mechanisms involved in periodontal disease development and progression and thus, show how NLRP3 activation can promote periodontal tissue destruction and bone loss.

Conflicts of interest and sources of funding

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Authors' contribution

Conceptualization, I.-I.S.-S., A.C.D; methodology, A.-S.D, D.M., B.-F.M; software, R.I., S.C.B; validation, T.-C.S., A.M.C..; formal analysis, D.M., S.C.B; investigation, T.-C.S., B.-F.M; resources, T.-C.S., A.M.C.; data curation, D.M., B.-F.M; writing-original draft preparation, I.-I.S.-S., T.-C.S., R.I.; writing-review and editing, I.-I.S.-S., D.M., A.-S.D, A.C.D; visualization, R.I., A.M.C.; supervision, A.-S.D, A.C.D; project administration, I.-I.S.-S., A.C.D.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Carol Davila University of Medicine and Pharmacy (22976/17.08.2022).

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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ORIGINAL ARTICLE

Evaluating Arbitrary and Histological Margins in High-Risk Nonmelanoma Skin Cancers: A One-Center Experience

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Abstract: Background: Nonmelanoma skin cancers (NMSCs), including basal cell carcinoma (BCC) and cutaneous squamous cell carcinoma (cSCC), often occur in high-risk areas like the face, ears, and scalp. Surgical excision is standard, but achieving tumor-free margins while preserving function and appearance is challenging. This study evaluates the adequacy of arbitrary surgical margins versus histopathological clearance. Methods: A retrospective analysis was conducted on 147 patients with BCC or cSCC in high-risk areas treated at Elias Emergency University Hospital (2020–2024). Data included demographics, tumor type, size, location, excision margins, and histological clearance. Margins were grouped from 0 mm to >10 mm. Results: Most patients were male (59.2%) with a mean age of 70.6 years. BCC accounted for 70.7% of cases. Arbitrary margins averaged 5.4 mm; mean histological margins were ~2.5 mm. Complete excision was achieved in 91.2%; positive margins were most common on the nose and scalp. Discussion: Arbitrary margins in high-risk areas are often narrower than guidelines recommend, especially where tissue is limited, increasing the risk of incomplete excision. Conclusion: While aesthetic and functional considerations often necessitate smaller margins, improved intraoperative margin assessment could enhance oncological outcomes in high-risk NMSC.

Keywords: Nonmelanoma skin cancer (NMSC), Basal cell carcinoma (BCC), Cutaneous squamous cell carcinoma (cSCC), Surgical margins, Histopathological clearance, High-risk anatomical areas, Mohs micrographic surgery, Arbitrary excision margins, Incomplete excision, Oncologic safety

INTRODUCTION

Basal cell carcinoma (BCC) and cutaneous squamous cell carcinoma (cSCC) represent the two most common forms of nonmelanoma skin cancer (NMSC), with BCC being the most prevalent malignancy globally. While BCC is characterized by slow growth and minimal metastatic risk, its potential for local destruction and disfigurement, especially in anatomically sensitive areas, underscores the importance of timely and adequate treatment. cSCC, although less frequent, poses a relatively higher risk of local invasion and lymphatic metastasis, particularly in immunosuppressed patients [1].

The cornerstone of NMSC treatment remains surgical excision, favored for its efficacy, cost-effectiveness, and accessibility across healthcare systems. Surgical excision allows for histopathological evaluation of the margins and is generally sufficient for low-risk lesions. However, for high-risk tumors located in functionally and cosmetically sensitive areas (defined as Area H: central face, ears, eyelids, nose, genitals, and hands), standard excision can be technically challenging. In these zones, even small tumors may have subclinical extension, increasing the risk of incomplete excision and recurrence [2].

A surgical margin refers to the outer edge of the tissue removed during surgery. The tumoral involvement of the resected margins is a crucial prognostic element. Evaluating the tumoral invasion of the margins is essential for determining whether the excision is sufficient or incomplete. In instances of cancer, follow-up management choices depend on whether the margins are devoid of tumors. Consequently, precise and timely margin evaluation is essential. Attaining clear surgical margins guarantees the complete removal of cancerous tissues, minimizing the chances of local recurrence [3].

While standard excision remains widely used, it has notable limitations in high-risk areas. One of the primary drawbacks is the inability to assess 100% of the peripheral

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and deep margins during surgery. Mohs micrographic surgery (MMS) is considered the gold standard for treating high-risk NMSCs, particularly recurrent or infiltrative lesions. MMS provides complete circumferential and deep margin control (CCPDMA), resulting in superior cure rates—over 98% for primary BCCs and above 95% for recurrent cases. Moreover, MMS allows for maximal tissue conservation, which is crucial in cosmetically sensitive areas. However, its disadvantages include longer operative times, higher costs, and limited availability, which restrict its universal application [4].

This study aims to demonstrate that standard surgical excision can yield acceptable oncological outcomes in the management of high-risk nonmelanoma skin cancers, particularly in under-resourced hospitals where Mohs micrographic surgery is not available.

MATERIALS AND METHODS

This study included 147 patients who underwent surgical excision of non-melanoma skin cancer at the Plastic Surgery Department in „Elias Emergency University Hospital", Bucharest, Romania, between 2020-2024.

Inclusion criteria – all patients admitted to our plastic surgery department with skin lesions with high suspicion of non-melanocytic skin cancer/histopathological confirmation, in a high-risk area, who accepted the surgical treatment. The high-risk areas were defined using NCCN guidelines depending on anatomical localization and size: head, scalp, genital area, trunk, and extremities >2cm.

Exclusion criteria – patients with BCC/cSCC in low-risk areas, premalignancies, melanoma, psychological disorders, and refusal of future treatments.

Arbitrary surgical margins were defined as preoperative markings based on local tissue availability, skin elasticity, and the surgeon's experience. Data regarding sex, age, comorbidities, type of anesthesia used, type of reconstruction, surgical dissection plane, arbitrary surgical margins, peripheral and deep clean margins, tumor diameter, and ulceration were collected and analysed. Surgical standard excision was performed using a no.15 blade in a full-thickness fashion. The deep dissection plane was chosen depending on the case. Excisions were performed under local, regional, or general anesthesia. For wound closure, depending on defect size and location, we used primary closure, skin grafts, and/or flaps. Specimens were oriented using sutures as markings for histopathological evaluation. Excised specimens were sent for histopathological examination to assess tumor clearance.

The histological clearance margins of the lesions were measured peripherally and in depth, recorded in millimeters to one decimal place. They were categorized into five groups:

- zero clearance (indicating margin involvement)
- clearance under 1 mm (0.1–0.9 mm)
- clearance between 1 and 4.9 mm
- clearance between 5 and 9.9 mm
- clearance greater than 10 mm.

Those with zero clearance were advised to have a re-excision following their histopathological results. Patients with clearance margins under 1 mm underwent dermatoscopy surveillance every 3 months; in case of recurrence, the tumors were re-excised. Patients were monitored for wound healing and recurrence.

RESULTS

Demographic

In the 4 years, there were 147 patients, predominantly men with 87 cases (59.2%), with an average age of 70.6 years (see Table 1). BCCs were the predominant cancer, with 104 excisions (70.7%), while cSCCs had 43 excisions (29.3%).

Regarding comorbidities, cardiovascular diseases were predominant in 68 cases (46,2%), followed by diabetes mellitus in 11 cases (7.4%) and other malignancies in 10 cases (6.8%). The anesthesia of choice was local in 143 cases (97.3%), regional and general anesthesias were less prevalent at 2 and 3 cases, respectively. The preferred method of reconstruction was the local flap, with 54 cases (36.7%), followed by full-thickness skin grafts with 38 cases (25.9%), and direct sutures with 36 cases (24.5%). On the other hand, reconstruction techniques such as regional flaps and split-thickness skin grafts were rarely used.

The surgical plane of choice was subcutaneous with 62 cases (42.2%), suprafascial with 42 cases (28.6%), while deeper planes such as supracondylar (14.3%) and supraperiostal (8.2%) have been rarely used.

Incomplete excisions

There were 13 cases of incomplete excisions (see Table 2). The areas with incomplete excisions were located on the nose, with 5 out of 13 cases, followed by the scalp, 4 out of 13 cases. The cheek and peri-orbital regions had a lower rate of incomplete excisions, accounting for 2 cases. Notably, no incomplete excisions were recorded for lesions located on the lips, limbs, or trunk.

Table 1: Demographics and Clinical Characteristics

Sex	
- female	60 (40.8%)
- male	87 (59.2%)
Age	70.6 years (29 – 94 years)
Type	
- BCC	104(70.7%)
- SCC	43 (29.3%)
Comorbidities	
- CV	68(46.2%)
- Diabetes Mellitus	11(7.4%)
- other malignancies	10 (6.8%)
Type of anesthesia	
- local	143 (97.3%)
- regional	2 (1.4%)
- general	3 (2%)
Type of reconstruction	
- direct suture	36 (24.5%)
- skin graft	
-STSG	6 (4.1%)
-FTSG	38 (25.9%)
- local flap	54 (36.7%)
- regional flap	3 (2%)
Surgical dissection plane	
- subcutaneous	62 (42.2%)
- suprafascial	42 (28.6%)
- supracondral	21 (14.3%)
- supraperiostal	12 (8.2%)
Arbitrary surgical margins (mean, mm)	5.4 mm (2-15mm)
Largest diameter (mean, mm)	21.6 mm (5 – 184mm)
Peripheral clearance margins (mean, mm)	2.52 mm (0-9.2mm)
Deep clearance margins (mean, mm)	2.65mm (0-20mm)

Table 2: Incomplete excisions expressed as % of all incomplete excisions depending on anatomic site

Anatomic site	Total Lesions	Incomplete Excisions	% of All Incomplete Excisions	Mean Diameter (mm)	Mean Arbitrary Margin (mm)	Presence of Ulceration (n)
Cheek	17	1	7.7%	17.11	4.3	16
Forehead	15	2	15.4%	18.6	4.4	9
Nose	33	5	38.5%	14.1	4.3	24
Peri-orbital	24	1	7.7%	15.45	5.2	15
Lips	8	0	0%	13.3	6.2	8
Scalp	26	4	30.8%	22.6	5.0	19
Limbs	14	0	0%	35.2	9.2	11
Trunk	10	0	0%	58.6	7.6	6
	147	**13**				

The majority of cancers (64.6%) had peripheral histological clearance margins between 1.0–4.9 mm, as we can see in Table 3. Margins with clearance under 1 mm were present in 15.6% of cases. Complete margin involvement (0 mm clearance) was identified in 4.8% of tumors, comparable to the 8.8% incomplete excision rate. No cases exceeded peripheral margins larger than 10 mm, balancing oncological safety with tissue preservation.

The most common deep margin range was 1.0–4.9 mm, present in 57.1% of cases. Deep margins between 0.1–0.9 mm were observed in 19% of cases. Complete deep margin involvement (0 mm clearance) was identified in 8.8% of cases, reinforcing the necessity of

achieving adequate excision depth to minimize recurrence risk. Only 4.1% of lesions had deep clearance margins exceeding 10 mm.

Table 3: Margin Clearance Distribution Among Excised Tumors

Margins (mm)	Peripheral total	% total lesions	Deep total	% total lesions
0	7	4.8%	13	8.8%
0.1-0.9	23	15.6%	28	19.0%
1-4.9	95	64.6%	84	57.1%
5.0-9.9	13	8.8%	16	10.9%
>10	0	0%	6	4.1%

Peripheral Margins >1 mm

The highest histological clearance rates, with peripheral margins over 1 mm, were noted in lesions located on the limbs (100%) and scalp (73%), suggesting that these regions allow a wider excision. In contrast, the lowest rates of peripheral clearance beyond 1 mm were documented in lesions on the forehead (6.1%) and lips (4.8%), revealing the technical difficulties of obtaining wider margins in these areas.

Table 4: Distribution of Peripheral and Deep Histological Clearance Margins depending on anatomic site

Anatomic site	Total lesions	Peripheral histological clearance margins > 1mm	%	Deep histological clearance margins (0.5-4.9 mm)	%
Cheek	17	13	8.8%	17	11.6%
Forehead	15	9	6.1%	11	7.5%
Nose	33	21	14.3%	27	18.4%
Peri-orbital	24	16	10.9%	22	15.0%
Lips	8	7	4.8%	8	5.4%
Scalp	26	19	12.9%	21	14.3%
Limbs	14	14	9.5%	13	8.8%
Trunk	10	8	5.4%	10	6.8%
	147	**107**	**72.8%**	**129**	**87.8%**

Deep Margins (0.5–4.9 mm)

The nasal region had the greatest number of cases with deep margins in the 0.5-4.9 mm range, with 27 cases (18.4%), as seen in Table 4, explaining its elevated rate of incomplete excisions. Areas such as the peri-orbital region, presented 22 cases (15.0%), the scalp with 21 cases (14.3%), reveal the difficulties of achieving sufficient deep margins in said locations.

DISCUSSION

Basal cell carcinoma is the most prevalent cancer, presenting with a slow growth rate and a very low metastatic risk, located predominantly on the head and neck, but also on the limbs and trunk [5]. BCCs tend to be locally destructive and may cause deformity or recurrences if not fully excised [6]. The most important risk factor is long-term/chronic sun exposure, followed closely by a history of either BCC or SCC, with patients being more likely to develop another BCC compared to patients without a history of NMSCs. The rate of developing BCC increases with age, with an average age of 68 years; men have higher rates of BCC than women [7]. The treatment of choice is removing the tumor through different methods, such as Mohs micrographic surgery, peripheral and deep en face margin assessment, cryosurgery, electrodesiccation, and surgical excision. Surgical excision remains the staple method of removing the tumor [8,9].

Cutaneous squamous cell carcinoma, while not as prevalent as BCC, is still a frequent malignancy with similar risk factors as the BCC, such as sun exposure, Fitzpatrick skin types I-II, male gender, and other etiologies such as immunosuppression and chronic wounds (e.g., Marjolin Ulcer). Regarding the recurrence rate following surgical excision, cSCCs located in areas prone to prolonged sun exposure yielded a higher rate of recurrence compared to areas with less sun exposure. The primary risk of recurrence is invasion of the dermis. On the other hand, there was no noticeable difference between the 2 areas regarding the rate of metastases [10,11]. SCCs pose a low metastatic risk, the preferred metastatic site being the lymph node [12,13]. They can be more aggressive than BCCs, with a higher likelihood of local invasion and potential for metastasis, especially in high-risk cases. Understanding the local invasiveness of NMSCs is crucial for determining appropriate treatment strategies and improving patient outcomes [14].

Surgical excision of NMSCs aims to completely remove the tumoral tissue while sacrificing as little normal tissue as possible. This can be achieved through simple elliptical excision. However, Mohs micrographic surgery is the gold standard for treating high-risk tumors. Its primary benefit lies in conserving healthy tissue, preserving anatomical structures, and ensuring tumor-free margins. A recent randomized clinical trial found that Mohs surgery yielded superior outcomes for recurrent BCC compared to direct excision, although no significant differences were observed for primary BCC treatment [15].

Area H refers to high-risk regions, including the central face, ears, genitals, and hands, which require balancing oncologic control with cosmetic and functional outcomes [16]. Mohs surgery is an excellent option for preserving function and enhancing cosmetic outcomes. From a reconstructive perspective, imaging techniques help to identify optimal blood supply for flaps, minimizing complications and improving success rates. As a result, the focus of skin cancer surgery has evolved from simple excision and flap coverage to superior functional and aesthetic outcomes [17].

Peripheral and deep en face margin assessment (PDEMA), also known as complete circumferential peripheral and deep margin assessment (CCPDMA), is associated with the highest cure rates for NMSCs, due to complete peripheral and deep margin visualization, and re-excision of residual tumor [18]. Mohs is the most common method of PDEMA; however, there are also other techniques, such as the Tubingen method, which differs from Mohs by having a histopathological examination in a 1-5 day interval over Mohs's rapid frozen section and interpretation [19].

The mean age of patients with NMSCs in our study was 70.6, with a tendency to be more represented in males. Ciążyńska et al. observed no significant differences between genders. However, the mean age of men in their group was 70.9 +/- 11,4 years, and for women 71.4 +/- 11.9 years [20].

According to NCCN Guidelines Version 2.2025, high-risk criteria for both BCCs and cSCCs are: tumor diameters >= 2 cm in the trunk and extremities, any size pertaining to the head, neck, feet, pretibial and anogenital area, recurrence, immunosuppression, and poorly defined borders [21,22].

The issue with arbitrary surgical margins is that they can lead to either removing more normal tissue than necessary or less than required, and thus leading to recurrence. A study from the Hospital Universitari Vall d'Hebron in Barcelona reviewed histopathological reports from all BCCs treated by different specialties, aiming to determine the influence of a surgical specialty on assessing and excising a tumor with proper safety margins. It revealed that dermatologists had the lowest rate of positive margins out of other specialties, such as plastic surgery, general surgery, etc. attributed to their broader knowledge and expertise in evaluating tumor margins macroscopically. Therefore, proper identification of macroscopic tumoral margins for excision must be emphasized [23].

Australian Journal of Plastic Surgery conducted a study in which they monitored subjects with different levels of experience, as they were drawing a 3 mm, 5 mm, and 10 mm margin around different lesions without using a measurement device, with acceptable margins being considered to be approximately within 0.5 mm of the recommended surgical margin. Margins were underestimated, no matter the level of experience, being smaller in size for each bracket. Margins of 3 mm have been the most accurately estimated out of the 3, increasing the margin size leads to an increase in inaccuracy. This serves to enforce the importance of proper safety margins and the risk of re-excisions caused by improper measurements [24,25].

The majority of excised tumors (64.6%) displayed peripheral histological clearance margins ranging between 1.0–4.9 mm. Narrow peripheral margins (0.1–0.9 mm) have been observed in 15.6% of cases. Complete margin involvement (0 mm clearance) was identified in 4.8% of tumors. No cases exceeded peripheral margins larger than 10 mm, balancing oncological safety with tissue preservation. The most common deep margin range was 1.0–4.9 mm, present in 57.1% of cases. Deep margins between 0.1–0.9 mm were observed in 19% of cases. Complete deep margin involvement (0 mm clearance) was identified in 8.8% of cases, reinforcing the necessity of achieving adequate excision depth to minimize recurrence risk. Only 4.1% of lesions had deep clearance margins exceeding 10 mm. Khalid-Raja M. suggested identifying the peripheral margin of excision by using the wet blotting technique and applying a 4-mm margin of excision to all NMSCs. Their results showed a 95% successful excision rate and a peripheral clearance margin of 0.1-14 mm, a mean peripheral margin of 3.73 in the case of BCCs, and a 96% successful excision rate with peripheral margins varying from 0.5 to 19.0 mm, a mean peripheral margin of 4.86. Comparatively, our study showed a 92% successful excision rate and a mean peripheral margin of 2.52 mm, with the mean average surgical margin being 5.4 mm. The highest number of incomplete excisions was located at the nose and scalp with arbitrary surgical margins of 4.3 and 5.0 mm, respectively. Lesions over 2 cm lead to a 91.2% complete excision with a 4 mm margin while using the wet blotting technique. This coincides with our findings [26,27].

J Kiely et al. recommend the excision of poorly differentiated cSCCs with a minimum peripheral margin of 6 mm, well-differentiated cSCCs were excised with an average peripheral margin of 4.72 mm, and the poorly differentiated ones with an average margin of 6.42 mm. This led to an involvement of peripheral margins in 3% of well-differentiated lesions and 13.2% of poorly differentiated ones. Deep margins were also close or involved in 50% of poorly differentiated cSCCs, demonstrating that poorly differentiated cSCCs are not properly excised in both peripheral and deep planes, in spite of the guidelines [28].

Australian Journal of General Practice recommends for high-risk lesions over 5 mm margins for BCCs and a 6 mm margin for cSCCs, claiming selection of surgical margin should aim to balance between incomplete excision of the tumor and normal tissue sacrifice, affecting cosmetic and functional outcomes [29].

Eva Van Loo et al. found the recommended excision margins for BCC too defensive, evaluating the Dutch Guideline, which states that the margin for low-risk BCC should be 3-4 mm, and the high-risk BCC up to 20 mm, incorporating the H-zone as a high-risk BCC. Currently, the Dutch and European consensus is that high-risk BCC should have a minimum of 5 mm, or 5-15 mm (European guideline). The findings indicate that adherence to the guideline in high-risk BCC was not adequate, yet it had a limited effect on the incomplete excision rate. A margin of 3 mm was sufficient for 99.6% of low-risk BCCs, and a 5 mm margin for high-risk BCCs up to 94% [30].

High incomplete excision rates (8.8%) suggest that in certain areas, such as the nose and scalp, wider margins might be needed. The lowest incomplete excision rates were found in the peri-orbital region and the cheek, while no incomplete excisions were documented on the lips, trunk, and limbs. Ceder H found higher incomplete excision rates located on the face and scalp, the highest ones being on the nose, ear, and peri-orbital area; also noteworthy, circular excisions were also more often incomplete compared to elliptical excisions [31].

Despite its high cure rates and tissue-sparing advantages, Mohs micrographic surgery (MMS) presents several notable disadvantages that limit its universal application. One of the primary concerns is the limited availability of specialized centers and trained personnel, making access difficult in under-resourced or rural healthcare settings. MMS is also more time-consuming than standard excision, as it involves intraoperative histological analysis of multiple tissue layers, requiring patients to remain in the facility for extended periods. Additionally, the procedure is costlier due to the need for specialized equipment and trained histopathologists, potentially placing a financial burden on healthcare systems and patients alike. From a logistical standpoint, not all lesions are suitable for MMS—tumors with extensive subclinical spread, poorly defined borders, or aggressive histologic subtypes may require complementary or alternative approaches. Lastly, in settings where rapid frozen-section pathology is not feasible, delays in diagnosis and treatment can occur, reducing the practicality of MMS outside high-volume dermatologic surgery centers [32]. While Mohs surgery offers excellent cure rates, its use in elderly patients requires careful consideration. The prolonged procedure time, need for patient immobility, and potential for postoperative care challenges may be poorly tolerated by those with comorbidities or cognitive impairment. Therefore, treatment should be individualized based on the patient's overall health, functional status, and life expectancy [33].

CONCLUSION

This study highlights the challenges of achieving adequate surgical margins in high-risk anatomical areas affected by nonmelanoma skin cancers. Although arbitrary margins based on surgeon experience averaged 5.4 mm, histopathological evaluation revealed that a significant number of excisions had suboptimal peripheral or deep clearance, particularly in constrained areas like the nose and scalp. Despite a 91.2% overall rate of complete excision, the 8.8% rate of positive margins underscores the limitations of visual and tactile margin estimation alone. The findings support existing guidelines recommending wider margins ≥5 mm for BCC and ≥6 mm for cSCC, while also emphasizing the need to balance oncologic safety with aesthetic and functional preservation. In high-risk regions, individualized surgical planning, improved intraoperative margin assessment, and consideration of techniques like Mohs micrographic surgery in selected cases may optimize outcomes and minimize recurrence. Ultimately, refining margin estimation and tailoring surgical strategy to both tumor characteristics and anatomical context are essential for improving the quality of care in patients with high-risk NMSCs.

Conflicts of interest and sources of funding

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Authors' contribution

Conceptualization, S.M.R and K.A.J, methodology, A.L.P.; formal analysis, A.L.P.; data curation, P.A.C; writing—original draft preparation, K.A.J, A.L.P; writing—review and editing, S.M.R..; visualization, R.D.S..; supervision, R.D.S.; project administration, A.L.P. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Elias University Emergency Hospital, Bucharest, Romania (protocol code 3606 and 19.06.2025).

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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REVIEW

Targeting Heart Disease in Cardiovascular–Kidney–Metabolic Syndrome: A Review of Novel Therapeutic Approaches with Prognostic and Quality-of-Life Impact

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Abstract: Cardiovascular-kidney-metabolic (CKM) syndrome describes the complex, bidirectional interplay among cardiovascular, renal, and metabolic dysfunctions, where impairment in one system accelerates decline in the others. This interconnected pathophysiology significantly elevates the risk and progression of heart disease, particularly heart failure, through mechanisms involving insulin resistance, systemic inflammation, neurohormonal activation, and endothelial dysfunction. In this context, therapeutic strategies targeting heart disease must address the multifaceted drivers of CKM. Recent advances highlight the need for an integrated, patient-centered approach that combines lifestyle interventions with pharmacological treatments tailored to individual cardiovascular risk. While foundational therapies such as RAAS inhibitors and statins remain essential, novel agents now offer additional prognostic and quality-of-life benefits. SGLT2 inhibitors have emerged as a cornerstone therapy, improving outcomes in heart failure with both preserved and reduced ejection fraction, independent of glycemic control. GLP-1 receptor agonists and dual GLP-1/GIP agonists like tirzepatide demonstrate cardiometabolic and renal protection, while finerenone shows promise in diabetic kidney disease and several heart failure phenotypes. These therapies not only target glycemic control but also reduce cardiovascular mortality, hospitalizations, and renal decline. Optimizing cardiovascular outcomes in CKM syndrome requires early, multifactorial therapeutic intervention, informed by evolving evidence and a deeper understanding of the heart–kidney–metabolism axis.

Keywords: heart failure, diabetes mellitus, obesity, chronic kidney disease, GLP-1 receptor agonists, SGLT2 inhibitors, mineralocorticoid-receptor antagonists, finerenone, cardiovascular-kidney-metabolic syndrome

INTRODUCTION

Heart disease is frequently observed in patients with CKM syndrome. There is a pathophysiological interplay between the cardiovascular, renal, and metabolic systems, where dysfunction in one organ can exacerbate impairment in others, which substantially increases the risk of developing heart failure. Patients with CKM syndrome usually present with overlapping conditions as type 2 diabetes, chronic kidney disease, and atherosclerotic cardiovascular disease, all of which contribute to functional and structural cardiac dysfunction.

Heart disease and cardiovascular mortality represent the main drivers of prognosis in all CKM syndrome comorbidities. Hence, special consideration should be directed toward understanding pathophysiological mechanisms through which all these metabolic risk factors converge toward cardiovascular impairment. Additionally, emphasis should be placed on the early and appropriate initiation of the right medication for the right patient, tailored to the estimated individual risk, particularly concerning the novel drug classes that have demonstrated beneficial effects across all components of the CKM syndrome: GLP1 receptor agonists, SGLT2 inhibitors, and the non-steroidal mineralocorticoid receptor antagonist finerenone.

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This review emphasises the role of the heart in the CKM spectrum, with particular interest in the therapeutic implications.

GLP-1 RECEPTOR AGONISTS

GLP-1 (glucagon-like peptide-1) is a natural hormone mainly released by intestinal L enteroendocrine cells residing in the distal jejunum, ileum, and colon, as a result to oral glucose intake. GLP-1 is classified as an incretin hormone because it stimulates insulin release from beta pancreatic cells, inhibits the release of glucagon and, along with that, hepatic glucose output, and reduces the rate of gastric emptying and promotes satiety by central appetite suppression. GLP-1 has a very short half-life of approximately 2 minutes, being quickly cleaved by dipeptidyl peptidase IV (DPP-4), a widely expressed enzyme, thereby limiting the strong insulin secretagogue effect of this hormone [1–3].

Owing to the very short half-life, the molecule of GLP-1 is not suitable for an adequate therapeutic agent. Nevertheless, researchers developed molecules capable of being adequate therapeutic agents: inhibitors of DPP-4, which are capable of prolonging the effects of natural GLP-1, and molecules that represent long-acting GLP-1 receptor agonists [1].

Apart from pancreas, GLP-1 receptors are expressed on various types of cells throughout the body and the finding of this widespread localization of receptors led to studies that demonstrated multiple effects beyond the well-known incretin actions, the so-called "pleiotropic effects": in central nervous system, being involved in the regulation of appetite, in the cardiovascular system, being expressed both in cardiomyocytes and vascular cells, in the musculoskeletal system, gastrointestinal tract, regulating the motility or secretion, interfering in the gut-brain axis, in adipose tissue influencing fat metabolism through interaction with leptin, in kidneys at multiple levels (arterial and arteriolar smooth cells, glomerulus, juxtaglomerular cells, proximal tubule), modulating renal function both directly by altering glomerular hemodynamics and indirectly through less known direct interactions between gastrointestinal system and kidneys known as gut-renal axis [3–7]. Even in beta pancreatic cells, besides altering insulin or glucagon levels, the stimulation of GLP-1R could enhance beta-cell proliferation, inhibit apoptotic pathways, and induce expansion of islet mass [4].

Considering that diabetes mellitus and obesity serve as risk factors in the context of cardiovascular diseases, the effects of GLP-1 receptor agonists on the cardiovascular system are, to a considerable extent, mediated through modulation of these metabolic conditions.

Regarding their role as antidiabetic medications, all GLP-1RAs proved effective in glycemic control by reliably reducing fasting plasma glucose and HbA1c (up to a reduction of 56.2 mg/dl FPG levels and 2.1% for HbA1c levels, respectively) [8].

Obesity is strongly associated with the development of T2DM as excessive adipose tissue, particularly visceral fat, contributes to insulin resistance [9]. The central downregulation of appetite, accompanied by slowing the gastric evacuation, which induces a feeling of fullness, facilitates the reduction of overall calorie intake and the maintenance of a favorable long-term energy balance [3]. Large meta-analyses of the available RCTs proved that GLP-1 RA are effective in inducing weight loss and a reduction in the body mass index and in the waist circumference [8,10].

Conversely, an increasing body of evidence suggests that GLP-1RAs exert direct beneficial effects on various components of the cardiovascular system, independently of their impact on glycemic regulation and weight control.

As far as cardiomyocytes are concerned, the presence and the exact extent of GLP-1R expression are unclear, corroborated by limited evidence regarding the cellular localization of GLP-1R protein [11]. However, in cellular and animal models, besides metabolic effects, which could indirectly improve cardiac function, there is evidence that GLP-1 could restore crucial signaling pathways that could be altered by external stressors [7]. For example, GLP-1 could ameliorate the extent of ischemia-reperfusion injury by activating specific signaling pathways and inhibiting cardiomyocyte apoptosis [3]. Moreover, GLP-1 signaling is involved in reducing hyperglycemia-induced apoptosis of the cardiomyocytes by optimizing the signaling pathways from the sarcoplasmic reticulum and in lowering the extracellular matrix remodeling [12].

There is also important evidence that GLP-1 receptor stimulation influences various pathogenic mechanisms that are involved in atherogenesis. First of all, GLP-1 can mitigate endothelial dysfunction by inducing NOS-mediated vasodilation and by limiting the formation of advanced glycosylation end-products [3,13]. Additionally, GLP-1 is involved in improving lipid metabolism at several levels: with respect to gene expression, it downregulates genes of lipogenic enzymes and upregulates genes of lipolytic enzymes, reduces lipotoxicity by lowering lipid peroxidation, stimulates fatty acid oxygenation, inhibits HMG-CoA reductase and lowers cholesterol levels, ameliorates hepatic steatosis, lowers serum triglyceride levels and even reduces intestinal lipid absorption [3,14]. There are also described effects of enhanced cholesterol efflux from foam cells or suppression of foam cell formation [13]. Even an anti-inflammatory pathway is proposed with the downregulation of genes involved in inflammation and oxidative stress. Moreover, GLP-1 promotes an atherosclerotic plaque remodeling towards stabilization by reduction of matrix metalloproteinases (MMP), proteolytic enzymes involved in weakening of plaque fibrous caps and in increasing the risk of rupture [3,13].

GLP-1RA proved effects of blood pressure reduction both in preclinical and clinical studies, by as much as 6 mmHg (as it was found in DURATION-1 trial) [11,15]. There are several mechanisms possibly involved in this effect: the capacity of GLP-1RA to counteract the sympathetic nervous system hyperactivation, both at the central and peripheral nervous system level, the improvement in the endothelial function and therefore in vasodilation, and the possible effect on renal tubular mechanisms by increased natriuresis, which

was observed in experimental models of hypertension [16,17]. The effect of lowering blood pressure was consistent throughout the entire spectrum of renal function, from normal to severely reduced [18].

Diabetes affected individuals are at high risk of chronic kidney disease (CKD) (as many as 40% diabetic patients develop chronic kidney disease), diabetic nephropathy representing the leading cause of chronic kidney disease worldwide. However, in the CKD population, cardiovascular events constitute the primary contributor to mortality, due to enhanced atherosclerosis, arterial and valvular calcification, and amplification of myocardial fibrosis [19]. There is a clear rationale that reducing both the risk and progression of chronic kidney disease could play a pivotal role in decreasing the burden of cardiovascular complications in diabetic patients.

Apart from indirect beneficial effects of glycemic control, weight loss and blood pressure reduction upon renal function, there are described direct effects: increased natriuresis and diuresis, at least partially explained by inhibiting sodium-hydrogen exchanger 3 in proximal tubular cells, anti-inflammatory effects by inhibition of cytokine production and chemotactic recruitment of inflammatory cells, free-radical scavenging effects and direct glomerular hemodynamic effects [5,17,19].

Another pathophysiological mechanism seemingly targeted by this therapeutic class is the modulation of the renin–angiotensin–aldosterone system, as there was been proven that GLP-1RA could induce a decrease in plasma levels of renin and angiotensin II. The effect of lowering the activity of this system could contribute to the effect of lowering blood pressure, the enhanced natriuresis, and could influence glomerular hemodynamics by vasodilation of the efferent arteriole and reduction of glomerular capillary pressure [18].

Beyond the pathophysiological effects demonstrated at the experimental level, the clinically relevant renoprotective effects include the reduction of albuminuria and, as evidenced by several studies, a reduction in eGFR decline [18,20].

Transitioning from experimental models and pathophysiological mechanisms to clinical evidence, GLP-1RA use in practice is supported by results from representative large-scale cardiovascular outcome trials. These studies have positioned GLP-1 receptor agonists as a distinct class among antidiabetic medications, with benefits that go beyond mere glycemic regulation.

There are 8 cardiovascular outcomes randomized controlled trials (CVOTs) for 7 different GLP-1RA (lixisenatide, liraglutide, semaglutide s.c., oral semaglutide, exenatide, albiglutide, dulaglutide, efpeglenatide) which included type 2 diabetic patients with high cardiovascular risk or previous cardiovascular disease. Four different GLP-1RAs, namely liraglutide, semaglutide s.c., dulaglutide, and efpeglenatide, proved efficient in reducing a composite end-point of major adverse cardiac events MACE (comprising cardiovascular death, non-fatal myocardial infarction, and non-fatal stroke)[21]. However, analyzing the hazard ratio for individual components of MACE, there is a clear heterogeneity between these molecules. A meta-analysis which included all 8 RCTs, comprising a total of 60.080 patients (72.4% having established cardiovascular disease), concluded that GLP-1RA class reduce MACE by 14% (HR = 0.86, 95% CI 0.79-0.94, P = 0.006), with significant effects on the risk of cardiovascular death by 13% (P = 0.016) and nonfatal stroke by 16% (P = 0.007) [22]. Another meta-analysis, which included the same RCTs but ELIXA (the RCT examining lixisenatide), because lixisenatide is a short-acting agent that raised concerns regarding the lack of sustained GLP-1 inhibition in patients, GLP-1 RA proved effective in reducing MACE by 14% (HR 0.86 [95% CI 0.80–0.93]; p<0.0001), cardiovascular death by 13% (HR 0.87 [0.80–0.94]; p=0.0010), stroke by 17% (0.83 [0.76–0.92; p=0.0002), and, in addition to the other meta-analysis, myocardial infarction by 10% (0.90 [0.83–0.98]; p=0.020) [23]. Additionally, both meta-analyses proved a significant 12% reduction in all-cause mortality [22,23].

Table 1: Major CVOTs of GLP-1RA [21] (Although promising results, albiglutide was withdrawn from the market by the manufacturer due to commercial reasons)

TRIAL	ELIXA	LEADER	SUSTAIN-6	EXSCEL	Harmony Outcomes	REWIND	PIONEER-6	AMPLITUDE-O	SELECT
DRUG	***Lixisenatide***	***Liraglutide***	***Semaglutide s.c.***	***Exenatide***	***Albiglutide***	***Dulaglutide***	***Semaglutide oral***	***Efpeglenatide***	***Semaglutide s.c.***
POPULATION	T2DM	T2DM	T2DM	T2DM	T2DM	T2DM	T2DM	T2DM	BMI>27 kg/m^2
MACE HR (95% CI)	1.02** (0.89–1.17)	0.87 (0.78–0.97)	0.74 (0.58–0.95)	0.91 (0.83–1.00)	0.78 (0.68–0.90)	0.88 (0.79–0.99)	0.79 (0.57–1.11)	0.73 (0.58–0.92)	0.80 (0.72–0.90)
CV DEATH HR (95% CI)	0.98 (0.78–1.22)	0.78 (0.66–0.93)	0.98 (0.65–1.48)	0.88 (0.76–1.02)	0.93 (0.73–1.19)	0.91 (0.78–1.06)	0.49 (0.27–0.92)	0.72 (0.50–1.03)	0.85 (0.71–1.01)
MI HR (95% CI)	1.03 (0.87–1.22)	0.86 (0.73–1.00)	0.74 (0.51–1.08)	0.97 (0.85–1.10)	0.75 (0.61–0.90)	0.96 (0.79–1.15)	1.18 (0.73–1.90)	0.78 (0.55–1.10)	0.72 (0.61–0.85)
STROKE HR (95% CI)	1.12 (0.79–1.58)	0.86 (0.71–1.06)	0.61 (0.38–0.99)	0.85 (0.70–1.03)	0.86 (0.66–1.14)	0.76 (0.61–0.95)	0.74 (0.35–1.57)	0.80 (0.48–1.31)	0.93 (0.74–1.15)

Taking into account the various pleiotropic effects of GLP-1, there has emerged the hypothetical idea of using GLP-1RA in individuals without diabetes. In 2023, the results of the SELECT trial were published, a randomized controlled trial that included overweight patients (BMI>27 kg/m^2) with a history of cardiovascular disease but without diabetes. Subcutaneous semaglutide proved efficient, compared to placebo, in reducing MACE by 20% (HR 0.80 [0.72 to 0.90]; p<0.001), mainly driven by a statistically significant reduction of myocardial infarction risk by 28% [24].

These solid results, from large, reputable RCTs, have been translated into guideline recommendations. According to the 2024 ESC Guidelines for the management of chronic coronary syndromes and the 2023 ESC Guidelines for the management of cardiovascular disease in patients with diabetes, GLP-1RAs have a class I (level of evidence A) recommendation in patients with diabetes mellitus type 2 and history of atherosclerotic cardiovascular disease to reduce incident MACE [25,26]. This recommendation stands regardless of the baseline or target HbA1c or any other antidiabetic medications the patient may be taking. Additionally, consistent with the SELECT trial, the 2024 ESC Guidelines for the management of chronic coronary syndromes (CCS) included a IIa (level of evidence B) recommendation for the utilization of subcutaneous semaglutide in overweight (BMI > 27 kg/m^2) CCS patients to mitigate the risk of cardiovascular mortality and myocardial infarction [24,25].

As far as kidney outcomes are concerned, there is an RCT specifically designed to assess renal endpoints. The FLOW trial included 3533 patients with type 2 diabetes mellitus and CKD (defined as eGFR 50-75 ml/min/1.73m^2 and macroalbuminuria UACR >300 mg/g or eGFR 25-50 ml/min/1.73m^2 and albuminuria UACR >100 mg/g) and compared subcutaneous semaglutide with placebo. The results showed that subcutaneous semaglutide reduced the risk of a primary outcome (defined as a composite of major renal adverse events comprising new onset of kidney failure, a reduction of eGFR of at least 50% from baseline, or death from kidney or cardiovascular-related causes) with 24% (HR 0.76; [0.66 to 0.88]; p=0.0003) [27]. There is also some evidence toward improving kidney outcomes emerging from the pivotal CVOTs of GLP-1RA and from the meta-analyses which assessed them, but with a lower degree of statistical power as the conclusions are derived from secondary outcomes or exploratory assessments, and the renal benefits were demonstrated through the reduction in the progression of albuminuria, with fewer effects observed on hard end-points [21].

With regard to heart failure, the aforementioned meta-analyses reported a 10% risk reduction of heart failure hospitalizations, but this result was driven only by the reduction reported in the AMPLITUDE-O trial, which investigated efpeglenatide [21–23]. As a result, this outcome could not be generalized as a class effect.

2 clinical trials specifically investigated GLP-1RA in HFrEF patients: FIGHT and LIVE. Neither of the two trials demonstrated the beneficial effects of GLP-1 receptor agonists in patients with HFrEF. Notably, the LIVE trial even raised concerns regarding a potential trend toward an increased risk of mortality, ventricular arrhythmias, and atrial fibrillation [21,28]. In terms of HFpEF, two complementary RCTs were published, which investigated subcutaneous semaglutide in obese patients (BMI>30 kg/m^2) without DM (STEP-HFpEF) and with DM (STEP-HFpEF DM). In both trials, there are reported similar results: significant reductions in primary end-points such as a reduction of 7.8 points in Kansas City Cardiomyopathy Questionnaire (KCCQ) (95% CI [4.8 to 10.9]; P<0.001) in STEP-HFpEF and a reduction of 7.3 points in KCCQ in STEP-HFpEF DM (95% [CI] [4.1 to 10.4]; P<0.001) and an average of 10.7% reduction in body weight (95% CI [−11.9 to −9.4]; P<0.001) in STEP-HFpEF and 6.4% reduction in body weight (95% CI [−7.6 to −5.2]; P<0.001) in STEP-HFpEF DM. There are also reported significant results concerning secondary end-points, such as an average improvement of 20.3 m at 6-minute walk distance (95% CI [8.6 to 32.1]; P<0.001) in STEP-HFpEF and 14.3 m (95% CI [3.7 to 24.9]; P=0.008] in STEP-HFpEF DM, respectively. Essentially, these two trials proved that semaglutide improves symptoms and exercise capacity in obese HFpEF patients with or without type 2 DM, but neither study was powered enough to evaluate the influence on hard clinical events, such as heart failure-related hospitalizations or urgent hospital visits [29,30]. It has been suggested that the clear weight reduction effect had a major contribution to the improvement of symptoms and exercise capacity in these patients [21].

Tirzepatide has recently emerged as a new star in the GLP-1 modulation field, being the latest discovered molecule in this class. More specifically, tirzepatide is a dual agonist that acts both on the receptors of GLP-1 and the receptors of GIP (glucose-dependent insulinotropic polypeptide). GIP represents another incretin hormone, naturally released by K-cells of the upper small intestine. Tirzepatide was validated as an effective antidiabetic medication (up to 2.4% reduction in HbA1c), as proved in the series of trials SURPASS 1-5, when tirzepatide was subsequently compared to placebo, or to active comparators, such as semaglutide, to which it was shown to be non-inferior or insulin degludec, to which it was shown to be superior in terms of glycemic control [31]. In terms of weight management, in SURMOUNT 1-4 clinical trials, tirzepatide proved a potent effect, with a mean weight loss of up to 26% [32]. In a recent network meta-analysis, compared to GLP-1 RA, tirzepatide proved higher efficacy in terms of glycemic control and weight control, probably owing to the dual incretin action [8]

Besides direct effects in the metabolic pathway, the SUMMIT trial yielded noteworthy findings in both the cardiovascular and, to some extent, the renal pathways of CKM syndrome. It included 731 obese patients (BMI over 30 kg/m^2) with HFpEF (LVEF of at least 50%), regardless of their diabetes status, who were randomized to receive either tirzepatide or placebo. The trial yielded positive results with regard to the both primary end-points: first one representing a composite end-point formed by death from cardiovascular causes or a worsening heart failure event (hazard ratio, 0.62; 95% confidence interval [CI], 0.41 to 0.95; P=0.026), a result mainly driven by a reduction in worsening heart-failure events (hazard ratio, 0.54; 95% CI, 0.34 to 0.85), and the second one a reduction in KCCQ score, with a between-group difference at the end of the study of 6.9 (95% CI [3.3 to 10.6]; P<0.001). There was no difference in

cardiovascular death [33]. Therefore, for the first time, the SUMMIT trial offered evidence derived from an RCT that influencing metabolic pathways with tirzepatide, regardless of diabetes diagnosis, we could influence hard cardiovascular end-points.

An interesting, recently published subanalysis of the SUMMIT trial found that the beneficial effects of tirzepatide upon worsening heart failure events or various metrics pertaining to functional capacity or quality of life are not influenced by the presence or absence of CKD, namely, eGFR does not seem to influence the effects on major adverse heart failure outcomes. In a head-to-head comparison pertaining to the primary composite end-point of death from cardiovascular causes or a worsening heart failure event, tirzepatide showed a larger absolute risk reduction in the CKD group (preventing 3.6 primary endpoint events for 100 patients treated for 1 year) compared to the group without CKD (preventing 1.6 primary endpoint events for 100 patients treated for 1 year). This may probably be an effect at least partially explained by the larger global risk of heart failure events in CKD patients. Moreover, tirzepatide seems to significantly ameliorate glomerular function at 52 weeks (+2.9 [95% CI: 0.9-4.9]; P = 0.004), an effect which proved constant throughout the broad spectrum of baseline eGFR (between 15 ml/min/1.73 m^2 and 70 ml/min/1.73m^2), as well as UACR at 24 weeks (−25%; 95% CI: −35.5% to −12.7%; P < 0.001) [34].

Although we have a very probable class effect of GLP-1RA in reducing MACE and unique evidence for tirzepatide in influencing hard end-points in HFpEF, currently, there is no direct evidence from RCTs that the dual GLP-1R/GIPR agonist reduces MACE. There exists an ongoing trial (SURPASS-CVOT) which currently evaluates the effect of tirzepatide in comparison to dulaglutide on MACE in patients with type 2 diabetes mellitus.

In general, GLP-1RAs are considered relatively well-tolerated medications, and evidence derived from clinical trials supports their effectiveness. However, there are described side effects such as hypersensitivity reactions (including, in rare cases, anaphylactic shock), a hypothetical enhanced risk of C-cell thyroid neoplasm (based on animal studies and theoretical risk), gastrointestinal adverse effects (such as nausea or vomiting, especially during initiation or dose escalation, increased ileus risk), an increased risk of cholelithiasis, cholecystitis and pancreatitis, and concerns of increased pancreatic cancer risk emerging from observational studies. Generally, gastrointestinal adverse effects are the most common and the most frequent cause of drug discontinuation [35].

SGLT2 INHIBITORS

SGLT2 inhibitors were created to target the SGLT2 transporter, a protein mainly located in the first segment of the proximal convoluted tubule, which is responsible for the absorption of 90-97% of the filtered glucose. The reabsorption of one molecule of glucose is coupled with one sodium ion, the latter being transported along the transmembrane concentration gradient created by the Na/K ATP-ase pump [36]. The residual glucose that escapes reabsorption is transported via SGLT1, which is located in the more distal parts of the proximal convoluted tubule. In case of SGLT2 blockage, SGLT1 could partially compensate for its effects, with only 50-60% of the filtered glucose excreted in the urine [37]. Although SGLT2 is mainly located in the kidney, it is also found in the brain, liver, thyroid, and striated muscle [36,37].

The development of SGLT2 inhibitors was inspired by phlorizin, a naturally occurring compound known to be a SGLT2 inhibitor, with the therapeutic objective of promoting natriuresis and glycosuria in order to obtain glycemic control [37]. In the context of efforts to preserve the biologically active properties of phlorizin, while also improving its oral bioavailability and stability, a series of novel molecules were developed: canagliflozin, dapagliflozin, empagliflozin, and ertugliflozin. Moreover, a dual inhibitor that blocks both SGLT1/SGLT2, namely sotagliflozin, was approved, which adds the effect of delaying intestinal glucose absorption by inhibiting SGLT1 at this level [37,38]. All molecules share very attractive pharmacologic properties: optimal oral absorption, once-daily administration as a result of the long half-life, metabolism occurring primarily in the liver, reduced renal clearance, and absence of clinically significant drug interactions [37].

Throughout studies, SGLT2 inhibitors have shown only modest effectiveness with regard to glycemic control, with reductions in HbA1c levels within the range of 0.5-1% [36,39]. Despite continuous glycosuria, inhibition of SGLT2 reduces insulin release and increases glucagon levels, which stimulates gluconeogenesis besides activation of lipolysis and lipid oxidation [40]. Similar to GLP-1 receptor agonists, and constituting an important observation for the largely overweight type 2 diabetes population, it was observed that SGLT2 inhibitors also exert a weight-reducing effect. This effect was observed to be approximately 2 to 4 kg, a consistent and durable reduction documented across all studies and for all molecules. To refer to specific figures, the inhibition of glucose reabsorption at the nephron level causes a loss of 75 g of glucose per day, which means nearly 300 kcal per day. It would be expected that a yearly weight loss of 10 kg, which is much more than was noted in clinical studies [40]. There are proposed mechanisms for this discrepancy between theory and practice, and the most prominent one is compensatory hyperphagia induced by a reduction in leptin, a hormone released by adipose tissue which inhibits appetite at a central level [40,41].

After the finding that rosiglitazone, a thiazolidinedione, is associated with an increased risk of adverse cardiovascular events, the scientific community requested that the new SGLT2i need to undergo cardiovascular outcome trials in order to prove their safety, similar to the aforementioned GLP-1RA.

The EMPA-REG OUTCOME trial tested empagliflozin in over 7000 patients with established cardiovascular disease and proved that this SLGT2i is associated with a significant 14% (HR 0.86; 95% confidence interval [CI], 0.74–0.99; p=0.04) reduction in the risk of major

adverse cardiovascular events, a 32% reduction of all-cause mortality (HR, 0.68; 95% CI, 0.57–0.82]; p<0.001), and a 35% reduction of HF hospitalization risk (HR, 0.65; 95% CI, 0.50–0.85; p=0.002) [42,43]. Similarly, the DECLARE TIMI trial, for dapagliflozin, showed non-inferiority to placebo with respect to MACE (p<0.001) and a reduction with 17% of the risk for cardiovascular death or hospitalization for heart failure (HR, 0.83; 95% CI, 0.73-0.95; p=0.005) [42,44]. The CANVAS trial, for canagliflozin, showed a reduction with 14% of the combined risk of death from cardiovascular causes, nonfatal myocardial infarction, or nonfatal stroke (HR, 0.86; 95% CI, 0.75 to 0.97; P<0.001 for noninferiority; P=0.02 for superiority) [42,45]. Ultimately, the VERTIS trial, which studied ertugliflozin, found a significant reduction in the risk of heart failure hospitalizations [42]. Based on these preliminary studies, the ESC guidelines for the management of heart failure mention a class I indication for the utilization of SGLT2i in diabetic patients at high risk for CV events to reduce the risk of hospitalization for heart failure events, MACE, end-stage renal dysfunction, or cardiovascular death [46].

These initial trials prompted the conduction of future dedicated trials in order to establish a potential role of SGLT2i in the management of heart failure patients.

The further DAPA-HF trial proved to be a pivotal study, which paved the way for the actual large indications of SGLT2i in heart failure with reduced ejection fraction (HFrEF). This trial included 4744 patients with II-IV HYHA class heart failure, with an ejection fraction of less than 40% and proved that dapagliflozin (10 mg single dose) is associated with the reduction of a primary composite outcome of cardiovascular death, HF hospitalizations, and worsening HF (HR, 0.74; 95% CI, 0.65 to 0.85; P<0.001), with all components of this composite outcome being positively and significantly influenced. This trial also found that the effect is not influenced by the diabetic status of the patient [42,47].

For empagliflozin, the EMPEROR-Reduced trial showed similar results: a reduced composite outcome of cardiovascular death and HF hospitalizations (HR, 0.75; 95% CI, 0.65–0.86; p<0.001), similarly independent of the diabetic status of the patient. In the case of empagliflozin, the effect on the primary outcome was mainly driven by the reduction of the rate of HF hospitalizations, as the reduction of cardiovascular death proved to be non-significant [42,48]. A meta-analysis of these 2 landmark trials found significant reductions in all-cause death (pooled HR 0.87, 95% CI 0.77-0.98; p=0.018) and in cardiovascular death (pooled HR 0.86, 0.76-0.98; p=0.027) [49].

These findings have been incorporated into guideline recommendations, as both the ESC guidelines and the ACC/AHA guidelines on heart failure mention dapagliflozin and empagliflozin as indicated in all HFrEF patients, irrespective of their diabetic status, to reduce the risk of heart failure-related death or hospitalization (class I level of recommendation A) [46,50].

Encouraged by the positive results in the HFrEF field and taking into account the lack of available medications with prognostic effects in HFpEF, the idea of investigating the efficacy of these medications in this prevalent HF phenotype has emerged. And initial evidence emerged from the SOLOIST-WHF trial, which evaluated the effects of sotagliflozin, the dual SGLT1/SGLT2 inhibitor, initiated soon after an episode of decompensation of heart failure; sotagliflozin proved a lower risk of a primary composite end-point formed by cardiovascular death or hospitalizations and urgent visits for heart failure (HR, 0.67; 95% confidence interval [CI], 0.52 to 0.85; P<0.001). The result, suggesting a potential effect of SGLT2i in HFpEF, was that the risk reduction of the primary end-point was consistent across the entire spectrum of ejection fraction [51].

Empagliflozin gained evidence for HFpEF and HFmrEF attributable to the results of the EMPEROR-Preserved trial (which included patients with an ejection fraction above 40%). Empagliflozin 10 mg once daily reduced a composite end-point of worsening heart failure or cardiovascular death (hazard ratio, 0.79; 95% confidence interval [CI], 0.69 to 0.90; P<0.001), a result driven by a significant reduction in the risk of worsening heart failure (with no difference in death from cardiovascular causes) [52]. Likewise, in the DELIVER trial (heart failure patients with an ejection fraction above 40%), dapagliflozin 10 mg od reduced the events of a similar primary composite end-point (hazard ratio, 0.82; 95% confidence interval [CI], 0.73 to 0.92; P<0.001). Once again, this effect is attributable to the reduction of the worsening heart failure events, without significant effects on cardiovascular mortality [53]. As a result, clinical guidelines have been harmonized and currently recommend empagliflozin and dapagliflozin (unique dose of 10 mg per day) in HFpEF and HFmrEF for the reduction of the risk of HF hospitalization or cardiovascular death (class I recommendation in European guidelines, class IIa recommendation in American guidelines) [50,54].

Important evidence for using SGLT2i in heart failure derives from the EMPULSE trial, in which empagliflozin was initiated in hospitalized acute heart failure after stabilization, regardless of ejection fraction or diabetes status. The primary analysis was a win ratio, being a composite of death, heart failure events, time to first heart failure event, and modifications in KCCQ. Empagliflozin proved a better win ratio, with a statistically significant result (win ratio 1.36, 95% CI 1.09-1.68, p=0.0054), with consistent results across the prespecified subgroups [55]. Besides this combination of prognostic and symptomatic benefits, empagliflozin also proved significant results on various indices of decongestion, suggesting that early initiation of empagliflozin in hospitalized acute heart failure patients also has the potential to contribute to an early and sustained decongestion [56].

Regarding the benefits of SGLT2 inhibitors in another acute setting—this time early after myocardial infarction—a recently published meta-analysis concluded that early administration of SGLT2i post-myocardial infarction may be beneficial by reducing the risk of recurrent major adverse cardiovascular events (MACE) or hospitalization for heart failure. However, this meta-analysis did not demonstrate a significant reduction in mortality among these patients [57].

Similar to GLP-1RA, SGLT2i is also an effective medication in chronic kidney disease. Based on findings from early studies on cardiovascular safety, specific trials have been designed to directly assess the potential beneficial effects of SGLT2i in CKD. 4 trials, CREDENCE (for canagliflozin), DAPA-CKD (for dapagliflozin), SCORED (for sotagliflozin), and EMPA-KIDNEY (for empagliflozin) proved that SGLT2i slow the progression of CKD. Whereas for canagliflozin and sotagliflozin, the effects were proven only in T2DM populations, in contrast, for empagliflozin and dapagliflozin, the effects were irrespective of the diabetic status, similar to HF trials. It is important to mention that the recommendation is to initiate dapagliflozin at an eGFR of at least 25 ml/min/1.73m^2 and empagliflozin at an eGFR of at least 20 ml/min/1.73m^2, but the administration may continue even if the renal function further deteriorates (this indication derives from the design of the studies and the characteristics of the included patients) [58].

Table 2: Major CVOTs of SGLT2i [42]

	Type 2 diabetes mellitus	Heart failure	Chronic kidney disease
Proved effects	In high-risk cardiovascular patients, it reduces the risk of major adverse cardiac events or cardiovascular death. (Besides improved glycemic control and weight loss)	Reduce the risk of heart failure-related death or hospitalization (across the entire ejection fraction spectrum) Improves decongestion in stabilized acute heart failure (empagliflozin)	Mitigate the progressive reduction of eGFR and lower the risk of renal or cardiovascular-related death
Drugs approved	Canagliflozin, Dapagliflozin, Empagliflozin, Ertugliflozin	Dapagliflozin, empagliflozin	Canagliflozin, Dapagliflozin, Empagliflozin
Guideline recommendations	Recommended as first-line add-on therapy to metformin, especially in cardiovascular high-risk, heart failure, or CKD patients	Class I recommendation in HFrEF Class I recommendation in ESC guidelines, class IIa recommendation in ACC/AHA guidelines for HFpEF	T2DM and CKD with eGFR>20 ml/min/1.73m^2 ACR>200 mg/g (and eGFR>20 ml/min/1.73m^2) ACR<200 mg/g with eGFR 20-45 ml/min/1.73m^2 CKD + heart failure

MINERALOCORTICOID RECEPTOR ANTAGONISTS (MRAs)

It is known that overactivation of the renin-angiotensin-aldosterone system and aldosterone receptors (mineralocorticoid receptors, MR) in particular, has effects beyond salt and water retention and hypertension. MR overactivation is capable of inducing inflammation and fibrosis in various organs, being involved in CKD and CV disease progression, situations in which RAAS is overly activated and aldosterone is in excess [73]. As far as metabolic derangement is involved, diabetes mellitus also induces a state of overactivation of MRs, and in obese patients there was observed a significant aldosterone production from adipose tissue was observed [74,75].

The deleterious effects of excess aldosterone are facilitated by the widespread expression of mineralocorticoid receptors throughout the body, as they are present not only in the kidneys but also in the heart, blood vessels, gastrointestinal tract, adipose tissue, and central nervous system [73].

The first therapeutic options to block MRs were the steroidal agents spironolactone and eplerenone, respectively.

The first results for these agents came from RCTs, which tested them in HFrEF, a common entity in advanced stages of cardio-renal-metabolic syndrome. In RALES trial, conducted in 1999, spironolactone reduced all-cause mortality by 30% (RR, 0.70; 95% CI, 0.60 to 0.82; P<0.001) and the risk of heart failure hospitalization by 35% (RR 0.65; 95% CI, 0.54 to 0.77; P<0.001), besides observed benefits on improvement regarding the symptoms of heart failure [76]. Eplerenone gained evidence from the EMPHASIS-HF trial, which proved a significant reduction of the primary composite endpoint of CV death or heart failure hospitalization (HR, 0.63; 95% CI, 0.54 to 0.74; P<0.001) [77]. Further trials provided evidence for steroidal MRAs, namely eplerenone, early after myocardial infarction. EPHESUS and REMINDER trials found that eplerenone, early initiated after MI, in patients with clinical signs and symptoms of heart failure and an ejection fraction of less than 40% could reduce all-cause mortality, length of hospitalization due to heart failure, ventricular arrhythmias, or the risk for deterioration of ejection fraction [78,79]. The beneficial effects of MR blockade in HFrEF and after myocardial infarction emphasize the important effects of aldosterone in cardiac adverse remodelling.

Regarding the HFmrEF and HFpEF, the available results are not as solid as in HFrEF. In the TOPCAT trial, 3,445 patients with symptomatic heart failure and a left ventricular ejection fraction of ≥45% were randomized to receive either spironolactone or placebo. Although the study narrowly failed to meet its primary composite endpoint (including cardiovascular death, aborted cardiac arrest, and hospitalization for heart failure), its results generated considerable debate [80]. The difference between a significant HR in American cohort (HR, 0.82; 95% CI, 0.69-0.98; p = 0.026) and a non-significant HR in Russian and Georgian cohort (HR, 1.10; 95% CI, 0.79-1.51; interaction p = 0.12) has led to speculation that the apparent neutral results of the TOPCAT trial may be due to issues

with enrollment and drug adherence in the Eastern European cohort [81]. Based on the significant results on American cohort, there is a 2b recommendation in ACC/AHA guidelines on heart failure for steroidal MRA use in HFpEF and HFmrEF [50].

Hypertension in general and resistant hypertension in particular are common entities in chronic kidney patients or in obese, insulin-intolerant, or diabetic patients. Resistant hypertension is associated with a poor prognosis, causing target organ damage. The PATHWAY-2 RCT established spironolactone as a first-line agent in resistant hypertension, on top of maximum tolerated doses of ACEI/ARB, calcium-channel blocker, and thiazide diuretic [82].

The main limitation of steroidal MRAs' use is the risk of hyperkalemia, a risk observed since the early trials and considered relatively significant, particularly in a population with chronic kidney disease. A meta-analysis that included the landmark RCTs of steroidal MRAs proved that the risk of hyperkalemia and the benefits are inversely proportional to the eGFR (with unclear benefits compared to high risk of hyperkalemia in patients with an eGFR<30 ml/min/1.73m^2) [83].

Gynecomastia and breast pain are other well-known and relatively frequent adverse effects of spironolactone (with a 10% to 1% ratio observed in the RALES trial) [73].

There is a limitation in the steroidal MRAs' potential for broader cardiorenal protection, given the fact that their widespread use is limited by hormonal adverse effects or, in advanced CKD, by a significant risk of hyperkalemia. Moreover, data regarding the direct effects of steroidal MRAs on slowing CKD are unconvincing. There is a meta-analysis that proposes some benefits on intermediate endpoints such as albuminuria or eGFR slope, albeit at the cost of significant risk of hyperkalemia, worsening renal function, or gynecomastia [84].

The novel class of non-steroidal mineralocorticoid receptor antagonists (nsMRAs) has emerged in an effort to overcome these limitations while preserving the beneficial effects of the steroidal MRAs.

Finerenone is the most prominent agent of this pharmacological class, with several advantages over its steroidal counterparts: a comparable potency of MR inhibition but with a much higher selectivity, virtually no sexual side-effects, a more balanced tissue distribution between kidneys and the heart (in comparison with sMRA which are more concentrated at the renal level) and a shorter plasma half-time, which might contribute to a lower risk of hyperkalemia [85,86].

The first RCTs investigating finerenone were FIDELIO-DKD and FIGARO-DKD. FIDELIO-DKD primarily enrolled diabetic patients suffering from advanced CKD with prominent albuminuria, and there was a prespecified primary composite kidney endpoint. FIGARO-DKD enrolled diabetic patients with moderate albuminuria associated with a wider spectrum of CKD and stated a primary composite cardiovascular endpoint [85]. The results from these 2 RCTs and from their pooled analysis (FIDELITY) form the basis for the KDIGO guideline recommendation of finerenone in CKD: in patients with diabetic nephropathy, with an eGFR>25 ml/min/1.73 m^2 and with an albuminuria >30 mg/g, despite a maximum dose of ACEI/ARB [72]. Apart from reducing the kidney disease progression, finerenone reduced cardiovascular risk (HR 0.86, 95% CI: 0.78-0.95) and the risk of heart failure hospitalization (HR 0.78, 95% CI 0.66-0.92) in diabetic CKD patients [86].

The latter FINEARTS-HF trial provides the first quality results for MRAs (particularly nsMRA, finerenone) in HF with EF above 40%. In this RCT, finerenone reduced the risk of a primary composite endpoint formed by worsening heart failure events and cardiovascular death with 16% (rate ratio, 0.84; 95% confidence interval [CI], 0.74 to 0.95; P=0.007), driven by a significant reduction in worsening heart failure events, similar to SGLT2i results in the same patient population [87]. Compared to the previous ones, FINEHEARTS was a revolutionary trial also by the population included, with approximately 60% not having CKD or diabetes, suggesting that there may be important direct effects on the heart [88].

The recent FINE-HEART meta-analysis allows for the formulation of global conclusions regarding finerenone as far as cardiovascular and renal outcomes are concerned. This meta-analysis included the three RCTs, FIDELIO-DKD, FIGARO-DKD, and FINEARTS-HF, encompassing a total of 18991 patients. 12.1% of the included patients concurrently had HF, CKD, and diabetes, the main conditions encompassed in the CKM syndrome. The results indicate that finerenone reduces the risk of heart failure hospitalization (HR: 0.83; 95% CI: 0.75-0.92; P < 0.001) and of a composite kidney outcome, thus delaying CKD progression (HR: 0.80; 95% CI: 0.72-0.90; P < 0.001). Although not succeeding in proving risk reductions in cardiovascular death (HR 0.89; 95% CI: 0.78-1.01; P = 0.076), finerenone seems to reduce the risk of all-cause death (HR: 0.91; 95% CI: 0.84-0.99; P = 0.027) [88].

Currently, the sole recommendation for finerenone within cardiology guidelines is the class IA recommendation in the 2023 ESC Guidelines for the management of cardiovascular disease in patients with diabetes, in patients with type 2 DM and CKD (UACR>300 mg/g or eGFR 25-60 ml/min/1.73m^2 and UACR>30 mg/g), in addition to ACE-I or ARB, to reduce the risk of cardiovascular events or kidney failure [26].

This recommendation, similar to those related to SGLT2i or GLP-1RA, empowers all medical doctors involved in the management of CKM syndrome to take proactive measures aimed at improving the prognosis of these patients. It remains to be seen what future heart failure guidelines will unveil regarding nsMRA indications, probably related to future trials that may investigate the effects of finerenone in this population.

CONCLUSION

Management of cardiovascular disease within the cardiorenal metabolic (CKM) syndrome requires a comprehensive, individualized approach, guided by cardiovascular risk stratification. While lifestyle interventions remain foundational, pharmacologic therapy plays a pivotal role in improving prognosis and quality of life.

Standard treatments such as RAAS blockade—with ACE inhibitors, ARBs, and steroidal MRAs—form the therapeutic backbone. However, recent advances support the early initiation of novel agents with proven benefits across all three CKM components.

GLP-1 receptor agonists and dual GLP-1/GIP agonists (e.g., tirzepatide) not only promote glycemic control and weight reduction but also lower the risk of major adverse cardiovascular events and may preserve renal function. SGLT2 inhibitors have demonstrated robust prognostic benefits in heart failure—regardless of ejection fraction—and chronic kidney disease, independent of glycemic status. Finerenone, a non-steroidal mineralocorticoid receptor antagonist, is recommended in diabetic kidney disease with albuminuria and shows emerging evidence of efficacy in HFpEF and HFmrEF, although formal guideline recommendations are still pending.

Optimizing therapeutic strategies with both established and novel agents is essential for altering the course of heart disease in CKM syndrome and addressing its complex, multisystem pathophysiology.

Conflicts of interest and sources of funding

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Authors' contribution

Conceptualization, A.G.F., S.H. and S.M.S., methodology, M.J..; formal analysis, M.F.; data curation, E.R.; writing—original draft preparation, A.G.F., S.A.P.; writing—review and editing, E.C., R.D.G., M.F., E.R.; visualization, R.D.G.; supervision, S.M.S.; project administration, S.H. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

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ORIGINAL ARTICLE

Investigating Digital Skills of Healthcare Professionals in Greek Military and Civil Hospitals: A Cross-Sectional Study

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Abstract: Background and Aim: Digital skills of healthcare professionals are recognized as critical for the successful digital transformation of healthcare. The present study aims to examine the relationship between the digital skills of healthcare professionals and the organizational structures of hospitals. Methods: In this cross-sectional study, using the convenience sampling method, 857 healthcare professionals, working in Greek military and civilian hospitals, voluntarily participated. Data were collected between July and November 2021 through a structured questionnaire, based on the Digital Competence framework. Digital skills were categorized into five subscales: information, communication, content creation, problem solving, and safety skills. Statistical analysis was performed with SPSS 26.0, with p-values <0.05 considered statistically significant. Results: Cronbach's alpha values for all subscales were (α)>0.8, indicating high reliability. Younger participants showed higher scores (p<0.001). The mean scores for information, communication, content creation, problem solving and safety skills in military hospitals were calculated as 52.1 (SD=32.3), 50.6 (SD=31.8), 48.4 (SD=33.3), 49.8 (SD =30.8) and 52.8 (SD=34.1) respectively, while in civilian hospitals they were calculated as 45.1 (SD=32.2), 44.5 (SD=29.5), 41.8 (SD=32.4), 41.5 (SD=30.8) and 47.1 (SD=34.5) respectively. Conclusions: Healthcare professionals in military hospitals exhibit higher digital competence compared to their counterparts in civilian hospitals. Identifying the organizational factors that promote greater digital competence among healthcare professionals provides essential information to policymakers and healthcare managers for implementing targeted organizational improvements.

Keywords: Digital skills, Healthcare professionals, Military hospitals, Digital Competence framework

INTRODUCTION

The management of competencies and skills in the healthcare sector has drawn significant attention from international organizations, recognizing that a well-trained workforce is essential for ensuring patient safety and delivering high-quality care [1,2]. In alignment with the continuous advancements in medical technology, the necessity for healthcare professionals to develop new skills has become increasingly urgent [3,4]. This need was starkly highlighted during the recent COVID-19 pandemic, where the heightened digital demands of healthcare services underscored the importance of sufficient digital capabilities [5,6].

In particular, digital skills are deemed critical not only for the effective management of healthcare organizations but also for medical diagnosis, treatment monitoring, patient care, and decision-making processes [7,8]. Within the healthcare domain, digital competence encompasses knowledge of digital literacy and technology as essential tools for the patient-centered provision of quality healthcare services. It reflects the dynamic interaction between healthcare professionals, patients, and colleagues, as well as the drive to acquire professional expertise in digital technology [9,10].

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It is widely recognized that digital competencies are essential for the effective integration of health technologies into routine clinical practice. Numerous processes are now carried out digitally, such as patient monitoring, where electronic health records and vital sign monitoring systems enable doctors and nurses to track patient status in real time. Material procurement is also facilitated through electronic systems, enhancing both accuracy and efficiency. Furthermore, telemedicine plays a pivotal role, particularly in military hospitals, by enabling the delivery of healthcare services to remote areas and military bases. Digital tools further support decision-making by allowing healthcare professionals to analyze data and make evidence-based decisions more efficiently [3,11,12].

In Greece, military hospitals offer more structured training programs for digital skills, supported by military frameworks that emphasize continuous professional development of their personnel. These programs encompass modules on the management of digital tools, telemedicine, and electronic patient record systems, reflecting the military's long-standing tradition of structured training and lifelong education. Additionally, military healthcare professionals are often encouraged to engage in e-learning programs to further enhance their expertise in healthcare technologies.

Conversely, in public hospitals, training programs for digital skills vary depending on the institution and the extent of technological integration. Significant advancements have been observed in large hospitals that have adopted electronic medical records; however, training opportunities remain limited due to resource constraints and time pressures. Healthcare professionals, including nurses and doctors, typically receive training through Ministry of Health initiatives or European-funded programs such as eHealth and Horizon 2020 [9,10].

Despite extensive research on digital skills, a literature review from the past decade indicates a scarcity of studies that examine the digital competencies of healthcare professionals within a specific and well-defined conceptual framework. One such framework is the European Digital Competence (DigComp) Framework, created by the European Union as a common umbrella of digital competences for its citizens, which defines digital competence domains, indicators for each domain, competence levels, examples of knowledge, skills, and attitudes, as well as examples of application [13]. Moreover, limited research has explored the associations between digital skills and the organizational work environment. To the best of our knowledge, no prior studies have investigated differences in this domain between military and civilian healthcare professionals.

Therefore, the objective of this paper is to address this research gap by examining the relationship between digital skills of healthcare professionals and the organizational structures of their work environments. We aim to contribute evidence on the organizational factors influencing healthcare professionals' digital competencies, which can inform policymakers and healthcare managers' efforts to implement targeted organizational improvements.

MATERIALS AND METHODS

Study Design

A cross-sectional survey utilizing a descriptive study design was conducted based on prior research, which demonstrated that the Digital Competence Indicator tool is a reliable and valid measurement scale applicable to Greek doctors and nurses [14]. The study addressed two primary research questions: (1) How are healthcare professionals' digital skills assessed? and (2) Are there differences in the digital skills of healthcare professionals between military and civilian hospitals?

The present study was conducted as part of the research activities of the Department of Nursing at the Hellenic Mediterranean University and received ethical approval from the Review Committee (Protocol No.: 4634/29-03-2021). Additionally, the necessary permissions were obtained from the Scientific Committees of all participating hospitals (Protocol No.: 5584/1973/26-06-2021). Data were collected using a self-assessment questionnaire, with the principal investigator maintaining sole access to the collected data. Participation was entirely voluntary, and all participants were informed both verbally and via an information/consent form that completing and returning the questionnaire constituted their consent to participate in the study. The information/consent form was approved by the above Scientific Committees. The research adhered strictly to the European Union's General Data Protection Regulation, ensuring full compliance with personal data protection standards.

Participants and settings

A convenience sample of doctors and registered nurses was selected from seven tertiary care hospitals in Greece. This particular sampling method was preferred because it offers time and cost savings, ease of access, and an abundance of qualitative data. However, it carries the risk of bias and systematic errors, and therefore, research findings cannot be accurately generalized beyond the study sample. The selection of hospitals was based on the diversity of organizational structures between military and civilian institutions, as well as the aim of achieving a large sample size. The study population size was calculated using a 95% confidence interval and a 5% margin of error, ensuring that the final sample accurately represented healthcare professionals in Greek hospitals. The target sample size was N>800. A total of 1207 questionnaires were distributed, and 876 were collected, of which 19 were invalid due to incomplete responses. Thus, the study population is N=857.

The inclusion criteria for participation were employment in either a military or civilian hospital, professional status as a doctor or nurse, and, for nurses, registration as per the European Qualifications Framework (EQF). The distribution of questionnaires was proportional to the size of each hospital, based on the number of medical and nursing staff within the institutions. Data collection

occurred during staff meetings, where participants from various work positions within the hospitals were approached, provided with explanations of the study objectives, and given clarifications on completing the questionnaire. Data collection was conducted between July and November 2021.

Research tool

Data were collected using a validated 37-item paper-based questionnaire. It is built on the European DigComp Framework, which comprehensively defines the elements of digital competence as a combination of skills, abilities and attitudes essential for incorporating data processing into the professional careers of healthcare providers [13,15].

The questionnaire consisted of two parts. The first part captured demographic characteristics, including gender, age, educational level, profession, specialty, hospital type, professional experience, administrative responsibilities, and possession of digital skills certification. The second part assessed 26 digital skills, organized into five competency domains (subscales): information, communication, content creation, problem-solving, and safety skills (Table 1).

Table 1: Competence area and digital skills of the questionnaire. Adapted from the European Commission's DigComp framework [13]

Competence Area	Digital Skills
Information Skills	1. Finding information about goods and services
	2. Obtaining information from public authority websites
	3. Reading or downloading online news/newspapers/news magazines
	4. Copying or moving a file or folder
	5. Seeking health-related information
Communication Skills	6. Sending/receiving emails
	7. Telephoning over the internet/video calls (via webcam) over the internet
	8. Participating in social networks
	9. Posting messages to chat sites
	10. Uploading self-created content to any website to be shared
Content Creation Skills	11. Creating websites or blogs
	12. Writing a computer programme using a specialized programming language
	13. Using copy and paste tools to duplicate or move information within a document
	14. Creating electronic presentations with presentation software (e.g., slides), including e.g., images, sound, video, or charts
	15. Using basic arithmetic formulae to add, subtract, multiply, or divide figures in a spreadsheet
Problem-Solving Skills	16. Connecting and installing new devices
	17. Installing a new or replacing an old operating system
	18. Modifying or verifying the configuration parameters of software applications
	19. Doing an online course
	20. Buying or ordering goods or services for private use (last 12 months) over the internet
	21. Selling online
	22. Job search or sending an application
	23. Internet banking
	24. Making an appointment with a practitioner via a website
Safety Skills	25. Using any kind of IT security software or tool (anti-virus, anti-spam, firewall, etc.) in order to protect private computers and data
	26. Updating one or more security products at least occasionally

Subscale values were calculated on a scale ranging from 0 to 100, with scores approaching 0 reflecting a more negative evaluation of the variable and scores approaching 100 reflecting a more positive assessment.

The questionnaire provided a concise yet compelling assessment of the Information Technology (IT) skills of hospital doctors and nurses. Designed for ease of use and brevity, it was particularly suitable for hospital settings often characterized by high workloads and limited time for activities outside core medical and nursing responsibilities. Participants rated the extent of their computer usage for each activity on a five-point Likert scale, ranging from 1 ("not at all") to 5 ("very much").

Data Analysis

Statistical analysis was performed using SPSS (Statistical Package for Social Sciences) version 26.0. Continuous variables were expressed as mean ± standard deviation (SD) and categorical variables were presented as absolute numbers (n) and percentages (n%). The normality of variables was assessed using the Shapiro-Wilks test, alongside graphical methods such as the Normal Q-Q plot, Detrended Normal Q-Q plot, and Box Plot. For all statistical tests, differences were considered significant at $p < 0.05$. Effect sizes weren't reported. The independent t-test was employed to compare the means of two groups, while one-way analysis of variance (ANOVA) was used for comparisons involving more than two group means.

RESULTS

Participant's characteristics

The study population comprised 857 Greek healthcare professionals employed across seven tertiary care hospitals. The results indicate that 538 participants (62.8%) were female, while 319 (37.2%) were male, with a mean age of 39.7 years (SD = 10.0). Of the total, 657 participants worked in military hospitals, while 200 worked in civilian hospitals. Indicatively, it is reported that the number of military doctors and nurses in hospitals, at the present time, amounts to approximately 1,700 people.

Specifically, among the military hospitals, 384 participants were from the 251 Hellenic Air Force General Hospital, 110 from the 401 General Military Hospital of Athens, 66 from the Athens Naval Hospital, and 97 from the General Military Training Hospital. Among civilian hospitals, 82 participants were from the Sismanoglio General Hospital of Attika, 51 from the Asklepieio Voulas General Hospital, and 67 from the Henry Dunant Hospital Center.

The majority of participants were registered nurses (70.2%), with doctors comprising 29.8% of the sample. Considering the nature of the study population, where nurses outnumber doctors by more than twice, the preponderance of the female gender can be explained by the fact that the nursing profession in Greece has traditionally been considered female-dominated for many years. The average professional experience was estimated at 15.3 years. Regarding educational levels, 528 participants (61.6%) held a Bachelor of Science (BSc) degree, 275 (32.1%) had also completed a Master's degree, and 54 (6.3%) held a PhD degree. Examining the level of education by profession, doctors have a higher proportion of PhD holders (5.4%) than nurses (0.9%). Conversely, nurses have a higher percentage of master's degree holders (22.8%) than doctors (9.3%). The demographic characteristics of the study population are summarized in Table 2.

Table 2: Demographic characteristics of participants (N = 857).

		Hospital				**Total**		
		Civilian hospital		***Military hospital***				
		N	N %	N	N %	N	N %	p-value
Age (years)*		40.6 (9.9)		39.4 (10)		39.7 (10)		0.143
Gender	Female	134	67.0%	404	61.5%	538	62.8%	0.158
	Male	66	33.0%	253	38.5%	319	37.2%	
Educational level	BSc	136	68.0%	392	59.7%	528	61.6%	0.092
	MSc	52	26.0%	223	33.9%	275	32.1%	
	PhD	12	6.0%	42	6.4%	54	6.3%	
Profession	Medical Doctor	53	26.5%	202	30.7%	255	29.8%	0.250
	Registered Nurse	147	73.5%	455	69.3%	602	70.2%	

**Note. Age expressed as mean (standard deviation)*

Digital Skills

Regarding digital skills, the results are overall. The mean scores of all digital skills subscales indicate an average level and are as follows: information skills, 52.1 (SD = 32.3); communication skills, 50.6 (SD = 31.8); content creation skills, 48.4 (SD = 33.3); problem-solving skills, 49.8 (SD = 30.8); and safety skills, 52.8 (SD = 34.1). Median scores, interquartile ranges (IQR), and p-values of the above subscales are presented in Table 3.

The reliability of the subscales was confirmed, with Cronbach's alpha values indicating high internal consistency: information skills (5 items; $\alpha = 0.95$), communication skills (5 items; $\alpha = 0.95$), content creation skills (5 items; $\alpha = 0.95$), problem-solving skills (9 items; $\alpha = 0.97$) and safety skills (2 items; $\alpha = 0.92$). Cronbach's alpha was calculated by group. It is worth noting that the high Cronbach's alpha values (>0.9) observed in the study results are likely due to the narrowness of the aspect structure of the measurements' tool subscales. However, the measurement scale was taken as an existing structure from the European DigComp Framework and was not developed by the researchers. Future survey to explore the factor structure by removing some potentially redundant items or adding

new ones is a challenge for the present investigators.

Table 3: Median scores, IQR, and p-values of subscales (information skills, communication skills, content creation skills, problem-solving skills, safety skills) per type of hospital

		Hospital		
		Civilian hospital	**Military hospital**	**Total**
Information skills	Median	45.00	60.00	55.00
	IQR	63.75	60.00	60.00
	p-value	<0.001		
Communication skills	Median	40.00	55.00	50.00
	IQR	50.00	65.00	60.00
	p-value	0.002		
Content creation skills	Median	40.00	50.00	50.00
	IQR	60.00	67.50	65.00
	p-value	0.001		
Problem-solving skills	Median	30.60	52.80	50.00
	IQR	58.30	52.80	55.60
	p-value	<0.001		
Safety skills	Median	37.50	62.50	62.50
	IQR	71.88	62.50	62.50
	p-value	0.007		

The mean scores across all five subscales of the measurement tool were found to differ significantly between military and civilian hospitals. Specifically, the mean score for the "Information skills" subscale in military hospitals was M = 54.2 (SD = 32.1), compared to M = 45.1 (SD = 32.2) in civilian hospitals, with $t(855) = -3.522$, $p < 0.001$. For the "Communication skills" subscale, the mean score in military hospitals was M = 52.4 (SD = 32.3), while in civilian hospitals it was M = 44.5 (SD = 29.5), with $t(855) = -3.088$, $p = 0.002$.

Similarly, the "Content creation skills" subscale showed a mean score of M = 50.4 (SD = 33.3) in military hospitals versus M = 41.8 (SD = 32.4) in civilian hospitals, with $t(855) = -3.236$, $p = 0.001$. For "Problem-solving skills," the mean score in military hospitals was M = 52.4 (SD = 30.4), compared to M = 41.5 (SD = 30.8) in civilian hospitals, with $t(855) = -4.395$, $p < 0.001$. Lastly, the "Safety skills" subscale yielded a mean score of M = 54.6 (SD = 33.8) in military hospitals and M = 47.1 (SD = 34.5) in civilian hospitals, with $t(855) = -2.740$, $p = 0.006$.

The mean values (SD) of digital skills (information, communication, content creation, problem-solving, and safety skills) by hospital type are illustrated in Figure 1.

DISCUSSION

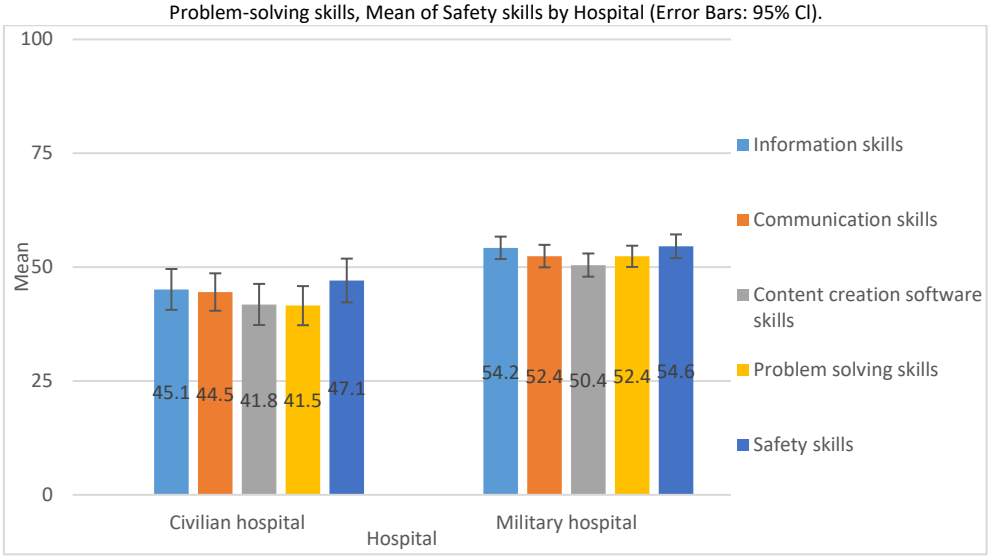
Based on the theoretical framework outlined, the findings of this study align with existing research emphasizing the growing importance of digital skills for healthcare professionals. Digital competence is essential not only for the effective delivery of healthcare services but also for ensuring patient safety and enhancing the overall efficiency of healthcare systems [16,17]. These competencies enable healthcare professionals to manage patient data effectively, navigate electronic health records [18,19], process large volumes of information[20], and utilize telemedicine tools [21,22]—capabilities that are no longer optional, but essential and fundamental to healthcare professionals' expertise [10,23,24].

In the present study, digital skills of the Greek study population can be characterized as good, as they are proven to be above the median level, with safety and information skills gathering the highest averages, communication and problem-solving skills being at the median level, and content creation skills being the weakest, with a score just below the median level. It is noteworthy that content creation skills require particular IT knowledge and technical skills, such as developing or maintaining computer systems, hardware, or software, and writing a computer program using a specialized programming language. Thus, they appear as the digital skills category with the lowest proficiency rates in the majority of occupations in Europe [25].

The above levels of digital skills are in line with a similar Greek study, where the digital competence of healthcare professionals is moderate to good [26]. Research conducted on healthcare professionals in European countries shows that the majority have medium levels of digital competence. A study carried out in Moldova found advanced levels of digital skills in the areas of information and communication and moderate levels in the areas of content creation, safety, and problem solving[6]. In Finland, the levels of digital

competence are relatively high[27], while in some regions in Finland and Sweden, digital skills are reported as adequate but with several shortcomings[16] and in Catalonia, they appear at a basic and intermediate level [28]. According to the European Centre for the Development of Vocational Training (Cedefop), in terms of digital intensity measurements in the EU for 2021, the level of digital skills for health professionals appears as basic to intermediate, and only in a percentage of 13.4% as advanced [29].

Figure 1: Clustered Bar Mean (95% CI) of Information skills, Mean of Communication skills, Mean of Content creation software skills, Mean of Problem-solving skills, Mean of Safety skills by Hospital (Error Bars: 95% CI).



Consistent with prior research, this study also revealed that younger healthcare professionals demonstrated higher levels of digital competence, particularly in domains such as communication and content creation. Literature suggests that younger professionals tend to exhibit greater eHealth literacy and are more adept at using digital tools [30,31]. This generational disparity in digital skills underscores the need for targeted interventions that provide older healthcare professionals with the necessary training and support to enhance their digital proficiency. Hospital management can organize continuing education programs that are easily accessible and tailored explicitly to older professionals, such as online courses, interactive sessions, or specially designed workplace courses.

It is worth noting that, in the measurement tool, all possible demographic confounding factors were included to avoid biased conclusions. According to the literature review, the demographic factors found to be associated with digital skills levels were age, type of occupation, gender, professional experience, and educational level, which were studied in our research [30,32,33]. However, the results of this study found no significant differences in digital skills based on gender, professional experience, specialty, or educational level.

In addition, research underscores the critical role of the working environment in shaping digital literacy. For instance, De Veer and Francke (2010) observed that attitudes toward electronic health records varied significantly depending on the type of healthcare organization [11]. Similarly, Jarva et al. (2023) as well as Rippen et al. (2013), emphasized that strong organizational support—through timely interventions and the provision of adequate resources—is essential for the successful adoption of digital technologies by healthcare professionals [9,34]. Similarly, the findings of the present survey revealed significant differences in digital skills between military and civilian hospitals, with the predominance of health professionals in military hospitals.

This superiority can be attributed to several factors, including the structured nature of military institutions, where digital training and protocols are more systematically integrated into daily operations [35,36]. Military hospitals often place a stronger emphasis on technical proficiency and operational readiness [37,38], which likely fosters the development of higher digital literacy among their staff. Moreover, they prioritize digital training for their personnel as part of their operational strategies and compliance with applicable protocols. In contrast, civilian hospitals may face a broader array of challenges, including inconsistent administrative policies and slower technology adoption due to resource constraints [35,39].

In Greece, in recent years, the Ministry of Digital Governance, in coordination with the Ministry of Health and other bodies, has been working to strengthen the digital competence of doctors and nurses through the implementation of a national e-health strategy. Among the objectives are to improve the continuous education of health professionals and to ensure access to reliable digital information and tools [40]. However, training programs for civilian health professionals are usually their personal responsibility, as organizational procedures are inadequate. In contrast, in military environments, such programs are supported by the respective military education sectors, financed with their own resources and means, and organized on a regular and periodic basis for the training of all their personnel.

The operation of military hospitals, with adherence to hierarchical procedures, is a significant factor in their ability to implement and

train staff in digital technologies efficiently. These institutions often adopt new systems at a faster pace, which may explain the consistently higher levels of digital proficiency observed among healthcare personnel in military settings. Such an organizational advantage aligns with the findings of Ludwick and Doucette (2009), who emphasized that organizational readiness and robust support systems are critical to the successful implementation of electronic health records [41].

Furthermore, the constant exposure to technology [36,42] and digital tools [43,44] observed in military settings provides healthcare professionals with more opportunities to enhance their digital skills. In contrast, civilian hospitals face a variety of challenges that may hinder the development of such competencies. In Greece, these challenges include diverse administrative structures, slower decision-making processes, and limited access to resources for digital training [45,46]. The inherent flexibility within civilian hospital operations may further contribute to inconsistencies in the application and development of digital skills among healthcare professionals. This discrepancy could reflect differences in organizational priorities and the availability of technological resources within these institutions.

A noteworthy area where military healthcare professionals excel is in safety skills, likely due to the stringent data protection and cybersecurity protocols mandated within military environments [47,48]. This focus on safeguarding sensitive patient and operational data, cultivates a more advanced understanding of cybersecurity measures among military hospital staff. Although civilian hospitals also prioritize data security, they often encounter greater challenges in implementing uniform protocols due to variations in organizational structure and resource constraints.

Another significant disparity lies in problem-solving skills. Military healthcare professionals appear to be better equipped to handle technical challenges, potentially due to the emphasis on problem-solving as an integral part of military training and operational effectiveness [49]. In contrast, the less regimented procedures commonly found in civilian hospital settings may provide healthcare professionals with fewer structured opportunities to develop and refine these skills.

We consider that the Ministry of Health or the EU frameworks could adopt military-style training modules emphasizing techniques such as extensive practical training, simulation exercises, the development of specialized centers of expertise, field training techniques, the use of existing training frameworks, needs- and context-based design, continuous harmonization with modern standards, and synergies between civilian and military professionals. One such model is the Common Security and Defence Policy (CSDP) Training Programme, an EU policy to provide senior civil and military officers with the necessary skills and knowledge in crisis management situations [50].

Future Implications

We believe that civil-military cooperation, by embracing strengths and avoiding weaknesses, could improve the overall digital capability of health professionals. As military hospitals provide a more conducive environment for developing digital skills, civilian hospitals could benefit from adopting some of the structured approaches used in military hospitals. With the continuous evolution of healthcare technology, it is essential that all healthcare professionals, irrespective of their work environment, are supported in acquiring and maintaining high levels of digital competence.

Digital skills among healthcare professionals represent a burgeoning field of research that has experienced rapid growth over the past decade. The findings of this study highlight the need to explore further the role of institutional support in fostering digital skills, as well as the factors contributing to digital competence gaps among healthcare professionals across different organizational settings. These results hold international relevance, given the global significance of digital transformation in the healthcare sector. With additional research conducted in similar settings across various countries, these findings could be generalized to further advance digital literacy and transformation in healthcare systems worldwide.

Limitations

This study is subject to three key limitations that may affect the generalizability of the findings. First, the participating nurses were all registered nurses (European Qualifications Framework - EQF 6) and not practicing nurses (EQF 5), and therefore, the measurement tool is validated and reliable only for registered nurses. Second, a self-assessment questionnaire was used as a measurement tool, which entails the risk of subjectivity due to overestimation or underestimation of the actual skills of the participants. Finally, the convenience sampling approach carries the risk of bias and systematic errors.

CONCLUSION

This study underscores the critical importance of digital skills among healthcare professionals, drawing attention to notable differences between staff in military and civilian hospitals. The findings indicate that military healthcare professionals generally demonstrate higher levels of digital competence, likely attributable to the structured and resource-rich environments characteristic of military hospitals. These organizational factors, which foster a more digitally skilled workforce, serve as a foundation for identifying and implementing necessary organizational improvements that healthcare managers can leverage.

However, it is important to note that both military and civilian healthcare professionals exhibited moderate levels of digital skills overall, signifying substantial room for improvement across both settings. Strengthening digital competence across the healthcare sector remains essential for meeting the demands of modern healthcare systems.

Conflicts of interest and sources of funding

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Authors' contribution

Conceptualization, A.K. and C.M.; methodology A.K. and C.M.; software, A.K. and C.C.; validation, A.K. and C.M.; formal analysis, C.C. and V.A.K.; investigation, A.K., D.K., V.A.K., M.M., V.T. and C.M.; resources, A.K. and C.M.; data curation, A.K., D.K., C.C. and C.M.; writing—original draft preparation, A.K.; writing—review and editing, A.K., D.K., C.C., V.A.K., M.M., V.T. and C.M.; visualization, A.K.; supervision, C.M., D.K. and C.C.; project administration, A.K., C.C. and C.M. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki and approved by the Hellenic Mediterranean University Ethics Committee (Nursing Department's Executive Board 4634/29-03-2021).

Patient consent for publication

Informed consent was obtained from all subjects involved in the study.

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Thank you for your interest in the *Romanian Journal of Military Medicine*. Please read the complete Author Guidelines carefully before submission, including the section on copyright.

To ensure fast peer review and publication, manuscripts that do not adhere to the following instructions will be returned to the corresponding author for technical revision before undergoing peer review. Note that submission implies that the content has not been published or submitted for publication elsewhere except as a brief abstract in the proceedings of a scientific meeting or symposium. Once you have prepared your submission following the Guidelines, manuscripts should be submitted online at office@rjmm.ro.

EDITORIAL AND CONTENT CONSIDERATIONS

Aims and Scope

Romanian Journal of Military Medicine (RJMM) is the official journal of the Romanian Association of Military Physicians. The Journal publishes peer-reviewed original papers, reviews, meta-analyses, systematic reviews, and editorials concerned with clinical practice and research in the fields of medicine. Papers may cover the medical, surgical, radiological, pathological, biochemical, physiological, ethical, and historical subject areas. Clinical trials are afforded expedited publication if deemed suitable. RJMM also deals with the basic sciences and experimental work, particularly that with clear relevance to disease mechanisms and new therapies. Case reports and letters to the Editor may not be considered for publication.

Editorial Review and Acceptance

The acceptance criteria for all papers and reviews are based on the quality and originality of the research and its clinical and scientific significance to our readership. All manuscripts are peer-reviewed under the direction of an Academic Editor. The Editor-in-Chief reserves the right to refuse any material for review that does not conform to the submission guidelines detailed throughout this document, including ethical issues, completion of an Exclusive License Form, and stipulations as to length.

ETHICAL CONSIDERATIONS

Principles for Publication of Research Involving Human Subjects

Manuscripts must contain a statement to the effect that all human studies have been reviewed by the appropriate ethics committee and have therefore been performed following the ethical standards laid down in an appropriate version of the Declaration of Helsinki (as revised in Brazil 2013), available at: http://www.wma.net/en/30publications/10policies/b3/index.html. It should also state clearly in the text that all persons gave their informed consent before their inclusion in the study. Details that might disclose the identity of the subjects under the study should be omitted. Photographs need to be cropped sufficiently to prevent human subjects from being recognized (or an eye bar should be used).

Registration of Clinical Trials

We strongly recommend, as a condition of consideration for publication, registration in a public trials registry. Trials register at or before the onset of patient enrolment. This policy applies to any clinical trial. We define a clinical trial as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g., phase 1 trials), are exempt.

We do not advocate one particular registry, but registration with a registry that meets the following minimum criteria:

– Accessible to the public at no charge;

– Searchable by standard, electronic (Internet-based) methods;

– Open to all prospective registrants free of charge or at minimal cost;

– Validates registered information;

– Identifies trials with a unique number; and

– Includes information on the investigator(s), research question or hypothesis, methodology, intervention and comparisons, eligibility criteria, primary and secondary outcomes measured, date of registration, anticipated or actual start date, anticipated or actual date of the last follow-up, a target number of subjects, status (anticipated, ongoing or closed) and funding source(s).

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These are invited by the Editor-in-Chief or their delegated editor and should be a brief review of the subject concerned, with reference to and commentary about one or more articles published in the same issue of RJMM. Editorials are generally 1200–1500 words, may contain one table or figure, and cite up to 15 references, including the source article. This should be cited as Rom. J. Mil. Med. (year); (vol): [issue].

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The Editors welcome contributions to the Education and Imaging section. The purpose is to present imaging for the evaluation of unusual features of common conditions or the diagnosis of unusual cases. Contributions will be reviewed by the Executive Editors. The format of the Images pages involves two parts, each of which will occupy up to one journal page. In Part 1, a case will be described briefly, including a summary of the presentation, clinical features, and key laboratory results. One to two key images will then be presented. It is helpful to the reader if the author responds to questions that follow from the images of the case, such as 'What is your diagnosis? What are the features indicated on the CT scan? What is the differential diagnosis?' Part 2 will briefly describe the imaging features, particularly those that lead to a diagnosis or are critical for management. Differential diagnosis should be mentioned. It will be useful to include either further images or pathological details that validate the imaging diagnosis. Occasionally, the presentation of analogous cases or related images from a similar case might be appropriate.

Please include between one and three references to definitive studies and appropriate reviews of the subject. The format of the Images page involves a brief background and description of the disorder of interest, together with two figures of high quality. Colored photographs are encouraged. The submission may take the form of a case report or may illustrate particular features from more than one patient.

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Manuscripts should follow the style of the Vancouver agreement detailed in the International Committee of Medical Journal Editors' revised 'Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication, as presented at http://www.ICMJE.org/.

Spelling

The journal uses US spelling and authors should, therefore, follow the latest edition of Merriam-Webster's Collegiate Dictionary.

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All measurements must be given in SI units as outlined in the latest edition of Units, Symbols, and Abbreviations: A Guide for Biological and Medical Editors and Authors (Royal Society of Medicine Press, London).

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Abbreviations should be used sparingly and only where they ease the reader's task by reducing the repetition of long technical terms. Initially, use the word in full, followed by the abbreviation in parentheses. Thereafter, use the abbreviation.

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Acknowledgments

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