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CONTENTS

Dear colleagues,

In the current issue, the editorial by Palaiologou et al. highlights the opportunities that artificial intelligence (AI) has primarily brought to public health and healthcare administration, while also exploring its potential future impact on disease prediction, epidemiology, and the improvement of healthcare quality. The editorial by Syrmas et al. reviews the current applications of AI in liver ultrasound elastography, with a particular focus on the assessment of chronic liver disease.

The original research article by Leontari et al. evaluates serum vitamin D levels among the residents of Karystos, investigating vitamin D status in a population with sufficient sun exposure throughout the year.

The first review, authored by Papageorgiou et al., discusses current diagnostic and therapeutic algorithms for primary angiitis of the central nervous system, emphasizing the need for continued research on this rare neurological condition. The review by Malliou et al. delves into the therapeutic benefits of thermal water therapy for patients with rheumatic and musculoskeletal diseases. Finally, the review by Zarkavelis et al. presents the latest insights on the optimal sequencing of therapies for metastatic colorectal cancer, emphasizing the role of molecular characteristics in shaping treatment strategies.

Yours sincerely,

C. Triantos
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and Gastroenterology Faculty of Medicine,
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Artificial Intelligence and Public Health in new Era

Athanasia Palaiologou, Rafail Fokas, Apostolos Vantarakis

INTRODUCTION

Artificial Intelligence (AI) has emerged as an effective and innovative technology in various and different sectors, including public health. It has rapidly been transformed into a tool revolutionizing numerous aspects of public health playing a significant role in it, mostly with its applications, benefits, several prospects but also with many challenges [1]. In this editorial, we explore the opportunities AI has predominantly offered in public health and healthcare administration and its future impact on disease prediction, epidemiology and healthcare quality (Table 1). Despite its capabilities in the healthcare domain, AI's evolvement in public health systems also poses various ethical and technical challenges that will be considerably addressed. In recent years, the focus of AI in public health has been expanded mostly because of its potential, regarding big data processing, recognizing patterns and making predictive analysis [1]. Therefore, using AI may result in the enhancement of disease surveillance, efficient health interventions and the optimization of healthcare delivery.

Disease Surveillance and Prediction

Disease surveillance is a major component of AI in public health, offering the potential improvement of our ability to predict the spread of infectious diseases enabling the health care officials to take preventive mechanisms with the appropriate public health measures. In parallel, AI plays a significant role in the limitation of disease outbreaks before they occur contributing to an efficient disease surveillance system in public health [2].

More specifically, machine learning algorithms which consist of a branch of artificial intelligence enable AI to imitate the way that humans learn, improving its accuracy in time. Those algorithms can analyse big datasets from various sources including electronic health records (EHRs), databases on a global scale and social media targeting not only the prediction of disease outbreaks but also the ability to monitor ongoing threats. For instance, AI and Machine Learning (ML) were adequately applied to COVID-19 issues, including the identification and evaluation of clinical and social factors linked with the risk of COVID-19 cases and deaths, the advancement of spatial risk maps and eventually, the development of vaccination approaches [3].

The implications in epidemiology are to predict the future spread of diseases accurately. Especially, traditional methods related to statistical techniques are not sufficient enough and struggle to evolve patterns and capture complex information. AI, particularly machine learning algorithms, address the issue by identifying hidden relationships and detecting health related trends, thus producing more accurate predictions. They aim to provide early warnings and strategies for mitigating disease outbreaks. A considerable advancement is related to Google AI, which has developed a model that can predict the number of COVID-19 cases in each region up to two weeks in advance [4].

Personalized Medicine and Health Interventions

AI may analyze individual health data to provide healthcare recommendations promoting personalized medicine. In public health, this involves personalized

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Key words: *Artificial intelligence; disease surveillance; prediction; risk analysis; personalized medicine; ethical frameworks; decision – making systems; healthcare management; resource optimization; telemedicine*

Table 1. *Public health domains and potential uses of AI-based measures.*

	Potential use of artificial intelligence	Example
Health protection	Analysing patterns of data for almost real-time surveillance and disease detection	Using Google search and phone GPS information to predict restaurants that are causing foodborne illness
Health promotion	Offering targeted and personalised health advice based on personal risk profile and behavioural patterns	Using machine learning to generate improved cardiovascular disease risk models
Increasing efficiency of health services	machine learning facilitated automated evidence synthesis	Human Behaviour-Change Project uses machine learning for evidence Synthesis and interpretation around behaviour change
Epidemiology	COVID-19 outbreaks surveillance	Identify COVID-19 outbreaks from contact-tracing interview forms Mask wearers facial recognition surveillance
	COVID-19 misinformation control	Use of social media companies like Facebook, Twitter, Instagram, TikTok, and LinkedIn to curb misinformation
	Transmission prediction	Machine learning models to forecast infectious disease spread
Prediction models	Forecast using HealthMap	Forecast COVID-19 spread from social media and other web data

interventions focused on disease prevention and health promotion at the population level. It aims to benefit personalized medicine by providing medical treatment to individuals based on factors like genetic profiles, lifestyles or environment.

Simultaneously, AI can contribute to the development of genetic analysis by processing genomic data to identify individuals at high risk of developing certain conditions. More specifically, the evaluation of vast amounts of genomic data gives the healthcare providers the opportunity to suggest measures regarding the prevention of the given situation. It can also efficiently contribute to early therapeutic protocols, especially when the aim is the management of chronic diseases [5]. An important application of AI in health intervention procedures are AI-tools applied in oncology which can analyze genetic tumors and can help to identify the best possible treatment plan on patients based on their genetic profiles.

Furthermore, AI plays an important role in enhancing the treatment precision by predicting how patients respond to various therapies. Based on the genetic profile of a specific individual, AI strengthens the possibility of predicting how this patient will respond to a particular drug. This fact is very beneficial for the options that are given to healthcare providers to select the most effective treatment, reducing multiple errors associated with the quest of the right treatment strategy [6].

Medical Diagnostics

Medical diagnostics evaluates medical conditions based on symptoms, medical history data, and test results. The main scope of medical diagnostics is the determination of the cause of a medical problem and provide an accurate diagnosis that benefits the patient, ensuring the correct treatment is administered.. AI plays a significant role in medical diagnostics, contributing to improvements to the prediction precision, accuracy and efficiency of the diagnostic procedures. AI algorithms can be utilized to analyse medical images and offer the opportunity for healthcare providers to effectively identify diseases as soon as massive amounts of patient data, demographic information, medical history data, and laboratory test results are being assessed. This is an important advantage considering the help healthcare providers can be provided to make more accurate

decisions about patient care [7]. Especially, utilizing multiple data sources, a more complete understanding of a patient's health can be succeeded as well as a more elaborating view regarding the causes of their symptoms. It is highly unlikely for misdiagnosis to occur by combining several and various data sources with the accuracy of diagnosis as a major result [7].

Health Systems and Resource Management

The optimization of healthcare delivery utilizing AI strategies can play a major role in improving resource allocation and management. Taking into consideration the needs in public health, this translates to more effective use of limited resources, such as hospital beds, medical supplies, and healthcare providers [8,9]. AI models give the possibility of the prediction of demands in healthcare services, aiming to supply public health personnel to allocate resources efficiently. At the same time, AI-driven decision support systems contribute to clinical decision-making resulting timely to appropriate patient care.

It is important that AI can be used in resource-poor settings as soon as AI systems can be utilized to benefit health programmes in various ways. AI has already played a major part in predicting, modelling and ceasing the spread of disease in epidemic situations worldwide, including in resource-poor settings. For instance, research has been made, leading to a ML tool for the identification of weather and land patterns linked with dengue fever transmission in Manila. That specific machine learning algorithm has learnt how to adjust its model to make predictions regarding dengue cases with high accuracy [10].

Prospects and Recommendations

One important perspective of AI in public health is related to its integration with other technologies. The evolvement of AI in the public health domain can strongly be associated with other imminent technologies, such as the Internet of Things (IOT) and big data analytics. Merging these technologies can effectively improve the capabilities of AI systems, enabling more comprehensive, secure and accurate public health interventions [11]. As an example, the IOT can offer real-time health data to AI systems, improving disease monitoring and intervention procedures.

Moreover, another asset of AI in public health is the empowerment of ethical and regulatory frameworks. This is a recommendation which can be established with the development of specific and relevant guidelines for the ethical use of AI, setting the seal on the fact that sensitive data privacy and bias in AI algorithms are being secured [12]. Similarly, regulatory agencies are obliged to adapt to the challenges introduced by AI, providing supervision for the secure and adequate use of AI systems.

In addition, developing an adequate workforce is significant for the beneficial implementation of AI in public health sectors. This is a strategy that involves training healthcare professionals working in AI technologies as well as partnerships between governments, universities and the private sector which can introduce and support knowledge innovation, resulting in the adoption of AI in public health [13,14].

Considering the great significance of AI in the domain of public health, it is understood that it improves healthcare accessibility. In many parts of the world, one of the challenges public health faces is the lack of remote and automated healthcare services. This obstacle can be buried with the development of telemedicine platforms powered by AI which can diagnose and recommend treatments for common health cases by minimising the need of in-person visits to healthcare facilities, mostly in rural areas where medical services are sometimes scarce [15].

Limitations and Challenges

One of the primary challenges of integrating AI into public health are ethical matters related to data privacy. The requirement of AI systems to have access to a huge volume of personal health data, does how the data is gathered, stored, and utilized quite sensitive [16]. It is a matter of paramount importance to secure that AI systems are transparent and accountable regarding the individuals' privacy.

Another limitation posed using AI in public health is the bias in AI algorithms. Specifically, AI algorithms are being used beneficially if the data they are trained on are accurate. In other words, considering the training data is biased, the AI system will also be biased, affecting negatively the patient outcomes and leading to unequal and untrustworthy results [17]. Google has developed a Testing with Concept Activation Vectors (TCAV) programme in which test decision-making algorithms are being used to reduce bias and gender discrimination [18]. As an example, to this issue is the fact that an AI model which is trained on data from a white population, it may not perform as well when applied to non-white patients.

The role of AI in public health systems introduces technical and logistical oppositions. More especially AI systems require significant computing analysis, massive data storage and skilled individuals to develop and maintain. Also, merging AI systems with existing health frameworks can be complex, rendering investment and coordination crucial [9].

AI in public health plays an important role in the addressing of regulatory and legal issues. As soon as current healthcare regulations are failing to present the challenges posed by AI, such as liability and accuracy for AI-driven decisions, the development of a regulatory base that assures the efficient utilization of AI in public health is excessively significant [19]. One important implication of facing such limitations is the procedure of training the application which may incorporate existing values and biases. Also, AI in healthcare can clash with data protection legislation, which in many situations requires only the collection of data associated with the purpose which is being examined [20].

CONCLUSION

Artificial intelligence promises a plethora of great and massive opportunities for public health, introducing new tools, approaches and strategies regarding disease surveillance, epidemiology, personalized medicine, and health systems management. Particularly, looking ahead, AI is set to evolve and expand even further if advances in AI technology will enable more accurate and sophisticated interventions. As follows it can play a major role in addressing global health challenges like climate change health impacts and it can help identify efficient strategies to combat such issues and improve population health on a global scale. All in all, AI promises great advances in public health from predicting disease outbreaks and generally improving healthcare to enhancing operational procedures. Considering the limitations concerning the ethical principles, it can be assumed that artificial intelligence can become a powerful ally in the quest for a healthier world.

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The Role of Artificial Intelligence in Liver Ultrasound Elastography: Challenges and Future Directions

Efstratios Syrmas[1], Ilias Gatos[1], Paraskevi F. Katsakiori[2], Stavros Tsantis[1], Stavros Spiliopoulos[3], George C. Kagadis[1]

INTRODUCTION

Chronic Liver Disease (CLD) is a leading public health concern [1]. CLD progresses through inflammation to fibrosis, and – if left untreated – to cirrhosis. Cirrhosis, the end-stage of the disease, may lead to hepatocellular carcinoma, liver failure, portal hypertension and eventually death. Accurate diagnosis of CLD is essential to secure effective clinical management and intervention strategies. Liver biopsy (LB) is considered the 'Gold Standard' for CLD diagnosis as it provides direct and detailed histological information. Nonetheless, LB is invasive and prone to sampling errors leading to significant inter- and intra-observer variability. These limitations have stimulated the quest for less or non-invasive diagnostic approaches leading to the adoption of elastography that demonstrates high correlation between liver stiffness and liver fibrosis [2]. Several literature reports aim to demonstrate the accuracy of this correlation. Their outcome is to calculate the liver stiffness cut-off values with the aid of ROC analyses. Fibrosis stages should be differentiated optimally to offer the radiologist a simple tool that corresponds stiffness values to fibrosis stages using a certain examination protocol [3].

Two modalities are mainly employing elastography, MR-Elastography (MRE) and Ultrasound Elastography (USE) [4]. Both have gained popularity due to their easy applicability and high-performance in differentiating various severity stages of CLD. Elastography constitutes a rather recent non-invasive modality for the assessment of liver fibrosis and has already started revolutionizing CLD diagnosis. Elastography basic principle is to generate a vibration within the tissue of interest, record the vibration's propagation through the tissue and subsequently deduce elasticity from the tissue response. Ultrasound (US) system manufacturers have used different technological interpretations of this principle leading to US systems that are different in terms of use in clinical practice.

While there is mainly one MRE variant, multiple USE variants are nowadays commercially available including Vibration Controlled Transient Elastography (VCTE), widely known as Fibroscan, Real Time Elastography (RTE), Acoustic Radiation Force Impulse (ARFI) Elastography, Shear Wave Elastography (SWE) and Sound Touch Elastography (STE) to name a few. All these techniques (except for RTE which makes a qualitative relative elasticity estimation) make a quantitative tissue stiffness estimation in an area of interest. Currently, most of these variants provide a colored elasticity map to visually guide the examiner to an optimum measurement. These techniques have been extensively studied and demonstrate USE's high diagnostic performance in CLD fibrosis stage differentiation.

USE techniques show certain limitations such as

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significant inter- and intra-observer variability, best stiffness cut-off values overlap between studies, and presence of non-liver fibrosis related factors that affect stiffness measurements leading to over- or under-estimation of patient's clinical condition. To overcome these limitations, Artificial Intelligence (AI) has recently been used and boosted the performance of computer aided diagnosis (CAD) systems in the pursuit of accurate CLD stage assessment. AI algorithms achieve - or even outperform - experts' accuracy in CLD assessment with USE, rendering them a useful tool in clinical practice [5]. In this editorial, the current state of AI applications along with their challenges and future perspectives in USE for CLD assessment are presented.

Main Body

AI applications can be categorized in Machine Learning (ML) and Deep Learning (DL) based ones [6]. ML requires feature extraction (radiomics in the case of radiological features) and manipulation from raw data to model input. DL directly evaluates raw data bypassing manual or semi-automated feature extraction and analysis for model input. In the case of CLD assessment various studies exist that deploy ML or DL models and attempt to address USE limitations or further improve diagnostic accuracy.

Machine Learning Studies

Few approaches with the use of image processing and analysis for feature extraction from US images have been proposed in ML studies. Gatos *et al*. made an inverse Red-Green-Blue (RGB) to stiffness mapping of 2D elastogram of SWE images and extracted and analyzed features from the resulting region of interest (ROI). They fed a Support Vector Machine (SVM) with the extracted features and differentiated CLD patients from healthy subjects with high accuracy surpassing clinical studies' performance [7, 8]. Furthermore, they suggested specific feature combinations and value ranges to indicate fibrosis existence [8]. More analyses on other fibrosis stage groups and their differentiation are deemed necessary to complete the deployed algorithms' potential on fully assessing CLD fibrosis staging.

Durot *et al*. also noted that SVMs, a multimodel ML algorithm, can effectively grade liver fibrosis through USE [9]. This approach showed diagnostic performance comparable to MRE, further broadening the scope of non-invasive liver fibrosis assessment tools. A hybrid ML methodology, combining a Convolutional Neural Network (CNN) with dual classifiers - SoftMax and SVM - was proposed by Sattar Jabbar *et al*. for the identification of liver fibrosis through the analysis of 700 US shear wave elastography images [10].

Deep Learning Studies

Several studies have recently shown that DL is a powerful tool with increased diagnostic accuracy over clinical or ML studies. Gatos *et al*. employed an elastogram reliability tool that temporally excluded unstable areas of the image [11]. Afterwards, they compared the examiners and DL performance on both filtered and full elastograms. Their results indicated that the examiners' measurements' accuracy was poor on the excluded areas. However, on the areas left intact, performance was relatively accurate. DL showed marginal improvement on performance when fed with the filtered images. Kagadis *et al*. further explored the diagnostic performance on the filtered and non-filtered images on a variety of settings and DL schemes validating their superior performance over clinical and ML approaches [12].

Subsequently, the implementation of DL radiomics of elastography demonstrated superior accuracy compared to traditional methods for accurately staging liver fibrosis in chronic hepatitis B patients through non-invasive 2D-SWE image analysis as shown by Wang *et al.* [13]. Xue *et al*. employed transfer learning to analyze elastogram and grayscale US images that further improved diagnostic accuracy, demonstrating the benefit of integrating both modalities over using them separately [14]. Meng *et al*. developed a liver fibrosis classification method using transfer learning with VGGNet and a deep classifier, FCNet, for ultrasound elastography images [15].

Challenges

Application of AI tools in medical imaging and diagnosis has been accelerated and facilitated the emergence of new pathways of optimizing CLD prognosis and management [16]. However, a few challenges need to be considered before such AI tools become fully operational in the clinical set-up. Although the integration of AI into hepatic elastography seems promising, it encounters several limitations. The robustness of AI models is often reduced when faced with data from diverse patient populations or imaging systems. These include data heterogeneity and quality issues arising from varied acquisition protocols and operator techniques, which challenge AI's ability to general-

ize. The 'black box' nature of DL models complicates their interpretability, a critical factor for clinical acceptance. Furthermore, the absence of standardized validation protocols for USE makes benchmarking AI tools challenging [17]. USE is used in real-time clinical procedures, requiring immediate analysis and interpretation. Integrating AI to enhance or automate this process demands high computational efficiency to provide instant feedback without disrupting the clinical workflow. Additionally, when introducing AI, it is important to deal with complicated rules and ethical issues to keep patients safe and their information private [18]. We also need to conclude on common rules for testing these AI systems in USE.

Future Directions

A complex and careful approach is deemed necessary to overcome the limitations faced by AI in hepatic elastography. Firstly, enhanced representability of the sample used can be achieved through inter-institutional collaboration to create large, diverse, and well-annotated datasets. This advancement could contribute to the development of AI models that exhibit improved generalizability and robustness, particularly in varying USE techniques and patient populations, ensuring consistent performance across different clinical settings. Secondly, enhancing AI model interpretability can involve incorporating explainable AI (XAI) techniques in the case of USE. These methods may explain the AI's decision-making pathways in elastographic analysis and offer a clearer understanding of its diagnostic predictions. Thirdly, a collaborative effort is needed to establish standardized validation protocols. These standards will help make sure that AI tools are reliable and useful in medical practices. Finally, general dealing with the detailed rules and ethical issues requires a firm commitment to privacy standards like the General Data Protection Regulation (GDPR), and a focus on ethical guidelines that prioritize patient safety and data security.

To conclude, current data demonstrate that AI could be implemented to improve the diagnostic accuracy of USE and avoid a great number of liver biopsies, which are related to low but not insignificant morbidity and mortality, and their use should be carefully reconsidered especially in more sensitive subgroups such as the pediatric population [19, 20]. Further investigation is required to validate these initial results and address current issues as to introduce AI-assisted hepatic elastography into everyday clinical practice.

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Evaluation of Vitamin D Levels in Karystos Residents: An Investigation of the Mediterranean Paradox

Iliana Leontari, George Kalapodas

Abstract

Background: According to recent studies, a considerable portion of the population in Greece demonstrates vitamin D insufficiency [25(OH)D < 30 ng/ml] and severe deficiency [25(OH)D < 12 ng/ml] [1]. The current study was designed based on the "Mediterranean Paradox," which highlights that residents of sunny regions, such as the Mediterranean, often exhibit vitamin D deficiency despite adequate solar exposure. The purpose of the study was to evaluate the serum vitamin D levels of the residents of Karystos and investigate vitamin D levels in a population where sun exposure is considered adequate throughout the year.

Materials and Methods: The research was conducted from September 2022 to September 2023 and included the analysis of serum vitamin D levels in Karystos residents undergoing routine examinations at the Biopathology Laboratory of the General Hospital of Karystos.

Results: The study results revealed that despite adequate sunlight throughout the year, a considerable percentage of Karystos residents exhibited vitamin D deficiency.

Conclusion: These findings support the "Mediterranean Paradox" phenomenon and indicate the need for further investigation and possible intervention to address vitamin D deficiency in populations with sufficient sunlight exposure.

Key words: *Vitamin D; mediterranean paradox; Karystos; vitamin levels*

INTRODUCTION

Vitamin D is one of the most important vitamins for human health, playing a multifaceted role in the body's physiology. Although primarily known for its contribution to calcium and phosphorus metabolism regulation, recent scientific studies highlight its broader significance in immune system function, cardiovascular health, and the prevention of various chronic diseases. Additionally, vitamin D deficiency has been linked to numerous pathological conditions, making it a considerable target for public health prevention and intervention [2].

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Vitamin D is unique because it is produced endogenously in the body through the effect of sunlight on the skin. Some researchers have proposed that sun exposure of 15-20 minutes a day is sufficient to produce the necessary amount of vitamin D for the body [3]. Vitamin D consists of several compounds, with the main representatives being ergocalciferol (D2), derived from plants and commonly added to foods, and cholecalciferol (D3), synthesized from 7-dehydrocholesterol in the skin.

During sun exposure, UVB radiation is absorbed by 7-dehydrocholesterol and converted into previtamin D3 (precalciferol). Over two-three days, previtamin D3 undergoes thermal isomerization leading to vitamin D3 (cholecalciferol). Once in circulation, any form of vitamin

D is hydroxylated to 25(OH)D3 in the liver and then in the kidneys, where it is hydroxylated to 1,25-dihydroxycholecalciferol (1,25(OH)2D3), the active metabolite of vitamin D (calcitriol) [4]. Calcitriol, once released into circulation, binds to a specific carrier protein and is transported to target organs to exert its effects. However, the best indicator for studying vitamin D is the circulating concentration of 25(OH)D, representing the vitamin obtained from both sun synthesis and diet. It has a half-life of two weeks, compared to 1,25-dihydroxycholecalciferol, which has a half-life of just four-six hours [4,5].

Additionally, vitamin D is provided through diet in the form of provitamin. Natural dietary sources include fatty fish (salmon, mackerel, tuna), cod liver oil, eggs, beef liver, and others. In the USA and some EU countries, certain foods such as milk, cereals, margarine, juices, and bread are fortified with vitamin D [6, 7].

Guidelines for vitamin D deficiency and insufficiency vary among organizations, reflecting different research methods and clinical experiences. According to some international clinical guidelines, serum 25(OH) D levels below 10 ng/mL (25 nmol/L) are considered deficient. However, the Institute of Medicine states that serum 25(OH)D levels should not fall below 20 ng/mL (50 nmol/L), while the Endocrine Society suggests that optimal skeletal health and muscle strength require serum 25(OH)D levels of at least 30 ng/mL (75 nmol/L) [8, 9]. The Endocrine Society defines vitamin D deficiency as 25(OH)D < 20 ng/mL and insufficiency as 21-29 ng/mL, with some authors recently advocating for values between 40 ng/ml and 60 ng/ml for better health [10].

The World Health Organization (WHO) has not issued specific guidelines for vitamin D but recommends ensuring adequate sun exposure and consuming vitamin D-rich foods to prevent deficiency. For most people, 90% of their vitamin D requirements are met through sun exposure and only 10% through their diet [10]. Therefore, one would expect that residents of sunny countries like those in the Mediterranean would have adequate 25(OH)D levels in their blood. However, studies in recent years highlight vitamin D deficiency in these populations, referring to the "Mediterranean Paradox" [11, 12].

This study was designed to evaluate the serum vitamin D levels of people living in the Karystos area, where sun exposure is considered adequate for most of the year. The study took place from September 2022 to September 2023 during routine examinations at the Biopathology Laboratory of the General Hospital of Karystos.

MATERIALS AND METHODS

Study Population

The study population included 1,199 Greeks, categorized by gender and age into three groups: 20-44, 45-64, and ≥65 years. No information was obtained on Body Mass Index (BMI), medical history, or current medication.

Sample Collection and Measurement

Vitamin D levels were measured using the Vit D Roche Elecsys reagent from Roche. Measurements were conducted using the electrochemiluminescence immunoassay method on the cobas e411 analyzer from Roche. Blood samples (3.5 - 5 ml) were taken from each volunteer in a special tube without anticoagulant, as serum is required for measuring 25(OH)D. The tubes were left to stand for a few minutes before centrifugation.

Definition of Levels

Vitamin D levels were defined as follows:

- Sufficient: 25(OH)D > 30 ng/mL
- Insufficient: 25(OH)D < 30 ng/mL
- Deficient: 25(OH)D < 20 ng/mL

A value of 3 ng/mL was set as the lower detection limit of 25(OH)D.

Statistical Analysis

Statistical analysis of the data was conducted using p-value and chi-square analysis to compare vitamin D levels based on age and gender, utilizing Python, the SciPy library and the statistical tool R. A significant level was set at $P<0.05$.

RESULTS

Demographic Characteristics

Participants included men and women aged 20 to 88 years. The sample distribution by age group was: 20-44 years (21.55%), 45-64 years (33.17%), and ≥65 years (45.27%). Women accounted for 64.49% and men for 35.51% of the participants (Figure1, figure 2).

Vitamin D Levels

The mean serum 25(OH)D concentration in the entire sample was below the laboratory's reference normal limit (30 ng/mL). Specifically, the mean vitamin D value for

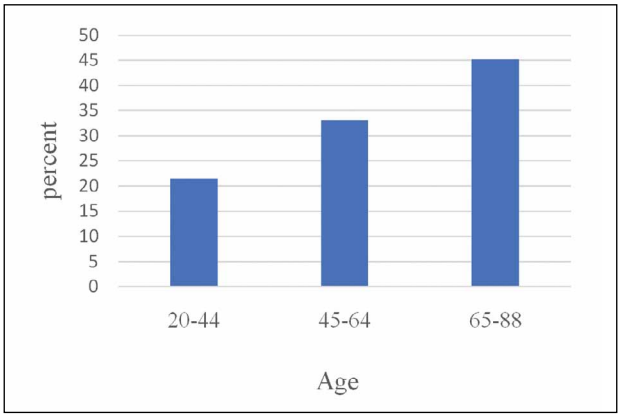

Figure 1. Distribution of the Sample by Age (%).

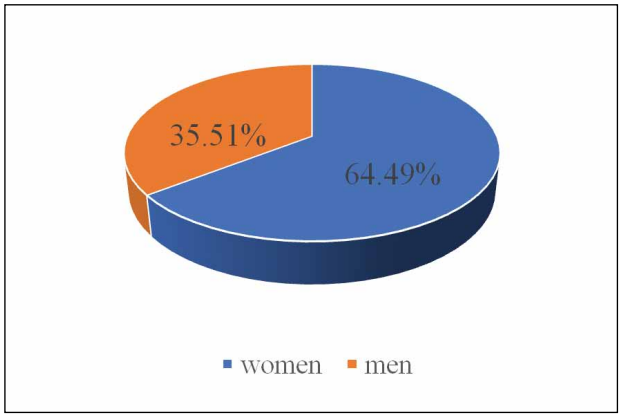

Figure 2. Distribution of the Sample by Gender (%).

the 2022-2023 period was 24.33±5.46 ng/mL, indicating insufficiency. The prevalence of vitamin D insufficiency (25(OH)D < 30 ng/mL) was 61.38%, while 23.32% of the population had vitamin D deficiency (25(OH)D < 20 ng/mL). Only 15.3% of the population had sufficient vitamin D levels (Figure 3).

Correlation with Age and Gender

Age was not considerably associated with the prevalence of vitamin D insufficiency, although a linear trend with increasing age was observed (t-statistic: -1.432, p-value: 0.37). The chi-square statistic based on the p-value and the degrees of freedom was calculated to 4.36. There was also a differentiation in vitamin D levels between women and men. Women had lower average vitamin D levels compared to men, but this difference was not statistically considerable (t-statistic:-1.016, p-value:0.16). The chi-square statistic based on the p-value and the degrees of freedom was calculated to 1.88. Overall, the effect of age and gender did not show a statistically significant impact on the results.

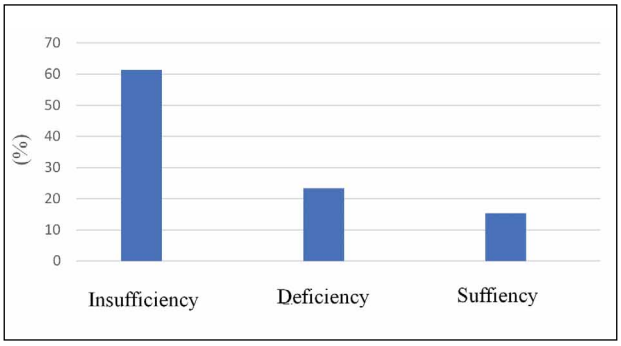

Figure 3. Percentage (%) of the Population with sufficient, insufficient, and deficient Vitamin D levels from 01/09/2022 to 31/09/2023.

Monthly Variation

During the first half of 2022-2023, the mean serum 25(OH)D concentrations were 23.58 (8.21; 66.46) ng/mL and 25.05 (3.00; 50.45) ng/mL for the second half, respectively. Significant seasonality effects were observed in vitamin D concentrations. The highest insufficiency and deficiency rates were recorded in February, with a mean vitamin D concentration of 19.40 ng/mL, while the lowest insufficiency rates and highest mean vitamin D concentrations were observed in September, with a mean value of 35.13 ng/mL. Below are the charts illustrating the levels of vitamin D by age group (Figure 4).

The variation in vitamin D levels seems to be explained by seasonality. February follows the month where sun exposure is limited, reducing vitamin D production in the skin. Conversely, September follows the summer months, where sun exposure is increased, leading to higher vitamin D levels.

DISCUSSION

This study aimed to evaluate vitamin D levels among adults living in a sunny region in Greece for most of the year. The Karystos area has a mild Mediterranean climate with approximately 270 sunny days per year.

The main finding was that a high percentage of participants (over 80%) had serum 25(OH)D concentrations below the reference limit of 30 ng/mL, while only 16% had 25(OH)D levels above 30 ng/mL. This result contradicts the expected relationship between sunlight and adequate vitamin D levels.

Despite Greece's known sunshine, even during win-

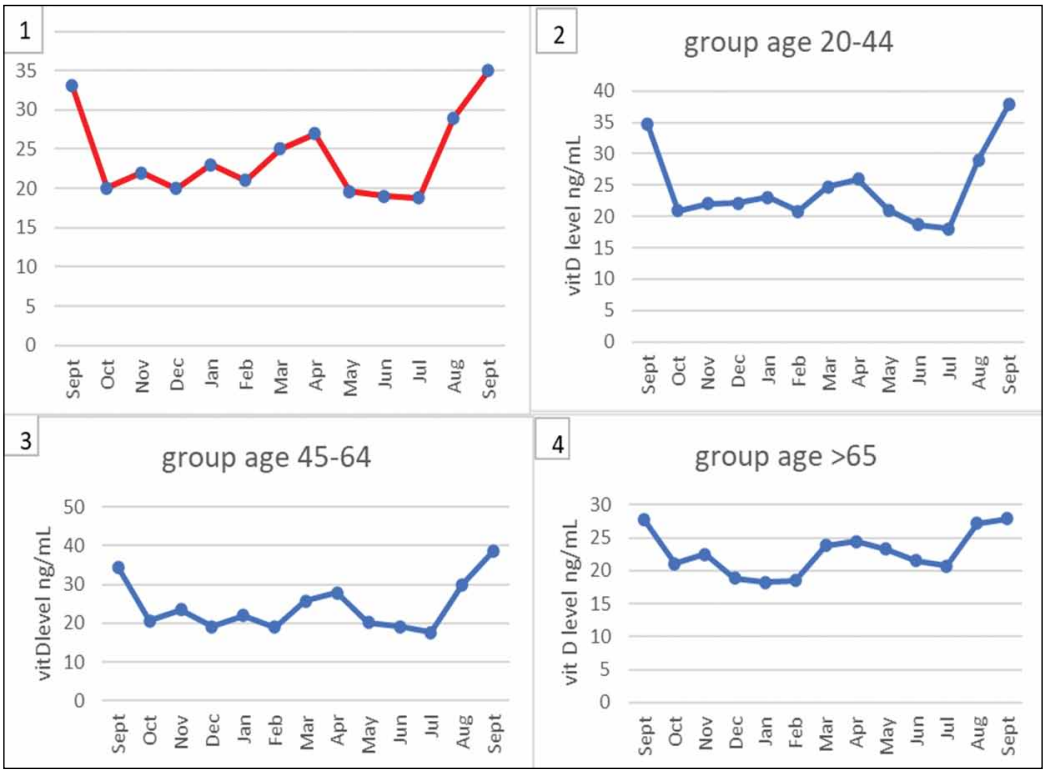

Figure 4. Distribution of vitamin level (ng/mL) in the total sample (1) and separately for each age group (2, 3, 4).

ter, previous research has shown that most of the population suffers from vitamin D deficiency [1]. Our study confirms this phenomenon, as more than two-thirds of the population did not have sufficient vitamin D levels. Significant study limitations include the absence of information on BMI, medical history, current medication, and lack of data on factors affecting vitamin D absorption, such as dietary habits and lifestyle.

It is known that there is a strong correlation between high body weight and vitamin D deficiency. Specifically, obese men and women (BMI > 30) are 75% more likely to present with vitamin D deficiency compared to those of normal weight, due to the deposition of vitamin D in subcutaneous fat tissue [1]. Additionally, reduced physical activity and a sedentary lifestyle limit sun exposure and may contribute to obesity. Dietary habits, such as insufficient consumption of fatty fish, eggs, and vitamin D-fortified products, may also contribute to inadequate vitamin D intake.

The lack of adequate sunlight exposure, especially during the winter months, appears to be the main reason for the low vitamin D levels in the Karystos region. Increased sun exposure is recommended, particularly during the hours when sunlight is most intense (morning and afternoon) throughout the year, to address this deficiency.

Furthermore, this study indicates that perhaps the cut-off levels of vitamin D should be re-evaluated and revised for a more accurate assessment. Revising the reference thresholds may provide a more accurate assessment of vitamin D status in the population and contribute to improving the prevention and management of deficiencies through increased sun exposure, appropriate supplementation, and dietary interventions.

In conclusion, this study demonstrates the need for regular monitoring of vitamin D levels and further research on the factors influencing these levels, even in populations with high sun exposure. The study highlights the importance of individualized health approaches, considering demographic, geographic, and seasonal factors to improve overall health.

Conflicts of interest: *None to declare*

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Author contributions: *Conceptualization, I.L. and G.K.; methodology, I.L.; formal analysis I.L. and G.K.; writing-*

original draft preparation, I.L.; writing-review and editing I.L. All authors have read and agreed to the published version of the manuscript.

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Primary angiitis of the central nervous system: A narrative review

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Abstract

Primary angiitis of the Central Nervous System (PANCS) stands out as a particularly demanding neurological disorder, presenting obstacles in both the diagnostic process and the subsequent treatment strategies. Two subtypes of PACNS have been described in medical literature: small vessel disease typically diagnosed by biopsy and large/medium-vessel disease typically diagnosed through angiography. Clinical manifestations vary widely, with most laboratory and imaging tests demonstrating low specificity in the diagnostic process. In clinical practice, brain Magnetic Resonance Imaging (MRI) is typically abnormal and further confirmation through histopathological or angiographic assessment is required. It is essential to differentiate PACNS from many inflammatory, infectious, vascular, and malignant neurological conditions that present similar clinical manifestations in order to ensure a definite diagnosis. Treatment strategies are developed based on prolonged administration of corticosteroids combined with immunosuppressive agents. As evidence arises only from observational data, a multidisciplinary team approach in a specialized center is recommended. Multicenter prospective clinical trials are also needed to standardize the diagnostic techniques and determine the optimal therapeutic strategies.

Key words: *Angiitis; stroke; angiography; biopsy; central nervous system*

INTRODUCTION

Primary Angiitis of the Central Nervous System (PANCS) is an uncommon neurological condition characterized by targeted inflammation of the small to medium-large vessel of the brain and/or spinal cord [1]. Although PANCS was first described by Harbitz in 1922 [2], the diagnostic process and treatment remains challenging, due to limited specificity of both its clinical manifestations and its primary diagnostic tests.

PACNS has an annual incidence of 2.4 cases per 1 million person-years with an equal sex distribution [3], while the condition affects predominantly median-aged patients, typically around 50 years of age [4]. According to Calabrese and Mallek, who first proposed diagnostic criteria in 1988, the diagnosis of PANCS is based on the presentation of a neurological or psychiatric manifestation along with characteristic angiographic or histopathological findings, while ruling out any other systemic vasculitis or other condition demonstrating similar clinical or imaging features [5]. Consequently, Birnbaum and Hellman proposed a revision of the previous diagnostic criteria in 2009, due to the necessity of distinguishing PANCS from Reversible Vasoconstriction Syndrome (RCVS) and other similar clinical conditions [6]. According to these criteria the terms "definite" and "probable" were suggested to define the strength of PANCS diagnosis. A "definite" diagnosis required histopathological evidence of vasculitis on cerebral biopsy, while "probable" diagnosis required the combination of a high-probability angiographic pattern with Magnetic Resonance Imaging (MRI) and cerebrospinal fluid (CSF) analysis indicating PACNS.

A probable diagnosis is considered when the follow-

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ing findings emerge on angiography [7]:

- Cerebral arterial areas with a pattern of smooth-wall stenosis followed by vessel dilatation
- Multiple arterial stenoses/occlusions
- Absence of proximal vessel atherosclerosis or other abnormalities

Based on the size of the affected arteries, PANCS can be categorized into two distinct types: small vessel disease (SV PANCS) and large/medium vessel disease (LV PANCS) [8]. Concordance between positive biopsy and positive angiography is observed in the minority of patients (<20%), probably reflecting the pathophysiological differences between LV-PACNS and SV-PACNS [3,9]. According to the current diagnostic criteria [6], SV-PACNS can be diagnosed only by biopsy, as Digital Subtraction Angiography (DSA) can detect abnormalities only in large and medium-sized cerebral vessels. Therefore, SV-PACNS diagnosis always meets the criteria of definite PACNS. Only LV-PACNS can correspond to probable PACNS [10,11].

In December 2023, the European Stroke Organization (ESO) published the first European guidelines on PACNS diagnosis and management, in order to establish an optimal approach in PANCS diagnosis and treatment [12]. A collaborative study group of specialists addressed 17 queries regarding SV-PANCS and LV-PANCS diagnosis and treatment. The lack of evidence-based diagnostic and treatment protocols emphasized the necessity for the development and implementation of standardized brain and vessel imaging examinations to enhance the diagnostic and therapeutic yield.

The aim of this narrative review is to outline current diagnostic and therapeutic algorithms in PACNS and to underline the importance for ongoing research on this rare neurological disease. We conclude by emphasizing the critical role of a multidisciplinary team approach in specialized centers for patients presenting with suspected PACNS [12].

DIAGNOSTICS

In medical daily practice PACNS' diagnosis is based on a combination of clinical, laboratory and neuroimaging findings, with brain biopsy remaining the gold standard as a diagnostic tool. However, biopsy is an invasive surgical procedure and carries the risk of a non-diagnostic or false (positive or negative) results [13-15].

Clinical

PACNS presents a wide variety of clinical symptoms, many of which are non-specific. Sarti et al, conducted a review of 24 case series with a total of 585 patients and categorized the clinical features into two groups, based on their frequency: major (≥42.7%) and minor (<42.7%). Major clinical features of PACNS include new onset or altered headache, focal neurological deficits, stroke/TIA, and subacute cognitive impairment, while minor clinical features comprise seizures, altered level of consciousness, and psychiatric disturbances. To suspect PACNS, the authors suggested that one clinical and one major neuroradiological or two clinical and one minor neuroradiological feature should exist and that other differential diagnostic considerations should be excluded. Major neuroradiological findings encompassed multiple parenchymal lesions with vessel occlusion, vessel wall enhancement and parenchymal or meningeal contrast enhancement, while hemorrhagic lesions and/or a single parenchymal lesion were considered as minor features [16].

PANCS clinical manifestations are diverse, most often subacute or chronic and insidious. However, acute symptomatology may occur occasionally [17]. The occurrence of thunderclap headache, i.e., very severe, explosive, abrupt onset headache reaching its maximum intensity within < 1 minute, may also be a feature of RCVS [18]. Clinical scenarios that arouse suspicion are subacute encephalopathy of unknown etiology, meningitis after exclusion of infection and neoplasms, and multiple ischemic strokes in different vascular territories. In a retrospective analysis of 187 patients of rapidly progressive dementia, [19], PACNS comprised 5.3% of cases. Often, the most common clinical manifestations are not present in some cases [3].

Imaging

Conventional brain MRI reveals abnormal findings in nearly all patients with PACNS [20]. MRI demonstrates high sensitivity of approximately 90-100% [6,21-23]. However, findings are nonspecific and include [12]:

- Acute/subacute infarcts, either single or multiple and affecting multiple arterial territories
- Small vessel disease (SVD)
- Intracerebral Hemorrhage (ICH)
- Subarachnoid Hemorrhage (SAH)
- Tumefactive pattern (t-PACNS)
- Parenchymal contrast enhancement
- Spinal cord involvement
- Leptomeningeal enhancement

The working group of the recent ESO guidelines [12] extracted data from 18 studies conducted between 1987 and 2020 that examined patterns of parenchymal abnor-

malities on MRI. Parenchymal contrast enhancement was the most frequent neuroimaging pattern (20.4%), followed by multiple ischemic lesions (18.6%). An ICH/SAH pattern was reported in 13.6%. SVD pattern was probably underreported (8.8%), and a single ischemic lesion pattern was found in 6.4%. The pseudotumoral pattern was rare (4.1%), and spinal cord involvement was even rarer (0.8%). The expert consensus committee concluded that there is no specific neuroimaging pattern of parenchymal signal change that can be attributed to any PACNS subtype. Therefore, it is questionable whether the description of neuroimaging patterns is crucial for the diagnosis [12].

During the last years, 3D-High Resolution Vessel Wall Imaging-MRI (HRVWI-MRI) has enabled the visualization of the vessel wall in three dimensions, thus facilitating the detection of internal pathological signs within it [24,25] (Figure 1). This MRI technique has been increasingly used to differentiate PACNS from intracranial atherosclerotic disease and other inflammatory or non-vasculopathies, by demonstrating concentric vessel wall enhancement (VWE) in the stenotic arteries. Although it seems a promising technique, further validation is needed, highlighting the necessity of combining the results with other imaging modalities in order to reach a diagnosis [12].

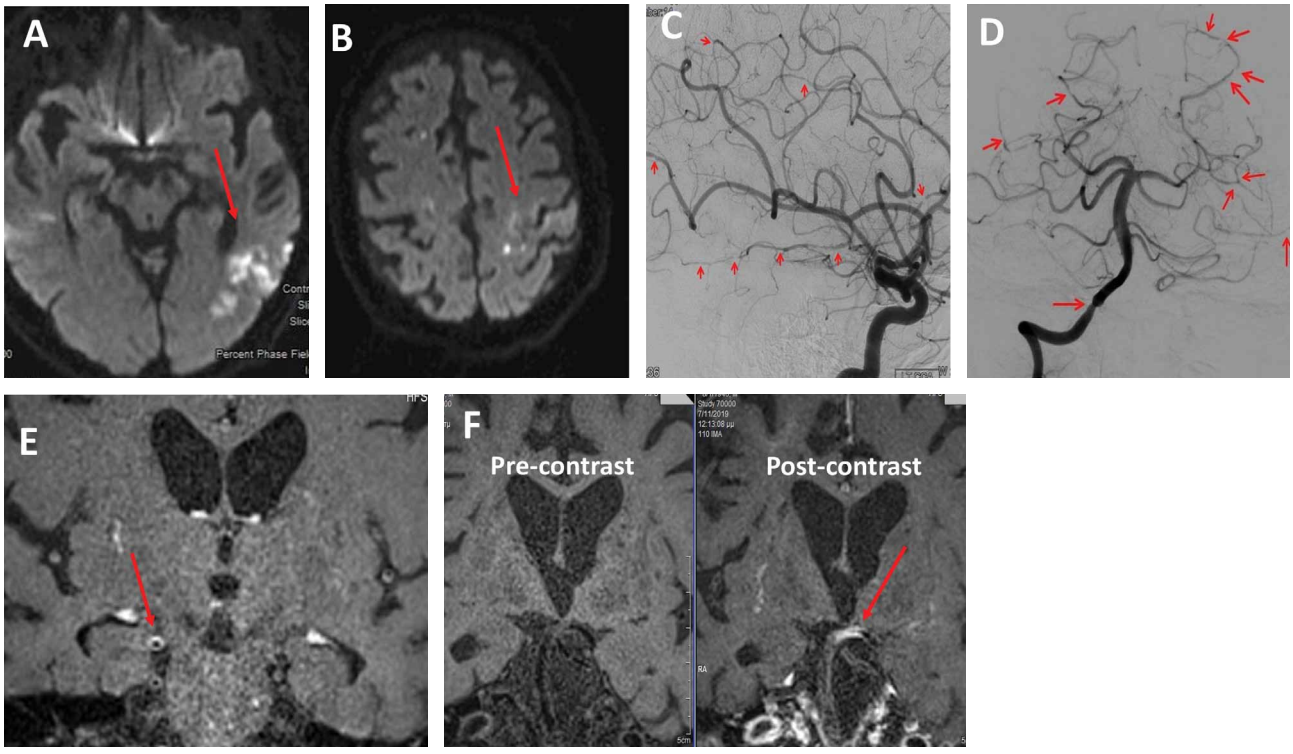

Figure 1. A 73-year-old male was transferred to our department with an eight-day history of limb weakness and gait unsteadiness. His medical history was remarkable for colon cancer surgically treated 20 years ago, coronary heart disease and diabetes. Neurological examination revealed paraparesis and left arm ataxia. Brain MRI demonstrated multiple infarcts of different time points and in several arterial territories (acute ischemic lesions in the left temporal lobe and subacute middle/anterior watershed ischemic lesions) (Panel A). An embolic mechanism was suspected, and a transesophageal echocardiogram demonstrated a mobile atheromatous plaque in the ascending aorta. The patient received double antiplatelet therapy (aspirin and clopidogrel) and rosuvastatin. Nine days later, he developed right facial palsy, right hemiparesis, and bilateral upper limb ataxia. A new MRI scan demonstrated new acute infarcts in the right parietal lobe and the left precentral area (Panel B) as well as prominent leptomeningeal enhancement. An extensive panel of blood test examinations were within normal limits. CSF protein concentration was 170mg/dl, and the rest of the CSF analysis was normal. DSA demonstrated the involvement of multiple large and medium-sized vessels, with multiple alternating areas of vessel stenosis and dilatations (Panels C&D). 3D HRVWI-MRI showed concentric vessel wall enhancement in the multiple stenotic arteries, namely the middle cerebral arteries and the basilar artery (Panels E&F). PACNS diagnosis was established based on the currently available diagnostic criteria and the patient was treated with 1g intravenous methylprednisolone for five days followed by oral prednizolone 100mg/day and monthly intravenous CYC pulse doses. However, his situation continued to deteriorate, so rituximab infusion as a rescue therapy was introduced, but unfortunately without significant response to treatment. This is a case of highly active and aggressive PACNS with minimal response to first- and second-line treatment regimens.

Non-invasive vascular imaging techniques, i.e., Cerebral Computed Tomographic Angiography (CTA) and Magnetic Resonance Angiography (MRA), have been increasingly utilized in recent years, while the implementation of DSA has been declining [12]. It is suggested that DSA, a less invasive procedure than brain biopsy, should be considered for all patients suspected of having PACNS, when MRA or CTA fail to indicate a high probability pattern and the clinical symptoms strongly suggest the presence of PACNS. DSA represents the gold standard technique for disclosing medium-sized vessel involvement in PACNS. Regarding CTA, its multislice technique exhibits similar diagnostic yield with MRA. Nevertheless, comparative analyses between the two imaging techniques are lacking. DSA remains the gold standard in PACNS diagnosis involving large and medium sized vessels, with a sensitivity reaching 70%, but a poor specificity as low as 30% [21,26]. The main limitations of DSA are its relatively low specificity and its limited sensitivity for SV-PACNS.

Biopsy

According to Birnbaum's criteria, SV-PACNS diagnosis requires a positive biopsy, whereas histopathological confirmation is needed in order to conclude to a "definite" PANCS diagnosis [12]. Biopsy exhibits sensitivity between 53-74%, and specificity between 90-100%, particularly when areas of imaging abnormalities are examined [1,27]. Although a negative biopsy cannot entirely rule out the diagnosis, it may be helpful to exclude PANCS mimics, especially infections and malignancies [17,28]. However, it should be noted that the histological features may resemble that of secondary vasculitis or of other immune-mediated inflammatory diseases [29]. The diagnosis is established through the identification of transmural lymphocytic infiltration and vascular wall destruction [30]. The literature describes the presence of three histopathological types: granulomatous (58%-27% of these cases associated with β-A4 amyloid deposition), lymphocytic (28%) and necrotizing (14%) [28].

Brain biopsy is an even less commonly used method to diagnose PACNS in clinical practice. This is due to the requirement for a surgical procedure and the fact that angiographic patterns are often highly indicative of PACNS [12]. Controversy exists about the utility of biopsy due to a high rate of false negative results, the risk of complications associated with an invasive diagnostic method, and the lack of standardized protocols. However, if all other diagnostic imaging methods do not yield a definitive diagnosis, a stereotactic brain biopsy should not be delayed in PACNS suspected cases. Samples from the meninges, superficial cortex, and lesion sites should be preferred to increase the diagnostic yield. Targeting acute gadolinium-enhancing MRI lesions may further improve the diagnostic process. If the affected lesion is not accessible for surgery, it is recommended to perform a biopsy from the non-dominant right frontal lobe [29].

The ESO guidelines highlight the requirement of CNS biopsy when SV-PANCS is speculated. Before proceeding to biopsy, DSA should be undertaken to demonstrate possible medium sized vessel involvement. SV-PACNS is associated with more gadolinium-enhancing lesions and fewer acute cerebral infarctions on MRI, compared to LV-PACNS. For patients exhibiting vascular abnormalities on DSA, CTA or MRA, a specialized medical team should be recruited in order to design a personalized management, including the assessment of the necessity for a brain biopsy. 12].

Laboratory tests and cerebrospinal fluid (CSF) analysis

As with clinical manifestations, there is no single laboratory test available to diagnose PACNS [22]. Therefore, the laboratory investigation involves a comprehensive screening of serological and immunological parameters, along with CSF analysis to rule out other potential diagnoses, such as infections, rheumatological diseases, and malignant disorders [1,17,21,32].

Lumbar puncture should be performed in all patients, as CSF is found abnormal in 80-90% of biopsy-proven PACNS [33]. Typically, CSF reveals mild lymphocytic pleocytosis (defined as >5 cells/ml) and/or elevated protein levels (defined as protein >45 mg/dl). Occasionally, oligoclonal bands or immunoglobulin IgG synthesis can be detected [21,34-35]. To exclude malignant vasculitis, CSF cytology and flow cytometry should be performed. If the number of cells exceeds 200/ml, an infection might be present and further analysis is required.

After analyzing data from 17 case-series and cross-sectional studies involving 763 patients, the ESO working group determined the sensitivity (77.7%), specificity (68.3%), positive predictive value (PPV: 86.6%), negative predictive value (NPV: 53.6%), and diagnostic accuracy (75.1%) of abnormal CSF analysis in PACNS patients. The working group determined that in addition to cell count and protein concentration measurement, oligoclonal bands detection and cytological analysis should be conducted. Results within the normal range should not solely exclude PACNS [12].

Differential diagnosis

An important non-inflammatory differential diagnosis to PACNS is RCVS. Formerly called benign angiopathy of the CNS [36], it occurs more frequently in young women, often presenting with sudden onset of focal neurologic deficit or thunderclap headache and is associated with a normal CSF analysis. Risk factors related to RCVS are exposure to vasoactive drugs and blood products, migraine and other headache disorders, eclampsia, pregnancy and early puerperium [37,38]. Diagnostic criteria include multifocal segmental cerebral artery vasoconstriction confirmed by angiography that must be reversed within three months, exclusion of aneurysmal SAH, a normal CSF, and severe acute headaches ("thunderclap headaches") [37-39]. Signs of focal neurological CNS disturbances, or seizures may occur, too. RCVS may be regarded as an underdiagnosed condition, and its differential diagnosis from PACNS is extremely important since corticosteroids worsen its outcome [37]. Angiogram shows multifocal cerebral artery vasoconstriction [40]. Black blood MRI usually demonstrates a short stenosis without wall thickening or enhancement, whereas in PACNS, long concentric arterial wall thickening with gadolinium enhancement is a characteristic finding [41]. On December 2023, the REversible cerebral Vasoconstriction syndrome intERnational CollaborativE (REVERCE) project was announced in European Stroke Journal, a prospective international observational study across multiple hospitals in four European and Asian countries, aiming to enhance the identification of the disease and to better understand its epidemiological and clinical characteristics [42].

Premature intracranial atherosclerosis' prevalence increases with age and is more likely associated with vascular risk factors (i.e. diabetes, hypertension). Unlike in PACNS, CSF analysis is typically normal, the infarcts are usually restricted to a single vascular territory and 3D-HRVWI-MRI demonstrates eccentric enhancement of atherosclerotic plaques, whereas brain CT/CTA exhibits calcified proximal cerebral arteries with irregular focal stenosis [1]. Other non-inflammatory vasculopathies as fibromuscular dysplasia and moyamoya disease are easily distinguished from PACNS based on their characteristic angiographic image and their effect on extracranial and proximal intracranial cerebral arteries [1,17,21]. Intravascular lymphomatosis (IVL) is a rare and aggressive form of extranodal non-Hodgkin's lymphoma that mainly affects elderly patients. It typically targets the brain, skin, and lungs, where malignant cells selectively invade the lumina of vessels. IVL is associated with high mortality, due to its challenging diagnostic process and its aggressiveness. Early diagnostic indicators include MRI ischemic lesions primarily located in subcortical regions, as well as elevated levels of serum lactate dehydrogenase (LDH), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), interleukins, microglobulin, and CSF protein. Apart from direct tissue examination, the diagnosis may be confirmed by CSF polymerase chain reaction analysis. [43,44]. Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a genetic disorder that presents with a variety of clinical manifestations including migraine, multiple strokes, psychiatric symptoms, seizures, motor, and cognitive deficits. Family history of stroke and dementia along with characteristic MRI features of bilateral external capsule and temporal pole hyperintensities usually raise suspicion of the diagnosis, which is further confirmed by genetic testing (NOTCH3 gene mutation) [45]. Characteristic MRI lesion topography and fundoscopic examination can contribute to the differential diagnosis of PACNS from demyelinating disorders, Susac syndrome and genetic disorders such as Hereditary Endotheliopathy with Retinopathy, Nephropathy and Stroke [21].

Importantly, infectious arteritis should be excluded, as most infectious diseases respond to antibiotic treatment. Varicella zoster virus (VZV) vasculopathy usually affects both large and small sized vessels [46]. However, in 37% of cases, VZV vasculitis affects only small-sized vessels, thus eliminating DSA's diagnostic efficacy. It's worth noting that a zoster rash does not always precede the clinical manifestations. Brain MRI usually demonstrates multiple cerebral infarcts at the grey-white matter junction. Confirmation of the diagnosis requires the detection of viral DNA or anti-VZV antibodies in the CSF, with the former being a more sensitive biomarker [47] (Figure 2).

Finally, PACNS should be distinguished from CNS secondary vasculitis related to rheumatological diseases. The latter typically present with systemic signs and symptoms affecting multiple organs (lungs, renal system, etc.) and are associated with elevated ESR and serum CRP, as well as specific autoantibodies that should be screened for (antinuclear, antineutrophil cytoplasmic, anti-MPO, anti-PR3) [1]. The data regarding the differential diagnosis are presented in Table 1.

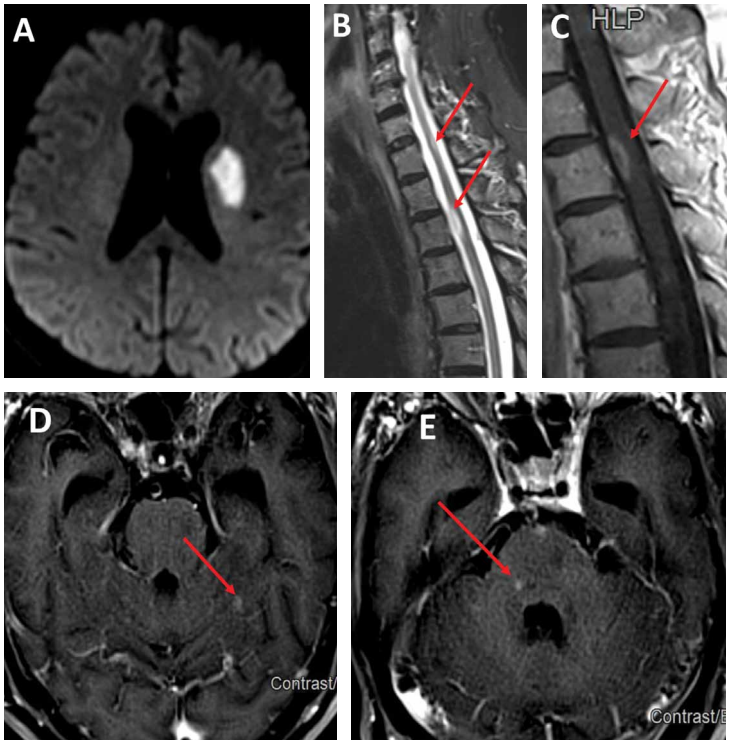

Figure 2. A 76-year-old patient was admitted at our department with a two-day history of diplopia and gait disturbance. Twenty days before admission he had been treated with empiric antibiotic therapy for an upper tract respiratory infection with concurrent headache and dizziness. His medical history was otherwise unremarkable. The neurologic assessment revealed mild confusion and deterioration in time, horizontal diplopia, right central type facial nerve paresis, right sided hemiparesis, and a sensory level at C7-C8. Brain MRI disclosed an acute infarct in the left middle cerebral artery territory (Panel A). In addition, a spinal cord MRI, with contrast injection, showed two enhancing intramedullary lesions in the cervical and thoracic spinal cord levels (Panel B&C). CSF disclosed 150cells/mm and elevated protein levels (126mg/dl), therefore an empiric antibiotic therapy with ceftriaxone, acyclovir and vancomycin for meningoencephalitis was initiated. A repeat brain MRI scan two days later demonstrated multiple foci of parenchymal enhancement in the pons and the left cerebellar hemisphere (Panels D&E), as well as diffuse leptomeningeal enhancement. High dose intravenous corticosteroid treatment was also introduced. CSF flow cytometry demonstrated multiple lymphoid cells suggesting possible lymphoproliferative disease. DSA revealed no abnormal findings, excluding large and medium vessel vasculitis. Finally, anti VZV IgG antibodies at high titers were detected in the CSF, whereas CSF PCR failed to demonstrate the presence of viral DNA. The diagnosis of VZV vasculopathy and myelopathy in an immunocompetent patient without rash was made and the patient was discharged ten days later with mild neurological deficits. One year later he remains stable, without any significant disability.

Treatment

Two primary therapeutic categories exist for PANCS: induction and maintenance therapies [20]. Induction therapy aims to achieve disease remission, usually using a combination of corticosteroids and an immunosuppressive agent. The maintenance phase aims to eliminate relapses and usually requires the addition of an immunosuppressive treatment along with corticosteroids in a tapering regimen [12]. Unfortunately, treatment is not based on randomized clinical trials [23] and is primarily derived from retrospective studies, with significant variations in the therapeutic strategies [8,12,48-50]. The clinical advantage of combining immunosuppressants and steroids still remains unclear. Given the potential severity as well as the relapsing course of PACNS, the ESO working group suggested adding an immunosuppressant to corticosteroid therapy to minimize the side effects from long-term corticosteroid administration. Developing a treatment protocol that is customized to the patient's specific clinical profile and medical history is crucial for achieving optimal results. Corticosteroid monotherapy might be considered in milder disease phenotypes [12]. The optimal administration route for corticosteroids is still questionable, but some experts suggest that it depends on the disease's initial severity [3,20,51]. Selecting the most suitable immunosuppressive agent is also a matter of debate. While cyclophosphamide (CYC) has been administered extensively, mycophenolate mofetil (MMF), studied in the Mayo Clinic series, was correlated with a better response to treatment, higher number of patients in remission off treatment, and lower disability scores [49]. Therefore, the ESO working group suggested initiation of treatment with either CYC or MMF in conjunction with corticosteroids. The decision between CYC and MMF should be personalized according to the patient's requirements [12]. CYC is preferably administered through intravenous monthly pulses over a period of up to 1 year [50,51]. The ESO working group also encouraged the use of aspirin in patients with large/medium vessel involvement, as it was found to be positively associated with long-term remission in

Table 1. *Differential diagnosis-mimics.*

VASCULAR DISEASES
Reversible cerebral vasoconstriction syndrome
Posterior Reversible Encephalopathy Syndrome
Fibromuscular dysplasia
CADASIL
Hereditary Endotheliopathy with Retinopathy, Nephropathy and Stroke
Moyamoya disease
Intracranial atherosclerosis
NEOPLASTIC DISEASES
Intravascular lymphoma Hodgkin's and non-Hodgkin lymphoma, leukaemia
Gliomatosis Cerebri Glioma/glioblastoma
Non-bacterial endocarditis Post irradiation intracranial vasculopathy
SYSTEMIC DISEASES
ANCA related vascilitis, Neuro-Behcet's disease, Sjogren syndrome, Polyarteritis nodosa, Antiphospholipid Syndrome, Systemic Lupus Erythematosis, Sarcoidosis, Crohn's disease
DEMYELINATING DISEASES
Multiple Sclerosis
Neuromyelitis optica
Acute Disseminated Encephalomyelitis
INFECTIONS
Viral (VZV)
Bacterial (Tuberculosis, Syphilis)
Fungal (Aspergillosis, Cryptococcus)

three retrospective studies [8,34,49].

The need for long-term use of immunosuppression is also controversial. In a Mayo clinic cohort, maintenance therapy did not appear to have any effect on long-term remission rates, however the risk of selection bias in these non-randomized studies is probably high [12,49]. In a French cohort, duration of corticosteroid treatment of approximately two years combined with prolonged immunosuppressive maintenance therapy were associated with prolonged remission and better functional status [50]. In this cohort, immunosuppression was added to corticosteroids about four months after their initiation and was continued for a mean duration of 2 years. The currently available data strongly supports the use of azathioprine as one of the favorable treatment options. Concerning patients with progressive disease who had previously received CYC or with a contraindication to CYC, rituximab proved to be highly effective [52,53]. It has been used both as induction as well as maintenance therapy [48-49,52,54-55] and demonstrated its efficacy in non-responders to azathioprine, MMF and methotrexate [49,55]. The latter is generally less preferable since it does not cross the blood-brain barrier effectively [56]. Importantly, if a patient relapses despite optimal treatment with corticosteroids and CYC, reconsidering the PACNS diagnosis is recommended [57].

Tumor necrosis factor-alpha blockers (Infliximab, Etanercept) have been also used in some patients with relapsing and/or refractory disease, but the evidence is extremely limited and only derived from isolated case reports [58-59].

Finally, the ESO working group suggested considering intravenous thrombolysis (IVT) in patients presenting with symptoms of acute ischemic stroke, according to ESO/ESMINT guidelines [12,60]. Endovascular thrombectomy (EVT) seems also a reasonable option in these patients when admitted within the eligible time window, since large vessel occlusion related stroke is typically associated with significant morbidity and mortality [12,61,62].

Prognosis

PACNS is an inflammatory vascular disease causing potentially significant morbidity and mortality [52]. Relapses may affect the 30-50% of patients, with high risk of residual disability [3,63-64]. In the Mayo clinic cohort, high disability scores and death were more frequently observed in patients with Aβ-related angiitis, cerebral infarction on initial MRI and angiographically proven large vessel involvement. In addition, advanced age and cognitive impairment at the time of the initial diagnosis were also negative prognostic factors [49]. According to Salvarani et al, PACNS represents a diverse group of diseases, each with unique clinical characteristics, outcomes, and treatment responses, depending mostly on the size of the affected vessels. Notably, LV-PACNS patients typically suffer from severe neurologic deficits, infarctions on MRI at diagnosis and have increased mortality rates, whereas SV-PACNS patients have overall better functional outcomes with lower mortality rates but

have higher recurrence rates [3]. Furthermore, the granulomatous and necrotizing histological patterns are associated with more aggressive disease phenotypes [65,66]. On the other hand, the lymphocytic histological pattern has better prognosis. However, the quality of evidence is low, therefore the ESO working group suggested that histological patterns should not guide treatment decisions [12].

Amyloid-β-related cerebral angiitis (ABRA) is a PACNS subtype that primarily affects older patients [67,68]. Histopathological examination reveals granulomatous vasculitis related to β amyloid infiltrated arterial vessels walls. It is typically associated with a high frequency of cognitive dysfunction and can manifest with seizures. CSF demonstrates high protein levels and brain MRI scan may reveal contrast-enhancing leptomeningeal lesions. However, this subtype is characterized by a good prognosis if treatment with corticosteroids and CYC is initiated without delay. Cerebral angiography is often negative in these patients, and biopsy is needed to establish the diagnosis [69]. ABRA is a pathological subtype of sporadic cerebral amyloid angiopathy (CAA), a common degenerative small vessel disease of the brain, characterized by the cerebrovascular deposition of β-amyloid, affecting mainly the cortical and leptomeningeal vessels [70,71].

In a single-center retrospective observational study assessing relapses, remission, and long-term outcome, male sex was the only significant predictor of relapse. Favorable outcome was evident in 80% of patients after two years of immunotherapy. The study underlined the need for further PACNS subtype stratification to evaluate predictors of response [48].

However, there are some limitations in the currently available literature data concerning the disease prognosis, since a definition of favorable outcome is lacking, and since most studies have included patients with non-biopsy proven vasculitis. Mortality rate may exceed 15% within the first three years after the diagnosis, and consequently, timely treatment is crucial [57].

To monitor response to treatment and disease activity, serial brain and angiographic imaging is required and according to the disease evolution [72]. Color duplex sonography is a bedside tool that might be useful to monitor patients with cerebral artery stenosis [73,74].

CONCLUSION

PACNS is a rare inflammatory disease affecting the central nervous system, characterized by a relapsing or progressive clinical course, and associated with significant morbidity and mortality. The diagnosis is challenging since various other diseases may present with similar clinical presentations and neuroimaging findings. Although biopsy is the gold standard diagnostic method, it may be negative in patients with isolated LV-PACNS, where the diagnosis is based on angiographic modalities, mainly DSA. Due to the lack of randomized control trials, there is no conclusive data and recommendations on the diagnostic process and the therapeutic strategies. However, precise diagnosis is vital due to the need of identifying the patients requiring prolonged and aggressive treatment. A specialized medical team with expertise in PANCS should ideally orchestrate this process. Prospectively designed controlled international studies and trials are needed to promote diagnostic accuracy and ensure the development of standardized treatment protocols.

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Therapeutic properties of thermal water in rheumatic diseases: A narrative review

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Abstract

The use of thermal water therapy or balneotherapy as a complementary form of non-pharmacological treatment is common in clinical practice and has sparked some renewed interest in research the past few years. Aim of this narrative review was to investigate the therapeutic properties of thermal water therapy in patients with rheumatic diseases. The keywords that were used were thermal water therapy, balneotherapy, spa therapy and rheumatic diseases and the search was done in databases such as PubMed, Cochrane, and Scopus for systematic and narrative reviews as well as for clinical trials and RCTs. Thermal water therapy or balneotherapy or spa therapy is used for its anti-inflammatory effect as a supplement to the pharmacological treatment of patients with rheumatic diseases with or without skin symptoms to improve pain, functionality and QoL and the patients' wellbeing. There is a consensus that double-blinded RCTs are missing to evaluate the primary and secondary outcomes of the trials. Researchers are reporting a high amount of heterogeneity in both research design and methodology as well as in the quality of samples. Further research is required to address the limitations and to verify the beneficial properties of this therapeutic modality to be used in the treatment of rheumatic diseases.

Key words: *Thermal water treatment; therapeutic properties; balneotherapy; rheumatic diseases*

INTRODUCTION

The use of water for medical purposes is probably as old as humanity itself. Spas, including hydrotherapy and bathing, remained popular until effective analgesics became available. However, no analgesic can eliminate pain, and severe adverse reactions led to renewed interest in spa therapy. There is some confusion about hydrotherapy and spa therapy. The former uses plain, cool water. The latter uses natural thermal mineral water. Due to methodological difficulties and lack of research funding, the effects of "water therapies" on pain have rarely been evaluated with randomized control trials-RCTs. However, existing RCTs show that pain can be relieved in inflammatory and non-inflammatory rheumatic diseases, chronic low back pain and fibromyalgia with results lasting from three to nine months [1]. The adjective "thermal" indicates that the water has a temperature of 20º C or higher. The term balneotherapy used interchangeably to thermal water is difficult to study, as it is usually part of the overall spa therapy treatment. However, it has been possible to compare the effects of balneotherapy with those of hot tap water therapy in double-blind trials in knee osteoarthritis and rheumatoid arthritis. In controlled studies, ambulatory bath therapy was tested in patients for pain due to chronic low back pain [2] and fibromyalgia [3] making an effort to exclude the "spa atmosphere". Balneotherapy (BT) is a popular treatment for many diseases. The mechanisms by which

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mineral or thermal water immersion, or in several cases combined with mud application, relieve symptoms, are not fully understood. The net benefit is probably the result of a combination of factors. Buoyancy, immersion, resistance and temperature all play important roles. According to the gateway theory [4], pain relief may be due to water pressure and temperature on the skin; hot stimuli may affect muscle tone and pain intensity, helping to reduce muscle spasm and increase pain threshold. Spa therapy has been found to cause an increase in insulin-like growth factor-1 (IGF1), which stimulates cartilage metabolism, and transforming growth factor-β (TGF-β). There is also evidence for the positive effect of mud baths and spas on the oxidative/antioxidant system, with a reduction in the release of reactive oxygen species (ROS) and nitrogen (RNS). Overall, heat stress has an immunosuppressive effect. Many other non-specific factors may also contribute to the beneficial effects observed after spa therapy in certain rheumatic diseases, including effects on cardiovascular risk factors and changes in environment, pleasant surroundings and absence of work obligations [5].

In BT the whole or part of the body is immersed in water bathing in individual or group baths or douches, i.e. for a certain period the body is exposed to thermometallic water, which comes at a low or high pressure. This is mainly of interest for skin diseases, arthropathies and rheumatic diseases. Peloid therapy is the application of cured clay (usually mixed with mineral water) to the points indicated for rheumatism and skin diseases. Three types of stimuli are applied during spa therapy. First, the mechanical stimuli, which are due to the physical properties of water. A mechanical stimulus is hydrostatic pressure, which is the effect of the water pressure on the body of the bather. Secondly, the thermal stimuli, which are due to the heat of the water and causes vasodilation or vasoconstriction. Finally, the chemical stimuli that facilitate the change of the internal state of the body through transdermal absorption [6].

One systematic review aimed primarily at evaluating whether BT, mud therapy and spa therapy can affect cortisol levels. The secondary aim was to understand whether these interventions can improve stress resilience. Five studies investigated the biological effects of spa therapy alone. Ten studies investigated the biological effects of spa therapy with or without the inclusion of mud/peloid treatment and all, but two studies reported significant changes in cortisol levels. The main findings suggest that spa therapy may have the potential to affect cortisol levels in healthy individuals in a way that improves stress resilience, hence bathing and spa therapy can be considered useful interventions for the management of stressful situations [7]. There is also increased interest in using preclinical models to investigate the effects of BT on inflammation, immunity, cartilage, and bone metabolism. The aim of another comprehensive analysis was to summarize current knowledge on in vitro studies in BT and to review the results obtained on the biological effects of thermal mineral waters. Particular attention was paid to main rheumatological and dermatological diseases, as well as to the regulation of the immune response. Human and animal samples were used. In particular, the properties of a thermal water, as a whole, of an inorganic molecule such as hydrogen sulfide, in different cell cultures (keratinocytes, erythrocytes, chondrocytes and peripheral blood cells), or of the organic component were analyzed. The results confirmed the scientific value of in vitro studies demonstrating the anti-inflammatory, antioxidant, chondroprotective and immunosuppressive role of thermal water therapy at the cellular level. However, the validity of the cell culture model is limited by several sources of bias, such as differences in experimental procedures, high heterogeneity among available studies, and difficulties in considering all chemical and physical factors of BT [8]. The validity of such results depends on the experimental procedure and the particular and complex composition of the mineral waters used to perform the preclinical studies. Analyzing the inorganic composition of the waters may not be adequate rather than considering including the organic composite that may play a role in the observed therapeutic effect and other biological mechanisms like toxicity [9]. Finally, BTs benefits for chronic back pain (cLBP) were shown to also induce changes in proteins involved in functions such as modulation of gene expression, differentiation, angiogenesis, tissue repair, acute and chronic inflammatory response [10]. 66 patients with cLBP secondary to OA were randomly enrolled and treated with daily mud packs and bicarbonate-alkaline mineral water baths, or a thermal hydrotherapy rehabilitation scheme, the combination of the two regimens for two weeks. Control group of patients received only meditation sessions. Clinical variables were evaluated at entry level, in 2- and 12-weeks' time. 1000 serum proteins were tested before and after a two-week mud bath therapy. Spa treatment groups showed clinical benefits, shown from improved VAS scores, Roland Morris disability questionnaire and

neck disability indexes. A few serum proteins were increased (≥2.5 fold) after spa treatment: inhibin beta A subunit (INHBA), activin A receptor type 2B (ACVR2B), angiopoietin-1 (ANGPT1), beta-2-microglobulin (B2M), growth differentiation factor 10 (GDF10), C-X-C motif chemokine ligand 5 (CXCL5), fibroblast growth factor 2 (FGF2), fibroblast growth factor 12 (FGF12), oxidized low density lipoprotein receptor 1 (OLR1), matrix metallopeptidase 13 (MMP13). Three proteins were found greatly decreased (≤0.65 fold): apolipoprotein C-III (Apoc3), interleukin 23 alpha subunit p19 (IL23A) and syndecan-1 (SDC1). Balneophototherapy (BPT), further enhances the anti-inflammatory effects. In a review [11], authors described BT and BPT use in three different treatment sites, with unique climates and chemical properties of the mineral water which proved to be an effective complementary therapy for inflammatory and autoimmune skin diseases; however, the burden of the travel to the site and the long duration of therapy could be prohibitive for a wider use of this form of treatment.

Methodology

The scope of this narrative review was to focus on the therapeutic effects of BT for rheumatic and musculoskeletal diseases. Therefore, this was set as the basic research question and the related key terms used for the bibliographical research were balneotherapy, spa therapy including mud and/or peloid therapy and thermal water treatment, therapeutic properties, following the aforementioned clarifications and definitions. In that aspect, some inclusion and exclusion criteria were set, even though in narrative reviews the search protocol is not as strict as in systematic reviews and meta-analyses [12]. The rationale for the databases' search was to include clinical trials, especially RCTs, double blind and randomized, to be able to comment on their results. Apart from that, multicenter trials and systematic reviews and meta-analyses were also included due to the added benefits they could offer to the discussion on the therapeutic effects of these modalities [13]. On the other, one basic exclusion criterion was the modality of thermal water (BT) was clearly defined and differentiated from the use of hydrotherapy, where the use of water in most cases pool water (no minerals, mud, peloid, sulphur or any other ingredient added) and normal (room/environmental) temperature. Databases that were searched were Scopus, PubMed and Cochrane. Initially, 110 research articles were identified. Research articles were removed based on relativity, presence of key terms, meeting or not the inclusion/exclusion criteria. Finally, 21 articles were the ones used for this narrative review focusing specifically on the therapeutic effects of these modalities on RMDs [12].

RESULTS

In many European countries and in Turkey [1, 14], BT is used in daily clinical practice however, there are few studies on the effectiveness of spa therapy in real life [15]. Naiade, an Italian national project, was a longitudinal observational study aimed at providing knowledge on the social, epidemiological, efficacy and economic characteristics of spa treatments in eight disease subgroups one of them being the rheumatic disease subgroup including 11,437 patients with osteoarthritis. The results showed that spa therapy is beneficial and could be cost-effective. A wider range of RMDs population was included in a similar study and provided more detailed information on the effectiveness of spa treatment in daily clinical practice. Findings showed that spa therapy is prescribed and practiced mainly for osteoarthritis, then fibromyalgia, lumbar/cervical disc herniation, and nonspecific low back pain; and less for ankylosing spondylitis, rheumatoid arthritis and improvements were basically for pain and function [2].

Rheumatoid arthritis (RA) is the most common inflammatory rheumatic disease. The symptoms of RA make the disease disabling and strongly affect quality of life (QoL). Spa therapy appears to be one of the most common forms of non-drug treatment for RA which benefits the QoL of patients. There seem to be positive effects in both mineral baths and sand or mud immersion [16]. The sulphur mineral water has special benefits during active inflammatory phases. A systematic review aimed to summarize the available evidence on the effects of balneotherapy on patients with RA. The systematic search was done in articles, published from 1980 to 2014, which have compared balneotherapy with other therapeutic modalities or with no intervention considered. Eight RCTs were found and included for full review involving a total of 496 patients. The studies highlighted an important improvement and statistically significant in several clinical parameters. Improvement on functional capacity up to six months of follow-up (FU) was emphasized in one article. Some of the studies reveal an improvement on morning stiffness (five studies), number of active joints (three studies), Ritchie index (two studies) and activities of daily living (two studies) up to three months of FU. Three studies revealed the

improvement on handgrip strength up to one month of FU. Three studies evaluating the parameter of pain (VAS) were inconclusive about any improvement. This review comments on differences of methodologies, treatment modalities, outcomes and their analyses as issues to be taken into account when considering the strength of the data collected. Homogeneity of the studied population (patient's clinical heterogeneity, diverse clinical course of the disease, variety of the medications received), natural mineral water composition and their potential specific biological effects [17]. A prospective, observational study in 49 Spanish patients aged 60-80 years with RA aimed at analyzing the influence of this modality on patients' functionality and QoL. After 10 sessions of BT and an additional technique (a circular shower, footbath, mobilization in the pool, steam room), the outcome variables were the Health Related QoL (HRQoL), EuroQoL 5d-5l and the Health Assessment Questionnaire (HAQ). The scores obtained in the variable "current health state" of EuroQol 5D-5L increased by 6.73 in the first and by 6.26 points in the third month. The EuroQol index decreased by 0.121 and 0.098 points in the first [t] and the third month. In all cases, the differences were statistically significant. Regarding functionality, the mean scores obtained in the HAQ decreased in all the follow up periods, although statistical significance was only reached at three months after the end of the balneotherapeutic treatment. The beneficial effects of balneotherapy on health-related quality of life and functionality in individuals with rheumatoid arthritis can be positive, although the effect size seems to be slightly lower than that found in RCTs [18].

Similarly, overall evidence of another systematic review [19] assessing benefits and harms of BT for RA patients in terms of pain, improvement, disability, tender joints, swollen joints and adverse events was not sufficient to prove that BT is more effective compared to no treatment or that one type of bath is more effective than another or that it's more effective than exercise or relaxation therapy. The review was the update from the previous one, from 2004 and updated in 2008. RCTs were included with participants having a RA diagnosis. Two new studies were included, in total nine studies involving 579 participants. One study involving 45 participants with hand RA compared mudpacks versus placebo. There was a very low level of evidence of reduction in the number of tender joints on a scale from 0 to 28 (MD -4.60, 95% CI -8.72 to -0.48; 16% absolute difference). Two studies involving 194 participants with RA evaluated the effectiveness of additional radon in carbon dioxide baths. There was some benefit of additional radon at six months in terms of pain frequency (RR 0.6, 95% CI 0.4 to 0.9; 31% reduction; improvement in one or more points (categories) on a 4-point scale; moderate level of evidence) and 9.6% reduction in pain intensity on a 0 to 100-mm VAS (MD 9.6 mm, 95% CI 1.6 to 17.6; moderate level of evidence). Some benefit was found in one study including 60 participants in terms of improvement in one or more categories based on a 4-point scale (RR 2.3, 95% CI 1.1 to 4.7; 30% absolute difference; low level of evidence). Study authors did not report physical disability, tender joints, swollen joints, withdrawals due to adverse events or serious adverse events. One study involving 148 participants with RA compared balneotherapy (seated immersion) versus hydrotherapy (exercises in water), land exercises or relaxation therapy. One study involving 57 participants with RA evaluated the effectiveness of mineral baths (balneotherapy) versus Cyclosporin A. Some benefit of balneotherapy was observed in overall improvement on a 5-point scale at eight weeks of 54% (RR 2.35, 95% CI 1.44 to 3.83) and some benefit of Cyclosporin A in the number of tender joints (MD 8.9, 95% CI 3.8 to 14; very low level of evidence).

Despite advances in pharmacological treatment, physical therapy is important for the management of AxSpA. Aim of a study was to evaluate the effects and tolerability of combined spa therapy and rehabilitation with physical therapy in a group of 30 patients treated with TNF inhibitors: 15 were prescribed 10 sessions of spa therapy (mud packs and thermal baths) and rehabilitation (exercises in a thermal pool) and the other 15 were considered controls. The patients in both groups had been receiving anti-TNF agents for at least three months. Outcome measures were scores of BASFI, BASDAI, BASMI, VAS for back pain and HAQ. Assessments were done at entry level, after three and six months. Most of the evaluation indices were significantly improved at the end of the spa treatment, as well as at the three- and six-month follow-up assessments. The control group patients showed no differences. Combined spa therapy and rehabilitation caused a clear, long-term clinical improvement in AS patients being treated with TNF inhibitors with no shown disease relapses [20]. BT was also compared to water-based exercise and land-based exercise regarding their effects on disease activity, symptoms, sleep quality, quality of life, and serum sclerostin level (SSL) in patients with axial spondyloarthritis (AxSpA) and

were all found effective and beneficial with a duration of the benefits of up to 12 weeks [21]. Between January 2019 and January 2020, a total of 60 patients (35 males, 25 females; mean age: 40.9±11.2 years; range, 18 to 55 years) who were diagnosed with AS were randomly divided into the balneotherapy (n=20), WBE (n=20), and LBE (n=20) groups (20 sessions of treatment in groups of five to six patients). Evaluations were done at entry level, at 4 and 12 weeks with BASDAI, BASFI, BASMI, ASDAS-CRP, Maastricht Ankylosing Spondylitis Enthesitis Score (MASES), Ankylosing Spondylitis Quality of Life (ASQoL) Scale, Fatigue Severity Scale (FSS), and Pittsburg Sleep Quality Index (PSQI). Serum sclerostin levels (SSL) were measured for all participants who had improved indices at four- and 12-weeks follow-up ($p<0.05$). A significant improvement in sleep latency was seen in the balneotherapy and WBE groups. Changes in SSL were not statistically significant in any group ($p>0.05$).

As discussed previously, BT is effective for fibromyalgia [16]. It has been found that patient education combined with a two-week application of BT has more beneficial effects in patients with fibromyalgia syndrome compared to patient education alone. Similarly, heat therapy may after all have a positive effect on specific symptoms. BTs effects for most patients appear after treatment, and are not noticeable after three months, mud bath therapy has longer lasting effects [22] when investigated in patients with primary fibromyalgia (FM) using rheumatological, psychiatric, biochemical, and proteomic approaches. 41 patients, 39 females and two males, with FM diagnosis, received a two-week thermal therapy once daily for six days/week. Twenty-one patients received mud-bath treatment, while the other twenty balneotherapy. Pain, symptoms, and quality of life were assessed. Oxytocin, brain-derived neurotrophic factor (BDNF), ATP and serotonin transporter levels during therapy were analyzed. Comparative whole saliva (WS) proteomic analysis was performed using a combination of two-dimensional electrophoresis (2DE) and mass spectrometry techniques. Both groups of patients showed reduction in pain, FIQ values and improvement of SF36, receiving either mud-bath or balneotherapy. The improvement of the outcome measures occurred with different timing and duration in the two spa treatments. Accordingly, patients in both groups showed a significant decrease in BDNF concentrations after twelve weeks, but no significant changes in oxytocin, ATP levels and serotonin transporter were detected. Significant differences were observed for phosphoglycerate mutase1 (PGAM1) and zinc alpha-2-glycoprotein 1 (AZGP1) protein expression.

In another RCT, a fibromyalgia-specific standardized spa therapy (SST) was assessed (through Fibromyalgia Impact Questionnaire-FIQ) for its efficacy and safety at six months in the context of a fibromyalgia-specific therapeutic patient education (TPE) program, compared to SST alone. The differences between groups were significant for primary parameters (pain and FIQ scores) at each time point. Similar results were obtained for the other secondary outcomes except for anxiety. Short- and long-term therapeutic efficacy of BT in FM is supported [23]. The beneficial effects of BT are supported also from a single blind RCT [3] that evaluated the effectiveness of BT in fibromyalgia management in fifty women under pharmacological treatment. Fifty women with FM under pharmacological treatment were randomly assigned to either the balneotherapy (n=25) or the control (n=25) group. The patients in the balneotherapy group had two thermomineral water baths daily/two weeks in Tuzla Spa Center. The patients in the control group received the standard of care for FM. Assessments were done four times, at entry level, at two weeks, in the first and third month. Outcome measures were pain intensity, Fibromyalgia Impact Questionnaire (FIQ), Beck Depression Inventory (BDI), patient's global assessment, investigator's global assessment, SF-36 scores, and tender point count. Balneotherapy was found to have an effect, and be a better modality, at the end of the cure period in terms of pain intensity, FIQ, BDI, patient's global assessment, investigator's global assessment scores, and tender point count as opposed to the standard of care. Benefits of BT lasted up to the end of the third month, except for the BDI score and the investigator's global assessment score [3].

Another rheumatic disease, Osteoarthritis (OA), is currently one of the leading causes of Disability Adjusted Life Year (DALY) indicators worldwide. BT is one of the most used non-pharmacological approaches for OA in many European countries, as well as in Japan and Israel. One review attempted to summarize the clinical effects and mechanisms of action of spa therapy in KOA. Several RCTs were conducted to evaluate the efficacy and tolerability of spa therapy and mud bath therapy in patients with KOA which support a beneficial effect of spa therapy on pain, functionality and quality of life that lasts over time, up to six-nine months after treatment. The net benefit is probably the result of a combination of factors, among which mechanical, thermal and chemi-

cal effects that are the most important and have been shown to be effective in the treatment and secondary prevention of KOA, reducing pain, consumption of NSAIDs and functional limitation while improving the QoL of patients [24]. Similarly, the results of another systematic review confirmed the beneficial effect for patients with chronic back pain, knee and hand osteoarthritis and chronic inflammatory pelvic disease of BT on pain with weight-bearing and at rest in patients with degenerative joint and spinal diseases. This review also revealed that spa therapy affects the antioxidant status and metabolic and inflammatory parameters [25].

A systematic review and meta-analysis on the effect of spa therapy and spa treatment on the QoL of patients with KOA when comparing spa therapy interventions with placebo interventions had results that favored the former in terms of long-term pain improvement, while no significant difference was found in terms of social functioning. The evidence suggested that spa therapy and treatment could significantly improve the quality of life of patients with KOA with reduction in medication consumption and improvement in algofunctional indicators [26]. The primary objective of a randomized RCT [27] was to evaluate the effectiveness of mud and thermal water baths compared with thermal water baths alone in Hand osteoarthritis-HOA and knee osteoarthritis-KOA. Investigators randomly assigned patients to either mud plus bath therapy (group 1) or balneotherapy (group 2). The primary outcome was a change in AUSCAN questionnaire for HOA and in the WOMAC for KOA at 12 months. Evaluations were performed at baseline (B), at week 2 (W2) after the interventions and after three (M3), six (M6), nine (M9) and 12 (M12) months. 37 patients with KOA and 52 with HOA participated. In HOA patients, AUSCAN pain improved more in group 1 compared to group 2 at M3, M6 and M12 ($p<0.001$, $p=0.001$ and $p=0.038$, respectively). AUSCAN stiffness improved more in group 1 at M3 ($p=0.001$). AUSCAN function improved more at M3, M6, M9 and M12 ($p=0.001$, $p=0.001$, $p=0.014$ and $p=0.018$, respectively). In KOA patients, WOMAC function decreased more prominently in group 1 compared to group 2 at M9 ($p=0.007$). The absolute values of WOMAC function at M6 and M9 were lower in group 1 compared to group 2 ($p=0.029$ and $p=0.001$, respectively). WOMAC pain absolute values were lower in group 1 at W2 ($p=0.044$) and at M9 ($p=0.08$). In conclusion, mud plus balneotherapy was more effective than balneotherapy alone on clinical outcomes of HOA. Differences in clinical outcomes of KOA were not significant, yet numerically higher [28]. Similarly, in a blind RCT, that evaluated the effectiveness of hot sulphur and non-sulphur waters in the treatment of KOA results showed that there was a significant reduction in VAS pain scores (pain on movement, at rest and at night) and use of pain medication, as well as improvement in WOMAC and LAFI scores ($P<0.05$) [29].

Among the available treatments, several health benefits of bathing in natural mineral water for a three-week bathing intervention in patients with KOA have been proposed for pain, functionality, emotional and social aspects and QoL. Those were the primary outcomes of this RCT in 120 patients with OA who were randomized in the experimental group (60 patients) and the control group (60 patients). Findings showed benefits for 45 patients of the experimental group who were found to benefit from the therapeutic intervention in terms of pain relief. Improvements were often clinically significant and, in most patients, persisted three months after the start of treatment [30]. Another review focused on preclinical studies, RCTs and clinical trials. The results of the clinical studies confirmed beneficial properties on various mediators and factors of inflammation, oxidative stress, cartilage metabolism and humoral and cellular immune responses in patients suffering from chronic degenerative musculoskeletal disorders. Data from mouse models of OA and RA revealed the efficacy of various BT therapies in reducing pain, inflammation and improving mobility, as well as reducing the expression of matrix-degrading enzymes and markers of oxidative stress damage. Different in vitro studies have analyzed the potential effect of a mineral water, as a whole, or a mineral element, demonstrating their anti-inflammatory, antioxidant and chondroprotective properties in OA cartilage, articular and chondrocytes, as well as osteoblast and osteoclast cultures. The data presented are promising and confirm BT as an effective complementary approach in the management of various low-inflammatory, degenerative and stress-related pathological conditions such as rheumatic diseases [31].

The short-term efficacy of different thermal modalities, such as BT, mud therapy and spa therapy in patients with OA were assessed in another systematic review. The primary outcomes for the included articles were pain, stiffness and QoL. BT was found to be effective in all three outcomes, mud therapy significantly reduced pain and stiffness, and spa therapy showed pain relief. However, heterogeneity of research designs, methodologies and quality of sample sizes raised serious consideration as

well as the lack of double-blind design RCTs. Still, some evidence was suggestive of these thermal modalities being effective at least for the long-term basis of treating patients with OA [32]. While the mechanism of spa therapy is yet to be defined clearly, it is suggested that adipocytokine, including leptin and adiponectin, may play an important role in the pathophysiology of OA. This RCT study [33] tried to assess whether during spa therapy there is evidence of plasma modified levels of leptin and adiponectin in thirty patients with knee OA treated with a cycle of a combination of locally applied mudpacks and bicarbonate-sulphate mineral bath water daily. Plasma levels of the adipocytokines leptin and adiponectin, which play an important mediating role on cartilage metabolism, were assessed at baseline and at week 2. Concentrations of leptin and adiponectin were measured by ELISA. At basal time, plasma leptin levels were significantly correlated with body mass index (BMI) and gender, but no significant correlation was found with age, disease duration, radiographic severity of knee OA, VAS score or Lequesne index as well as for the plasma adiponectin levels. For plasma adiponectin levels a correlation was found only with the Lequesne index. At the end of the mud-bath therapy cycle, serum leptin levels showed a slight but not significant increase, while a significant decrease ($P < 0.05$) in serum adiponectin levels was found. Data showed that spa therapy can modify plasma levels of the adipocytokines leptin and adiponectin. Whether this effect may play a potential role in OA needs to be further investigated and clarified.

BT applied in combination with physical therapy (PT) could have a more positive effect in patients. That was the aim of a study on 305 patients aged 65 years and older with knee osteoarthritis (KOA) compared to physical therapy (PT) alone. Findings supported the original hypothesis that BT plus PT would be more effective than PT in KOA for patients aged over 65 years supporting the notion that reducing pain is a treatment goal that positively contributes to functionality, quality of life, fatigue and sleepiness of patients living with knee osteoarthritis [34]. Similar findings showed that spa treatment reduced the level of pain in the majority of patients in the short- and long-term follow-up and contributed to improving the quality of life both in their social relations and their environment [35]. In this study, the aim was to evaluate the short- and long-term effects of spa therapy on quality of life and pain in 70 patients aged 60 years and older with OA. Spa treatment lasted three weeks (15 days of treatment) and was applied during a session lasting 120 to 150 minutes a day. BT can become an alternative to pharmacological treatment for KOA patients who do not tolerate pharmacological treatments well. In this prospective, single blind RCT BT with mineral sulphate-bicarbonate-calcium water was used for its potential to offer substantial symptomatic improvement, and to create any changes in the QoL of 60 patients with symptomatic KOA. A significant reduction of drug consumption was noted. The differences between the two groups were significant for all considered parameters already from the 15th day and persisted during follow-up. Tolerability of BT seemed to be good, with light and transitory side effects. Results confirmed that BT with mineral sulphate-bicarbonate-calcium water has beneficial effects on pain, function and QoL in patients with KOA who last over time [36]. On the same notion, BT as well as terrain therapy is making use of microclimate factors. In a study designed to assess the short- and long-term effects of spa therapy, 102 patients with osteoarthritis (OA) of the spine received treatment in health resorts. The main conclusion was that spa therapy reduces pain, improves functionality, and enhances life satisfaction in these patients. Notably, the positive effects were sustained for at least six months. Spa therapy was more effective long-term, than outpatient treatment for OA of the spine [37].

CONCLUSION

From all the above (Table 1), it becomes clear that there is evidence to support that thermal water therapy or balneotherapy or spa therapy have therapeutic properties and are beneficial for people living with rheumatic diseases. BT in particular is a beneficial therapeutic modality and can be used as a supplement or complementary treatment, but its mechanisms are yet to be defined [38]. There is also evidence to support the notion that BT has proven to be beneficial mainly for patients with OA, hand OA and/or knee OA [15,33,35,36,37,39]. Patients with rheumatoid arthritis also benefit from the use of BT alone or in combination with mud packs, peloid therapy or other therapeutic modalities such as physiotherapy [11,16,17,18,19]. There is evidence of BT being effective for FM patients alleviating pain and functionality having a positive effect even on mood indices [3,23.40,41]. For AxSpA patients BT has shown some efficacy when combined with physiotherapy or even alone and positive effects have been shown to last in time, for at least three months [42,20,21]. Still, there are not enough systematic reviews and RCTs for this group of patients. General consensus among researchers is that there is a lack of double-blind RCTs

Table 1. *Data from patient groups using BT alone or in combination with other modalities.*

Authors	Type of study	Aim of study	Methodology	Outcome measures	Conclusion
Coccheri et al. 2008	Multicenter Study	Investigate whether appropriately applied spa therapy in several indications could be associated with a subsequent fall in the need for costly health services and missed working days due to sick-leave	39,943 patients divided into eight diseases subgroups (rheumatic, respiratory, dermatologic, gynecologic, otorhInologic, urinary, vascular and gastroenteric) underwent entry inquiry and appropriate spa treatment. 11,437 patients with OA	The Naiade project was a multicenter observational, longitudinal, questionnaire-based study comparing an "entry" inquiry addressed to patients before an entry thermal cycle, and a "return" inquiry after 1 year. Was carried in 297 Italian spa centers	The results showed that spa therapy is beneficial and could be cost-effective
Fraioli et al. 2018	Review	Investigate the evidence of the efficacy of BT, Mud-Pach therapy, Mud-Bath therapy on pain, functional limitation, drug use, and quality of life	Studies published between 2002 and 2017	35 studies were examined among which 12 were selected and included	The mud-pack therapy, balneotherapy, mud-bath therapy, and spa therapy have proved effective in the treatment and in the secondary prevention of knee osteoarthritis, by reducing pain, nonsteroidal anti-inflammatory drug consumption, and functional limitation and improving quality of life of affected patients
Antonelli et al. 2018	Meta-Analysis	Assess if balneotherapy and spa therapy can significantly improve Quality of Life (QoL) of patients with knee OA	Searched for articles about trials involving patients with knee OA and measuring the effects of balneotherapy and spa therapy on study participants' QoL with validated scales	Seventeen studies were considered eligible and included. Fourteen trials reported significant improvements in at least one QoL item after treatment	Evidence shows that BT and spa therapy can significantly improve QoL of patients with knee OA. Reduction of drug consumption and improvement of algofunctional indexes may be other beneficial effects.
Benini et al. 2021	RCT	Assess the efficacy of mud plus bath therapy in comparison to bath therapy alone in hand and knee osteoarthritis (HOA and KOA)	Patients were randomly assigned to either mud plus bath therapy (group 1) or balneotherapy (group 2)	The primary outcome was a change in AUSCAN questionnaire for HOA and in WOMAC for KOA at month 12. Evaluations were performed at baseline (B), immediately after the interventions (week 2, W2) and after 3 (M3), 6 (M6), 9 (M9) and 12 (M12) months. 37 patients with KOA and 52 with HOA were randomized in the study	Mud plus balneotherapy was more effective than balneotherapy alone on clinical outcomes of HOA. Differences in clinical outcomes of KOA were not significant, yet numerically higher
Cantista & Maraver 2020	RCT	(1) to identify possible health benefits (in terms of effects on dimensions of pain, functionality, emotional and social aspects, and quality of life) of a 3-week balneotherapy intervention in patients with knee osteoarthritis; (2) to assess the clinical relevance of any benefits detected; and (3) to determine if these effects persist	120 patients randomly assigned to (1) an experimental group (3 weeks of balneotherapy consisting of daily whirlpool baths, hydrokinesitherapy sessions, and knee shower/massages) or (2) control group in which no form of treatment apart from their usual analgesia medication was given	(1) visual analogue scale (VAS) of pain, (2) Timed Up & Go Test (TUG), (3) WOMAC osteoarthritis questionnaire, and (4) SF 36 health survey questionnaire. In the experimental group, these tests were conducted immediately before treatment, immediately after treatment, and at 3 months of follow-up. Patients assigned to the control group were assessed at the study start and 3 months later	Out of 60 patients in the experimental group, 45 were found to benefit from the treatment intervention in terms of pain relief among other aspects, and also when test scores were compared to those obtained in the control group. Improvements were often clinical relevant and in most patients persisted 3 months after treatment onset.

Table 1. *Data from patient groups using BT alone or in combination with other modalities (continued).*

Authors	Type of study	Aim of study	Methodology	Outcome measures	Conclusion
Branco et al. 2016	RCT	Evaluate the effectiveness of hot sulfurous and non-sulfurous waters in the treatment of knee osteoarthritis	140 patients, both genders, mean age of 64.8±8.9 years, with knee osteoarthritis and chronic knee pain. Patients were randomized into three groups: the sulfurous water (SW) group (N.=47), non-sulfurous water (NSW) group (N.=50), or control group (N.=43) who received no treatment. Treatment groups received 30 individual thermal baths (three 20-minute baths a week for 10 weeks) at 37-39 °C	Pain (visual analog scale, VAS), physical function (Western Ontario and McMaster Universities Osteoarthritis Index, WOMAC; Lequesne Algofunctional Index, LAFI; Stanford Health Assessment Questionnaire, HAQ), and use of pain medication. Patients were assessed before treatment (T1), at treatment endpoint (T2), and two months post-intervention (T3)	Both therapeutic methods were effective in the treatment of knee osteoarthritis; however, sulfurous baths yielded longer-lasting effects than non-sulfurous water baths.
Fernandez-Gonzalez et al. 2021	Review	Explore the effectiveness of balneotherapy for improving the quality of life of patients with RA	Spanish, English RCT or CCT until May 2021	A total 535 records were retrieved, and seven met the inclusion criteria	Balneotherapy benefits the quality of life of people with RA. Positive effects for both mineral bathing and immersion in sand or mud on the quality of life of people who suffer from RA
D' Angelo et al. 2021	Review	Evaluate the short-term efficacy of different thermal modalities in patients with osteoarthritis	Systematic Reviews from inception until October 2020, with no language restrictions, including: pain, stiffness and quality of life	Seventeen systematic reviews containing 27 unique relevant studies were included	BT was effective in reducing pain and improving stiffness and quality of life, mud therapy significantly reduced pain and stiffness, and spa therapy showed pain relief. The evidence supporting the efficacy of different thermal modalities could be seriously flawed due to methodological quality and sample size, to the presence of important treatment variations, and to the high level of heterogeneity and the absence of a double-blind design
Dilekçi et al. 2019	RCT	Investigate whether balneotherapy (BT) applied in combination with physical therapy (PT) has a more positive effect in patients aged 65 years and older with knee osteoarthritis (KOA) compared to PT alone	305 individuals were randomized into two groups. Group I was applied PT alone; group II was applied PT + BT	Assessments were made using the Pain (VAS), EQ-5D-3L Scale, Western Ontario And McMaster Universities Osteoarthritis Index (WOMAC), Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT-F) Scale, Epworth Sleepiness scale (EPWORTH) and the Outcome Measures in Rheumatology-The Osteoarthritis Research Society International set of responder criteria for osteoarthritis (OMERACT-OARSI) at the beginning (T0) and at the end (T1) of treatment	Balneotherapy plus physical therapy was more effective than physical therapy alone in KOA patients aged over 65 years. Reducing pain, especially, positively contributes to functionality, quality of life, fatigue and sleepiness of KOA patients

Table 1. *Data from patient groups using BT alone or in combination with other modalities (continued).*

Authors	Type of study	Aim of study	Methodology	Outcome measures	Conclusion
Fioravanti et al. 2011	RCT	Assess whether spa therapy modified plasma levels of leptin and adiponectin in thirty patients with knee OA treated with a cycle of a combination of daily locally applied mud-packs and bicarbonate-sulphate mineral bath water	30 patients with KOA	Leptin and adiponectin plasma levels were assessed at baseline and after 2 weeks, upon completion of the spa treatment period. The concentrations of leptin and adiponectin were measured by ELISA. Parameters: BMI, age, disease duration, radiographic severity of OA, VAS score, Lequesne index	Data showed that spa therapy can modify plasma levels of the adipocytokines leptin and adiponectin, important mediators of cartilage metabolism.
Zwolińska et al. 2018	CT	Evaluate the short- and long-term effects of spa therapy on quality of life and pain in patients aged 60 years and older with osteoarthritis	70 patients with generalized osteoarthritis were enrolled in the study. Spa treatment lasted 3 weeks (15 days of treatment) and was applied during a session lasting 120 to 150 minutes a day	Visual Analogue Scale (VAS) for pain, the Laitinen scale, and WHOQOL-BREF questionnaire were used to assess the condition of the patients. The examinations were performed three times: at the beginning of the spa treatment, after three months, and one year after the first examinations	Spa treatment reduced the level of pain in majority of the patients in short- and long-term follow-up and contributed to improving the quality of life in the domain of social relations and environment.
Zwolińska & Gasior 2022	CT	Assess short- and long-term effects of spa therapy administered to patients with osteoarthritis of the spine who received treatment in health resorts located in Poland	102 patients receiving treatment in health resorts, a group of subjects receiving outpatient treatment (100 patients) and a group receiving no therapy (100 patients)	Pain VAS and Laitinen, LISAT-9 and HAQ-20 questionnaires. Assessments three times: at the start of the therapy program, as well as one month and six months after the end of the program	Spa therapy reduces pain, improves functional efficiency and increases the level of life satisfaction in patients with osteoarthritis of the spine. Its effects are sustained for at least six months. Spa therapy is more effective long-term, than outpatient treatment.
Santos et al. 2016	Review	Summarize the available evidence on the effects of balneotherapy on patients with rheumatoid arthritis	Articles published from 1980 to 2014, RCTs, English, French, Spanish, Italian, Portuguese, participants with RA	A total of eight articles documenting RCTs, involving 496 patients	There are very few published studies about the use of natural mineral water in RA. International multicentre studies, using the same methodologies, could be achieved by carrying the scientific arguments
Verhagen et al. 2015	Review	Evaluate the benefits and harms of balneotherapy in patients with RA	Search various databases up to December 2014, BT the intervention under study, compared with another intervention or with no intervention. Pain, improvement, disability, tender joints, swollen joints and adverse events among the main outcome measures	Nine studies were included involving 579 participants	Overall evidence is insufficient to show that balneotherapy is more effective than no treatment; that one type of bath is more effective than another or that one type of bath is more effective than exercise or relaxation therapy
Ciprian et al. 2013	RCT	Evaluate the effects and tolerability of combined spa therapy and rehabilitation in a group of AS patients being treated with TNF inhibitors	30 AxSpA patients being treated with TNF inhibitors for at least 3 months were randomized: 15 were prescribed 10 sessions of spa therapy (mud packs and thermal baths) and rehabilitation (exercises in a thermal pool) and the other 15 were considered controls	BASFI, BASDAI, BASMI, VAS for back pain and HAQ. Assessments at entry, end of treatment, 3 months, 6 months	Combined spa therapy and rehabilitation caused a clear, long-term clinical improvement in AS patients being treated with TNF inhibitors. Thermal treatment was found to be well tolerated and none of the patients had disease relapse

Table 1. *Data from patient groups using BT alone or in combination with other modalities (continued).*

Authors	Type of study	Aim of study	Methodology	Outcome measures	Conclusion
Bestaş et al. 2021	prospective randomized study	Compare the effects of balneotherapy, water-based exercise (WBE), and land-based exercise (LBE) on disease activity, symptoms, sleep quality, quality of life, and serum sclerostin level (SSL) in patients with ankylosing spondylitis (AS)	Between January 2019 and January 2020, a total of 60 patients (35 males, 25 females; mean age: 40.9±11.2 years; range, 18 to 55 years) who were diagnosed with AS were randomly divided into the balneotherapy (n=20), WBE (n=20), and LBE (n=20) groups (20 sessions of treatment in groups of five to six patients)	Evaluations before treatment and at 4 and 12 weeks using the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), Bath Ankylosing Spondylitis Functional Index (BASFI), Bath Ankylosing Spondylitis Metrology Index (BASMI), Ankylosing Spondylitis Disease Activity Score-C-reactive protein (ASDAS-CRP), Maastricht Ankylosing Spondylitis Enthesitis Score (MASES), Ankylosing Spondylitis Quality of Life (ASQoL) Scale, Fatigue Severity Scale (FSS), and Pittsburg Sleep Quality Index (PSQI), and SSL were measured	Balneotherapy, WBE, and LBE are effective in the treatment of AS, and the beneficial effects may last for at least 12 weeks.
Ozkurt et al. 2012	RCT	Effectiveness of balneotherapy in fibromyalgia management	BT group (n=25) Control group (n=25) BT daily/2 weeks vs standard of care patients with FM	Pain intensity, Fibromyalgia Impact Questionnaire (FIQ), Beck Depression Inventory (BDI), patient's global assessment, investigator's global assessment, SF-36 scores, and tender point count.	Balneotherapy was found to be superior. The superiority of balneotherapy lasted up to the end of the 3rd month, except for the Beck Depression Inventory score and the investigator's global assessment score.
Bazzichi et al. 2013	RCT	Study the effects of both balneotherapy and mud-bath therapy treatments in patients affected by primary fibromyalgia (FM) using rheumatological, psychiatric, biochemical and proteomic approaches	41 FM patients (39 female and 2 male) received a 2-week thermal therapy programme consisting of therapy once daily for 6 days/week. Twenty-one patients received mud-bath treatment, while the other twenty balneotherapy	Pain, symptoms, and quality of life were assessed. Oxytocin, brain-derived neurotrophic factor (BDNF), ATP and serotonin transporter levels during therapy were assayed. Comparative whole saliva (WS) proteomic analysis was performed using a combination of two-dimensional electrophoresis (2DE) and mass spectrometry techniques	Thermal treatment might have a beneficial effect on the specific symptoms of the disease. In particular, while balneotherapy gives results that in most patients occur after the end of the treatment but which are no longer noticeable after 3 months, the mud-bath treatment gives longer lasting results.
Fioravanti et al. 2018	RCT	Assess the efficacy and tolerability of balneotherapy (BT) in patients with primary fibromyalgia syndrome (FS)	100 FS patients were randomized to receive a cycle of BT with highly mineralized sulfate water (BT group) or with tap water (control group)	Primary outcome measures were the change of global pain on the Visual Analogue Scale (VAS) and Fibromyalgia Impact Questionnaire total score (FIQ-Total) from baseline to 15 days. Secondary outcomes included Widespread Pain Index, Symptom Severity Scale Score, Short Form Health Survey, State-Trait Anxiety Inventory (STAI), and Center for Epidemiologic Studies Depression Scale	The differences between groups were significant for primary parameters at each time point. Similar results were obtained for secondary outcomes except for the STAI outcome. Our results support the short- and long-term therapeutic efficacy of BT in FS

and studies to confirm the mechanisms of this type of therapy and its therapeutic properties [9,16,17,18,37,33]. In addition to that, lack of homogeneity in study designs, methodologies, quality of sample sizes and analysis and parameters to be considered create discrepancies in the findings and the need for further research on the subject area to confirm findings [17,31,32]. Despite all that, evidence suggests that there are benefits and apparent therapeutic potential to this modality for patients with rheumatic diseases and could become a valid supplement to the available pharmacological treatment to be used in daily clinical practice.

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Treatment sequencing in metastatic colorectal cancer

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Abstract

Colorectal cancer management remains a significant challenge in contemporary oncology. Although current therapies have improved median survival rates, the overall prognosis for patients with stage IV disease remains dismal. Over the past decade, significant advances have expanded the available therapeutic options. The molecular characteristics of the tumor now play a pivotal role in therapy selection, both in the first line and subsequent lines of treatment in the metastatic setting. Chemotherapy remains the cornerstone of therapeutics for the disease while immunotherapy and targeted therapies implementation in the treatment algorithm continues to evolve rapidly. The idea of a "continuum of care" is currently fundamental, aiming at providing all the available therapeutic options to our patients to maximize the derived clinical benefit. Ongoing research efforts aim at further elucidating the underlying molecular biology of the tumor and eventually addressing the optimal sequencing of therapies in patients with metastatic colorectal cancer.

Key words: *Metastatic colorectal cancer; molecular characteristics; sequential therapy; pMMR tumors; dMMR tumors*

INTRODUCTION

Colorectal cancer ranks third among the most common types of cancer and is the second leading cause of cancer deaths globally [1]. Current estimates foresee an increase of incidence reaching up to 3 million deaths by 2040 with a concurrent decline in the age standardized mortality rate [2,3]. Although current therapies have led to improved median overall survival (OS) rates in patients with early colon cancer, a considerable proportion will eventually develop metastatic disease. Additionally, approximately 25% of patients are diagnosed with metastatic spread of the disease upon presentation [3]. The 5-year OS of patients with stage IV disease is estimated at 15%. However, with the advent of new therapeutic strategies, patients may achieve a median survival that exceeds 30 months according to current published data [4,5].

During the last decade, major advances have been made towards expanding the available therapeutic choices for patients with metastatic colorectal cancer. Tumor sidedness, extent of the disease, molecular characteristics of the tumor, aim of the applied therapy, patients' overall needs, and performance status are some of the key factors to be considered when deciding on the optimal therapeutic approach, ideally in the context of a multidisciplinary team [6]. When the disease is deemed incurable, the aim is to prolong life expectancy and palliate symptoms. The continuum of care concept, where all available therapies are applied serially, may improve survival rates and extend disease control [7].

First line therapy for pMMR tumors

It has been historically established that 5-fluorouracil (5-FU) is the cornerstone of stage IV colorectal cancer

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treatment. Both intravenous 5-FU and oral capecitabine have been shown to provide comparable results regarding their estimated survival benefit [8]. Further improvements in survival rates and progression-free survivals (PFS) estimates can be achieved by incorporating oxaliplatin and/or irinotecan with 5-FU in the first-line setting, using doublets or triplets i.e., FOLFOX, FOLFIRI or FOLFOXIRI [9].

Numerous clinical trials have explored the role of anti-EGFR targeted therapy in therapeutic regimens for metastatic disease. Results have clearly demonstrated that the presence of KRAS/NRAS and BRAF mutations precludes the use of EGFR monoclonal antibodies due to lack of efficacy and even detrimental effects in this population. On the other hand, in patients with tumors harboring RAS/RAF wild-type tumors, the addition of either cetuximab or panitumumab provides significant clinical benefits. The combination of FOLFIRI plus panitumumab in patients without KRAS mutations led to a significantly reduced risk of disease progression and improved OS compared to FOFLIRI alone [10]. Furthermore, the combination of FOLFOX plus cetuximab led to improved response rates and PFS in patients with RAS wild-type metastatic colorectal cancer [11]. Thus, the combination of 5-FU based doublets with the addition of anti-EGFR targeted therapy has been established as a valid first-line option for patients with KRAS/NRAS/BRAF wild type tumors.

Apart from targeting the EGFR axis, the benefits of antiangiogenic compounds have also been explored and incorporated in current treatment algorithms. Bevacizumab, a selective VEGF-A inhibitor, has been investigated in numerous clinical trials. When combined with capecitabine, its addition led to improved PFS, and when combined with irinotecan plus 5-FU plus leucovorin positive results in terms of OS were demonstrated [12,13]. Taken together, although bevacizumab provides modest results, it is a valid option in the treatment of patients with metastatic colorectal cancer, as it can be used for all patients regardless of molecular status.

At the time point of first-line therapy selection, the absence of mutations in both KRAS/NRAS and BRAF genes allows for the addition of EGFR monoclonal antibodies whereas their presence precludes their administration, and then bevacizumab is selected along with backbone cytotoxic chemotherapy if not contraindicated. However, primary tumor sidedness has also emerged as a key factor that affects the treatment strategy. Combined analyses from studies investigating anti-EGFRs highlighted the benefit of cetuximab or panitumumab addition in patients with left sided primary tumors [14]. On the contrary, patients with right-sided primary tumors fared better with the addition of bevacizumab to first-line therapy, while the use of anti-EGFR antibodies had limited impact in this population [15]. Recently, the question of how primary tumor sidedness impacts the selection of optimal therapy was investigated in a phase III clinical trial. PARADIGM confirmed the added benefit of anti-EGFR antibodies in left sided primary tumors while also providing better results in the entire population of the study regardless of primary tumor location. Improved OS was recorded mainly in patients with left sided primaries. Thus, it is wise to provide anti-EGFR targeted therapy in patients with left-sided primaries that are KRAS/NRAS/BRAF wild type based on the aforementioned data [16]. For patients with right-sided tumors or those harboring KRAS/NRAS/BRAF mutations, bevacizumab can be incorporated into the applied regimen.

First line in dMMR tumors

Current guidelines advise checking the microsatellite instability (MSI) status at presentation of any patient with colorectal cancer. MSI, when present, is the result of either sporadic events or Lynch syndrome. Genetic testing to confirm or exclude possible Lynch syndrome is of utmost importance for both patients and their families. Apart from familial and personal medical history, MSI status is characteristic of hypermutated tumors that usually also have higher tumor mutational burden, making them the perfect candidates for immunotherapy application [6,17,18].

In cases of early colon cancer, MSI status may have both prognostic and predictive role when it comes to adjuvant therapy selection [6,17]. However, this molecular feature, encountered in approximately 5% of patients with metastatic disease, is indicative of immunotherapy application in the first-line setting, or in later lines if not previously given. The results of KEYNOTE 177, where patients with MSI metastatic colorectal cancer tumors had significantly improved PFS with pembrolizumab versus chemotherapy, established immunotherapy as the ideal option for this subset of patients [18]. Furthermore, recent data of CHECKMATE 8HW confirmed the superiority of immunotherapy versus chemotherapy, in patients with MSI/dMMR stage IV colon cancer [19].

In addition, DNA polymerase epsilon (POLE) mutations, which lead to DNA-repair deficiencies, have been associated with high tumor mutational burden. POLE mutated colorectal cancers have a hypermutated profile, and their microenvironment is rich in neoantigens. They are usually present in male patients of younger age, with a higher incidence reported in right-sided primaries [20]. Somatic POLE mutations are present in less than 1% of colon cancer cases and are associated with microsatellite stable (MSS)/pMMR tumors whereas germline POLE mutations correlate with MMR germline variants in MSI high/dMMR tumors. According to recently published data, patients with tumors harboring POLE mutations may be candidates for immunotherapy due to the expected high response rates [21.22].

To conclude, in the first line setting of therapy for patients with metastatic colorectal cancers, Either chemotherapy plus targeted therapy or immunotherapy alone can be offered according to the molecular characteristics of the tumor. For pMMR/MMS tumors backbone chemotherapy consisting of 5-FU-based doublet may be selected with either anti-EGFR antibody addition if KRAS/NRAS/BRAF wild-type tumor is identified, especially if left-sided, or bevacizumab when mutations are present. If MSI/dMMR status is identified during molecular testing, then immunotherapy is the optimal treatment choice.

Further lines of therapy

After the failure of first-line therapy, patients proceed with second and further lines while aiming to receive all available antineoplastic agents under a continuum of care in order to prolong their life expectancy and palliate their symptoms. The optimal treatment choice and sequence rely on tumor mutational profile that is ideally acquired at presentation. Recent advances in colorectal cancer therapeutics have led to the incorporation of further targeted agents according to druggable mutations discovered when sequencing the tumor. BRAF inhibitors, anti-HER2 targeted agents, KRAS inhibitors, NTRK gene fusion therapies are among the available further treatment choices [23-26].

Targeting BRAF V600E mutation

BRAF mutations are usually present in right-sided colorectal cancers with an estimated incidence of 10-15%. BRAF mutant right-sided cancers may also have a MSI high/dMMR status due to MLH1 promoter methylation. For patients with metastatic disease, this mutation confers a poor prognosis and relative resistance to chemotherapy, while clinical trials conducted in the past, clearly demonstrate a lack of anti-EFGR antibody efficacy in this subset of patients. The most common BRAF mutation is V600E accounting for approximately 80% of all BRAF mutations in metastatic colorectal cancers [23,24].

Until today, patients presenting with BRAF-mutated tumors undergo standard first-line therapy, as already analyzed, mainly the combination of backbone cytotoxic chemotherapy plus bevacizumab with chemotherapy triplets leading to higher response rates in numerous clinical trials [6]. Although anti-EGFR resistance is expected when BRAF V600E mutation is present, the combination of a BRAF inhibitor plus anti-EGFR therapy has become a standard treatment in the second or further line. The combination of a BRAF inhibitor plus a MEK inhibitor and anti-EGFR antibody overcomes the reported resistance in patients with BRAF V600E mutated tumors. Based on the results of BEACON study, encorafenib plus cetuximab plus binimetinib versus FOLFIRI or irinotecan plus cetuximab resulted in improved OS and response rates [25]. Moreover, the doublet consisting of encorafenib plus cetuximab provided comparable median OS to the triplet combination with fewer toxicities. Both the doublet and triplet combination therapy are approved choices for patients harboring BRAF V600E mutated tumors [25,26].

As expected, triplet targeted therapy has also been investigated in the first line setting in a phase II clinical trial with promising results, while phase III clinical trials are underway to explore the administration of encorafenib plus anti-EGFR as the first-line treatment choice. Initial data have also emerged regarding the combination of BRAF inhibitor plus anti-EGFR plus immune checkpoint inhibitors in an attempt to investigate the impact of immunotherapy when combined with BRAF inhibitors in this population [27,28].

Targeting KRAS G12C mutation

Novel potent KRAS inhibitors have come into the spotlight recently, rendering the once undruggable KRAS mutation sensitive to selective inhibition. KRAS G12C mutated metastatic colorectal cancers account for approximately 2-4% of all metastatic colon cancers. Both sotorasib and adagrasib have been investigated in clinical trials with positive results. In CodeBreak 100 phase I clinical trial sotorasib provided low response rates in patients with KRAS G12C tumors [29]. How-

ever, when combined with the anti-EGFR antibody panitumumab improved response rates of 30% and a 5,7-month median PFS were recorded in CodeBreak 101 clinical trial [30]. In parallel, adagrasib alone provided a response rate of 19% in pretreated patients, but when combined with cetuximab the responses improved, climbing up to 46%, with a median PFS of 6,9 months [31].

Thus, the combination of targeted KRAS G12C inhibition along with anti-EGFR monoclonal antibodies seems to overcome the upstream signaling of EGFR activation. Recently the results of CodeBreak 300 phase III clinical trial depicted that the combination of sotorasib plus panitumumab resulted in tumor shrinkage in 81% of the patients and led to 51% reduction in the risk of disease progression. Skin-related toxicities and hypomagnesemia were the most common adverse events noted, with no new safety signals raised [32]. The scientific data provide a new therapeutic option for previously treated patients with KRAS G12C mutated metastatic colon cancer.

HER2 amplification targeting

HER2 amplification can be detected in 1-5% of all metastatic colorectal cancers, primarily in RAS/RAF wild-type tumors. Both immunohistochemistry (IHC) and next generation sequencing (NGS) can be applied to tumor specimens as detection methods [33]. Multiple clinical trials have explored the potency of sole anti-HER2 directed therapies with initially modest results. On the other hand, dual anti-HER2 targeting is approved for previously treated patients having HER2-positive tumors. The MOUNTAINEER clinical trial investigated the efficacy of tucatinib plus trastuzumab in 117 patients with response rates of 38% [34]. In addition, the combination of trastuzumab plus lapatinib provided an overall response rate of 30% in patients with HER2-positive tumors that were previously treated according to the results of HERACLES [35]. In the MyPathway phase II clinical trial, trastuzumab plus pertuzumab combined resulted in 32% overall response rates [36].

In DESTINY-CRC01 phase II clinical trial, previously treated patients with HER2 IHC +3 metastatic colorectal cancer underwent therapy with trastuzumab deruxtecan (T-Dxd), an HER2 antibody and topoisomerase inhibitor conjugate. The results were very promising with overall response rates of 45,3% and a median PFS of 6,9 months. Approximately one-third of those patients had already received prior anti-HER2 targeted therapy. Apart from the positive results, higher rates of T-Dxd-related pneumonitis were also announced [37]. The overall positive results of these studies, indicative of anti-HER2 targeted therapy efficacy, led to the approval of trastuzumab with either pertuzumab, lapatinib or tucatinib in the treatment algorithm of metastatic colon cancer, along with trastuzumab deruxtecan for chemotherapy-refractory HER2-positive patients. Ongoing clinical trials are aiming to assess the efficacy of anti-HER2 therapy in the first line setting [34-37].

NTRK and RET gene fusions

NTRK and RET gene fusions are rare, accounting for less than 1% of mutations in metastatic colorectal cancer. When present, targeted therapy can lead to high objective responses [38]. In particular, for patients harboring NTRK fusions that lead to activation of the chimeric tropomyosin receptor kinase, larotrectinib and entrectinib provide promising results. In 19 patients with metastatic colon cancer, larotrectinib resulted in responses reaching up to 47%, a median PFS of 5,6 months, and OS of 12 months [39]. Furthermore, entrectinib, tested in 10 patients, provided response rates of 20%, a PFS of three months and 16 months OS [40]. Both agents have been approved for use in patients with previously treated metastatic colon cancers. The LIBRETO-001 basket trial investigated the efficacy of selpercatinib in patients with RET gene fusions, resulting in an overall response rate of 20% and median duration of response of 9,4 months, leading to approval for RET-positive pretreated patients with metastatic colorectal cancer [41].

MSI in the non-first line setting

Since MSI high/dMMR tumors respond to immunotherapy, monoclonal antibodies targeting the PD1/PD-L1 axis have been incorporated in the first-line therapy setting of metastatic colorectal cancer therapy. However, if for any reason patients with dMMR tumors were not initially treated with immune checkpoint inhibitors, immunotherapy should be provided in the second or further lines of therapy. According to KEYNOTE 164, in pretreated patients with metastatic colon cancer pembrolizumab resulted in overall response rates of 33% [42]. Nivolumab also led to response rates of 30% when tested alone, while the combination of nivolumab plus ipilimumab provided higher responses of 55% with significantly improved quality of life [43]. Dostarlimab-gxly was also investigated in the COARNET phase I clinical trial where the cohort of

patients with dMMR or POLE mutated tumors achieved response rates of 36% [44]. Hence, immune checkpoint inhibitors should be part of the treatment sequence if not contraindicated, in previously treated metastatic colorectal cancer patients who have not already received immunotherapy.

Anti-EGFR targeted therapy in subsequent therapy lines

Patients who have KRAS/NRAS/BRAF wild-type tumors and who have not, for any reason, received anti-EGFR targeted therapy in the first line, the combination of cetuximab or panitumumab with chemotherapy is a valid option in further lines. The addition of panitumumab to FOLFIRI as a second line option resulted in improved PFS with evident differences in OS versus chemotherapy alone [45]. When tested as monotherapy, panitumumab led to improved OS versus best supportive care (BSC) after oxaliplatin and irinotecan failure [46]. Cetuximab, when combined with irinotecan also led to improved PFS however, without OS benefit versus irinotecan alone [47].

Whether anti-EGFR therapy is more effective after prior anti-angiogenesis administration or vice versa has been an area of investigation and controversy, with more recent studies indicating that anti-EGFR after a prior bevacizumab-containing regimen results in no difference in PFS compared to bevacizumab continuation therapy [48]. The concept of anti-EGFR rechallenge strategies in further lines of therapy has been extensively investigated in recent years due to the advent of liquid biopsies. Several clinical trials have led to the conclusion that acquired anti-EGFR resistance may be overcome upon anti-EGFR therapy withdrawal since resistant clones tend to decay exponentially [49]. The use of liquid biopsies for applying anti-EGFR is compelling and data demonstrating high response rates and prolonged PFS, compared to historical benchmarks, have come to spotlight further supporting the notion of anti-EGFR rechallenge when patients are found to carry RAS/Raf wild-type tumors using peripheral blood [48,49].

Anti-angiogenesis in subsequent lines of therapy

Bevacizumab continuation combined with standard second-line chemotherapy led to improved OS when compared to chemotherapy alone, according to the results of ML18147 study [50]. The same conclusion has been drawn by the GONO phase III BEBYP clinical study. Although modest, these results indicate that continuing anti-VEGF therapy can lead to marginal improvements in survival. The addition of bevacizumab after the failure of the first-line bevacizumab-free regimen also led to modest improvements [51]. Apart from bevacizumab, ziv-aflibercept, which functions as a VEGF trap and inhibits angiogenesis, is also approved as a component of second and further line of therapy in metastatic colorectal cancer patients. The VELOUR trial met its primary endpoint, providing improved OS even in patients previously treated with bevacizumab-containing regimen [52,53].

Ramucirumab, a monoclonal anti-VEGFR2 antibody, blocks VEGF signaling. The phase III RAISE clinical study investigated the addition of ramucirumab to FOFLIRI versus FOLFIRI plus placebo and resulted in improved OS in the experimental arm [54]. It is of note that angiogenic agents may lead to thromboembolic events, hypertension, or bleeding. However, a meta-analysis performed on this subject concluded that ramucirumab administrations did not increase the probability of thromboembolic or bleeding events [55].

Regorafenib is a multiple kinase inhibitor including VEGFR, that is incorporated in the treatment algorithm in the metastatic setting. In the phase III CORRECT trial, regorafenib led to improved survival (rates) compared to placebo, as well as in the phase III CONCUR trial. Main adverse events included hand-foot skin reaction, fatigue, hypertension, and diarrhea [57]. The subsequent phase II ReDOS trial evaluated an alternative dose-escalating schedule for regorafenib making it a valid option [58].

FRESCO and FRESCO2 are two phase clinical studies that explored the use of fruquintinib, an oral VEGFR 1,2,3 inhibitor, in patients with metastatic colon cancer who had undergone at least two prior lines of therapy, with a median of four lines in FRESCO2. When compared to placebo, fruquintinib improved OS including patients with prior anti-VEGF therapy exposure. Hypertension was the most reported adverse event [59,60].

TAS-102

Trifluridine tipiracil consists of trifluridine and tipiracil hydrochloride which block the degradation of the trifluridine component. This oral combination was evaluated in the phase III RECOURSE trial providing improved overall survival compared to placebo [61]. Furthermore, the combination of TAS-102 plus bevacizumab led to improved PFS when tested in a phase I/II clinical trial in patients previously treated with chemotherapy and

anti-VEGF therapy or anti-EGFR monoclonal antibodies [62,63].

Continuum of care

Recent advances in colorectal cancer therapy have improved patents' OS with metastatic disease. In some studies, survival that exceeds 32 months has been reported. However, the overall prognosis of patients with stage IV disease remains dismal. Contrary to previous scientific research aimed at addressing the optimal sequence of therapies, the idea of a "continuum of care" is gaining grounds, aiming to provide all the available therapeutic options to maximize the derived clinical benefit [64].

The issue of first-line therapy has been extensively studied and clearly addressed. It is wise to test patients at presentation for possible KRAS/NRAS/BRAF mutations, as well as for MSI status, to implement the appropriate therapy. It would be wise for both time and cost-effectiveness to evaluate a larger gene panel for existing mutations before the initiation of therapies, which can also assist in determining a median or long-term therapeutic plan. The molecular signature of the tumor seems to evolve as the cornerstone for both first and subsequent lines of therapy [6].

Among strategies to prolong patients' PFS, one might also opt for maintenance therapy after first-line application. This subject has been evaluated in different clinical trials, including CAIRO3, AIO 0207, PRODIGE9 amongst others [65,66,67]. According to the results of a systematic review, maintenance therapy with a fluoropyrimidine combined or not with bevacizumab improves PFS but has no impact on OS of patients [68]. Thus, one might opt for chemotherapy break based on scientific data from eleven randomized clinical trials where intermittent versus continuous systemic therapy was investigated. Opting for therapy holidays and intermittent treatment delivery did not lead to significant effects on OS when compared to continuous therapy administration [69].

Ongoing research

Despite the significant advances in the therapeutic landscape of colorectal cancer with the application of a more personalized pattern of therapy, unexplored areas still exist. Clustered regularly interspaced short palindromic repeat (CRISPR)- associated protein 9 can be used to identify previously undiscovered cancer genes in colorectal cancer based on genome editing techniques [70]. Epigenetic modifications have also been associated with colorectal cancer incidence and development [71]. Recently, scientific data highlighted the impact of microbiota in colorectal cancer and may unfold to a field of active research to further expand therapy armamentarium [72].

The relatively accessible next-generation sequencing platforms make it much more possible to identify new targets and unravel ways to inhibit signaling pathways and proteins such as TGFβ, p53, PI3K/AKT/mTOR [73]. It is of note though, that apart from the ongoing attempts to identify new targets and refine metastatic colorectal cancer therapy, resistance to currently available regimens remains a big subject. A gap in our knowledge regarding biomarker-based therapy sensitivity should be a primary focus of scientific research [74].

Under this scope, circulating tumor DNA (ct-DNA) may become a valuable tool in metastatic colorectal cancer therapeutics. Although of currently limited clinical use, the possible applications in patients' management are further investigated. Apart from mutation identification in cases where little time to therapy initiation is available or when anti-EGFR rechallenge strategies are applied, ct-DNA may be used to identify mechanisms of resistance to applied therapy or even detect the upcoming disease progression months before it is clinically evident. Monitoring the patient's journey from diagnosis of metastatic colorectal cancer throughout the application of different therapeutic regimens is an area of intense scientific research that aims to highlight ct-DNA as a useful biomarker in everyday clinical practice [75].

CONCLUSION

Metastatic colorectal cancer therapeutics have substantially evolved over the years, with new treatment options now readily available in clinical practice. Despite these advancements, conventional chemotherapy remains the mainstay of treatment across the various lines of therapy. Table 1 shows the line of therapy depending on the tumor mutations. However, with the advent of immunotherapy as well as new targeted therapies, regimen sequencing in the era of personalized metastatic colorectal cancer therapy can be rather challenging. In order to facilitate therapy sequencing and application, an in-depth comprehension of underlying molecular biology of the tumor as well as patients' characteristics and available treatment options is a fundamental prerequisite. Exposing the patient to all accessible options

Table 1. *Therapy depends on gene mutations.*

	pMMR		dMMR/MSI-H or POLE mut
	KRAS/NRAS/BRAF wt and left sided tumors	KRAS/NRAS/BRAF mut OR right-sided tumors	
1st line	ChT + Cetuximab or Panitumumab	ChT + Bevacizumab	Pembrolizumab OR Nivolumab OR Nivolumab + Ipilimumab OR Dostarlimab-gxly
2nd line	ChT + Bevacizumab or Ziv-aflibercept or Ramucirumab) **HER 2 amplified:** Trastuzumab + (Pertuzumab OR Lapatinib OR Tucatinib)	**BRAF V600E mut:** Engorafenib + (Cetuximab OR panitumumab)	As pMMR 1st line
Subsequent lines	Fruquitinib OR Regorafenib OR Trifluridine + tipiracil +/- Bevacizumab **HER2 amplified (IHC 3+):** Fam-trastuzuamb deruxtican-nxki **NTRK gene-fusion positive:** Entrectinib or Larotrectinib Or Repotrectinib **RET gene fusion positive:** Selpercatinib **KRAS G12C mut:** Adagrasib OR Sotorasib + Cetuximab OR Panitumumab		

should remain the primary focus of clinicians in order to maximize the derived benefit for their patients while waiting for results of continuing research to refine the optimal sequencing of therapies and improve outcomes for patients with metastatic colorectal cancer.

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