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HIGHLIGHT

SYSTEMATIC REVIEW AND META-ANALYSIS

Omega-3 Polyunsaturated Fatty Acids and Ventricular Arrhythmias

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TABLE OF CONTENTS

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JORNAL DA SOCIEDADE DAS CIÊNCIAS MÉDICAS DE LISBOA

EDITORIAL

Medical Education: Challenges in a World That is no More at the Time of *The Anatomy Lesson* of Dr. Nicolaes Tulp

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Firstly, it is amazing that it is not yet defined what is expected from a physician in Portugal within the next ten years: what skills should medical doctors have?

The answer to this question is obviously a very complex task which, in order not to be based solely on readings from a good crystal ball or the entrails of a sacred bird, must have a scientific approach, led by physicians and universities. However, it must also include the perspectives of other stakeholders in the process: industry, other health professionals, patient associations, and the general population, among others. It is a process that, once started, is already behind schedule, but even so, it would still provide a basis. The translation of these societal needs into pedagogical and clinical competencies requires the technical grounding that only those immersed in medical practice and education can provide.

In the absence of this crucial definition, some speculation on these putatively important skills can be done. Emerging skills are numerous and varied, some more focused on medical care and others less specific. A physician is not expected just "to know," but "to do," and do well. As prime examples, we can cite technologies and the new epidemiological reality. Additional relevant changes include the paradigm shift in the lifestyle of the new generations, the need to intervene in public health policies and management skills.

The inclusion of skills related to technological evolution, including artificial intelligence, precision medicine, and continuous monitoring systems, seems to be the most obvious. Some examples include the ability to interpret and validate diagnoses assisted by artificial intelligence, knowing how to deal with the limitations and biases of

> *"Medical education, specifically how to teach, must be scientifically informed. The scientific evolution of learning sciences must be taken into account in curriculum design."*

clinical machine learning models, adequate use of the data-driven clinical decision support tools, integration of remote monitoring data and molecular biomarkers into clinical practice, or working with robotic surgery systems and advanced telemedicine.

It also seems that epidemiological changes (population aging, chronicity and comorbidity, and the increasingly cosmopolitan nature of the Portuguese society) cannot be reflected solely in the inclusion of these theoretical aspects in the curriculum ("four additional lectures and the issue is solved"). To cope with these challenges skills or competences are needed, such as teamwork, leadership, communication, and shared decision-making, which, in addition to including how to deal with radically different cultures, must be taught in Portuguese medical schools.

Other important skills, would be those related to management, how to maintain professional and care integrity in a complex media ecosystem, recognizing and dealing with the emerging public health threats, and integrating the impact of climate change on health, among others.

Human relations, compassion, and empathy are the foundation of every medical act. While Portuguese medical schools recognize these as core competencies, the challenge lies not in acknowledging their importance but in ensuring they survive the pressures of an increasingly technology-driven and overburdened curriculum, and that they are genuinely assessed rather than merely assumed.

Medical education, specifically how to teach, must be scientifically informed. The scientific evolution of learning sciences must be taken into account in curriculum design. The methods that have proven effectiveness, such as problem-based learning, clinical simulation, or structured feedback should be implemented. The assessment needs a dramatic shift. In addition to an improvement in curricular alignment, assessment must include competency-based assessments, especially in the workplace, and transforming assessment into a learning tool. The validity and reliability of quantitative assessment must also be discussed - what does a grade of 16 or 17 mean in the assessment of a patient with a red eye?

Medical schools have made remarkable efforts to face these new realities, but constraints are also significant. As mentioned before, the lack of definition of competencies is probably the most important one. However, there are other factors such as curricular overload (and a need to remove content - something comparable to cleaning the Augean Stables), a pedagogical culture that still confounds exposition with learning and the alignment of assessment with competencies (decrease the impact of high stakes assessments, e.g. - comparable to slaying the Lernaean Hydra). The pressure on university hospitals, the scarcity of clinical teachers with dedicated time for teaching, or the difficulty in attracting students to specialties and regions with greater need, among others.

> *"A physician is not expected just 'to know,' but 'to do,' and do well."*

> *"Medical schools must themselves be pedagogical research communities."*

Of course, none of this implies abandoning the scientific rigor that should underlie any medical training. It implies recognition that this rigor must also be applied to teaching itself. Medical schools must themselves be pedagogical research communities capable of studying, investigating, evaluating, and reforming their own practices with the same critical spirit that they demand from their students when analyzing a clinical trial.

The Journal of the Sociedade das Ciências Médicas could play an important role in this process, by accompanying and helping structure this national conversation. Not only as a space for publishing research in medical education, but as an active platform for the debate, dissemination, and critical reflection.

Frederico Simões do Couto

Co-Editor JSCMed; President of the Pedagogical Council, Católica Medical School

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JORNAL DA SOCIEDADE DAS CIÊNCIAS MÉDICAS DE LISBOA

VIEWPOINT

Senile Dementia: An Old-Fashioned Concept

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ABSTRACT: The concept of *senile dementia* was widely accepted at the transition of the 19th to the 20th century. The pioneering researchers found different pathologies in the brains of old demented patients, apparently linked to the clinical severity of the disease. The scientific communication by Alois Alzheimer describing a 51-year-old woman with dementia led to the establishment of a distinction between *senile dementia* and *presenile dementia* or Alzheimer's disease that persisted for several decades. Later in the 20th century, research on clinicopathological series found that there were no significant differences between senile and presenile cases except for age, and a single designation was adopted – Alzheimer's disease. The term *senile dementia* was essentially dropped.

Recently, the presence of different pathologies in the brains of demented old patients has been emphasised. The view that in older patients the presence of brain co-pathologies is very common and is associated to cognitive decline seems to have come intriguingly close to the pioneering researchers' concept of *senile dementia.*

KEY WORDS: Presenile Dementia; Senile Dementia; Alzheimer's Disease; History

The concept of dementia, in the present clinical meaning, was established at the end of the 18th century, largely due to the works of the French physicians Philippe Pinel (1745 - 1826) and his disciple Jean-Étienne Dominique Esquirol (1772 - 1840)[1,2]. Pinel in 1801 explicitly used the term *démence sénile* as one of the causes of dementia or abolition of thought (Fig. 1), *La démence sénile, souvent accélérée par l'épuisement des plaisirs, se rapproche de celle qui vient d'être décrite [manie avec délire]; mais on y remarque bien moins d'effervescence.*[3] At the transition of the 19 to the 20 century, it was widely recognized that elderly people would suffer from dementia, and important findings on the pathology of *senile dementia* were described by the great pioneering researchers of that time. According to German Berrios,[4] senile plaques, called miliary foci or Redlich-Fischer's plaques, were identified as early as 1892 by Paul-Oscar Blocq and Gheorghe Marinesco. The discovery of neurofibrillary tangles is usually attributed to Alois Alzheimer, although they were probably identified previously, as proposed by Frank Ashall.[5] Alois Alzheimer mentioned the terms

FIGURE 1. "Senile Dementia". Engraving published in Medical Times and Gazette. 1858;37:506a, from a photograph by Dr. Diamond. Courtesy of Wellcome Collection.

Dementia senilis and *senile Demenz* as early as 1894.[6]

Remarkably, the initial researchers described the co-existence of multiple pathologies in the senile brain. As early as 1863 Louis Victor Marcé reported a neuropathologic series of 40 senile dementia cases from the hospice of Bicêtre.[7] He consistently described *atrophie des circonvolutions*, but also other different brain lesions. Remarkably, he noted that *dans la démence sénile, les lésions organiques sont toujours proportionnées au degré de l' affaiblissement de la motilité et de l' intelligence.* Alfred Campbell described in 1894 the neuropathology of the *aged insane*, identifying, beyond neurodegenerative changes, hemorrhages and thrombotic softenings.[8] Adolf Meyer introduced in 1896 the work of Jean Noetzli presenting a clinicopathological series of 70 cases of *senile dementia*, most cases studied by Auguste Forel.[9] Notably, he attributed senile dementia to a list of different etiologies, namely focal lesions, alcoholism and several psychiatric conditions, beyond what he called *typical senile dementia*. As noted by Engelhardt and Grinberg,[10] Alois Alzheimer in 1898 also considered that mental disorders of old age would probably correspond to clinically and histologically different forms, when he wrote *Die meisten dieser Krankheitsbilder bedürfen noch weiterer klinischen und anatomischen Erforschung, doch ist zu hoffen, dass wir bald die Seelenstörungen des Greisenalters in eine vielleicht noch grössere Anzahl klinisch und histologisch scharf trennbare Formen zergliedern können.*[11] Francesco Bonfiglio described in 1908 an interesting case with both neurodegenerative findings and syphilitic neuropathology.[12] At the same time, the intriguing absence of a clear-cut distinction between senile dementia and normal ageing was recognized, as emphasised by Gaetano Perusini in 1911.[13]

We may conclude that at the transition of the 19 to the 20 century *senile dementia* was a widely accepted concept (Figure), different pathologies could be found in the brains of patients with *senile dementia*, they were linked to the clinical severity of the disease, but could not be definitely separated from the normal ageing process. The eminent psychiatrist Emil Kraepe-

lin concluded in his widespread book *Psychiatrie. Ein Lehrbuch für Studierende und Ärzte* in 1910 that *senile dementia* might reflect vulnerability of the brain to the ageing process. Following the English translation made by the *Italian Study Group on Brain Aging* and the *World Federation of Neurology Research Group on the Dementias*, he wrote *the involutional processes, known in man as old age, can also influence mental health seriously is most clearly demonstrated by the well-known fact of senile dementia,* and importantly admitted that *senile dementia* would be caused by different disorders when proposing that *very probably there exists a series of diseases, not yet well-known, that might be causally related to involutional processes.*[14]

However, the clinical concept of *senile dementia* was deemed to be dramatically challenged by Alois Alzheimer's scientific communication entitled Über *eine eigenartige Erkrankung der Hirnrinde* at the 37 Meeting of the South West German Psychiatrists, in Tübingen, Germany, 1906, published the following year in the Berliner journal *Allg Z Psychiatrie Psychisch-Gerichtl Med*, describing a 51-year-old woman with dementia.[15] This uncertainty became after Emil Kraepelin, in his above cited book *Psychiatrie. Ein Lehrbuch für Studierende und Ärzte*, in 1910, advanced the eponym of Alzheimer based on the previous description of the case. He wrote *While the anatomical findings suggest that we are dealing with a particularly serious form of senile dementia, the fact that this disease sometimes starts already around the age of 40 does not allow this supposition. In such cases we should at least assume a "senium praecox" if not perhaps a more or less age-independent unique disease process.*[14] The dichotomy between *senile dementia* and *presenile dementia* or Alzheimer's disease was launched, and persisted for several decades.

Later in the 20 century, research on clinicopathological series (reviewed in Amaducci *et al.*, 1986)[16] would contest the distinction between *senile dementia* and *presenile dementia*. To this regard, the position of the influential American neurologist Robert Katzman might have been determinant, by claiming in 1976 that *neither the clinician, the neuropathologist, nor the electron microscopist can distinguish between the two disorders, except by age of the patient*, and further on asserted *we believe it is time to drop the arbitrary age distinction and adopt the single designation, Alzheimer's disease.* In the same line, Professor Carlos Garcia assumed in 1988 *recentemente, com base em estudos de índole clínica, anatomopatológica e bioquímica, concluiu-se não haver razão para considerar que a doença de Alzheimer e a demência senil fossem doenças diferentes e passou a ser uso falar-se só da doença de Alzheimer.*[17]

Interestingly, in the last decades, the presence of different pathologies in the brains of demented, and even non-demented people, has been emphasised as the rule and not the exception. By reviewing several series of demented elderly brains, including their own, Kurt Jellinger and Johannes Attems in 2015 stated *it is obvious that cognitive and other neuropsychiatric impairment in the aged result from a multimorbid condition in the central nervous system rather from a single disease, and the number of complex pathologies progresses with increasing age*.[18] A more recent study, recruiting more than four thousand cases by combining six cohorts with decedents aged 80 years or older having autopsy data, found that 91% had more than one of six key neuropathologies (neuritic plaques, Braak neurofibrillary tangle stage, microinfarcts, macroinfarcts, Lewy bodies, and limbic-predominant age-related TDP-43 encephalopathy neuropathologic change (LATE-NC)) and 41% had three or more, co-pathology being strongly associated with the dementia status.[19]

The view that cognitive decline in old people is distinct, due to presence of brain co-pathologies, seems to have come intriguingly close to the pioneering researchers' concept of *senile dementia*.

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JORNAL DA SOCIEDADE DAS CIÊNCIAS MÉDICAS DE LISBOA

ONE LIFE IN MEDICINE

Catarina Resende de Oliveira

Neurologist and Scientist

By
Victor Oliveira

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She was born in Coimbra in February 1946 into a family originating from the nearby village of Murtosa. Given that her father was a professor of medicine at the University of Coimbra (Full Professor of Microbiology and Parasitology), her choice of medicine comes as no surprise. She graduated from the University of Coimbra in 1970, became a specialist in neurology in 1976 at the Hospitais da Universidade de Coimbra (HUC), and attained the position of Full Professor in 1984.

Her primary scientific mentors were Professor António Nunes Vicente (a neurologist) and Professor Arsélio Pato de Carvalho (a biologist), who directed a research laboratory at the University of Coimbra. This laboratory would later evolve into the Centro de Neurociências e Biologia Celular, where Catarina Oliveira became its President in 2003.

As a neurologist, she committed herself to the study of neurodegenerative diseases, which pose a significant burden on the elderly population.

She believes that neurodegenerative disorders are multifactorial conditions of largely unknown causes, although factors such as oxidative stress, excitotoxicity, calcium dysregulation, and mitochondrial dys-

FIGURE 1. Professor Catarina Resende de Oliveira participating in a round-table discussion during the 39th Annual Meeting of the Study Group on Cerebral Aging and Dementia, 2025.
Foto: © Esfera das Ideias.

> *Intellectual activity is an important tool for preserving a healthy brain.*
>
> **Catarina Resende de Oliveira**

function are thought to play a critical role in the cell damage associated with these disorders.

It is also recognized that amyloidogenic peptides, including beta-amyloid, prion peptide, and alpha-synuclein, contribute to neuronal death and glial activation during this process. Indeed, her primary research interests revolve around investigating these factors, as well as assessing antioxidant defenses and neuronal repair mechanisms in aging and neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, and Huntington's disease.

Additionally, she is interested in studying biomarkers and genetics related to such diseases. During her career, she has supervised more than 20 young investigators who obtained their PhD degrees at the University of Coimbra and led a research group focused on advanced diagnostics and biomarkers associated with aging and brain diseases, particularly the study of new biomarkers for neurodegenerative disorders at the Centro de Neurociências e Biologia Celular at the University of Coimbra.

She has been an active member of JPND, a European consortium dedicated to investigating biomarkers for Alzheimer's and prion diseases. The European Union Joint Programme – Neurodegenerative Disease Research (JPND) represents the world's largest collaborative research effort aimed at addressing the challenges posed by neurodegenerative diseases, especially Alzheimer's disease.

It is no wonder that Professor Catarina Oliveira has held several leadership roles, including serving as President of the Scientific Council of the Faculty of Medicine at the University of Coimbra and contributing to curricular reforms within her Medical School. She has also served as President of the Scientific Council for Life Sciences and Health at the Portuguese Founda-

tion for Science and Technology (FCT) and coordinated the Inter-University PhD Program in Aging and Chronic Diseases, among several other responsibilities. She was also the first President of the Portuguese Agency for Clinical Research and Biomedical Innovation (AICIB).

Throughout her career, she has received numerous accolades, including: the Portuguese Government's Grande-Oficial da Ordem da Instrução Pública (Order of Public Instruction) in 2014; the Portuguese Ministry of Health distinction "Serviços Distintos" (Distinct Services), gold medal, in 2016; the gold medal of the Faculty of Medicine of the University of Coimbra in 2016; the European Society for Clinical Investigation (ESCI) Award and Albert Struyvenberg Medal in 2022; and the Prémio Ciência (FCT) in 2024.

Her contribution to medical research in Portugal, together with the international recognition she achieved, provides an example for young investigators in her country and abroad.

Married to a physician (Orthopaedic Surgeon), mother of four and grandmother of six grandchildren, she managed to reconcile family responsibilities with scientific activity at the highest level, which is internationally recognized in her field, making her a reference for Portuguese investigators.

Although retired from her academic duties since 2016, she remains active not only due to the various scientific invitations she receives but also because she believes that intellectual activity is an important tool for preserving a healthy brain, as she states.

FIGURE 2. Tribute to Professor Catarina Resende de Oliveira by the Portuguese Society of Neurology, 2024. *(Foto: © Esfera das Ideias.)*

RESEARCH ARTICLE

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JORNAL DA SOCIEDADE DAS CIÊNCIAS MÉDICAS DE LISBOA

First Exposure to Virtual Reality in Medical Education: Insights From *Meta Quest 2* Use

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ABSTRACT: Introduction: Virtual reality (VR) is increasingly integrated into medical education, yet its impact on student engagement and learning outcomes is not fully defined. This study evaluated medical students' experiences with VR (Meta Quest 2) for procedural skills training. **Methods:** A cross-sectional survey was conducted with third-year medical students at the Faculty of Medicine, University of Lisbon over one semester. Participants trained in intubation, arterial line placement, thoracentesis, paracentesis, and central line insertion using Meta Quest 2 headsets. The survey assessed familiarity, engagement, perceived realism, quality, effectiveness, helpfulness for understanding procedures, and preference for VR over traditional methods. Descriptive statistics were calculated, and chi-square tests explored gender differences. **Results:** Of 298 respondents (81% response rate), 5% were very familiar with VR, while 60% were not familiar at all. Engagement was high, with 54% feeling very engaged and 37% somewhat engaged. Realism was rated somewhat realistic by 56% and very realistic by 20%. VR was considered somewhat effective by 58% and very effective by 30%. Fifty-five percent preferred VR over traditional methods, and 67% supported its integration into the curriculum. For procedural understanding, 64% found VR very helpful and 31% somewhat helpful. **Conclusion:** VR training improved students' confidence and procedural understanding despite limited prior exposure. While improvements in realism and technical accuracy are needed, the high engagement and positive perceptions support VR's role as a valuable adjunct to traditional teaching in medical education.

KEYWORDS: Virtual reality; Medical education; Meta Quest 2; Simulation Training; Clinical Competence.

INTRODUCTION

Virtual Reality (VR) is a digital educational tool that leverages computer technology to create immersive three-dimensional (3D) environments that users can interact with in seemingly real ways[1]. Virtual worlds are three-dimensional settings built around multiplayer online games that let players communicate without time or place restrictions[2]. These environments can simulate clinical settings for training emergency personnel in managing mass-casualty incidents [3], with avatars representing patients to create realistic simulations. VR provides a more immersive experience than traditional methods[1,4], enhancing spatial understanding and student motiva-

tion. Studies show that VR-based learning can be as effective, if not more so, than traditional methods like lectures, textbooks, and cadaveric dissection[1,4,5].

Several studies have underscored the potential of VR in medical education, highlighting its ability to improve student engagement, knowledge retention, and the acquisition of clinical skills[4-6]. The immersive nature of VR makes it a powerful tool for enhancing the learning experience, offering a dynamic and interactive platform for students to practice and refine their skills[4,6-8].

VR in medical education primarily focuses on two areas: developing technical competencies and teaching soft skills. Meta Quest 2 mainly focuses in developing technical competencies, allowing learners to engage in surgical procedures with simulated patients[4-7].

This research intends to build on these findings by providing a comprehensive analysis of MS feedback on VR use to teach technical skills[9]. The study aims to systematically gather and analyze feedback from medical students on their firsthand experiences with virtual reality (VR) technologies, focusing on the use of Meta Quest 2.

METHODOLOGY

This study was designed as a cross-sectional observational study conducted during a semester at the Faculty of Medicine, University of Lisbon. A total of 368 third-year medical students were enrolled in the "Introduction to Clinical Practice" course and were included without exclusion criteria.

During the teaching period, students participated in a 50-minute VR training session using Meta Quest 2 headsets with NP Skills Lab software. Students could choose to practice one of the following procedures: intubation with direct laryngoscopy, arterial line insertion, thoracocentesis, paracentesis, or central line placement. All procedures were performed in a VR environment with visual support from ghosted hands, clear procedural protocols, and a guidance mode. These techniques were also taught during in-room lectures and integrated into clinical case discussions over the semester.

The survey instrument was developed with the support of artificial intelligence (chatgpt, GPT-4) to evaluate familiarity with VR, engagement level, perceived realism, and preference for VR compared to traditional teaching methods. The questionnaire contained both closed-ended items using 5-point Likert scales and open-ended questions for qualitative feedback. Participation was voluntary and responses were anonymous.

The survey was distributed immediately after the VR session via a QR code, allowing real-time data collection to capture impressions while the experience was fresh. Data were analyzed using IBM SPSS Statistics, version 29.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics (means, standard deviations, frequencies, and percentages) were calculated, and Pearson's chi-square test was used to examine associations between categorical variables. A significance level of $p < 0.05$ was adopted. [Institutional Ethics Committee (Reference No. 241/24).]

RESULTS

The study population consisted of 297 third-year medical students, with a response rate of 81%. The majority of participants were under 21 years of age, and 70% were female, reflecting the overall demographic profile of third-year students.

Survey responses are summarized in Table 1. The results indicate that only 5% of the students were very familiar with VR technology, while the majority (60%) were not familiar at all. A closer look at gender distribution shows that 40 women and 139 men rated themselves as not familiar at all, while only 9 women and 6 men reported being very familiar. Chi-square analysis confirmed a statistically significant association between gender and familiarity. The p-value of <0.0001 for this question indicates a highly significant difference between female and male participants in their familiarity with virtual reality technology, with male respondents more frequently reporting being "very familiar" compared to their female counterparts.

Regarding engagement with VR, 54% of the students reported being very engaged, 37% somewhat engaged, 6% neutral, and 2% somewhat disengaged. Among them, 110 women and 51 men reported being very engaged, while 77 women and 32 men were somewhat engaged.

When it comes to the perceived realism of VR technology, 56% of the students found it somewhat realistic, 20% very realistic, 15% neutral, and 9% not very realistic. Perceived quality was also explored, with 50% rating the experience as good and 41% as excellent (Figure 1). Gender analysis showed that 106 women and 43 men rated it as good, **while** 83 women and 38 men rated it as excellent.

TABLE 1. Survey responses

Survey question		N	Percentage (%)	Female n(%)	Male n(%)	P-value
How familiar are you with virtual reality technology?	Very familiar	15	5	6 (3,4%)	9 (10,2%)	< 0,0001
	Somewhat familiar	102	35	62 (29,8%)	40 (44,9%)	
	Not familiar at all	179	60	139 (66,8%)	40 (44,9%)	
How often do you use virtual reality technology?	Daily	0	0	0	0	0,403
	Weekly	5	1	3 (1%)	2 (2,2%)	
	Monthly	8	3	5 (2,4%)	3 (3,4%)	
	Rarely	80	27	51 (24,5%)	29 (32,6%)	
	Never	205	69	150 (72,1%)	55 (61,8%)	
How do you rate the quality of virtual reality experience?	Excellent	121	41	83 (39,9%)	38 (42,7%)	0,9
	Good	149	50	106 (51,9%)	43 (48,3%)	
	Average	23	8	16 (7,7%)	7 (7,9%)	
	Poor	2	0	1 (0,5%)	1 (1,1%)	
	Very poor	0	0	0	0	
How effective do you think virtual reality is as a tool for practicing surgical procedures?	Extremelly effective	88	29	62 (29,8%)	26 (29,2%)	0,286
	Somewhat effective	173	58	123 (59,3%)	50 (56,2%)	
	Neutral	20	7	13 (6,3%)	7 (7,9%)	
	Somewhat ineffective	13	4	9 (4,6%)	4 (4,5%)	
	Completely ineffective	2	1	0	2 (2,2%)	
How engaged did you feel during the virtual reality experience?	Very engaged	161	54	110 (52,9%)	51 (58,6%)	0,557
	Somewhat engaged	109	37	77 (37%)	32 (37%)	
	Neutral	16	6	14 (6,7%)	2 (2,2%)	
	Somewhat disengaged	7	2	5 (2,4%)	2 (2,2%)	
	Very disengaged	1	0	1 (1%)	0	
Did you find the virtual reality surgical experience helpful in understanding the procedure better?	Yes, very helpful	191	64	133 (64%)	58 (65,2%)	0,696
	Somewhat helpful	92	31	63 (30,5%)	29 (32,6%)	
	Neutral	7	3	6 (3%)	1 (1,1%)	
	Not very helpful	6	2	5 (2,5%)	1 (1,1%)	
	Not helpful at all	0	0	0	0	
Would you prefer to learn using virtual reality technology instead of traditional methods such as textbooks or lectures?	Yes, definitely	164	55	113 (54,3%)	51 (57,4%)	0,954
	Somewhat	79	27	56 (26,9%)	23 (25,8%)	
	Neutral	30	10	22 (10,6%)	8 (9%)	
	Not really	21	7	15 (7,2%)	6 (6,7%)	
	No, not at all	2	1	1 (1%)	1 (1,1%)	
Would you prefer to practice procedures using virtual reality technology instead of traditional methods such as cadavers or animal models?	Yes, definitely	53	18	32 (15,4%)	21 (23,6%)	0,075
	Somewhat	85	29	67 (32,2%)	18 (20,2%)	
	Neutral	69	23	43 (20,7%)	26 (29,2%)	
	Not really	62	21	47 (22,6%)	15 (16,9%)	
	No, not at all	28	9	19 (9,1%)	9 (10,1%)	
How would you rate the realism of the virtual reality experience?	Very realistic	58	20	41 (19,7%)	17 (19,1%)	0,516
	Somewhat realistic	167	56	119 (57,2%)	48 (53,9%)	
	Neutral	45	15	29 (14%)	16 (18%)	
	Not very realistic	26	9	19 (9,1%)	7 (7,9%)	
	Not realistic at all	0	0	0	1 (1,1%)	
How likely are you to recommend virtual reality technology for practicing surgical procedures to your peers?	Very likely	144	48	104 (50%)	40 (44,9%)	0,396
	Somewhat likely	112	38	72 (34,5%)	40 (44,9%)	
	Neutral	23	8	17 (8,2%)	6 (6,7%)	
	Not very likely	16	5	13 (6,3%)	3 (3,5%)	
	Not likely at all	2	1	2 (1%)	0	
Do you think virtual reality technology for practicing procedures should be incorporated into medical school curriculums?	Yes, definitely	200	67	141 (67,8%)	59 (66,3%)	0,131
	Somewhat	73	25	48 (23,1%)	25 (28,1%)	
	Neutral	15	5	14 (6,7%)	1 (1,1%)	
	Not really	8	3	5 (2,4%)	3 (3,4%)	
	No, not at all	1	0	0	1 (1,1%)	
In your opinion, at what stage of the medical curriculum is this technology most useful?	Since 1st year	143	49	100 (48%)	43 (50,3%)	0,907
	Since 2nd year	3	1	2 (1%)	1 (1,7%)	
	In clinical years (> 3rd year)	147	50	106 (51%)	41 (48%)	

In terms of VR's effectiveness as a learning tool, around 90% of students responded positively. Regarding helpfulness, 64% found it very helpful and 31% somewhat helpful, with 133 women and 58 men in the first category **and** 63 women and 29 men in the second.

A majority of 55% of students indicated they would prefer to learn using VR instead of traditional methods. Interestingly, 50% believed VR is most useful in the clinical years (after the third year), while 48% found it useful from the first year. For curricular integration, 141 women and 59 men stated VR should definitely be incorporated into medical education.

When asked if they would recommend VR for practicing surgical procedures to peers, 48% were very likely and 38% somewhat likely to do so. Furthermore, 67% felt VR should be incorporated into medical school curriculums. These findings reinforce the perception of VR as a credible and desirable adjunct to traditional medical training.

Regarding qualitative feedback, students particularly appreciated the ability to repeatedly practice procedures, which significantly boosted their confidence before real-life applications. They felt more prepared and less anxious about performing actual procedures.

Students suggested several improvements to improve the VR experience. 40% recommended increasing the realism of procedures, particularly in gesture recognition, while 30% emphasized the need for more dynamic elements such as responsive vital signs. Additionally, 35% suggested that the tools should be more interactive and less guided to enhance realism and learning value, as their experience was based on the learning mode.

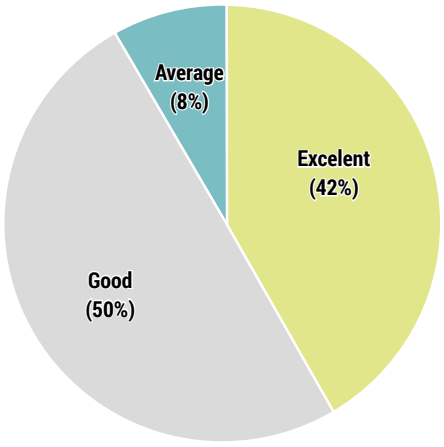

FIGURE 1. Medical students' ratings of the quality of the virtual reality experience.

Several students also mentioned the need for better ergonomic design of VR equipment to reduce discomfort during long sessions.

Non-structured feedback included recommendations to incorporate finer details and precise movements in VR simulations to mimic real-life procedures better. Many students emphasized the need for more VR practice hours and suggested integrating VR into the curriculum from the first year to familiarize them with procedural steps early on. There were also calls for simulating real-life complications to teach how to handle them effectively, making the learning experience more comprehensive and realistic.

These findings underscore a strong interest in and positive reception of VR as a learning tool among medical students. They also highlight specific areas where improvements could further enhance student satisfaction and realism.

DISCUSSION

The results of this study provide insightful data on medical students' familiarity, engagement, and perceptions of VR technology as a learning tool. The study identifies three main points: first, there is a high level of student engagement with VR despite limited prior experience with the technology. Second, students believe that VR can significantly enhance learning. Third, several limitations were identified that must be addressed when planning to implement VR in medical education.

Although only 5% of students were very familiar with VR, 54% reported high engagement with the technology. Despite limited initial familiarity, this high level of engagement suggests that VR has an intrinsic appeal and usability that can quickly draw students in. The findings support the idea that VR can be an engaging educational tool even for those with minimal prior exposure. The p-value of < 0.0001 for familiarity indicates a highly significant difference between female and male participants, with male respondents more frequently reporting being "very familiar" compared to their female counterparts. Interestingly, gender-based analysis for engagement showed no statistically significant difference between genders ($p = 0.557$), suggesting that once exposed to VR, both male and female students demonstrated comparable levels of engagement.

The students considered VR to have improved learning, with 58% finding it somewhat effective and 29% highly effective in increasing their knowledge of the

procedure. No statistically significant gender differences were found for perceived effectiveness (p = 0.286) or helpfulness (p = 0.696), indicating a shared perception of VR's educational value across genders. Although we did not test the student performance in these skills, this survey suggests that VR can significantly enhance confidence in learning, particularly in developing technical competencies. The preference for VR over traditional methods was clear, with 55% of students prefer VR-based learning.

The ability to repeatedly practice procedures in a VR environment was particularly valued, as it boosted confidence and reduced anxiety before performing actual procedures. This repeated practice and hands-on experience are critical in medical education, where confidence and preparedness can significantly impact performance [4,6-8,10].

Regarding limitations, VR in medical education has specific challenges that need to be addressed. One significant limitation is the lack of tactile feedback, crucial for performing real-life medical procedures. This highlights the need for advancements in haptic technology to enhance the realism of VR simulations. Additionally, some students noted the need for better gesture recognition and more dynamic elements, such as responsive vital signs, to increase realism. Prolonged use of VR equipment also caused discomfort for some students, indicating a need for better ergonomic design for longer training sessions.

Compared to similar studies on VR platforms in education, our findings align with existing research demonstrating immersive technologies' effectiveness in increasing student engagement and learning efficacy. *Cheung et al.* Emphasise that VR allows students to train under safe and controllable conditions, which aligns with our findings that Meta Quest provides a secure environment for learning[7]. We found similar levels of satisfaction and increased learning perspective compared to previous studies[4,5,7,11]. Our study also reflects high engagement and positive perceptions among students using Meta Quest, underscoring its effectiveness as a learning tool. However, unlike some reports, our results did not show significant gender differences in engagement or perceived usefulness, except for familiarity, where male students had higher prior exposure.

However, gender-based differences in perception should be considered in future implementations, as our data show that male and female students may experience and evaluate VR-based tools differently.

Mistry et al. (2023) highlighted that VR enables students to learn at their own pace and enhances procedural confidence, which aligns with our findings that students appreciated the flexibility and repeated practice opportunities provided by Meta Quest. However, while our study also noted students' preference for VR over traditional methods, it suggests room for improvement in enhancing the realism of VR simulations—a point that complements Mistry et al.'s report on the need for ongoing development in VR technology[5,7].

The strength of this study lies in its comprehensive approach and robust dataset, derived from nearly 300 third-year medical students, which provides a broad and representative sample for analysis. The methodical collection and analysis of feedback offer detailed insights into various aspects of VR technology in medical education, such as familiarity, engagement, perceived realism, and effectiveness as a learning tool. This rigorous evaluation ensures that the findings are well-grounded and reliable, highlighting the significant potential of VR to enhance medical training. The study's large sample size and thorough methodology contribute to its credibility and the applicability of its results to similar educational settings. Gender differences were also found in preferences for curricular integration, with more men than women stating VR should definitely be included in the curriculum.

Despite providing valuable insights, this study has several limitations. Notably, it lacked a control group for comparing feedback from participants who did not use the system or used alternative learning tools. As a result, we cannot determine whether this method is superior to traditional teaching approaches, such as lectures and case discussions. Additionally, the study's findings are based on participant evaluations, which may not always provide an objective measure of learning effectiveness. The results may only apply to medical students at the University of Lisbon and may not be generalizable to other populations. Moreover, despite efforts to maintain consistency in the study environment, there could have been unavoidable teaching variations, including differences in students' engagement levels and concentration. Although we achieved a high response rate of 81%, it is possible that students who were more motivated or engaged were more likely to participate, introducing a potential response bias.

CONCLUSION

This study offers important information about the efficiency and practicality of VR technology for University of Lisbon medical students. It thoroughly assesses how virtual reality improves their educational experiences and looks at their opinions on incorporating this technology into the medical curriculum. From the students' perspective, we have identified areas of strength and need for progress, including specific VR components that require further development. By emphasizing these results, the study hopes to encourage the broader use of VR as a meaningful teaching tool. It also provides valuable suggestions for planning medical education activities integrating VR.

In summary, our findings support the integration of VR into medical curricula as a complementary tool, while highlighting the importance of addressing technical, ergonomic, and pedagogical aspects to optimise its impact on learning outcomes.

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The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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JORNAL DA SOCIEDADE DAS CIÊNCIAS MÉDICAS DE LISBOA

Obesity Medical Treatment: A Retrospective Analysis of Clinical Outcomes in Patients with Obesity in a Real-World Setting

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ABSTRACT: **Background**: Obesity is a chronic multifactorial disease characterized by excessive fat accumulation, influenced by genetic and environmental factors. It is associated with numerous health complications and significant reductions in life expectancy. Effective weight loss of at least 5% of total body weight (TBW) significantly reduces these risks. While lifestyle changes are pivotal, they often result in modest and difficult-to-maintain weight loss. Pharmacological treatments have emerged as effective strategies for achieving weight loss, yet data from real-world settings remain limited. **Objective:** This study aims to evaluate the effectiveness of pharmacological treatments for weight loss in real-world clinical practice. **Methods:** This observational, retrospective cohort study analyzes data from patients enrolled in a multidisciplinary obesity treatment program at a Portuguese public hospital, from July 2022 to November 2024. Inclusion criteria were age >18 years, body mass index (BMI) >25.0 kg/m², prior failed lifestyle interventions, and ≥3 months of pharmacological treatment (including semaglutide, liraglutide, bupropion/naltrexone and orlistat). For treatment goals we stablished a weight loss of ≥5% TBW at 6 months and ≥10% TBW at one year. Demographic, clinical, and treatment data were collected from medical records, and weight loss outcomes were assessed at three-month intervals. **Results:** Forty-three patients were included, with a mean age of 53±15 years and a mean BMI of 39.1±7 kg/m². After six months of treatment, 81% achieved the ≥5% TBW reduction target, with 45.9% surpassing ≥10% TBW. After one year, 54.5% lost ≥10% TBW, and 41% achieved ≥15% TBW reductions. Semaglutide users experienced the highest mean TBW loss (-10.8%). A total of 43% of patients were reclassified to a lower obesity class. Treatment goals were met by 81.1% of patients at 6 months and by 54.5% at 12 months. **Conclusions:** Pharmacological treatment, combined with lifestyle interventions, resulted in clinically significant and sustained weight loss in the majority of patients, highlighting its effectiveness in real-world clinical settings even amidst challenges like medication shortages and financial constraints. Further research is needed to optimize individualized treatment strategies and enhance long-term outcomes.

KEYWORDS: Obesity; Weight Loss; Pharmacological Treatment; Semaglutide; Liraglutide.

INTRODUCTION

Obesity is a chronic multifactorial disease characterized by the excessive accumulation of fat, resulting from a strong genetic predisposition and several environmental factors (diseases, drugs, stress, menopause, sleep deprivation, microbiome, viruses and toxins).[1-3]

It is associated with numerous health complications, impacting nearly every organ system. Activates the renin-angiotensin-aldosterone system, promotes insulin resistance, changes the lipidic metabolism and generates a pro-inflammatory state, contributing to conditions such as hypertension, type 2 diabetes, dyslipidemia, coronary heart disease, obstructive sleep apnea, hypoventilation syndrome and symptomatic degenerative osteoarthritis, and also raises the susceptibility and severity of infections and cancer.[4-6] This significantly increases morbidity and mortality, resulting in up to 10 years reduction in life expectancy.[7-8]

Prevalence has reached alarming levels, especially in developed countries. In Portugal, 68% adults are overweight and 29% obese.[5] The worldwide economic impact is huge, almost US$2 trillion in 2023[6]. Recent data shows, for the first time, a 2% reduction in obesity in the United States of America between 2020 and 2022.[7]

Weight losses of at least 5% of total body weight (TBW) have been shown to alleviate complications such as diabetes, hypertension, and cardiovascular disease, with larger losses producing even greater health benefits and further reducing these risks.[4-5,12-15]

Although lifestyle interventions (e.g., dietary changes and increased physical activity) are the cornerstone of treatment, they often lead to modest and difficult-to-maintain weight loss.[4-5,13-14] This is mainly because the body weight is regulated mainly in the brain, where physiological adaptations counteract changes, leading to stability. As a result, weight loss leads to a reduction in resting metabolism and hormonal changes affecting appetite and thyroid function.[12]

In recent years, pharmacological treatments have proven to be highly effective in achieving weight loss, though data outside clinical trial settings remains limited.[13–16]

The goal of this study is to evaluate the effectiveness of pharmacological treatments for weight loss in real-world clinical practice.

MATERIALS AND METHODS

Patients (inclusion and exclusion criteria)

This observational, retrospective cohort study uses a convenience sample of the first consecutive patients enrolled in a multidisciplinary medical obesity treatment program within the internal medicine department in a Portuguese public hospital. Data was obtained from the medical electronic health record (EHR), between july 2022 and november 2024.

Participants were required to meet the following inclusion criteria: age over 18 years old, BMI over 25.0kg/m^2, a history of unsuccessful attempts at lifestyle changes, and at least three consecutive months of pharmacological treatment aimed at weight loss. Data was collected from the medical electronic health record (EHR) for all eligible patients. The multidisciplinary team involved in the program included internal medicine doctors, dedicated nurses, nutritionists, physiatrists, physiotherapists, and psychologists. Weight loss outcomes were assessed every three months following treatment initiation.

Study design and treatment

Demographic data collected included sex, age, previous comorbidities, weight and BMI. The study assessed patient weight at the beginning and trimonthly during treatment. Weight loss was analyzed, including the mean weight loss of the population, BMI and mean BMI of the cohort, obesity classification and percentage of patients who achieved ≥5%, ≥10%, ≥15% and ≥20% losses of TBW.

Endpoints and measurements

Based on previous data, treatment goals were set to achieve a ≥5% TBW loss at 6 months and ≥10% TBW loss after 12 months.[2,8,12,14,15,17,18]

The primary endpoint was to evaluate the percentage of patients that achieve ≥5% TBW loss after 3 months of treatment and ≥10% TBW loss after 12 months. The secondary endpoint was to evaluate the safety of medical treatment of obesity.

Patients' obesity class category was monitored throughout treatment. According to the body mass index (BMI), obesity can be classified in: overweight (25-29.9kg/m^2), class I obesity (30-34.9kg/m^2), class II obesity (35-39.9kg/m^2) and class III obesity (≥40kg/m^2).[19] Any changes in the obesity class were analyzed.

According to response to treatment, patients were classified into three groups: **responders**, who sustained weight loss throughout the treatment period; **initial responders,** who achieved at least the first goal of ≥5% TBW loss at the beginning but experienced weight regain after a few months; and **nonresponders**, who did not lose weight or did not reach any goal despite treatment.

Data regarding adverse effects and intolerance to medication were collected. The treatment was adjusted as needed.

According to the program's protocol, treatment for comorbidities such as hypertension, dyslipidemia, diabetes and hypothyroidism was optimized. Regular blood samples were taken to monitor dyslipidemia, renal function, glucose and HbA1C and hepatic markers. Abdominal echography was performed at baseline to assess for liver disease such as nonalcoholic fatty liver disease (NAFLD) and choledocholitiasis. Several patients were previously followed by pneumology, psychiatry, gastroenterology, orthopedy and neurosurgery for pathologies as obstructive sleep apnea, depressive syndrome, NAFLD or symptomatic osteoarthritis; when appropriate, patients were referred to these specialties for evaluation or follow up. Throughout the program, chronic medications were reviewed, optimized, and when possible, medications known to promote weight gain were adjusted to maximize treatment outcomes.

Patients with type 2 diabetes (T2D) were preferentially prescribed semaglutide in a maximum dose of 1.0 mg weekly (the recommended dose for T2D) due to market shortages. Prescription of medication for the nondiabetic patients was preferentially liraglutide 3.0mg daily, as well as bupropion/naltrexone180/16mg twice daily preferred for smokers or alcohol abusers and orlistat for economic reasons, chosen by clinical criteria combined with patient preference.

Regarding lifestyle, patients were classified as sedentary if they spent more than half of the day without walking. Patients were referred to exercise sessions led by physiatrists and physiotherapists, and encouraged to start or intensify physical exercise according to their capacities. In collaboration with nutritionists, patients' dietary habits were assessed. Those with inadequate habits, such as excessive intake of carbohydrates and fats, were closely monitored to promote education on healthy eating. Patients who reported compulsive overeating or expressed a need for psychological support were referred to psychological consultations and, when necessary, to psychiatry.

Statistical analysis

All patients that fulfilled the inclusion criteria were included. Statistical analysis comprised calculation of means, standard deviations, medians, minimum and maximum values and frequency distributions. Data was presented as the percentage of patients meeting various treatment goals. Data collection and initial analysis were performed using Microsoft Excel.

Data analysis was performed using the STATA program version 14.0. Descriptive analysis was conducted.

Sign tests were utilized to assess paired variables before and after the initiation of medication, and Spearman's correlation coefficient was used to evaluate the relationship between results obtained during the follow-up period concerning blood glucose and weight.

RESULTS

Baseline characteristics of the study population

A total of 43 patients, followed in the multidisciplinary medical obesity treatment program, met the eligibility criteria.

They were mostly women (n=35, 81.4%) with a mean (± standard deviation (SD)) age of 53±15 years old. The mean (± SD) weight at first consultation was 105±20Kg, and mean (± SD) BMI was 39.1±7 Kg/m^2.

One third of the cohort had class III obesity (BMI ≥40 Kg/m^2) and 44% had class II obesity.

Dyslipidemia and arterial hypertension were the most common comorbidities (74% and 57%), however T2D and depression syndrome were also frequent (35% and 40%, respectively). Almost 90% of the population had inadequate diet, 72% had a sedentary lifestyle and 44% referred binge eating. Demographic and clinical characteristics of the study population are summarized in Table 1.

Body weight and BMI

Table 2 presents data on patient under treatment at each stage and corresponding weight loss goals over a 21-month treatment period. During the time of the study, 43 patients were enrolled (100%). Over time, there was a gradual decline in the number of patients that reached the different trimonthly observation stage due to different follow-up time.

Detailed information on individual weight loss throughout the treatment period is provided in Figure 1, each line showing the weight evolution of one patient over time. Not all patients responded with consistent

TABLE 1. Demographic and clinical characteristics, obesity class I.

Demographic and clinical characteristics	
Number of patients, n	43
Sex, n (%)	
Women	35 (81.4%)
Men	8 (18.6%)
Age, mean ± SD (minimum, maximum) | median (Q1 – Q3), years	53±15 (22, 79) | 54 (42 – 63)
Weight measurements	
Weight, mean ± SD (minimum, maximum) | median (Q1 – Q3), Kg	105±20 (69, 148) | 102 (91 – 122)
BMI, mean ± SD (minimum, maximum) | median (Q1 – Q3), Kg/m^2	39,1±7 (25.6, 59.3) | 38 (35.4 – 42.75)
Obesity class III, n (%)	14 (33%)
Obesity class II, n (%)	19 (44%)
Obesity class I, n (%)	8 (18.7%)
Excessive weight, n (%)	2 (4.7%)
Overweight since infancy	25 (58%)
Comorbidities n, %	
Dyslipidemia	32 (74%)
Arterial hypertension	24 (57%)
Depressive syndrome	17 (40%)
Type 2 diabetes	15 (35%)
Symptomatic osteoarthrosis	14 (32%)
Osbtructive sleep apnea	11 (26%)
Nonalcoholic fatty liver disease	10 (23%)
Lifestyle, n (%)	
Inadequate diet	38 (88%)
Sedentarism	31 (72%)
Binge eating	19 (44%)
Taking weight promoting medication	8 (19%)

LEGEND – **BMI:** body mass index; **n:** number of patients; **SD:** standard deviation; **Q1:** Quartile 1; **Q3:** Quartile 3.

weight loss: 22 (51%) responders (weight loss consistent through treatment); 12 (27.9%) initial responders but regained weight, and 9 (20.9%) nonresponders (no goal met or weight loss was observed).

At 3 months of treatment, nearly half (46.5%, n=20) of the population reduced their TBW by more than 5%. As expected, the mean BMI at this stage decreased as well from 39.1 kg/m^2 to 37.19 kg/m^2 (-1.91Kg/m^2) – Table 2.

At 6 months of treatment the primary goal (≥5% TBW loss) was attained by 81% of all patients (n=30), and 45.9% (n=17) exceeded this goal, achieving losses above 10% TBW. The overall mean weight losses was 10.22Kg, corresponding to a mean loss of 9.7% TBW. At this point, 16 patients (43.2%) were down reclassified in their class of obesity.

At one year follow-up, the treatment revealed the same tendency with the majority of this population

TABLE 2. Body weight changes during treatment.

Body weight change during treatment							
Body weight and BMI measures	**Time of treatment, months (m) | n= patients at time of treatment (%)**						
	3 m | n=43 (100%)	6 m | n=37 (86%)	9 m | n=28 (65%)	12 m | n=22 (51%)	15 m | n=12 (28%)	18 m | n=7 (16%)	21 m | n=4 (9%)
Predefined ΔTBW goal	≥ 5 %	≥ 5 %	≥ 10 %	≥ 10 %	≥ 10 %	≥ 10 %	≥ 10 %
Percentage body weight reduction, n (%)							
At least ≥ 5 % TBW	20 (46.5)	30 (81.1)	22 (78.6)	18 (81.8)	8 (66.7)	5 (71.4)	3 (75.0)
At least ≥ 10 % TBW	5 (11.6)	17 (45.9)	15 (53.6)	12 (54.5)	7 (58.3)	3 (42.9)	2 (50.0)
At least ≥ 15 % TBW	1 (2.3)	7 (18.9)	9 (32.1)	9 (40.9)	5 (41.7)	2 (25.0)	2 (50.0)
At least ≥ 20 % TBW	0 (0)	2 (5.4)	1 (3.6)	1 (3.6)	3 (25.0)	1 (12.5)	1 (20.0)
Mean ΔTBW (%)	-4.9	-9.7	-11.1	-11.2	-11.7	-9.9	-11.8
Body weight							
Mean (Kg)	100.2	96.9	96.3	94.3	94.5	91.6	92.0
standard deviation (Kg)	19.84	20.07	20.78	16.60	17.70	14.65	19.70
mean loss (Kg)	-5.2	-10.2	-11.9	-12.3	-12.8	-9.9	-11.9
BMI (kg/m^2)							
Minimum	25.8	26.9	24.2	24.7	24.7	33.2	25.6
Maximum	60.1	50.3	61.7	45.8	41.3	40.9	40.8
Mean	37.2	37.0	36.0	35.3	34.9	36.7	34.8
Standard deviation	6.7	5.4	7.8	5.7	5.6	3.8	7.2
Obesity class, n (%)							
Excessive weight	3 (7)	6 (16.2)	6 (21.4)	5 (22.7)	2 (16.7)	1 (14.3)	1 (25.0)
Class I	18 (41.9)	13(35.1)	6 (21.4)	5 (22.7)	4 (33.3)	3 (42.9)	1 (25.0)
Class II	11 (25.6)	9 (24.3)	8 (28.6)	7 (31.8)	2 (16.7)	1 (14.3)	0 (0.0)
Class III	11 (25.6)	9 (24.3)	8 (28.6)	5 (22.7)	4 (33.3)	2 (28.6)	2 (50.0)
Patients reducing class, n (%)	15 (34.9)	16 (43.2)	13 (46.4)	10 (45.5)	5 (41.7)	4 (57.1)	1 (25.0)

LEGEND – **Excessive weight:** BMI 25.0-29.9 Kg/m^2; **Obesity Class I:** BMI of 30.0-34.9kg/m^2; **Class II:** BMI of 35.0-39.9 Kg/m^2 and **Class III:** BMI >40.0 Kg/m^2. **QI:** first quartile; **ΔTBW:** variation of total body weight; **BMI:** body mass index; **n:** number.

(81.8%) losing at least 5% TBW, more than half (n= 12, 54.5%) achieved more than 10% TBW loss and 41% (n=9) losing more than 15% TBW. The mean loss of weight at this stage of treatment was 12,33Kg and the mean ΔTBW was -11% surpassing the general goal stablished for this stage.

Through time, the declining sample size due to different follow up period preclude more robust interpretation, however between 15 and 21 months the mean body weight reduction varies between 10 and 12% TBW. Figure 2 represents the percentage of patients that attained various levels of weight loss (as a percentage of total body weight, TBW) over the course of treatment. During treatment, the percentage of patients that had lost at least 5% TBW is superior to 70% after 6 months. The loss of 10% TBW increases after 6 months treatment with more than 40% of the cohort reaching the goal of treatment.

The tendency of weight loss is present even after more than one year of treatment (Figure 2 and 3). While the most significant weight loss occurred in the initial months, statistical reductions in weight persisted even after 12 months. Some patients showed either weight regain or stabilization in their weight loss trajectory, as detailed in Figure 1 and 2.

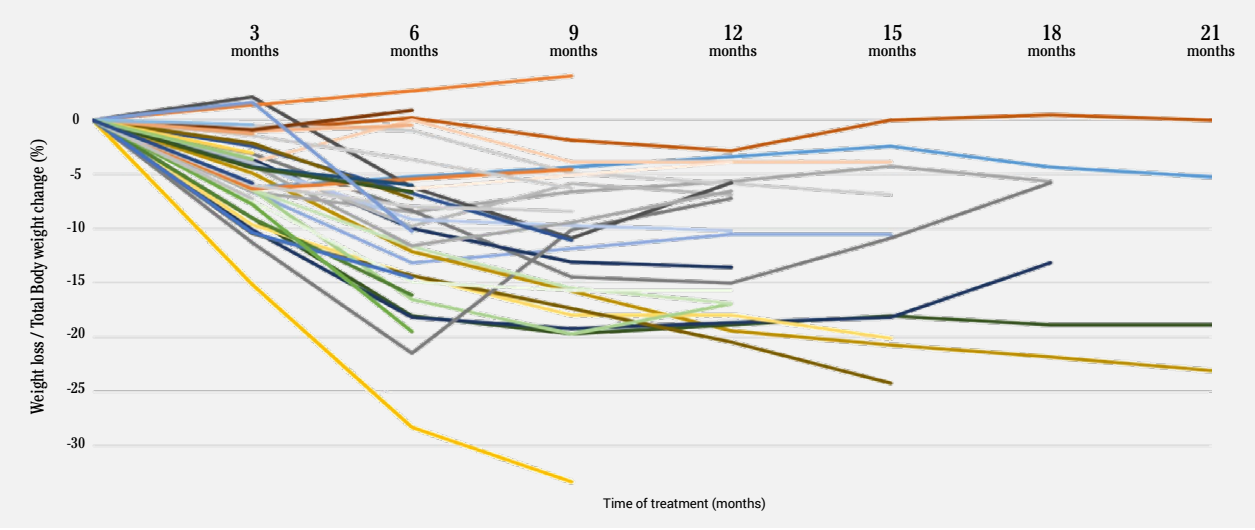

FIGURE 1. Individual weight loss during treatment

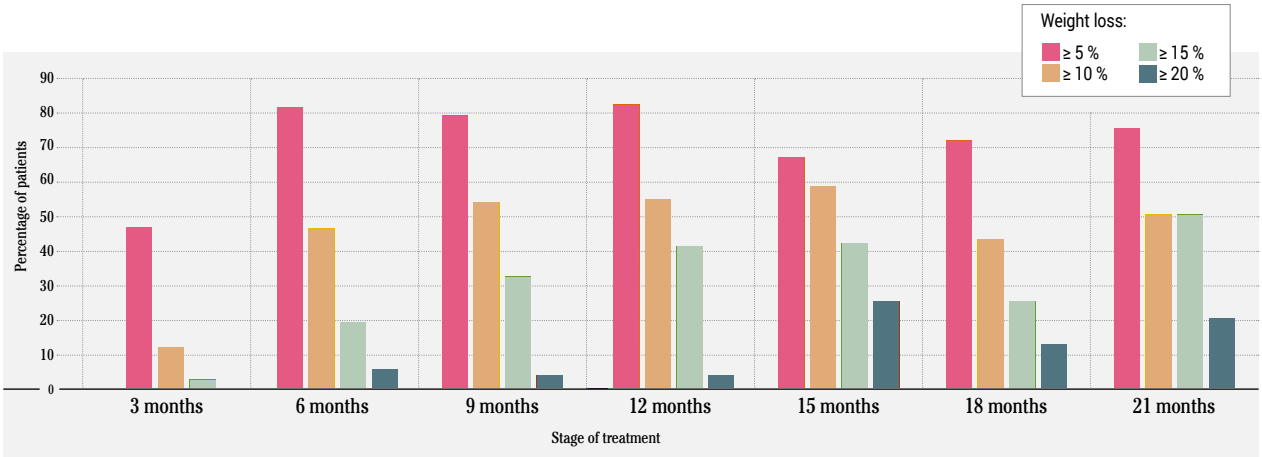

FIGURE 2. Weight loss during treatment.

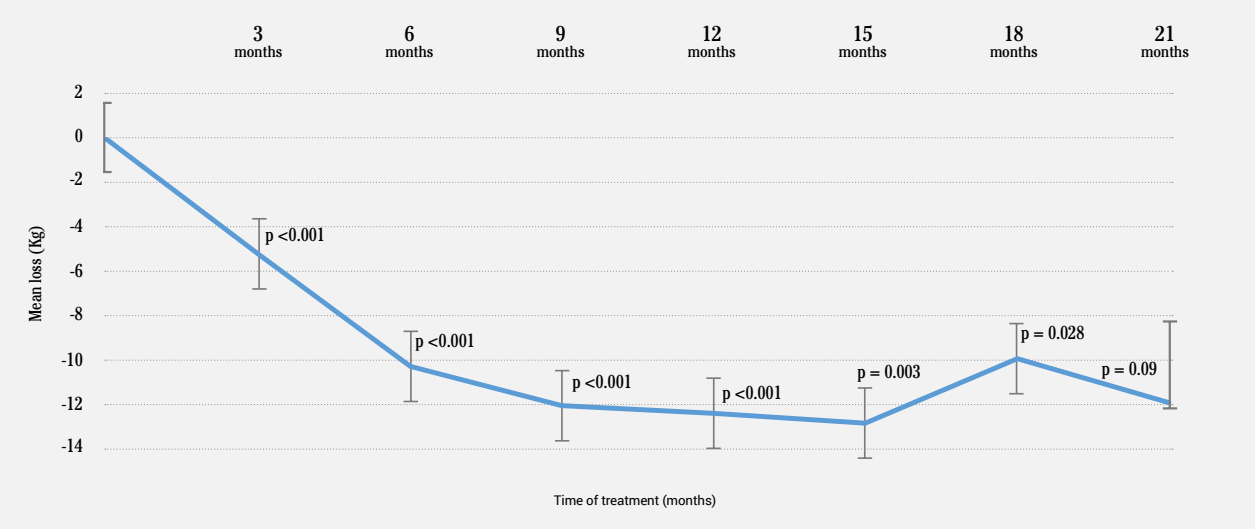

FIGURE 3. Mean weight loss of patients on medical treatment for obesity.

Medication used during treatment

Table 3 describes the different drugs used in the program, their outcomes, adverse effects and motive for drug alteration. For analysis purposes, when one patient tried more than one drug, we considered the one used for the longest time. None of the patients was under more than one drug at the same time. The majority of the patients (n=29, 67.4%) were treated with semaglutide, liraglutide was the second most used medication (n=11, 25.6%) and only 3 patients (6.9%) were treated with bupropion/naltrexone.

Patients treated with semaglutide had better results of weight loss with a mean loss of 10.8% TBW over 21 month. The population under liraglutide had a mean loss of 4.2% TBW. The few patients under buproprion/naltrexone achieved a mean loss of 7.4% TBW. Orlistat was only used for short periods in 2 patients under other drugs the majority of time. (Table 3)

During treatment, 13 patients changed medication: due to market shortages (the most common reason (n= 6, 13.9%)); 3 patients (6.9%) due to poor results; gastrointestinal intolerance to liraglutide (n=1, 2.3%); 1 patient (2.3%) interrupted treatment because of a new diagnose of neuroendocrine tumor and another for a planned pregnancy; 2 patients (4.7%) stopped medication altogether for economical insufficiency. The

TABLE 3. Analysis of drugs used during patients treatment.

Treatment	Semaglutide	Liraglutide	Bupropion/naltrexone	Orlistat
N prescribed drug	30	17	3	2
Main used drug during treatment, n (%)	29 (67.4)	11 (25.6)	3 (6.9)	0
Weight Δ, mean ± SD (minimum, maximum), Kg	-11.8 ± 9.3 (-33, +2.3)	-4.4 ± 4,76 (-11.7, +6.0)	-6.2 ± 2.0 (-8.4, -4.5)	0.5 ± 2.12 (-1.0, +2.0)
		Weight Δ, Kg		
Mean mean ± SD, Kg	-11.8 ± 9.3	-4.1 ± 5.5	-6.2 ± 2.0	0
Minimum, maximum, Kg	-33.0, +2.0	-11.7, +6.0	-8.4, -4.5	0
Median (Q1, Q3), Kg	-8.0 (-19.7, -5.2)	-3.2 (-10.2, -0.5)	-5.8 (-8.4, -5)	0
		ΔTBW, %		
Mean mean ± SD, %	-10.9 ± 8.1	-4.2 ± 5.0	-7.4 ± 0.9	0 (0)
Minimum, maximum, %	-33.0, +1.9	-11.1, +4.1	-8.4, -6.5	0 (0)
Median (Q1, Q3), %	-7.2 (-17.4, -5.6)	-3.6 (-10.3, -0.4)	-7.3 (-8.4, -7)	0 (0)
Loss ≥ 5 % TBW, n (%)	24 (82.8)	4 (36.4)	2 (66.7)	0 (0)
Loss ≥ 10 % TBW, n (%)	14 (48.3)	3 (27.3)	0 (0)	0 (0)
Loss ≥ 15 % TBW, n (%)	10 (34.5)	0 (0)	0 (0)	0 (0)
Loss ≥ 20 % TBW, n (%)	3 (10.3)	0 (0)	0 (0)	0 (0)
		Reported adverse effects, n (%)		
Nausea and vomiting	5 (11.6)	3 (6.9)	0 (0)	0 (0)
Constipation	1 (2.3)	0 (0)	0 (0)	0 (0)
Malaise	0 (0)	1 (2.3)	0 (0)	0 (0)
Steatorrhea	0 (0)	0 (0)	0 (0)	1 (2.3)
		Motive of suspension/drug alteration, n (%)		
Intolerance	0 (0)	1 (2.3)	0 (0)	0 (0)
No response	2 (4.7)	1 (2.3)	0 (0)	0 (0)
Contraindication by new diagnose	1 (2.3)	0 (0)	0 (0)	0 (0)
Planned pregnancy	1 (2.3)	0 (0)	0 (0)	0 (0)
Market shortage	6 (13.9)	2 (4.7)	0 (0)	0 (0)
Economic insufficiency	0 (0)	2 (4.7)	0 (0)	0 (0)

LEGEND – **Δ:** variation; **QI:** first quartile; **Q3:** third quartile; **n:** number; **SD:** standard deviation

adverse effects reported were mainly gastrointestinal, namely mild nausea and vomiting attributed to semaglutide (n=5, 11.6%) and liraglutide (n=3, 6.9%); mild malaise was reported by 1 patient and another had steatorrhea due to orlistat.

DISCUSSION

Our study provides real-world data from an obesity outpatient clinic in Portugal. To the best of our knowledge, this is the first published evidence in a Portuguese population. The results demonstrate positive outcomes in weight loss, with patients surpassing the 6-month target for total body weight (TBW) reduction.

In this cohort treated with weight-loss medications — semaglutide, liraglutide, bupropion/naltrexone and orlistat — the predefined TBW reduction goals were consistently achieved throughout the treatment stages. At 6 months, the majority of patients exceeded the -10% TBW target typically set for 9 months, and by one year, the average weight loss corresponded to an 11% reduction in TBW continuing to accomplish the proposed goals. Remarkably, 41% of patients surpassed weight-loss goals, achieving reductions of 15% to 20% in TBW.

Although weight loss fluctuated during the treatment period, more than half of the patients sustained a reduction of over 10% in TBW above one year follow up. These results underscore that, even in real-world settings, combining pharmacological treatments with lifestyle modifications can lead to significant and lasting weight loss.

Data denoted different patterns of response. Most patients (51%) were classified as responders, achieving sustained weight loss during treatment. Some patients (27.9%) lost weight only at the beginning (initial responders), while others (20.9%) did not lose significant weight (nonresponders). It would have been important to recognize the treatment response early and, if necessary, change the type of medication used and control external modifiable factors. Given the retrospective design and missing data, it was not possible to identify specific characteristics of patients in each group, limiting our ability to draw conclusions that could help predict outcomes earlier. Additionally, some patients were still in the 3-month stage of treatment and might later become initial responders, which could introduce a potential bias in this analysis.

The most used drug in our study was semaglutide (n=30, 69.8%). Patients treated with it lost an average of 10.8% of their total body weight (TBW), similar to the -10.6% TBW loss reported at 20 weeks in the STEP 4 trial for semaglutide 2.4 mg weekly.[8,20] However, as expected since our study concerns a small size population with the real-world setbacks, our results were lower than the -17.4% TBW loss seen at 68 weeks in STEP 4.

In our study, 83.3% (n=25) of patients lost at least 5% TBW, compared to 88.7% in STEP 4. Additionally, 46.7% (n=14) lost at least 10% TBW, while 79.0% in STEP 4 and 70.9% in STEP 8 achieved this goal. For higher weight-loss targets, 33.3% (n=10) lost at least 15% TBW, compared to 63.7% in STEP 4 and 55.6% in STEP 8, while only 10% (n=3) lost at least 20% TBW, compared to 39.6% in STEP 4 and 38.5% in STEP 8.[8,13,20]

The lower results in our study may be explained by the lower semaglutide dose used (1.0 mg weekly). STEP 2 showed better outcomes with higher doses (2.4 mg weekly), with TBW losses of 9.6% compared to 7.0% for 1.0 mg.[8,19] Other factors that may have influenced our findings include varying treatment durations among patients, the inclusion of diabetic patients in our study (unlike STEP 4), and the need to switch medications for 13 patients due to earlier-mentioned reasons.

Liraglutide was used by a quarter of the patients (n=11), who lost an average of 4.8% TBW. This is lower than the weight loss reported in the SCALE IBT trial (-7.5% at 15 months) and STEP 8 (-6.4% at 15 months).[13,17,21] In our study, 36.4% (n=4) achieved ≥5% TBW loss compared to 61.5% in SCALE IBT, while 27.3% (n=3) achieved ≥10% TBW loss compared to 30.5% in SCALE IBT and 25.6% in STEP 8. None of our patients achieved ≥15% TBW loss, whereas 18.1% in SCALE IBT and 12.0% in STEP 8 did.[8,13,20] Despite these lower results, and considering the small sample size and the non-randomized nature of our study, 4 patients showed consistent weight loss throughout the treatment, supporting liraglutide is an option for patients that are not candidates for treatment with semaglutide.

The differences in weight loss outcomes in this study may be partly due to including patients at different treatment stages and changes or interruptions in medication, which could have introduced bias. During treatment, one patient had a new diagnose of a neuroendocrine tumor and all data available suggests the tumor origin was completely independent from the obesity treatment and another one was planning a pregnancy, both reasons contraindicated the continuation of the treatment. Only mild adverse reactions were reported

by patients (mainly gastrointestinal), which is consistent with the results in the previously mentioned trials.[13,17,20,22] Despite these challenges, the results show a general trend of TBW loss over time. Overall, our findings demonstrate significant weight loss and suggest that, even with medication limitations and real-world challenges, patients can achieve meaningful reductions in body weight.

During the study, tirzepatide was not available in Portugal and therefore was not used in the program. However, trials show that tirzepatide leads to greater weight loss compared to semaglutide. In the SURMOUNT trial, 85% and 91% of patients lost ≥5% TBW with 5 mg and 15 mg doses, respectively, at 72 weeks, while 50% (5 mg) and 57% (10 mg and 15 mg) lost ≥20% TBW.[15] These promising results suggest that the program's outcomes could improve significantly now that tirzepatide is available in Portugal.

Strengths and limitations

This study has several strengths, including its real-world setting, which reflects the challenges and barriers patients face outside of controlled clinical trials. The long-term data presented here provide insight into the sustainability of weight loss over time. However, there are notable limitations. The sample size is small, as the study only includes patients from a single hospital in Lisbon, where the multidisciplinary medical obesity treatment program was still in its early stages and not widely replicated. This limits the generalizability of our findings.

Additionally, we were unable to track the exact duration of treatment interruptions due to market shortages of GLP-1 medications, which may have introduced bias in the analysis. Some patients also discontinued treatment or switched medications due to intolerance, responsiveness, clinical reasons or patient preferences, including financial constraints, further complicating the interpretation of long-term outcomes. Another limitation is that we focused primarily on the medical treatment, and did not quantify the effects of lifestyle changes, such as diet and exercise, which undoubtedly played a role in weight loss alongside the pharmacological interventions.

CONCLUSIONS

Pharmacological treatment, combined with lifestyle interventions, resulted in significant and sustained weight loss, highlighting its effectiveness in real-world clinical settings even amidst challenges like medication shortages and financial constraints. Further research is worthwhile to better tailor treatment and improve results.

AUTHOR CONTRIBUTIONS: *Rita Gano and Miguel Ardérius were responsible for study design, data collection, interpretation and analysis, revising and approving the final version for submission. Mariana Alves was responsible for interpretation, analysis, statistical analysis, revising and approving the final version for submission.*

CONFLICT OF INTEREST STATEMENT: *Miguel Ardérius holds stock market shares in the following companies: Amgen, Bayer AG, BioNTech SE, Eli Lilly, GE Healthcare Technology, Gilead Sciences, GSK, Merck, Moderna, Novartis AG, Pfizer, Teladoc; Mariana Alves has participated in educational meetings and/or attended a conferences or symposia (including travel, accommodation and/or hospitality) with Boehringer-Ingelheim, AstraZeneca, Bayer, Bristol-Myers-Squibb, Grünenthal, Tecnimede, Merck Sharp & Dohme.*

ETHICS: *Confidentiality of Data: The authors declare that they have followed the protocols of their work center on the publication of data from patients.*

PROTECTION OF HUMAN AND ANIMAL SUBJECTS: *The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki as revised in 2013).*

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CASE SERIES

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Maps of Death in Patients With Covid-19

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ABSTRACT: Introduction: Coronavirus disease (COVID-19) is a highly lethal infectious disease caused by the SARS-CoV-2 virus, a novel coronavirus that led to a pandemic. We know that COVID-19 affects the lungs, but it can also affect vital organs, damage blood vessels, and lead to ARDS. We hypothesized that this toxic plasma leads to mechanical destruction of the microvascular capillary endothelium, especially in lung tissues, and contributes to mortality caused by ARDS due to diffuse pulmonary capillary endotheliitis. We aimed to present the common specific radiologic findings of deceased patients from COVID-19. **Methods:** We retrospectively re-evaluated the radiological findings of 15,373 patients diagnosed with COVID-19 at VM / Medical Park Samsun Hospital, Samsun, Türkiye, between March 2020 and January 2023, and we aimed to present the specific radiologic findings of deceased patients from COVID-19. **Results:** The majority of patients died due to complications of hypoxemia caused by lung involvement, known as ARDS with or without tissue damage, resulting in barotrauma and other mechanical complications. **Conclusion:** COVID-19 starts as an infectious, contagious disease and leads to death in infected individuals because it may render blood plasma toxic. We hypothesized that this toxic plasma leads to mechanical destruction of the microvascular capillary endothelium, especially in lung tissues, and the mortality caused by ARDS due to diffuse pulmonary capillary endotheliitis.

KEYWORDS: Covid-19, ARDS, Death, Radiology, Toxic plasma

INTRODUCTION

In December 2019, cases of pneumonia of unknown cause were reported in Wuhan/China, and in January 2020, a newly identified coronavirus, which had not been detected in humans before, was identified. Initially referred to as 2019-nCoV, the disease was later named COVID-19. After emerging in China, it rapidly spread worldwide within about three months. As of March 2020, the COVID-19 outbreak, declared a pandemic by the World Health Organization, had evolved into a globally deadly epidemic and continues to be observed as an endemic. Many parameters have been studied to understand this disease and the relationship between severity and death worldwide, and efforts have been made to prevent mortality and elucidate the cause of death [1,2].

Death from COVID-19 is mainly caused by lung involvement, known as ARDS (Acute Respiratory Distress Syndrome), with severe hypoxemia and tissue damage resulting in barotrauma, namely pneumothorax, as mechanical complications. We believe that the underlying cause of this is the tissue damage caused by a fundamental pathology known as toxic plasma, which is characterized by an increase in circulating proinflammatory cytokines stemming from endotheliitis [3].

We hypothesized that this toxic plasma leads to mechanical destruction of the microvascular capillary endothelium, especially in lung tissues, and the mortality caused by ARDS due to diffuse pulmonary capillary

endotheliitis, and aimed to review and present the common specific radiologic features of deceased patients from COVID-19.

MATERIALS AND METHODS

We retrospectively examined the radiological findings of 15,373 patients diagnosed with COVID-19 at VM / Medical Park Samsun Hospital, Samsun, Türkiye, between March 2020 and January 2023, and we aimed to identify shared radiologic features among the deceased patients.

The study was performed in accordance with the principles of the Declaration of Helsinki. The written informed consents were obtained from patients.

Patient Selection

Patients who were suspected to be infected with COVID-19 were confirmed with clinical, laboratory, and/or radiologic results and included in the study. The radiological features were obtained and re-viewed from files of deceased patients with COVID 19.

COVID-19 testing

Respiratory samples were obtained by nasopharyngeal and nasal swabs and analyzed by RT-PCR assay. Confirmation of a COVID-19 tests were made according to the positive results of reverse-transcriptase-polymerase-chain-reaction (RT-PCR) assay and with the consistent high-resolution CT(HRCT) findings. Also, we included in the study those whose multiple nasopharyngeal swab samples were negative for COVID-19 by RT-PCR, but then serological IgM/IgG antibodies against SARS-CoV-2 were detected by a rapid antibody test.

CASES AND RESULTS

Between March 2020 and April 2021, a total of 15,373 COVID-19 patients who applied to the Chest Diseases Clinic of Samsun Medicalpark Hospital were retrospectively evaluated. The mean age was 60.1 ± 18.1 years.

We lost the majority of the patients due to complications related to hypoxemia caused by lung involvement known as ARDS, with or without tissue damage resulting in pneumothorax and mechanical complications. We believe that the underlying cause of this is the tissue damage caused by a fundamental pathology known as toxic plasma, which is characterized by an increase in circulating proinflammatory cytokines stemming from endotheliitis. We present radiological images that support this hypothesis, especially in our deceased patients (Figures 1-8).

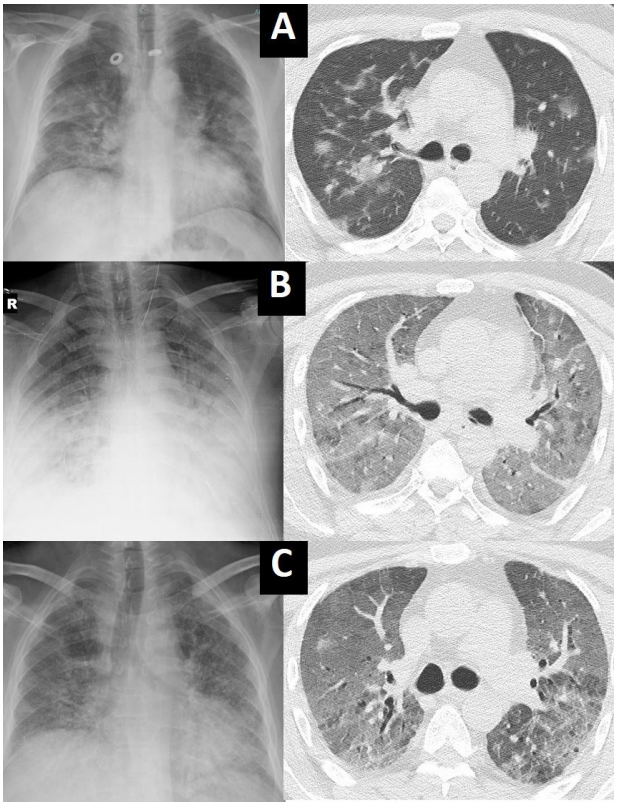

FIGURE 1. 53 years-old male patient with COVID-19 PCR positive. Early phase lung involvement is shown in Figure 1A as small patchy GGO's and consolidations just around the pulmonary capillary. The progressive (1B) and severe phases (1C) are showing the bilaterally diffuse GGOs and fibrotic changes consistent with "white lungs" due to chemically "baked or boiled" lung parenchyma as a result of toxic plasma.

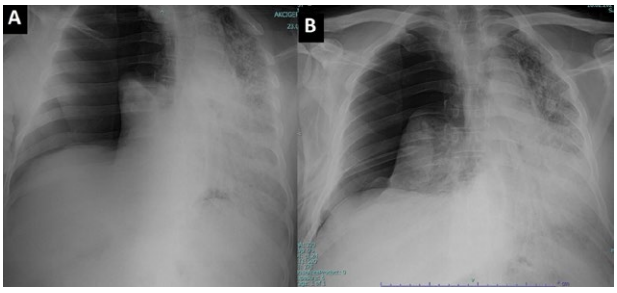

FIGURE 2. Pneumothorax and totally collapsed right lung (2A) is shown in 1st patient with COVID-19. Also, there is no expansion despite the chest tube insertion due to the loss of elastic recoil of the pulmonary parenchyma is shown in Figure 2B.

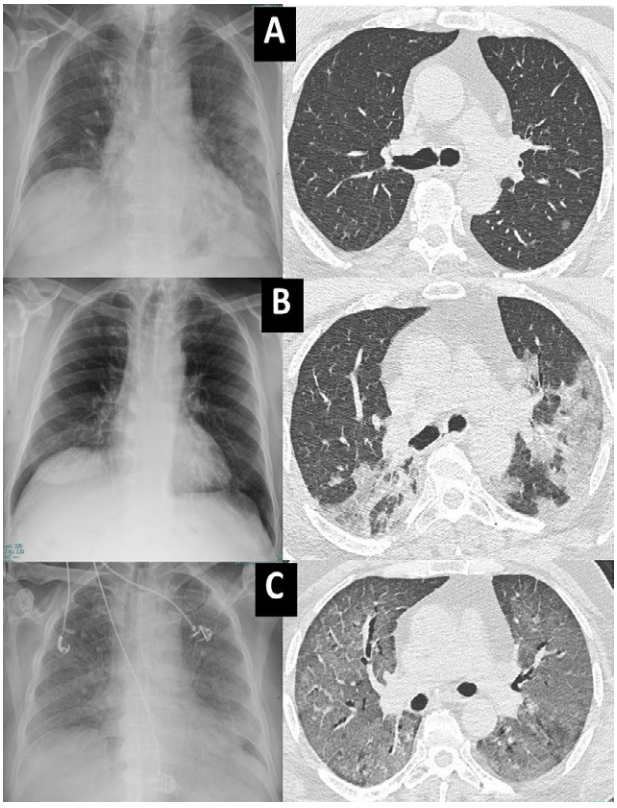

FIGURE 3. The small nodular GGO (3A) is shown as a first involvement in a 69-year-old male patient with PCR-positive COVID-19. And, bilaterally alveolar consolidations were noted after 6 days (3B), and "white or cooked lung" developed (3C) 2 weeks later. He died from Hypoxemic respiratory failure.

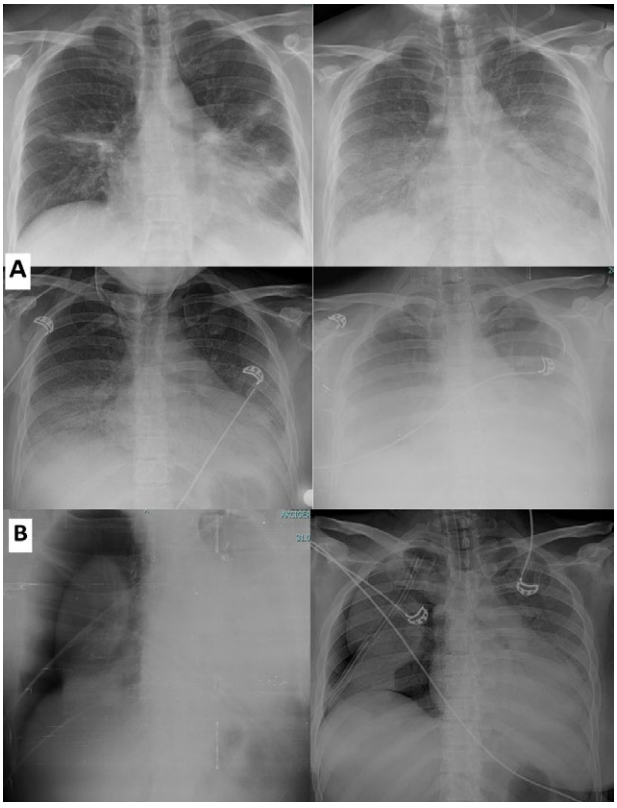

FIGURE 4. Chest X-ray images are showing the early, progressive and severe phases of COVID 19 in a 33 years-old female patient within two weeks (4A). Pneumothorax was noted and there is no expansion despite the chest tube insertion due to loss of elastic recoil of pulmonary parenchyma is shown in Figure 4B. And, she died from pneumothorax and respiratory failure.

One of the most common causes of death in COVID-19 patients is pneumothorax. When a pneumothorax occurs in a COVID-19 patient, partial or complete expansion does not occur despite the insertion of a chest tube. This condition results from the loss of elastic recoil as a result of chemical cooking of the lung parenchyma by toxic plasma.

DISCUSSION

As of May 2023, a total of 687 million COVID-19 cases have been reported worldwide, and the number of deaths has reached 6.8 million. Although the pandemic situation has ended, COVID-19 continues to be observed in society as a disease that still causes death[4]. This disease has four stages [3]:

Stage 1 – In the transmission and infection stage, COVID-19 generally spreads through respiratory transmission, typically when an infected person coughs, sneezes, talks, or breathes. An infected person can also be asymptomatic or show symptoms such as fever, sore throat, etc.

Stage 2 – The stage of systemic inflammatory response; It depends on the viral load in the infected individual, the pathogenicity of the virus, and the immune response of the infected person. Various chemokines and cytokines, primarily including ferritin, are produced. These cytokines transform the plasma of the infected person into a toxic and destructive chemical fluid.

Stage 3 – The endothelial damage(endotelitis) stage; The plasma of the infected person transforms into a toxic and destructive chemical fluid, starting from the innermost layer of the vascular system, known as the endothelial layer, and vascular wall damage occurs. The thin walls of microvascular capillaries break down, the toxic and destructive plasma exits the

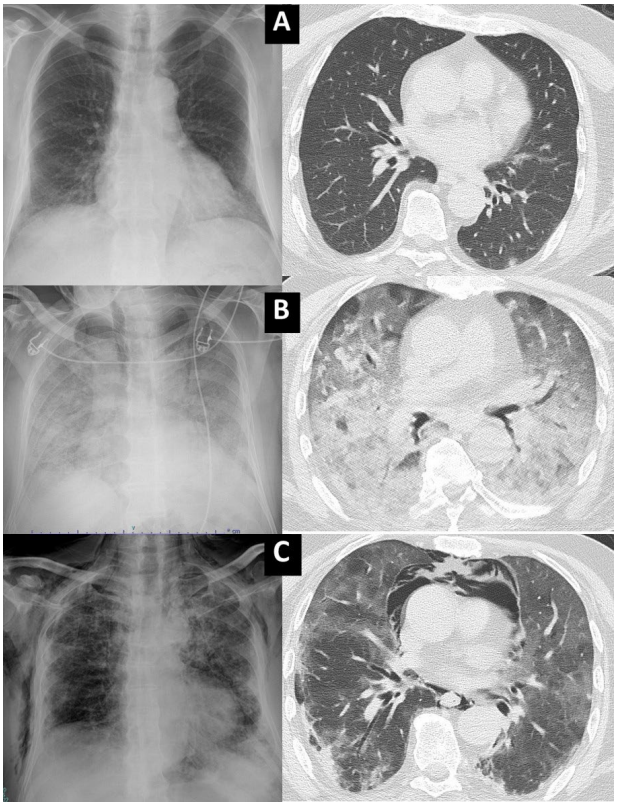

FIGURE 5. A 67-year-old male patient with COVID-19 is PCR positive. Early phase lung involvement is shown in Figure 5A as small patchy GGO's and consolidation. The severe phase (5B) is showing the bilaterally diffuse GGOs and consolidations consistent with "white lungs" due to chemically cooked lung parenchyma as a result of toxic plasma. Also, pneumomediastinum is seen in Figure 5C, and he died from respiratory failure and pneumomediastinum within 3 weeks of the early phase.

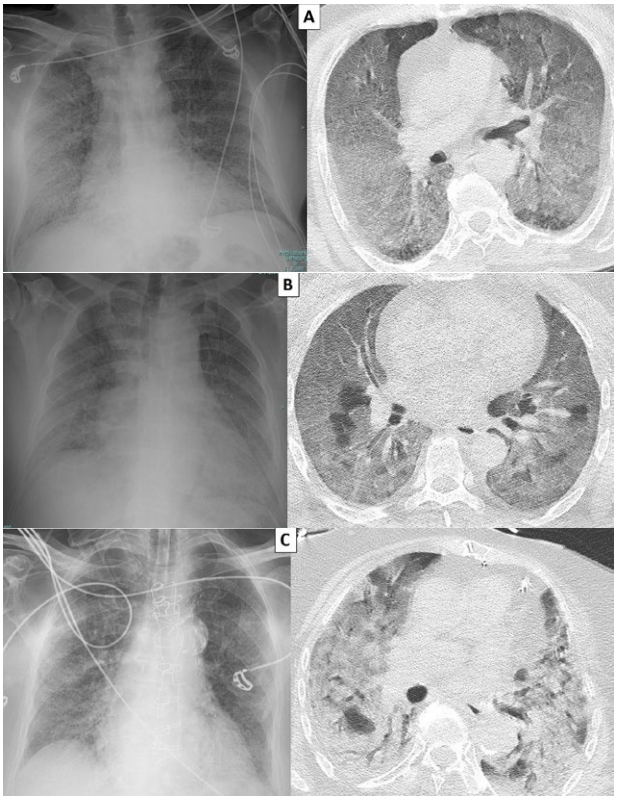

FIGURE 7. The "white or cooked lungs" were seen in 58-year-old male (7A), 58-year-old male (7B), and 84-year-old female (7C) patients with COVID-19. Diffuse bilaterally GGOs and alveolar consolidations are called "white or cooked lungs". It is caused by toxic plasma, and this intravascular toxic plasma leads to diffuse endotheliitis, vascular and tissue damage in patients with COVID-19.

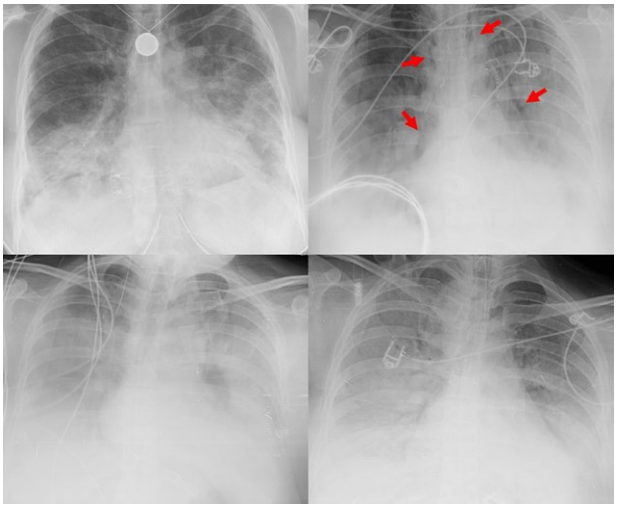

FIGURE 6. A 33-year-old female patient was admitted to the ICU with COVID-19 and respiratory failure. Chest X-ray images show the early, progressive, and severe phases within two weeks. Also, pneumomediastinum was developed (arrows). And, she died from respiratory failure.

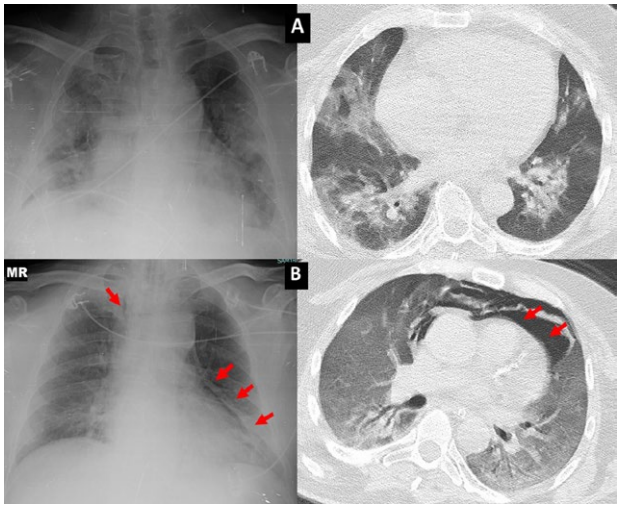

FIGURE 8. Bilateral alveolar consolidations and diffuse interstitial and GGO's were seen in Figure 8A and B. He died from pneumomediastinum (arrows) and hypoxemic respiratory failure.

blood vessels and causes tissue damage. In this context, the vascular system with the most concentrated microvascular capillaries and the thinnest wall structure is the pulmonary capillaries, primarily in the initial lungs, as a result of the endothelium (endotelitis), radiologically detectable ground-glass opacities are identified (Figures 9-14).

Stage 4 – Tissue damage and severe illness; The toxic and destructive plasma leads to tissue damage depending on the infected individual's response and the host's immune reaction. As a result of this, primarily respiratory failure, along with organ failure and death due to inadequate tissue perfusion related to endothelial dysfunction, arises.

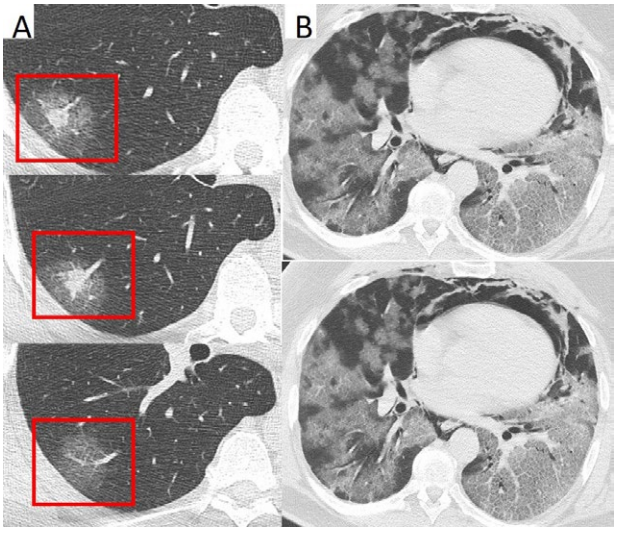

FIGURE 9. 56 years-old male presented with PCR-positive COVID-19. The first and early lesions start from pericapillary GGO and consolidation (9A). This pericapillar GGO is the best evidence of endotheliitis due to chemically destroyed to endothelium in pulmonary capillaries consisting only of the endothelial layer. And, it leads to chemically cooked lung parenchyma as a result of toxic plasma.

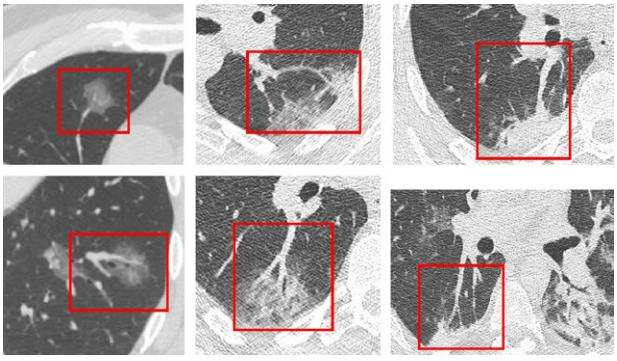

FIGURE 10. The pericapillary GGOs (early lesions) and consolidations (10 days later), like "lightning strikes," are shown in a 53-year-old male with PCR-positive COVID-19.

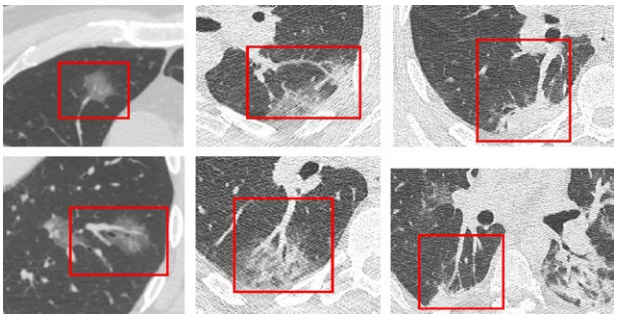

FIGURE 11. The GGOs are showing around the capillary in a 52-year-old female with COVID-19 in the early phase.

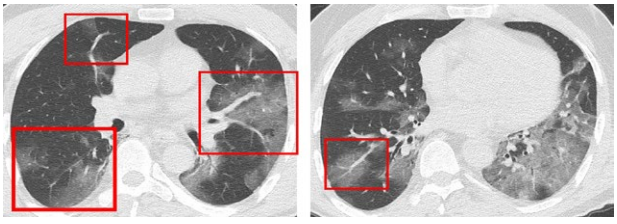

FIGURE 12. Bilaterally diffuse GGOs are showing, especially around the capillary in a 50-year-old male with COVID-19 in the progressive phase.

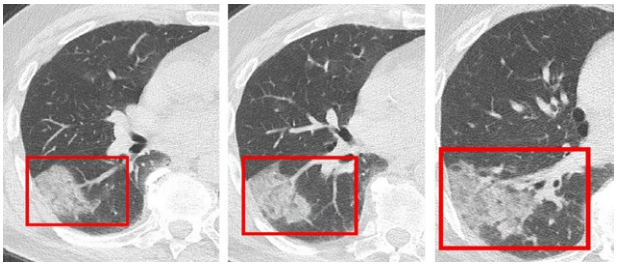

FIGURE 13. Localized GGOs with widening of the vessels are showing in a 74-year-old male with COVID-19.

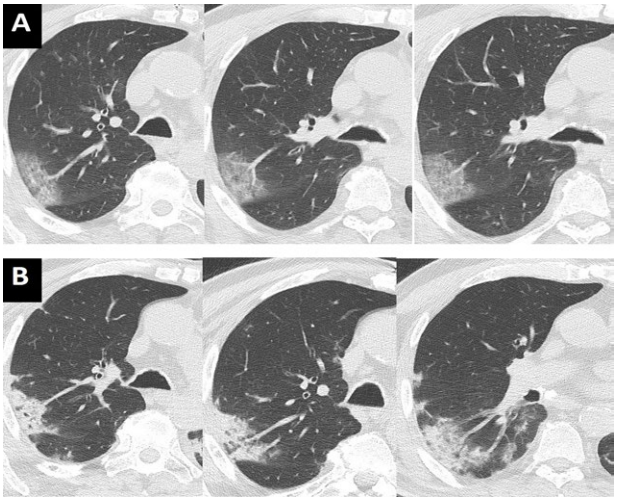

FIGURE 14. A 85 years old-male admitted to the hospital with PCR-positive COVID-19. Pericapillar GGOs were noted (14A), and fibrotic changes and consolidation were seen around the capillary, like "lightning strikes".

The lung involvements associated with COVID-19 can vary according to the radiological stages of the disease; they can primarily be observed as ground-glass opacities (GGOs), in addition to consolidations, linear interstitial infiltrations, reverse halo sign, and fibrotic areas[3,5].

We can interpret COVID-19 disease findings on CT imaging in 4 phases (early, progressive, severe, and dissipative). In the early phase, numerous small patchy shadows and interstitial changes appear and exhibit a distribution starting near the pleura or bronchi rather than the pulmonary parenchyma. When the lesions increase and enlarge, developing into multiple GGOs and infiltrating consolidation in both lungs, it corresponds to the progressive phase. In the severe phase, massive pulmonary consolidations and "white lungs" are recognized, but pleural effusion is uncommon. As the lesions began to change into fibrosis, the GGOs and pulmonary consolidations were completely absorbed, which implies the dissipative phase[3].

Our patients with diffuse interstitial involvement had higher mortality rates. We named this radiological appearance "baked or boiled lungs". Patients with this radiological appearance often experienced exitus due to hypoxemic respiratory failure. The most common complication leading to mortality was pneumothorax. In cases of pneumothorax, despite the presence of a chest tube, in many cases, lung expansion did not occur due to the loss of lung elasticity caused by the "baked or boiled lungs" effect. Pneumomediastinum is also a frequently observed complication, and despite its high frequency, it has better survival rates compared to patients with pneumothorax in terms of mortality. According to reports and articles, pneumothorax and pneumomediastinum are the most common causes of mortality in patients with COVID-19 due to mechanical complications [3,6-8].

We know that COVID-19 continues to persist as a fatal disease among us, and lung involvement, along with hypoxemic respiratory failure, continues to be the most frequent cause of death. Stage 2 of the disease plays a key role in patients with COVID-19. The stage of systemic inflammatory response depends on the viral load in the infected individual, the pathogenicity of the virus, and the immune response of the infected person. Various chemokines and cytokines, primarily including ferritin, are produced [3,9-16]. It has been shown that Interleukins (such as IL-1, IL-4, IL-6, IL-7, IL-10, IL-12, IL-17, and IL-18), IFN-γ, TNF-α, TGF-β, and NF-κB play major roles in the body's inflammatory response to SARS-CoV-2 infection [15]. These cytokines transform the plasma of the infected person into a toxic and destructive chemical fluid. This stage leads to the endotheliitis stage (stage 3) in depends on the patient's immune response.

One typical radiological finding in patients with COVID-19 is vascular enlargement within areas of ground-glass opacity. In our patients, the earliest pulmonary lesions appeared as GGOs surrounding the pulmonary capillaries (Figure 15). These findings may support the presence of endotheliitis caused by chemical damage to the endothelium of pulmonary capillaries, whose walls consist primarily of an endothelial layer. This process may contribute to chemically "cooked" lung parenchyma as a result of toxic plasma.

If the viral load and systemic inflammatory response are high, endotheliitis may affect all pulmonary capillaries and cause diffuse lung lesions. We identified diffuse interstitial involvement, which we refer to as "baked or boiled lungs," as the most lethal form of lung involvement, and pneumothorax as the most fatal complication.

We showed to endotheliitis in the biopsy section of the lung parenchyma. Haematoxylin&Eosin-stained sections from representative areas of lung parenchyma were seen with the mixed-type inflammatory-cell infiltration of lung tissue and exudative capillaritis with thickened microvascular walls, as reported in articles[15-17].

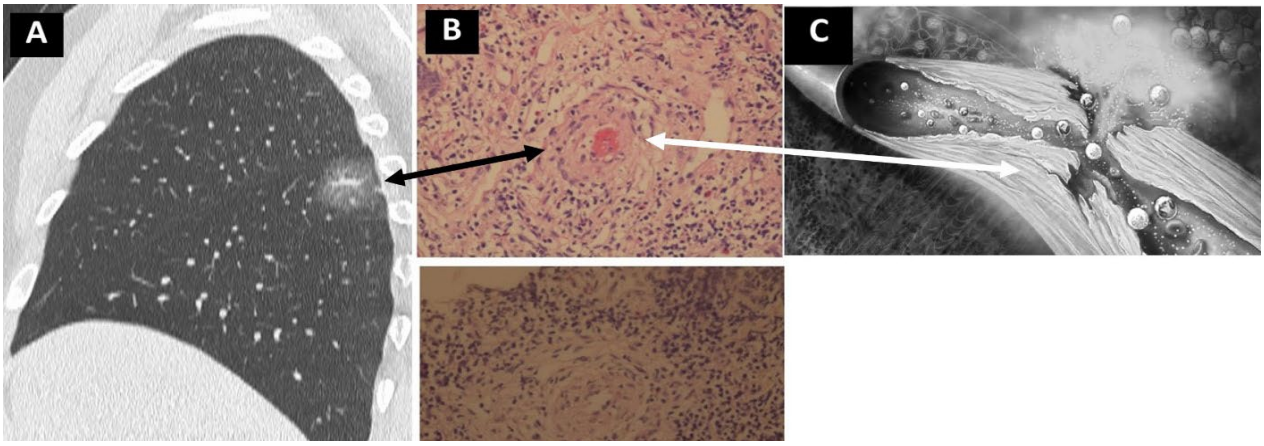

FIGURE 15. A perivascular ground-glass opacity with widening of the pulmonary vessels is seen in Figure 15A. The pulmonary histopathologic features and illustration of endotheliitis are shown in patients with COVID-19 (Arrows in Figure 15B and C*).
*Permission of Kenneth Warrington, MD. Mayo Clinic Minute: How vasculitis affects the body. June 21, 2023.

As a result, COVID-19 starts as an infectious contagious disease and leads to death in infected individuals because it renders the blood plasma toxic. We hypothesized that this toxic plasma leads to mechanical destruction of the microvascular capillary endothelium, especially in lung tissues, and the mortality caused by ARDS due to diffuse pulmonary capillary endotheliitis.

COMPETING INTERESTS: *No potential conflict of interest was reported by the authors.* **AUTHORS CONTRIBUTIONS:** *AD, SO, and TC contributed to the design and implementation of the research, to the analysis of the results, and to the writing of the manuscript. SO and AD contributed to the patient's care. SO and ZK contributed to the hypothesis of pathophysiological features. CB and DO contributed to the collection of data and figures from the files of cases. All authors have read and agreed to the published version of the manuscript.* **FUNDING STATEMENT:** *This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.* **FINANCIAL SUPPORT:** *The authors declare that no financial support was provided for the conduct of this study.*
DATA AVAILABILITY STATEMENT: *All data generated or analysed during this study are included in this article. Further enquiries can be directed to the corresponding author.*

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SYSTEMATIC REVIEW AND META-ANALYSIS

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JORNAL DA SOCIEDADE DAS CIÊNCIAS MÉDICAS DE LISBOA

Omega-3 Polyunsaturated Fatty Acids and Ventricular Arrhythmias in Patients with Implanted Cardioverter-Defibrillator: Systematic Review with Meta-analysis

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ABSTRACT: **Background:** Omega-3 polyunsaturated fatty acids (PUFA) are one of the most common supplements taken around the world, due to many beliefs in its positive effect on cardiovascular disease and cardiovascular related death. Nevertheless, despite showing promising results on *in vitro* and *in vivo* animal studies, PUFA's cardiovascular and, more specifically, antiarrhythmic role is still not well established in humans. Patients with implanted cardioverter-defibrillator (ICD) are a subset of individuals at a greater risk of suffering deadly arrythmias, which have a device that can detect, register and intervene in those arrhythmias. We aimed to understand the antiarrhythmic influence of omega-3 PUFA in patients with ICD. **Methods:** In this systematic review we searched randomized controlled trials regarding the arrhythmic effects of omega-3 PUFA supplementation on ICD patients, comparing to either placebo or no intervention at all. Results were pooled using a random effects model and reported using Hazard ratio (HR) with a 95% confidence interval (CI). **Results:** Of the 5 retrieved studies for review, 4 (n=1714) were included in the meta-analysis. Compared to placebo, there was not a significant risk reduction of ventricular arrhythmia or death (HR =0.88, 95% CI 0.71-1.10). The presence or absence of coronary disease as well as the severity of left ventricular systolic dysfunction did not influence the results. However, when excluding the first published study (n=200), a significant risk reduction of ventricular arrhythmias or death was observed with n-3 PUFA on ICD patients (HR 0.80, 95% CI 0.67-0.96; p= 0.014). **Conclusion:** The best available evidence does not support the recommendation of using omega-3 PUFA on ICD patients to reduce death or significant arrhythmias.

KEYWORDS: Fish-oil; Omega-3; n-3; Polyunsaturated fatty acids; PUFA; Eicosapentaenoic acid; EPA; Docosahexaenoic acid; DHA; Sudden cardiac death; SCD; Ventricular arrhythmias; Ventricular tachycardia; Ventricular fibrillation; Implanted Cardioverter-defibrillator; ICD.

BACKGROUND

Omega-3 polyunsaturated fatty acids (PUFA), present in fish oils, have been the target of many studies trying to find specific effects on both cardiovascular (CV) and metabolic systems. A Cochrane systematic review on the role of omega-3 fatty acids, namely long-chain omega 3 (LCn3) and/or alpha-linolenic acid (ALA), on primary and secondary prevention of CV disease, included 86 randomized controlled trials (162,796 participants). It was found little or no effect of increasing LCn3 and ALA on reduction of all-cause mortality and CV mortality. long-chain omega 3 appears to slightly reduce coronary heart disease (CAD) mortality and events, even though ALA showed little to no effect. On the other hand, CV events and arrhythmia seem to have a slight reduction in risk with ALA and little to no effect with increase in LCn3[1].

Some studies regarding the CV effect of these molecules showed a reduction in mortality due to a decrease in fatal arrhythmias rather than other nonfatal CV diseases. Some examples are the DART trial and more recently the GISSI Prevenzione trial. They studied patients with recent myocardial infarction, showing a reduction in all-cause mortality and death from CV causes on the patients, either with a diet containing fish oils or supplemented with n-3 PUFA, respectively. However, they failed to show a reduction in nonfatal myocardial infarctions. This reduction in all-cause mortality seemed to be a result of a major decrease in sudden cardiac death (SCD), reigniting an interest in the potential antiarrhythmic properties of fish oil[2–5]. Nevertheless, the evidence is still inconclusive as for the role of n-3 PUFA, particularly their potential antiarrhythmic benefits[2].

The aim of this review was to evaluate the effects of n-3 PUFA supplementation on the incidence of arrhythmias in patients with Implantable Cardioverter Defibrillators (ICD).

METHODS

This systematic review with meta-analysis was developed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines for reporting systematic reviews[6].

Eligibility criteria

In this systematic review and meta-analysis, we included randomized controlled clinical trials in which dietary supplementation was used to increase n-3 PUFA intake, compared to either placebo or no intervention, in patients with ICD, to assess its effect on ventricular arrhythmias.

Trials were included irrespective of follow-up time, n-3 PUFA supplementation amount or respective serum levels of n-3 PUFA quantification. These eventual differences in study design and subsequent results will be further analysed and described in the results and discussion of this review. The studies had to include patients with ICD irrespective of its implantation indication, duration, comorbidities, age or sex. The intervention had to be dietary supplementation of n-3 PUFA in a form of fish oil or its refined components, such as eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) or alpha-linolenic acid (ALA), irrespective of dose.

Trials were eligible if comparing n-3 PUFA supplementation, of any form, versus a control group comprised of patients with ICD taking either placebo or no intervention at all.

Information sources and search strategy

The bibliographic databases Ovid MEDLINE(R) and Epub Ahead of Print, In-Process, In-Data-Review & Other Non-Indexed Citations, Daily and Versions were searched with results from January 2023 and updated in November 2025. The EBM Reviews - Cochrane Central Register of Controlled Trials was also searched for relevant material for inclusion in the review.

The supplementary data regarding the search strategy is further detailed in this review's appendix (*Appendix 1. Search strategy*).

Study selection and data collection

Two reviewers independently screened the titles and abstracts retrieved from the electronic search. Duplicated publications were manually screened and excluded. The articles that met criteria or were unclear were further assessed through full-text analysis. Reasons for study exclusion were recorded in both screening stages.

Subsequently, study characteristics and corresponding outcomes were independently collected into a standardized form. These include the following: author, year of publication, number of participants, population demographics, comorbidities and concurrent medication, follow-up time, patient compliance, daily n-3 supplementation dose, control used, primary endpoint and resulting statistical results.

Study risk of bias assessment

The risk of bias of the included studies was independently assessed by two reviewers using the second version of Cochrane Risk of Bias Tool for randomized controlled trials (RoB-2)[7]. We used the six predefined domains (randomization process; deviations from the intended interventions; missing outcome data; measurement of the outcome; selection of the reported result). An additional domain (risk of bias arising from period and carryover effects) was used to assess the bias of the crossover study using the specific RoB2 tool for crossover randomised controlled trials (RCTs).

Both the domains and overall risk of bias were qualitatively classified as low, some concerns or high risk of bias, in accordance with the respective tools' algorithm.

Statistical analysis

A random-effects meta-analysis was performed based on the data retrieved from four out of the five selected studies. The pooled forest plots and statistic results were obtained using Stata 17.0 software. Since the primary outcome is a time-to-event outcome, hazard ratio (HR) presents as the most suitable form of presenting the results of the intervention at study[8]. These results were presented using 95% confidence intervals (CI).

The heterogeneity was evaluated primarily by visual inspection of forest plots and statistically using the Chi^2 for heterogeneity (threshold P > 0.10) test and I^2. The I^2 statistic indicates the percentage of the considered variability which is due to heterogeneity and not by chance or sampling error. An I^2 value below 40% indicates that heterogeneity may not be important, between 30 and 60% may be moderate, between 50 and 90% may be substantia and above 75% it is considerable. Note that in low numbered studies the Chi^2 for heterogeneity and I^2 statistics may raise some uncertainty in their respective interpretations[9].

Continuing the investigation on heterogeneity between studies and their respective demographic and intervention characteristics differences, both a subgroup analysis and a meta-regression were performed. The subgroup analysis on the primary outcome was performed according to the differences in study population, namely in patients with and without CAD and the lower left ventricular ejection fraction (LVEF) subgroups of each study. The meta-regression was performed according to the differences in each studies' main demographics (age, sex, comorbidities such as CAD, mean LVEF and intervention differences in omega-3 supplementation dosage.

Assessment of confidence in Evidence

The assessment of the certainty in evidence was conducted by two reviewers resorting to the Grading of Recommendations, Assessment, and Evaluation (GRADE) norms[9]. The GRADEpro software was used to generate a summary table of evidence with the corresponding grading of the quality of evidence assessment.

RESULT

Study selection

The databases search resulted in a pool of 336 articles. After manually removing 97 duplicates, 239 studies were screened by their title and abstract. Subsequently 38 studies were selected for full-text assessment. The reasons for exclusion were recorded in both screening stages. At the end of full-text evaluation phase, five randomized controlled trials were included (figure 1).

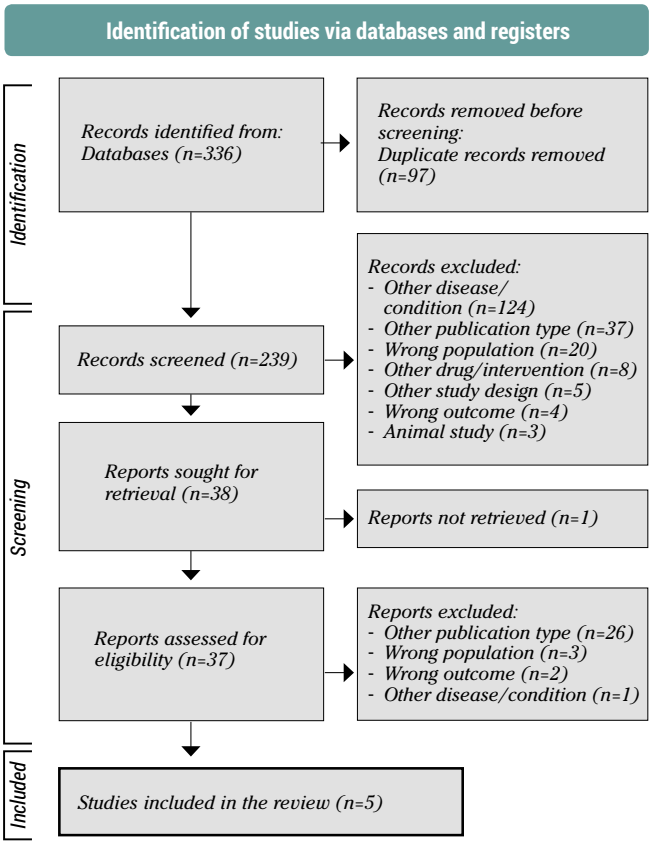


FIGURE 1. Flow diagram of database search, screening and study selection based on PRISMA 2020 flow diagram for new systematic reviews[6]

TABLE 1. Demographic characteristics of included patients.

Authors (Year)	Experimental groups	Participants	Age, yr, mean (SD)	Male sex	White	CAD/IHD	Previous MI	HTN	DM2	Current smoker	NYHA class					Ejection fraction					Medication								
											I	II	III	IV	NA	QM % (*SD*)	<30%	<35%	<40%	≥40%	ACEi	Amd	β-Blocker	CCB	Digoxin	Diuretic	Sotalol	Statin
Raitt et al (2005)[13]	Fish oil	n=100	63 (13)	86	94	75	55	46	24	NR	25	13	48	14	0	36 (16)	46	NR	57	33	66	0	74	9	NR	54	0	54
	Placebo	n=100	62 (13)	86	97	71	56	55	23		28	14	50	8	0	34 (15)	37		56	34	66	0	73	13		52	0	41
Leaf et al (2005)[23]	Fish oil	n=200	65.7 (0.82)	169	191	151	NR	NR	NR	30	47	66	20	0	51	32.9 (1.00)	102	NR	142	45	121	31	132	16	NR	104	23	NR	
	Placebo	n=202	65.3 (0.82)	165	195	163				23	54	75	10	1	45	34.2 (1.05)	99		138	58	114	31	118	15		99	33		
Brouwer et al (2006)[21]	Fish oil	n=273	60.5 (12.8)	231	NR	187	167	143	45	44	23	65	4	0	0	36.9 (15)	87	NR	NR	NR	151	59	145	15	NR	109	21	NR	NR
	Placebo	n=273	62.4 (11.4)	228		197	175	134	42	23	39	47	5	2	0	37.0 (15)	95				160	50	155	13		116	15		
Finzi et al (2011)[22]	Fish oil	n=278	64.9 (9.5)	250	NR	NR	161	113	65	39	0	176	101	1	0	28.1 (6.5)	NR	NR	273	5	209	106	214	12	NR	254	NR	95	
	Placebo	n=288	64.8 (9.8)	250			162	143	77	50	0	186	100	2	0	28.7 (6.9)			283	5	223	98	223	17		270		83	
Weisman et al* (2017)[12]		n=105	0 (9.6)	99	NR	105	NR	39	27	NR	27	53	18	0	7	NR	NA	68	NA	NA	NR	35	89	11	NR	NR	14	NR	83	NR

*Cross-over study with a duration of 6 months per intervention with a 4 month wash out period between Fish oil supplementation and Sunflower oil with corn oil control. **ACEi:** angiotensin converting-enzyme inhibitor; **Amd:** amiodarone; **CAD:** coronary artery disease; **CCB:** calcium-channel blocker; **DM2:** type 2 diabetes mellitus; **HTN:** hypertension; **IHD:** ischemic heart disease; **MI:** myocardial infarction; **NA:** not applicable; **NR:** not reported; **NYHA:** New-York Heart Association; **QM:** quantitative mean; **SD:** standard deviation; **Yr:** year

Study characteristics

Each study's main demographic characteristics are listed below (Table 1). Out of the five selected randomized controlled trials, four are parallel in design and one is a cross-over study. Each study sample size ranged from 105 to 566 patients, with a total, across all studies, of 1819 participants constituted mostly by males. There was also a high prevalence of ischemic heart disease and, to a lesser degree, hypertension. As an indication for ICD, in specific circumstances, heart failure is also well represented and characterized in each study with a reduced mean LVEF across all the four earlier trials. Duration of follow-up ranged from one year to approximately two and a half years in the parallel designed studies and six months per intervention in each arm, with a 4-month washout period, in the cross-over study. Moreover, daily doses of n-3 fatty acids ranged from 0.8 grams to 3.6 grams, with different concentrations of DHA, EPA and other refined components. Olive oil was used as a control in *Raitt et al*[13] and *Leaf et al*[23], whereas sunflower oil was used in *Brouwer et al*[21] and combined with corn oil in *Weisman et al*[12]. Finally, *Finzi et al*[22] used a placebo as control.

The primary end point in the studies of *Raitt et al*[13], *Leaf et al*[23], *Brouwer et al*[21] and *Finzi et al*[22] was time to first ICD intervention, in response to an episode of either Ventricular tachycardia (VT) or Ventricular fibrillation (VF) and all-cause mortality. On the other hand, *Weisman et al*[12] presented its results in number of VT recorded by the ICD and device therapy/shocks as primary and secondary outcomes, respectively. All studies' primary outcomes were performed based on intention to treat analysis. *Leaf et al*[23] also presented an adjusted result for probable ICD interventions (events without an electrogram documentation of ventricular arrhythmia available but which had other data suggesting successful VF/VT termination), corresponding to a significant re-

duction in relative risk (RR) to 0.69 (95% CI 0.49-0.97; p=0.033). We chose to use the unadjusted RR, where only recorded and reviewed episodes were taken into account (RR= 0.72 CI 95%, 0.51-1.01; p=0.057).

The remaining study characteristics and summary of statistical results are further detailed in Table 2.

The risk of bias of the primary outcome (performed based on intention to treat) of every selected study was done using Cochrane's RoB2 tool, as presented in the table below (Table 3). The period and carryover effects domain is only applicable to the cross-over study of *Weisman et al*[12].

TABLE 2. Summary of study characteristics and results.

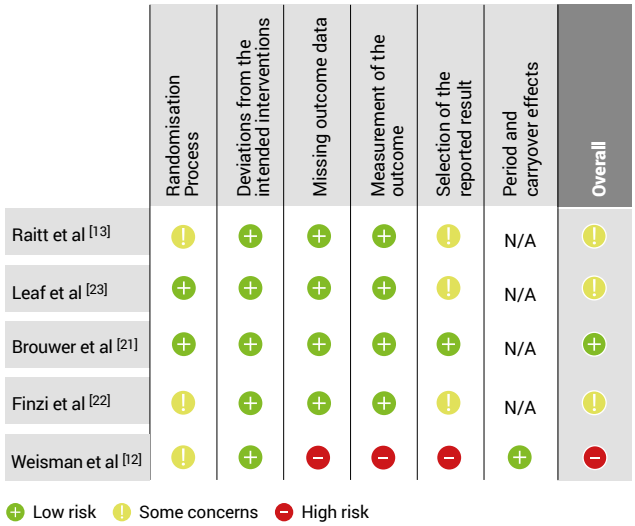
Authors	Experimental groups	Participants	Duration of follow-up, yr	Compliance, No (%)	Daily dose of omega-3 (EPA, DHA and other PUFAs), grams	Control	Primary endopoint	Event rate, %		
								6M	12M	24M
Raitt et al[13]	Fish oil	n=100	2	98 (98)	1.3	Olive oil	Time to 1st episode of VT/VF leading to ICD therapy	46	51	65
	Placebo	n=100		94 (94)				36	41	59
Leaf et al[23]	Fish oil	n=200	1	127 (64)	2.6	Olive oil	Time to first ICD discharge for VT/VF; All-cause mortality	NR	28	NA
	Placebo	n=202		133 (66)					39	
Brouwer et al[21]	Fish oil	n=273	1	244 (89)	0.9	High-oleic sunflower oil	ICD intervention for VT/VF; All-cause mortality	NR	30	NA
	Placebo	n=273		248 (91)					33	
Finzi et al[22]	Fish oil	n=278	2.5	278 (100)	0.8	Placebo	Time to ICD intervention due to VF/VT; No of VT and VF episodes	NA		
	Placebo	n=288		288 (100)						
Weisman et al*[12]	Cross-over study	n=105	0.5	87 (83)	3.6	Sunflower and corn oil	Number of ICD-recorded TV	NA		

*Cross-over study with a duration of 6 months per intervention with a 4 month wash out period between Fish oil supplementation and Sunflower oil with corn oil control.
CI: confidence interval; **DHA:** docosahexaenoic acid; **EPA:** eicosapentaenoic acid; **ICD:** Implantable cardioverter defibrillator; **M:** months; **No:** number; **NR:** Not reported; **PUFA:** Polyunsaturated fatty acids; **VF:** ventricular fibrillation; **VT:** ventricular tachycardia; **Yr:** year

Risk of bias

The overall risk of bias amongst the studies raised some concerns. The 3 studies of *Raitt et al*[13], *Finzi et al*[22] and *Weisman et al*[12] raised some concerns regarding the randomization process domain, due to absent information in the reports regarding the concealment of allocation. Unlike *Brouwer et al*[21], the four studies of *Raitt et al*[13], *Leaf et al*[23], *Finzi et al*[22] and *Weisman et al*[12] did not report a pre-specified analysis plan before the unblinded outcome data was available for analysis, raising some concerns in the selection of the reported results domain. The later presented its results mostly in mean values of each outcome, represented in bar graphs without appropriate listing of each analysis results, and did not present any detailed results regarding any differences between cross-over arms. Therefore, it was classified as a higher risk of bias in the missing data, measurement of outcome and selection of the reported results domains.

TABLE 3. Risk of Bias Analysis using Cochrane Risk of Bias Tool for randomized controlled trials (RoB-2).



N/A: not applicable.

Primary outcome – Time to ICD intervention or death

A meta-analysis was performed with the results pooled from four (*Raitt et al*[13], *Leaf et al*[23], *Brouwer et al*[21], *Finzi et al*[22]) out of the selected five studies for review, as a consequence of *Weisman et al*[12] not reporting the required outcome.

Overall, there was no statistically significant difference in time to first ICD intervention due to VF/TV or all-cause mortality between fish oil and placebo in our 1714 ICD patient meta-analysis (combined HR=0.88, 95% CI 0.71-1.10; overall effect p-value = 0.26). Although not statistically significant (Chi^2 for heterogeneity p=0.13), there was moderate heterogeneity between the four analyzed studies (I^2= 45.45%) (Figure 2).

When performing the meta-analysis omitting successively each of the studies, it was noted a statistically significant difference in time to first ICD intervention, benefiting n-3 supplementation, when excluding the *Raitt et al*[13] trial (HR=0.80, CI 95%; 0.67-0.96; p=0.014). There was no significant deviation from the overall meta-analysis when excluding each one of the other included studies (Figure 3).

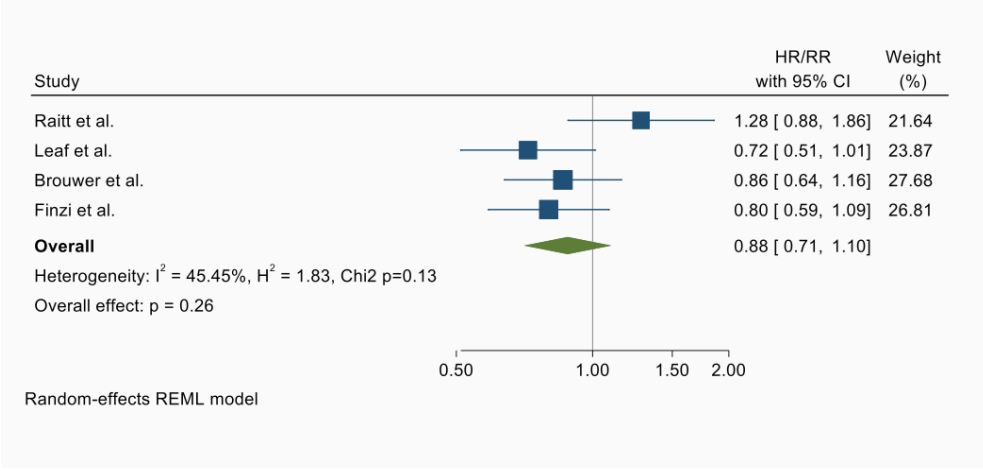


FIGURE 2. Forest plot for time to ICD intervention in ICD patients with omega-3 supplementation.

CI: confidence interval; HR: hazard ratio; RR: relative risk. Raitt et al[13]; Leaf et al[23] ; Brouwer et al[21]; Finzi et al[22] ; Weisman et al[12] .

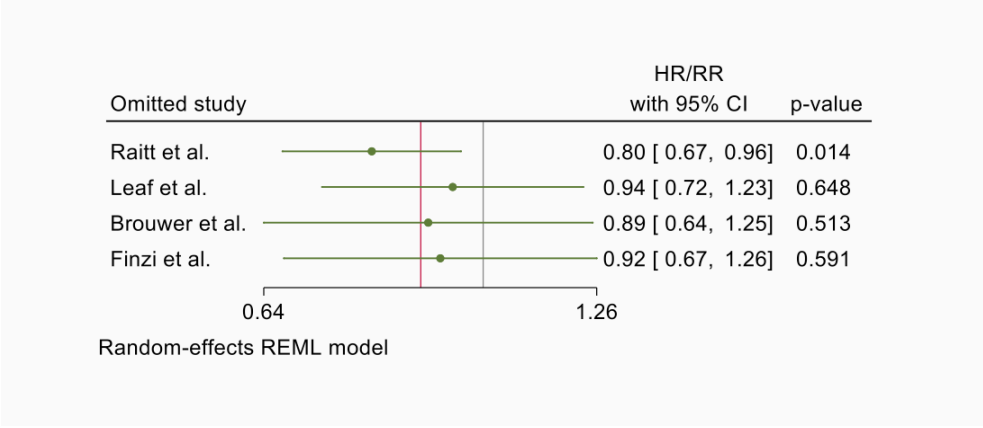


FIGURE 3. Omitted studies analysis.

CI: confidence interval; HR: hazard ratio; RR: relative risk. Raitt et al[13]; Leaf et al[23] ; Brouwer et al[21]; Finzi et al[22] ; Weisman et al[12] .

Subgroup analyses and meta-regression

In respects to the differences in demographics presented by each of the studies, we conducted a subgroup analysis between patients with CAD or its absence and between the subgroups with lower ejection fractions in every trial.

Three of the included studies in the meta-analyses (*Raitt et al*[13], *Leaf et al*[23], *Brouwer et al*[21]) reported the number of patients with CAD included. It was not found any statistically significant difference between fish oil supplementation and placebo in patients with ICD, regarding time to first ICD intervention either in each individual study or in the overall analysis (overall HR=0.92, CI 95%, 0.58-1.44; p value = 0.70) (Figure 4). Although not statistically significant and contrasting with the other studies, *Raitt et al*[13] showed a proarrhythmic effect in patients with CAD taking fish oil supplementation. However, we cannot ignore that in this specific subgroup analysis there is a substantial heterogeneity (I^2=77.45%) between the considered trials (Figure 4).

The absence of CAD and its respective results was only reported in *Raitt et al*[13] and *Leaf et al*[23], each without significant results regarding the beneficial antiarrhythmic effect of n-3 PUFA in patients without CAD. This subgroup analysis resulted in an unsignificant heterogeneity (I^2=0%), which can be the result of few included studies (Figure 4).

Regarding patients with low LVEF, in the Leaf et al[23] trial ICD patients with LVEF lower than 30%, we observed a significant antiarrhythmic benefit in supplementing with omega-3 PUFA over placebo. However, when considering the overall lower LVEF subgroups reported, we did not find a statistically significant difference between the intervention and control groups. Heterogeneity was moderate (I^2=50.34%) (Figure 5).

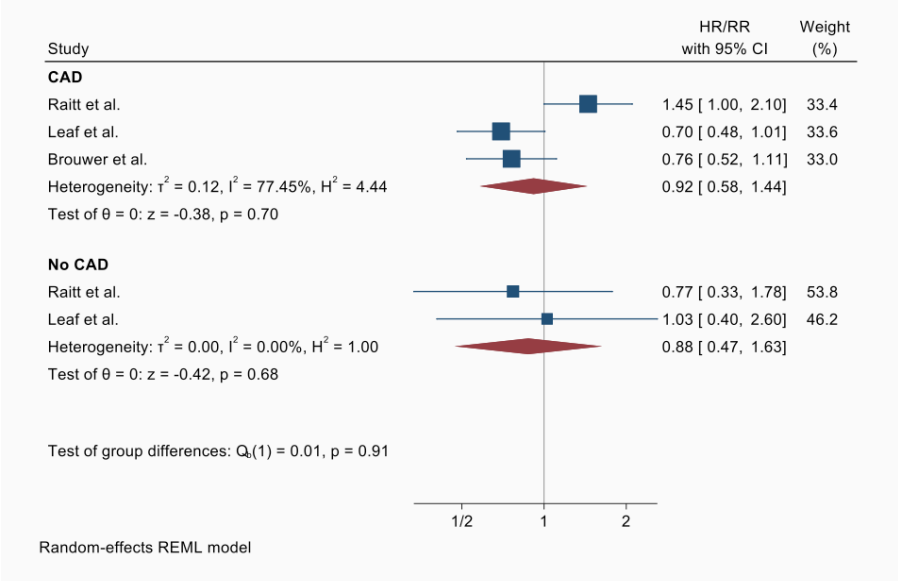


FIGURE 4. Subgroup analysis by presence or absence of coronary artery disease (CAD).

CI: confidence interval; HR: hazard ratio; RR: relative risk. Raitt et al[13]; Leaf et al[23]; Brouwer et al[21].

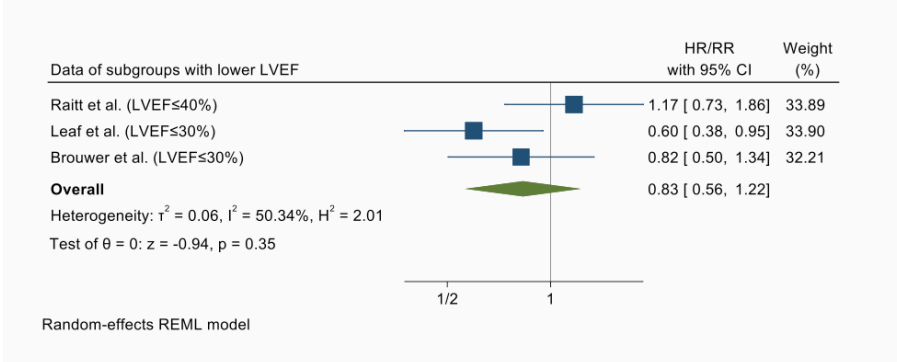


FIGURE 5. Subgroup analysis by lower left ventricle ejection fraction (LVEF).

CI: confidence interval; HR: hazard ratio; RR: relative risk. Raitt et al[13]; Leaf et al[23]; Brouwer et al[21].

In respect of the meta-regressions, we considered patients mean age, percentage of male patients, percentage of participants with CAD, mean LVEF in each study and finally the omega-3 supplementation dosage in in each study. Reviewing each of the generated meta-regression scatter plots (Suppl data 2), we can see a decrease of the predicted HR with the increase of each studies population mean age, with the lowering of LVEF and with the augmentation of omega-3 supplemented dosage. On the other hand, we note a progressive but discreet increase in predicted HR with the increase in the percentage of male population and CAD patients in each study. None of the observed meta-regression results prove to be significant as their corresponding 95% CI crosses the no effect line.

Reporting bias and certainty of evidence

The supplementary summary of findings table was generated according to the GRADE approach to evaluate the time to first ICD intervention or mortality outcome analyzed in the meta-analysis.

We accessed a very low certainty of evidence as a result of the concerns raised by the risk of bias of three of the four studies selected for meta-analysis. Inconsistency was also downgraded due to the moderate heterogeneity between studies' results. Finally, we also downgraded imprecision owing to the small effect of the intervention which has the possibility of no effect as the confidence interval crosses the no-effect line (Table 4).

DISCUSSION

This meta-analysis of four randomized controlled trials did not demonstrate a statistically significant protective role of n-3 PUFA supplementation on the incidence of life-threatening ventricular arrhythmias.

Even though our study is, to our knowledge, the first meta-analysis to include four studies regarding the potential antiarrhythmic effect of n-3 PUFA in patients with ICD, our results are still in line with previous reports[10–12]. The previously described unsignificant tendency to an antiarrhythmic effect of PUFA on ICD patients was corroborated by our study. In addition, our study found a narrower confidence interval and an even lower heterogeneity between studies than had ever been reported before[10–12].

One interesting finding was the omitted study analysis where, when excluding the *Raitt et al* trial[13], we obtained a statistically significant result concerning the protective effect of omega-3 PUFA. This trial, in contrast with any other studies reviewed, reported that there was no risk reduction of VT/VF in patients with ICD taking omega-3 supplementation and even found a significant proarrhythmic effect in patients with recent sustained VT[13]. Being the only one of four analyzed

TABLE 4. Summary of findings table according to the GRADE approach.

Certainty assessment							Summary of findings		
Participants (studies) Follow-up	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	Overall certainty of evidence	Study event rates (%) With Placebo	Study event rates (%) With Omega-3	Relative effect (95% CI)
Time to ICD intervention or mortality (follow-up: median 12 months)									
1714 (4 RCTs)[10, 12-14]	serious[a]	serious[b]	not serious	serious[c]	none	⨁◯◯◯ Very low	863 participants	851 participants	**HR 0.88** (0.71 to 1.10) [Time to ICD intervention or mortality]

CI: confidence interval; **OR:** odds ratio.

[a] Of the 4 randomized controlled trials assessed for risk of bias using ROB2 tool, only one represents a low risk of bias, while the other 3 represent an unclear risk of bias due to unclear information mainly regarding the randomization process and the selection of reported results. Thus, we considered the overall risk of bias as serious.

[b] In order to judge inconsistency we used I^2 and Chi^2 for heterogeneity statistical tests. Due to a non-statistically significant Chi^2 for heterogeneity (p=0.13) and a moderate heterogeneity represented by a I^2 of 45.45% we chose to consider inconsistency as serious.

[c] In our meta-analysis the results include the possibility a small effect of the intervention (HR=0.88, CI 95% 0.71 - 1.10) with the possibility of no effect. Therefore, we chose to classify imprecision as serious.

studies reporting a tendency of proarrhythmic effect of n-3 PUFA and the consequent significant effect of its exclusion from the analysis, we must ask what differences this study may bare which considerably impacted its results. A previous meta-analysis found an unsignificant increase in risk of VT/VF in patients taking fish oil, who simultaneously had VT at entry and did not take any antiarrhythmics[11]. Since the Rait trial included patients with VT at entry and excluded patients taking any class I or III antiarrhythmics, this could have influenced its results. Further studies with better discrimination of subgroup results in patients with and without VT at entry and class I and class III antiarrhythmics, may help us understand omega-3 PUFAs' true effect on these populations and clarify not only the differences in results in previous studies, but also comprehend the true effect of this intervention on fatal ventricular arrhythmias.

Due to the higher incidence of SCD resulting from ventricular arrhythmias (VT/VF) in patients with CAD, it may prove to be fundamental to understand the true effect of n-3 PUFAs on this specific population[14–16]. The protective antiarrhythmic effect of PUFA had been demonstrated before in animal models namely with ischemic insults in dogs' hearts resulting in VF which were promptly prevented when injecting omega-3 PUFA[17]. However, this evidence in humans is still ambiguous and many hypothesize if the early described protective effect of n-3 on CV mortality is due to an overall reduction in CV events rather than an antiarrhythmic effect operating amongst the mechanisms of SCD[3,4,18–21].

In our systematic review, *Weisman et al*[12] was the only study to just include patients with ICD with concomitant ischemic cardiomyopathy. Even though it analyzed the mean number of interventions and not the time to first intervention, which impeded us from comparing to our other studies in our meta-analysis, the outcome results were rather interesting. In this 105-patient cross-over study, there was a significantly lower incidence of ICD interventions in patients taking 3.6 grams of omega-3 PUFA than patients in the control arm with sunflower and corn oil (mean number of VT registered 1.7 Vs. 5.6; p= 0.035).

Some have hypothesized that particularly in individuals who suffered MI, there is a greater risk for reentry arrhythmias in the surrounding ischemic tissue which now has different rates of conduction and n-3 PUFA can then hyperpolarize these partially depolarized cells preventing the initiation of arrhythmic events in the ischemic scars[5]. *Leaf et al*[23] also argued that, even though the partially depolarized cells around the ischemic tissue may become unexcitable in the presence of n-3 PUFA, this effect is not seen, at least to a same degree, in the heathy myocardium, which can continue to function normally[5]. In our meta-analysis we could not find a significant protective antiarrhythmic benefic of using omega-3 on the subgroup of patients with CAD (HR=0.92, CI 95%, 0.58-1.44; p value = 0.70). However, we did not find a proarrhythmic effect on ICD patients without CAD either (HR=0.88, CI 95%, 0.47-1.83; p value= 0.68). The data collected did not permit a subgroup analysis of patients with previous MI, which should be interesting to compare with the positive results reported by *Weisman et al*[12] in this specific ICD population.

Regarding lower LVEF, despite overall results not being statistically significant (HT=0.83, CI 95%, 0.56-1.22; p= 0.35), when we observe the groups with LVEF lower than 30% we see a tendency to a benefit in the use of n-3 PUFA for preventing ventricular arrhythmia in this subgroup of ICD patients, and a clearly significant protective effect on the *Leaf et al*[23] trial population. However, we must not ignore the fact that in this subgroup analysis we used each studies' reported results for each considered LVEF, that resulted in some studies as *Raitt et al*[13] also including patients between 30 and 40% LVEF. This heterogeneity could have impacted results but is also an indication to further study not only the potential antiarrhythmic effect of PUFA on patients with reduced LVEF, but also potential differences within this population which can impact the indication or contraindication of the intervention being studied.

Some of the limitations we found regard differences between inclusion criteria in each study. For example, patients enrolled in *Finzi et al*[22] had a considerably lower mean LVEF (28.4%), and all its included patients had heart failure with LVEF below 35%[22]. Also in this study, more than half of the patients had ICD for primary prevention, while the other three considered trials only included patients with ICD for secondary prevention, i.e. had a previous episode of ventricular arrhythmia. This may mean that these patients had a lower risk of arrhythmia than patients which endured a VF/VT event.

Another limitation of this meta-analysis was the wide amplitude of fish oil daily supplementation between studies (0.8-2.6 grams), and their respective differences in PUFA refined components concentration (EPA and DHA). All the included trials measured red blood cell and plasma n-3 PUFA levels in both intervention and control groups, with a statistically significant increase

across all participants taking fish oil supplementation versus control. Although the *Finzi et al* study[22] had the lowest daily intake of fish oil supplement (0.8g), in its main trial – the GISSI trial – patients with a recent myocardial infarction taking the same daily amount of fish oil still had a significant reduction in total deaths, CV deaths and SCD[3].

Additionally, compliance in studies was another limitation found, majorly in Leaf et al, where it never surpassed 64 and 66% in the fish oil and placebo arms, respectively[23].

All these factors might have contributed to the lack of statistically significant results obtained. Furthermore, the differences in reported results between studies, particularly the results in each subgroup, hampered their comparative analysis. If available in the future, this additional data could help us better understand the true role of omega-3 PUFAs on ventricular arrhythmias.

In conclusion, this meta-analysis did not find a significant antiarrhythmic effect of n-3 PUFA on ICD patients. A significant result was only evident after excluding the first 200 patient published study which had different exclusion criteria than the others included. Despite previously reported positive results in patients with ischemic cardiomyopathy and in those with low LVEF, our subgroup analysis did not find significant results in these subsets of participants. These results do not support the systematic prescription of n-3 PUFA for patients with ICD. However, this systematic review raised new potential hypothesis regarding potential interactions with antiarrhythmic drugs that merit further studies.

DECLARATION OF INTERESTS:

DC has participated in educational meetings and/or attended a conferences or symposia (including travel, accommodation and/or hospitality) with Bial, Bristol-Myers Squibb, Bayer, Boehringer Ingelheim, Daiichi Sankyo, Merck Serono, Ferrer, Pfizer, Novartis and Roche. The remaining authors have nothing to declare.

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SUPPLEMENTARY DATA

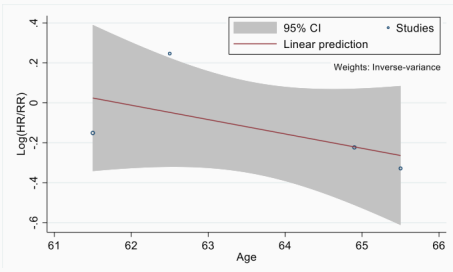
1. SEARCH STRATEGY

#	Searches
1	exp Fatty Acids/
2	exp fatty acids, unsaturated/
3	exp Fatty Acids, Omega-3/
4	exp Fatty Acids, Omega-6/
5	alpha-Linolenic Acid/
6	Docosahexaenoic Acids/
7	Eicosapentaenoic Acid/
8	(omega 3 or omega 6).tw.
9	polyunsaturat$ fatty acid$.tw.
10	PUFA.tw.
11	exp Fish Oils/
12	exp linseed oil/
13	(EFA or EPA or MaxEPA or DHA or ALA).tw.
14	(oil$ adj3 (fish$ or flax or linseed)).tw.
15	omega 3 fatty acid/
16	(fish adj3 (diet* or nutrit* or oil* or supplement*)).ti,ab.
17	(fish adj3 capsul*).ti,ab.
18	exp salmoniformes/ or tuna/
19	(eicosapentaen* or icosapentaen*).ti,ab.
20	(eicosapentaen* or icosapentaen*).ti,ab.
21	Linolen*.ti,ab.
22	alpha*linolen*.ti,ab.
23	alphalinolen*.ti,ab.
24	exp Defibrillators/
25	(icd or icds).af.
26	(defibrillat* or defibrilat*).af.
27	(crt or crts).af.
28	electrover*.af.
29	((resynch* or re*synch*) adj3 (therap* or treatment* or device*)).af.
30	(cardiover* or (cardio adj ver*) or cardioconver* or (cardio adj conver*)).af.
31	1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23
32	24 or 25 or 26 or 27 or 28 or 29 or 30
33	31 and 32
34	randomized controlled trial.pt.
35	controlled clinical trial.pt.
36	randomized.ab.
37	placebo.ab.
38	clinical trials as topic.sh.
39	randomly.ab.
40	trial.ti.
41	34 or 35 or 36 or 37 or 38 or 39 or 40
42	33 and 41
43	exp animals/ not humans.sh.
44	42 not 43

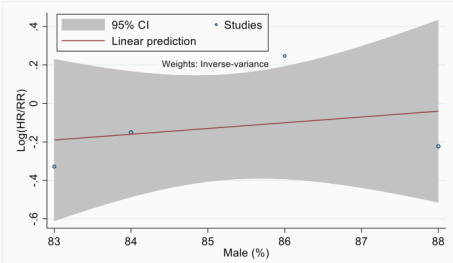
#	Database: Web of Science Core Collection
#1	(defibrillat* or defibrilat*) (All Fields) OR (icd or icds) (All Fields) OR (cardiover* or cardioconver*) (All Fields)
#2	(ALL=((omega3 OR omega-3 OR omega6 OR omega-6 OR fish oil)))
#3	ALL=(EFA or EPA or MaxEPA or DHA or ALA or PUFA)
#4	#3 OR #2
#5	#4 AND #1
#6	TS=(random* OR allocat*)
#7	#6 AND #5

2. META-REGRESSION SCATTER PLOTS

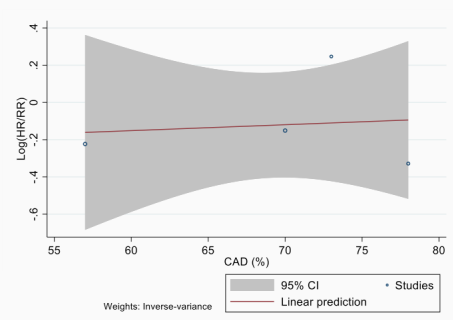
2.1. Age



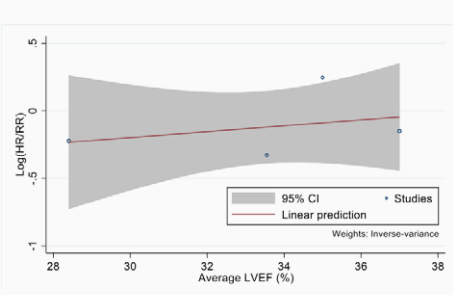
2.2. Male (%)



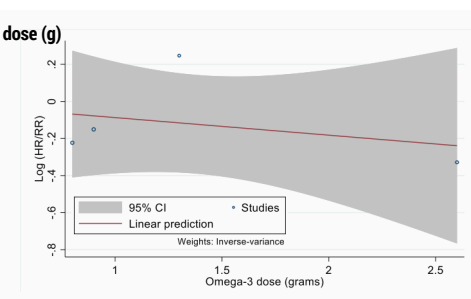
2.3. CAD (%)



2.4. LVEF (%)



2.5. Omega-3 dose (g)



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HISTORICAL ARTICLE

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JORNAL DA SOCIEDADE DAS CIÊNCIAS MÉDICAS DE LISBOA

The XV International Congress of Medicine (Lisbon, April 1906): 120 Years Later

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ABSTRACT: The XV International Congress of Medicine, held in Lisbon in April 1906, was the most important international scientific event ever organized in Portugal prior to the First World War, bringing together more than 2,000 international participants, not including accompanying persons. Many of the leading figures in world medicine were present. This congress provided Portuguese physicians with direct contact with the most advanced medical knowledge of the time. This article revisits the circumstances that led to the organization of the congress, highlights its scientific and social program and discusses its impact on the development and internationalization of Portuguese medicine. One hundred and twenty years later, this congress remains a landmark in the history of Portuguese medicine.

KEYWORDS: History of Medicine; XV International Congress of Medicine; 1906; Lisbon; Miguel Bombarda.

In the second half of the nineteenth century, awareness of health challenges implied an increasingly active and effective response from medicine to the serious problems being faced worldwide. The development of medicine was progressively gaining momentum. The exchange of knowledge needed to be enhanced, and personal contact became imperative to promote the sharing of ideas.

In France, annual medical congresses had already been held since 1863 in various cities. At the 1865 congress held in Bordeaux, the possibility was raised of taking advantage of the 1867 Paris Universal Exposition (April–November) to organize an international medical congress, as it would certainly attract a large number of health professionals from all over the world. This congress, which would become the *I Congrès International de Médecine*, took place in August with around 1,500 participants and was an extraordinarily successful event. This success stimulated the organization of new editions every three years, held in several European cities and, on one occasion, in the United States (Washington, 1887), becoming the largest global medical events of their era, attended by physicians and other health professionals from all over the world. (Table 1)

TABLE 1. International Congresses of Medicine (1867–1913).

Number	Year	Location
1st	*1867*	*Paris, France*
2nd	*1869*	*Florence, Italy*
3rd	*1873*	*Vienna, Austria*
4th	*1875*	*Brussels, Belgium*
5th	*1877*	*Geneva, Switzerland*
6th	*1879*	*Amsterdam, The Netherlands*
7th	*1881*	*London, United Kingdom*
8th	*1884*	*Copenhagen, Denmark*
9th	*1887*	*Washington, D.C., United States*
10th	*1890*	*Berlin, Germany*
11th	*1894*	*Rome, Italy*
12th	*1897*	*Moscow, Russia*
13th	*1900*	*Paris, France*
14th	*1903*	*Madrid, Spain*
15th	***1906***	***Lisbon, Portugal***
16th	*1909*	*Budapest, Hungary*
17th	*1913*	*London, United Kingdom*

We thus arrive at the XIV International Congress of Medicine, which took place in Madrid in 1903. Among the approximately 10,000 participants, there were about 200 Portuguese delegates. The idea of bringing the next congress to Portugal was discussed among some of the Portuguese participants. Miguel Bombarda, Professor at the Medical School of Lisbon and an active politician, was one of the most enthusiastic supporters of the Portuguese bid. The first steps were taken by telegraphing Lisbon, communicating these intentions to Prime Minister Hintze Ribeiro and requesting the necessary support. While the Madrid congress was still in session, a positive response arrived, and the Portuguese delegation therefore proposed that the XV International Congress of Medicine should take place in Lisbon in April 1906. Bombarda was unanimously appointed Secretary-General of the future congress.

Despite the initial enthusiasm, the difficulties involved in organizing such an event at the beginning of the twentieth century were immediately apparent in a country facing serious economic difficulties, political instability, and no previous experience in organizing events of this magnitude. The conditions required for hosting the congress and all the associated logistics were, from the outset, a source of concern that urgently needed to be addressed: how could thousands of participants and their companions be accommodated? Where would the scientific sessions take place? How could a programme be organized that was not only scientifically valuable but also socially engaging, leaving participants with lasting memories? Upon returning to Lisbon, Miguel Bombarda urged the government to complete the building intended to house the Medical-Surgical School, as this would be the ideal venue for the congress. He made it clear that this was a *sine qua non* condition for proceeding with the project, since construction work was progressing slowly. Furthermore, these brand-new and fully functional facilities, enriched by frescoes, tiles, paintings, and sculptures, would provide the dignity and prestige appropriate to an event of such importance (Fig. 1).

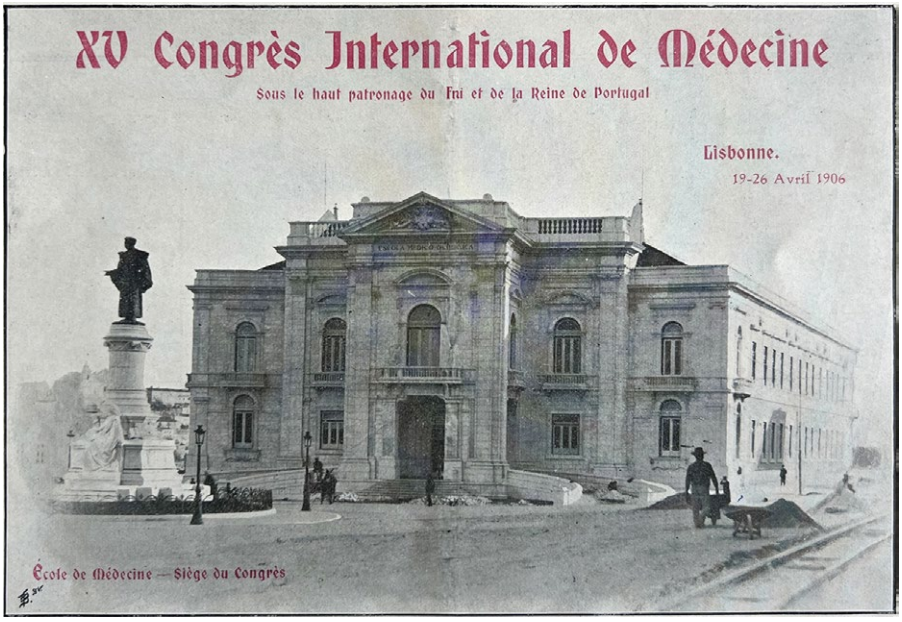

Fig 1. Official announcement of the XV International Congress of Medicine, Lisboa, depicting the new building where the congress would be headquartered

To ensure the success of the congress, it is worth highlighting the excellent coordination and the almost general mobilization that guaranteed everything proceeded smoothly. Miguel Bombarda worked tirelessly, contacting and inviting many Portuguese physicians to participate in various capacities, including chairing sessions in different medical specialties, while simultaneously having to overcome certain personal rivalries and conflicts.

This congress was of decisive importance for the progress of Portuguese medicine, which remained largely isolated from the main currents of European medicine and, therefore, out of step with the rapid advances emerging in Europe and the United States. [3] A collective effort was successfully mobilized to properly welcome such an important and numerous group of professionals, totalling about 2,000 people, in addition to their companions. [4]

The inaugural session took place in the Portugal Hall, the main hall of the Lisbon Geographical Society (*Sociedade de Geografia de Lisboa*), which was filled well beyond its capacity. It was presided over by King Carlos I, accompanied by Queen Amélia of Orléans and the Queen Mother, Maria Pia of Savoy. (Fig. 2) The congress included 17 sections and approximately 500 meetings and sessions. [2]

The facilities included several amphitheatres and multiple smaller rooms where hundreds of meetings took place. Special mention should be made of the Projection Room (Fig. 3), an innovation in which surgical procedures performed by Dr Eugène Doyen were shown, creating a considerable impact and leading to repeated screenings at the request of attendees. The Cancer Section debated the prevailing theory that attributed these diseases to infectious causes. It was likely from this point onward that the Portuguese physician Francisco Gentil began the path that would eventually lead him to head the fight against cancer in Portugal. Communicable diseases were also widely debated in both the Infectious Diseases and Public Health sections. Professor Ricardo Jorge, a renowned Portuguese authority in public health, chaired the latter, and the principal topics included the scourge of tuberculosis, together with diphtheria, smallpox, leprosy, and other diseases affecting the world at the time.

In the Colonial and Tropical Medicine section, advances in combating the tsetse fly attracted considerable attention, an area in which Portugal played an important role, particularly in São Tomé and Príncipe under the leadership of Dr. Ayres Kopke.

In addition to lectures and scientific sessions, the programme featured study visits to hospital units, the Barbadinhos water pumping station, the Lisbon Cen-

Fig 2. Opening session of the XV International Congress of Medicine, presided over by the Portuguese Royal Family, April 19th 1906. (Source: *Ilustração Portugueza*)

Fig 3. The Projections Room
(Source: *Ilustração Portugueza*, April 20th, 1906 p315)

Fig 4. Social program for the visit to Vila Franca de Xira, April 22nd, 1906
(Source: Library - Faculdade de Medicina - Universidade de Lisboa)

tral Prison, the Royal Bacteriological Institute, and the naval quarantine station, among other institutions, according to participants' interests. Of particular note was the lively daily social programme.

Besides the inaugural session on 19 April, on 20 April a garden party was hosted in Sintra by the Count of Monserrate, a wealthy English gentleman settled in Lisbon. On 21 April, King Carlos hosted a dinner for the delegates of the various countries. Particularly memorable was the of 22 April, which featured a boat trip from Lisbon to Vila Franca de Xira, where an equestrian display took place, followed by a traditional bullfight. The return journey was made by train to Rossio Station in the centre of Lisbon (Fig. 4). On 23 April, a garden party hosted by the King took place at the Necessidades Royal Palace, for which, according to contemporary press reports, more than five thousand invitations were issued. On 24 April, a reception was again held at the *Sociedade de Geografia de Lisboa*, and finally, on 25 April, a reception took place at Lisbon City Hall, hosted by the Mayor of Lisbon.

It is worth noting that Lisbon at that time lacked adequate hotel infrastructure to accommodate so many visitors. In addition to the few suitably equipped hotels, it was necessary to distribute congress participants among private residences and the homes of several Lisbon physicians. However, a providential solution came in the form of a commercial vessel which, besides bringing delegates from the United Kingdom, served as a floating hotel during the congress (Fig. 5).

The congress was remembered by most participants not only for its scientific quality but also for the excellence of its organization, hospitality, and social programme. This congress constituted a milestone for Portuguese medicine, enabling contacts with leading figures of international medicine and giving rise to exchanges and training opportunities abroad.

The number and distinction of participants were impressive. Among them were Paul Richer, Édouard Brissaud, Ramón y Cajal, Karl Landsteiner, Élie Metchnikoff, Albert Neisser, Wilhelm Waldeyer, Pierre Robin, Adolphe Pinard, Paul Ehrlich, and Oswaldo Cruz, among many others.

In Portugal, the following years witnessed a development to which this congress was not unrelated. In 1911, with the creation of the Universities of Lisbon and Porto and their respective Faculties of Medicine, this new direction was confirmed. In Lisbon, in particular, what became known as the "medical generation of 1911" emerged. The importance attributed to Miguel Bombarda's contribution to the organization and success of the congress is illustrated by the commemorative medal issued in his recognition (Fig. 6).

The International Congresses continued to be held at approximately three-year intervals: Budapest in 1909 and London in 1913. However, the outbreak of the Great War permanently interrupted these events. It was the end of an era.

As 120 years have passed since this remarkable event, it is only fitting to remember it and reflect upon its impact on Portuguese medicine.

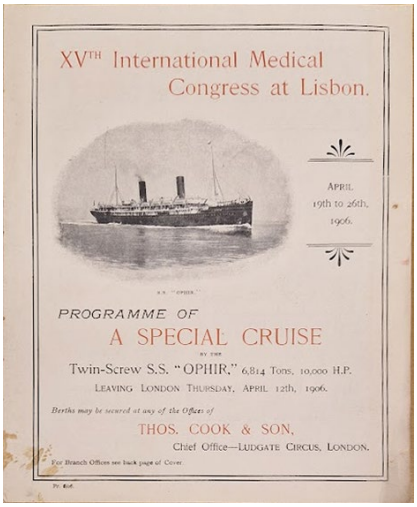

Fig 5. The ship *Ophir,* which brought delegates from the United Kingdom and served as a floating hotel during the Congress (Source: Library - Faculdade de Medicina - Universidade de Lisboa)

Fig 6. Commemorative medal in honor of Professor Miguel Bombarda for his contribution to the organization and success of the congress. Inscription: *"To Professor Miguel Bombarda, from the Portuguese Doctors and Congress Participants"*. (property of the author)

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Diplexil R

Valproato Semisódico

CONFIANÇA NUMA VIDA COM QUALIDADE[1,2]

INFORMAÇÕES ESSENCIAIS COMPATÍVEIS COM O RESUMO DAS CARACTERÍSTICAS DO MEDICAMENTO

▼Este medicamento está sujeito a monitorização adicional. Isto irá permitir a rápida identificação de nova informação de segurança. Pede-se aos profissionais de saúde que notifiquem quaisquer suspeitas de reações adversas. Para saber como notificar reações adversas, ver secção 4.8 do RCM.

NOME, COMPOSIÇÃO QUALITATIVA E QUANTITATIVA e FORMA FARMACÊUTICA DO MEDICAMENTO: Diplexil-R 250 mg (269,1 mg de valproato semisódico) comprimidos gastrorresistentes cor de pêssego. Excipiente(s) com efeito conhecido: Amarelo sunset (E110) – 0,2 mg, Sódio: 18,5 mg por comprimido. Diplexil-R 500 mg (538,2 mg de valproato semisódico) comprimidos gastrorresistentes cor-de-rosa. Excipiente(s) com efeito conhecido: Carmoisina (E122) - 0,104 mg, Vermelho de ponceau 4R (E124) - 0,091 mg, Sódio: 37,0 mg por comprimido. **INDICAÇÕES TERAPÊUTICAS:** Epilepsias generalizadas e parciais: Generalizadas primárias: Pequeno e grande Mal, epilepsias mioclónicas; Parciais: simples e complexas. Generalizadas secundárias: síndroma de Lennox-Gastraut, síndroma de West; Formas mistas. Epilepsias especiais: Convulsões febris na criança. Privação do sono. Alterações do comportamento associados à epilepsia. Tratamento de episódios maníacos na doença bipolar quando o lítio está contraindicado/ não é tolerado. Considerar a continuação do tratamento após a ocorrência de episódios maníacos em doentes que responderam ao valproato semisódico na mania aguda. Em: Doentes com ciclos rápidos; bipolares difíceis (idosos e doentes em situações de comorbilidade com abuso de substâncias). - Tratamento profilático dos seguintes tipos de cefaleias: Enxaqueca, Cefaleia Crónica Diária (Enxaqueca Transformada e enxaqueca Persistente e Resistente) e Cefaleia em salvas. **POSOLOGIA E MODO DE ADMINISTRAÇÃO:** Via oral. Se tratamento prévio com Ácido valpróico, iniciar a terapêutica com a mesma dose diária e esquema posológico. Após estabilização do doente poderá instituir-se um esquema posológico de 2 a 3 tomas diárias. A frequência de efeitos adversos (particularmente enzimas hepáticas elevadas) pode estar relacionada com a dose. Avaliar benefício-risco de doses mais elevadas. Concentrações de Fenitoína no sangue podem ser afetadas com aumento de dosagem. Para os doentes que se queixam de irritação gastrointestinal, recomenda-se a administração do fármaco durante as refeições, e aumento gradual da dose a partir de um nível inicial baixo. Epilepsia: dose inicial recomendada:15 mg/kg/dia 3 x dia, com aumentos de 5 a 10 mg/kg/dia em intervalos de uma semana até controlo das crises ou até dose tolerável. A dose máxima recomendada é de 60 mg/kg/dia. Se exceder os 2500 mg, esta deverá ser administrada em doses repartidas. Para a maior parte dos doentes, as concentrações séricas terapêuticas de Valproato situam-se no intervalo de 50-100 mcg/ml. Caso a posologia diária seja igual ou superior a 50 mg/Kg/dia, recomenda-se monitorizar níveis sanguíneos. Episódios maníacos na doença bipolar: Em adultos: A dose diária deve ser estabelecida e controlada pelo médico. Dose diária inicial recomendada é de 750 mg. Em ensaios clínicos, a dose inicial de 20 mg de valproato/kg de peso corporal também demonstrou um perfil de segurança aceitável. A dose deve ser aumentada tão rapidamente quanto possível, de forma a atingir a dose terapêutica mais baixa que produza o efeito clínico desejado. Ajustar a dose à resposta clínica. A dose média diária varia, habitualmente, entre 1000 a 2000 mg de valproato. Se doses superiores a 45 mg/kg de peso corporal, monitorizar. A continuação do tratamento deve ser adaptada individualmente usando a dose mínima eficaz. Em crianças e adolescentes: A segurança e a eficácia de Diplexil-R para o tratamento de episódios maníacos na doença bipolar não foram avaliadas em doentes com idade inferior a 18 anos. Crianças do sexo feminino e mulheres em idade fértil: O valproato deve ser iniciado e supervisionado por um especialista com experiência no tratamento da epilepsia, perturbação bipolar ou enxaqueca. O valproato não deve ser utilizado em crianças do sexo feminino e mulheres em idade fértil a não ser que outros tratamentos sejam ineficazes ou não tolerados. O valproato é prescrito e dispensado de acordo com o Programa de Prevenção do valproato na Gravidez. O valproato deve ser prescrito preferencialmente em monoterapia e na dose eficaz mais baixa, se possível numa formulação de libertação prolongada. A dose diária deve ser dividida pelo menos em duas tomas únicas. Homens: Recomenda-se que Diplexil seja iniciado e supervisionado por um especialista com experiência no tratamento da epilepsia ou perturbação bipolar ou enxaqueca. Cefaleias: A dose mínima eficaz é de 250 mg 2x dia e o tratamento deverá ter a duração mínima de 3 meses. A dose média é de 1000 a 1500 mg/dia. Em doentes com insuficiência renal: pode ser necessário diminuir a dosagem, ou aumentar a dosagem em doentes em hemodiálise. Valproato é dialisável. A dosagem deve ser modificada de acordo com a monitorização clínica do doente. Diplexil-R só deve ser iniciado e supervisionado por um especialista com experiência no tratamento da enxaqueca. O tratamento só deve ser iniciado se outros não forem eficazes ou tolerados e os benefícios e riscos devem ser cuidadosamente reavaliados em revisões regulares do tratamento. **CONTRAINDICAÇÕES:** Diplexil-R está contraindicado nas seguintes situações: - Hipersensibilidade às SA ou excipientes. - Doença hepática ou disfunção significativa. - Antecedentes pessoais ou familiares de hepatite grave, nomeadamente medicamentosa. - Porfiria hepática. - Doentes que tenham doenças mitocondriais causadas por mutações no gene nuclear que codifica a enzima mitocondrial polimerase gama (POLG), por exemplo a síndrome de Alpers-Huttenlocher, e em crianças com menos de 2 anos de idade em que se suspeita de terem doenças relacionadas com a POLG. - Doentes com distúrbios do ciclo da ureia. Tratamento da epilepsia: - Na gravidez, a não ser que não exista um tratamento alternativo adequado. - Em mulheres em idade fértil, a não ser que as condições do programa de prevenção da gravidez sejam cumpridas. Tratamento da perturbação bipolar e profilaxia de crises de enxaqueca: - Na gravidez. - Em mulheres em idade fértil, a não ser que as condições do programa de prevenção da gravidez sejam cumpridas. **ADVERTÊNCIAS E PRECAUÇÕES ESPECIAIS DE UTILIZAÇÃO:** trombocitopénia, alterações da hemostase/coagulação, hiperamonemia com ou sem letargia, alterações nos testes de função da tiroide, insuficiência hepática, muito raramente pancreatites graves, reações adversas cutâneas graves e angioedema. **Programa de Prevenção de Gravidez:** O valproato tem um elevado potencial teratogénico e as crianças expostas ao valproato in utero têm um elevado risco de malformações congénitas e perturbações do desenvolvimento do sistema nervoso. Ver RCM. Foram relatados casos de ideação e comportamentos suicidas em doentes tratados, com medicamentos antiepiléticos, para várias indicações terapêuticas. Não é recomendado o uso concomitante de ácido valpróico/ valproato de sódio com os antibióticos do grupo dos cabapenemos. Doentes com doença mitocondrial conhecida ou presumida: O valproato pode desencadear ou agravar sinais clínicos de doenças mitocondriais subjacentes causadas por mutações do ADN mitocondrial bem como do gene nuclear que codifica a POLG. Se há suspeita de uma deficiência no ciclo enzimático da ureia, devem ser feitos estudos metabólicos antes do tratamento, devido ao risco de hiperamoniemia com o valproato. Poderá ocorrer aumento de peso no início do tratamento. Doentes com uma deficiência tipo II em carnitina palmitoiltransferase subjacente (CPT) devem ser advertidos do risco aumentado de rabdomiólise. Nos insuficientes renais pode ser necessário proceder-se a uma diminuição da dosagem. Excipientes. **INTERAÇÕES MEDICAMENTOSAS E OUTRAS FORMAS DE INTERAÇÃO:** Efeitos do valproato semisódico nos outros medicamentos: Neurolépticos, antidepressivos, benzodiazepinas e barbitúricos; Fenobarbital e Primidona; Fenitoína; Carbamazepina; Etossuximida; Lamotrigina; Zidovudina; Felbamato; Olanzapina; Rufinamida; Propofol; Nimodipina. Efeito de outros medicamentos sobre o valproato de sódio: Antiepiléticos; Mefloquina; Fármacos com ligação elevada às proteínas plasmáticas; Anticoagulantes; Cimetidina ou eritromicina; Fluoxetina; Carbapenemos; Rifampicina; Inibidores de protéase; Colestiramina; Medicamentos que contêm estrogénio; Metamizol; Outras interações: Topiramato ou acetazolamida; Quetiapina; Álcool; Lítio; Clonazepam; Clozapina. Ver RCM. **EFEITOS INDESEJÁVEIS:** Os efeitos indesejáveis notificados mais comuns para o valproato são perturbações gastrointestinais, as quais ocorrem em aproximadamente 20% dos doentes. Têm sido observados casos de lesões hepáticas graves (ou mesmo fatais), especialmente em crianças tratadas com doses elevadas ou em combinação com outros antiepilépticos. Os efeitos indesejáveis foram classificados por ordem de frequência segundo a seguinte convenção: Neoplasias benignas, malignas e não especificadas (incl. quistos e polipos): Raros: Síndrome mielodisplásico. Doenças do sangue e do sistema linfático: Frequentes: Trombocitopenia, leucopenia. Pouco frequentes: Hemorragia. Raros: Anemia macrocítica, macrocitose. Muito raros: Perturbações da medula óssea, concentração reduzida de fibrinogénio e/ou de fator de coagulação VIII, alteração da agregação plaquetária, tempo de coagulação prolongado, linfocitopénia, neutropénia, pancitopénia, anemia ou aplasia da linhagem de células vermelhas. Desconhecido: Agranulocitose. Doenças do sistema imunitário: Pouco frequentes: Angioedema. Raros: Lúpus eritematoso, erupção medicamentosa com eosinofilia e sintomas sistémicos (síndrome DRESS). Desconhecido: Reações alérgicas (ver também "pele e perturbações dos tecidos subcutâneos"), síndrome da resposta inflamatória sistémica. Doenças endócrinas: Raros: Hiperandrogenismo (hirsutismo, virilismo, acne, alopécia com aparência típica masculina e/ ou aumento dos níveis de androgéneos), hipotiroidismo. Doenças do metabolismo e da nutrição: Frequentes: Hiperamonemia, aumento do peso (fator de risco para a síndrome do ovário poliquístico, requer monitorização cuidadosa) ou diminuição de peso; aumento ou diminuição de apetite. Pouco frequentes: Síndrome de secreção inapropriada de hormona antidiurética (SIADH). Raros: Hiperinsulinemia, baixos níveis da IGFBP-1 (insulin-like growth factor binding protein 1), obesidade. Muito raros: Foram relatadas anomalias das provas da função tiroideia, com relevância clínica duvidosa. Hiponatremia. Vasculopatias: Raros: Vasculites. Perturbações do foro psiquiátrico: Frequentes: Agressividade*, agitação*, atenção alterada*. Raros: Irritabilidade, alucinações, confusão, comportamento anormal*, hiperatividade psicomotora*, perturbação da aprendizagem*. * Estas reações adversas são observadas principalmente na população pediátrica. Doenças do sistema nervoso: Frequentes: Sonolência, tremores, parestesias, defeito de memória, nistagmo, torturas. Pouco frequentes: Coma transitório, em alguns casos associado a aumento da frequência das crises, ataxia. Raros: Cefaleias, hiperatividade, espasticidade, estupor, perturbação cognitiva, diplopia. Muito raros: Encefalopatia, demência associada a atrofia cerebral (reversível após a descontinuação do tratamento), distúrbios extrapiramidais p. ex. síndrome parkinsónica (reversível). Desconhecido: Agravamento das crises, sedação, letargia. Afeções do ouvido e do labirinto: Muito raros: Perda de audição (reversível ou irreversível), acufenos. Doenças respiratórias, torácicas e do mediastino: Pouco frequentes: Derrame pleural (eosinofílico). Doenças gastrointestinais: Muito frequentes: Dores, náuseas, vómitos. Frequentes: Diarreia, perturbação gengival (principalmente hiperplasia gengival), estomatite. Raros: Pancreatite (por vezes fatal), hipersalivação, ileus, obstrução intestinal. Afeções hepatobiliares: Frequentes: Alterações nas provas da função hepática. Raros: Lesão hepática grave que inclui insuficiência hepática. Afeções dos tecidos cutâneos e subcutâneos: Frequentes: alopecia, enfraquecimento do cabelo e aparecimento de cabelo encaracolado, alterações nas unhas e leito ungueal. Raros: Exantema, eritema multiforme. Muito raros: Síndrome de Stevens-Johnson, Síndrome de Lyell. Desconhecido: Hirsutismo (por ex. resultante da síndrome do ovário poliquístico), hiperpigmentação. Afeções musculosqueléticas e dos tecidos conjuntivos: Raros: Rabdomiólise. Desconhecido: Foram notificados casos de densidade mineral óssea diminuída, osteopenia, osteoporose e fraturas ósseas em doentes sob tratamento prolongado com valproato. Ainda não se conhece o mecanismo pelo qual o valproato afeta o metabolismo ósseo. Doenças renais e urinárias: Frequentes: incontinência urinária. Muito raros: Síndrome de Fanconi (com acidose metabólica, fosfatúria, aminoacidúria, glicosúria, reversíveis após a descontinuação do tratamento), enurese nas crianças. Desconhecido: Insuficiência renal, nefrite intersticial, deterioração da função renal. Doenças dos órgãos genitais e da mama: Frequentes: Amenorreia, dismenorreia. Raros: Síndrome de ovário poliquístico, infertilidade masculina. Desconhecido: Espermatogénese anormal (com contagem reduzida de espermatozóides e/ou motilidade). Afeções congénitas familiares e genéticas: Malformações congénitas e alterações no desenvolvimento. Perturbações gerais e alterações no local de administração: Raros: Hipotermia, edema. Exames complementares de diagnóstico: Raros: Redução dos fatores de coagulação (pelo menos um), testes de coagulação anormais (tais como prolongamento do tempo de protrombina, prolongamento do tempo parcial de tromboplastina ativada, prolongamento do tempo de trombina, valor de INR aumentado), carência de biotina/biotinidases. População pediátrica: Existe risco particular de lesão hepática grave em bebés e crianças pequenas, especialmente com idade inferior a 3 anos, e de pancreatite em crianças pequenas. Estes riscos diminuem com o aumento da idade. Transtornos psiquiátricos como agressão, agitação, perturbação da atenção, comportamento anormal, hiperatividade psicomotora e transtorno de aprendizagem são observados principalmente na população pediátrica. **Para mais informações deverá contactar o Titular da AIM:** TECNIFAR - Indústria Técnica Farmacêutica, S.A. Rua José Da Costa Pedreira, Nº 11 – B - Torre Sul - 1750-130 Lisboa. **Medicamento sujeito a receita médica.** Regime de comparticipação: Escalão A. **RCM aprovado a 05-02-2025 (versão 24.0)**

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Targeted Therapeutic Approaches in Hypertrophic Cardiomyopathy

This article was prepared as the final work for the Master's Degree in Medicine at the Faculty of Medicine, University of Lisbon.

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ABSTRACT: Hypertrophic Cardiomyopathy (HCM) is a common autosomal dominant inherited myocardial disease characterised by left ventricular hypertrophy, hypercontractility and diastolic dysfunction. Most cases are caused by mutations in sarcomeric protein genes, such as *MYH7* or *MYBPC3*. Clinical manifestations are heterogeneous, varying from none or mild exercise intolerance to severe lifestyle-limiting symptoms, heart failure, ventricular arrhythmias or sudden cardiac death. Diagnosis relies on multimodal imaging techniques and genetic testing. Current management includes symptom control with β-blockers, calcium channel blockers, and antiarrhythmics, as well as invasive strategies like septal myectomy in obstructive forms. Recently, myosin inhibitors (e.g., Mavacamten, Aficamten) have demonstrated significant improvements in functional status and left ventricular outflow tract (LVOT) obstruction gradients in obstructive HCM, although their benefits in non-obstructive HCM remain limited.

Target therapies are emerging as a potentially disease-modifying approach that targets the underlying genetic defects, even before the onset of phenotype. *In vitro* and pre-clinical studies using animal models and human induced pluripotent stem cell–derived cardiomyocytes (iPSC-CMs) have demonstrated that techniques such as gene replacement, gene editing, allelic silencing, and other RNA based therapies can restore normal cardiomyocyte function.

These approaches are designed to target cardiomyocytes, employing viral vectors or nanoparticle-based delivery systems to reduce off-target effects. Despite significant progress, many challenges remained unsolved, including efficient delivery, immune system responses, long-term safety, and determining the optimal timing for intervention. Even so, gene and RNA based approaches may represent a transformative shift in HCM treatment, moving from symptom management to directly targeting the underlying cause of the disease.

KEYWORDS: Hypertrophic cardiomyopathy, Sarcomeric mutations, Gene therapy, RNA-based therapies, Cardiac myosin inhibitors.

1. INTRODUCTION

Hypertrophic cardiomyopathy (HCM) is the most common inherited cardiac disease, with an estimated prevalence ranging from 1:200 to 1:500 individuals in the general population. It is defined by the presence of left ventricular hypertrophy in the absence of abnormal loading conditions sufficient to explain it, such as long-standing hypertension or aortic stenosis[1]. HCM is usually inherited as an autosomal dominant disorder and is characterised by marked phenotypic heterogeneity, ranging from asymptomatic individuals to patients with heart failure, atrial or ventricular arrhythmias, and sudden cardiac death [2].

In most cases, HCM is caused by pathogenic variants in genes encoding sarcomeric proteins that integrate the contractile unit of heart muscle, the sarcomere (Fig. 1). Rather than causing primary structural instability, these variants predominantly alter sarcomere function. Although the underlying mechanisms are not yet fully elucidated, two major pathophysiological hallmarks have consistently emerged: hypercontractility with impaired relaxation, and increased myofilament calcium sensitivity, both of which contribute to excessive contractile activity and diastolic dysfunction [3].

At the molecular level, three-dimensional structural studies have shown that the motor domain of β-myosin heavy chain (β-MHC) can adopt distinct conformational states that regulate the availability of myosin heads for actin binding. In the super-relaxed state (SRX), the two myosin heads fold back against the thick filament backbone, forming the interacting-heads motif (IHM), an energy-conserving "OFF state". In the disordered-relaxed state (DRX), one head remains associated with the thick filament backbone, whereas the other becomes more accessible for actin interaction. In the active "ON state", both heads are available to bind actin and generate force [4]. Cardiac myosin-binding protein C (cMyBP-C) contributes to stabilisation of the IHM, thereby limiting myosin head mobility and reducing the number of heads available for contraction [5].

Cardiac contraction depends on ATP binding and hydrolysis, followed by phosphate release, which induces conformational changes in the myosin head and powers actin filament sliding. Phosphorylation of the regulatory light chain weakens head–tail interactions, destabilises the IHM, and shifts myosin heads towards the "ON state" [6]. In HCM, pathogenic variants in thick-filament genes, particularly *MYH7* and *MYBPC3*, disrupt this regulatory equilibrium and increase the proportion of myosin heads in the "ON state", thereby enhancing actin–myosin interaction [5,7,8]. By contrast, mutations affecting thin-filament proteins, such as troponin T and troponin I, generally increase myofilament calcium sensitivity and prolong thin- filament activation. Together, these abnormalities promote hypercontractility and impair myocardial relaxation, contributing to diastolic dysfunction [6,9].

Both preclinical and clinical studies indicate that the proportion of myosin heads in the energy-conserving "OFF state" is markedly reduced in HCM, falling to approximately 15–20%, compared with 40–50% in healthy

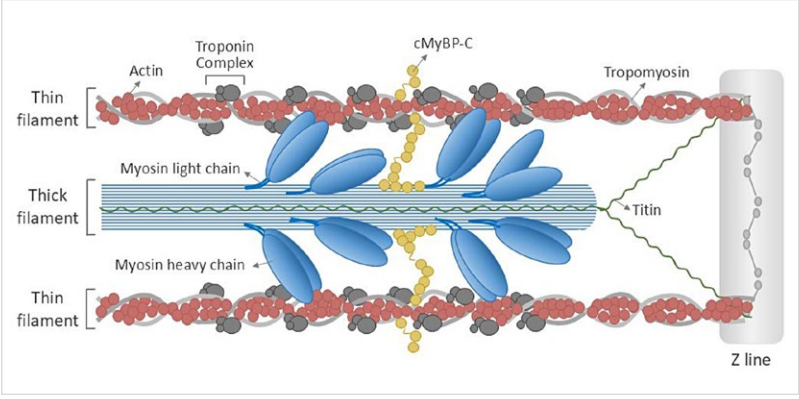

FIGURE 1. The cardiac sarcomere and its components.
Adapted from Lehman, 2022 [4]

myocardium (Fig. 2). This shift leads to increased ATP consumption, excessive cross-bridge formation during both systole and diastole, and energetically inefficient contraction [10].

The resulting hypercontractility, together with altered calcium handling and metabolic stress, promotes mitochondrial dysfunction and activates stress-responsive and pro- hypertrophic signalling pathways, including calcineurin–NFAT, MAPK, PI3K–mTOR, and TGF-β. These cascades drive pathological remodelling and contribute to the classical histopathological hallmarks of HCM, namely cardiomyocyte hypertrophy, myofibrillar disarray, interstitial fibrosis, increased myocardial stiffness, and small-vessel disease [11–13].

These molecular and cellular abnormalities arise from pathogenic variants in genes encoding sarcomeric proteins. Among these, the two most frequently implicated genes are *MYH7*, which encodes β-myosin heavy chain, and *MYBPC3*, which encodes cMyBP-C [14].

Together, pathogenic variants in these genes account for approximately 55–70% of genetically confirmed cases [15,16]. Other well-established sarcomeric genes include *TNNT2*, *TNNI3*, *TPM1*, *ACTC1*, *MYL2*, and *MYL3*, although each account for a smaller proportion of cases.

As an autosomal dominant disease, HCM is characterised by incomplete penetrance and variable expressivity, such that individuals carrying the same pathogenic variant may exhibit markedly different phenotypes[17]. Importantly, genetic abnormalities are thought to initiate myocardial dysfunction long before overt structural changes become evident. This concept is particularly relevant to the development of targeted therapies, since it supports the possibility of intervening early in the disease course, before irreversible remodelling has occurred [17–19].

Backwell and Marsh classified pathogenic variants in autosomal dominant diseases according to three principal molecular mechanisms: haploinsufficiency, gain-of-function, and dominant-negative effects [20]. Haploinsufficiency usually results from nonsense, frameshift or splicing variants that introduce premature stop codons and activate nonsense-mediated decay, thereby reducing the amount of functional protein. Gain-of-function mutations, often missense, produce a protein with increased or novel activity. On the other hand, dominant-negative mutations generate a mutant protein that interferes, directly or indirectly, with the normal function of the wild-type protein [20].

These concepts are highly relevant in HCM. Most *MYBPC3* variants are nonsense, frameshift, or splice-altering mutations that lead to haploinsufficiency, reduced levels of functional cMyBP-C, and impaired sarcomeric regulation; clinically, they are often associated with later disease onset [16,17,21]. By contrast, many pathogenic *MYH7* variants are missense mutations that exert dominant-negative effects, directly altering myosin head function and frequently producing a more severe and earlier phenotype [22,23]. The principal sarcomeric genes involved in HCM, together with their predominant mutation types and pathogenic mechanisms, are summarised in Table I [3,16].

Hypertrophic cardiomyopathy, however, is not exclusive to sarcomeric HCM. It may also occur in a range of genetic phenocopies, including Fabry disease caused by mutations in *GLA*, PRKAG2 syndrome associated with *PRKAG2* mutations, Danon disease caused by *LAMP2* mutations, and transthyretin cardiac amyloido-

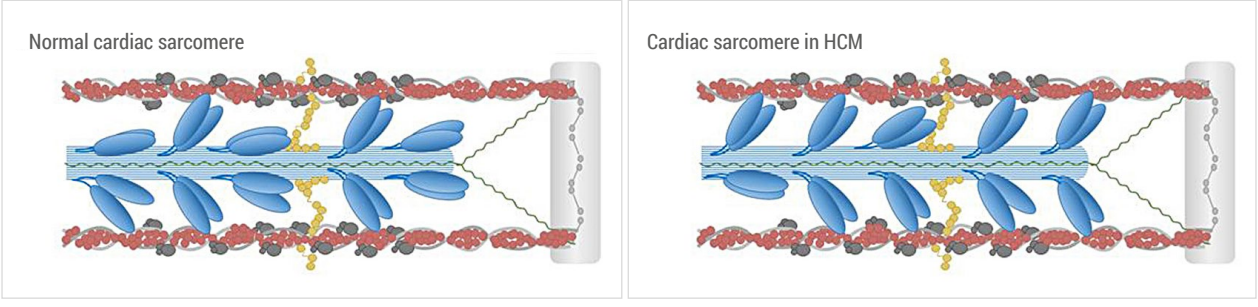

FIGURE 2. Comparison of sarcomeric structure under normal conditions and HCM. Adapted from Lehman, 2022 [4]

sis associated with *TTR* mutations [19,24–26]. These conditions may resemble HCM morphologically, but differ significantly in pathogenesis, extracardiac manifestations, prognosis, and treatment. Their main distinguishing features are summarised in Table II.

From a clinical perspective, HCM is commonly classified according to the presence or absence of left ventricular outflow tract (LVOT) obstruction, which results from the interaction between septal hypertrophy and systolic anterior motion (SAM) of the mitral valve [2]. In obstructive HCM, LVOT obstruction is dynamic and may be present at rest or provoked by physiological manoeuvres such as Valsalva or exercise. In some patients, the Valsalva manoeuvre may accentuate a systolic crescendo–decrescendo murmur. Non-obstructive HCM is diagnosed when characteristic hypertrophy is present

TABLE I. Major sarcomeric genes implicated in HCM and their pathogenic mechanisms

Gene	Encoded protein	Variant type	Main pathogenic mechanism	Typical phenotypic features
MYH7	β-myosin heavy chain	Missense	Dominant-negative effect; increased actin–myosin cross-bridge cycling and hypercontractility	Early onset, marked hypertrophy, variable arrhythmic risk
MYBPC3	Cardiac myosin-binding protein C	Frameshift, Nonsense, splicing	Haploinsufficiency leading to reduced cMyBP-C levels and impaired sarcomeric regulation	Later onset, milder hypertrophy, progressive diastolic dysfunction
TNNT2	Cardiac troponin T	Missense	Increased myofilament Ca^{2+} sensitivity with minimal hypertrophy	Mild hypertrophy, disproportionate arrhythmic risk
TNNI3	Cardiac troponin I	Missense	Impaired inhibitory regulation of actin–myosin interaction, delayed relaxation	Diastolic dysfunction, variable hypertrophy
TPM1	α-tropomyosin	Missense	Altered thin-filament regulation and Ca^{2+} sensitivity	Variable hypertrophy, familial clustering
ACTC1	Cardiac actin	Missense	Disrupted actin–myosin interaction and force transmission	Early-onset hypertrophy, variable severity
MYL2 / MYL3	Myosin regulatory and essential light chains	Missense	Altered modulation of myosin head activity	Hypertrophy with variable systolic and diastolic involvement

Adapted from Argirò, 2025 and Nogueira-Garcia, 2025.[3,16]

TABLE II. Major genetic phenocopies in the differential diagnosis of HCM

Condition	Key features	Distinguishing elements
Sarcomeric HCM	Asymmetric septal hypertrophy; SAM; dynamic LVOT obstruction	Pathogenic variants in sarcomeric genes; patchy mid-wall LGE
Fabry disease (GLA	Concentric LVH; inferolateral fibrosis	Low native T1; angiokeratomas; α- galactosidase A deficiency
Cardiac Amyloidosis (TTR)	Increased wall thickness; HFpEF	Apical sparing strain; global subendocardial LGE; extracardiac involvement
PRKAG2 syndrome	LVH with pre-excitation	WPW pattern; glycogen storage; PRKAG2 mutation
Danon disease (LAMP2)	Severe early LVH; systolic dysfunction	Skeletal myopathy; intellectual disability; X-linked inheritance

Abbreviations: HCM, hypertrophic cardiomyopathy; **LVH,** left ventricular hypertrophy; **SAM,** systolic anterior motion; **LVOT,** left ventricular outflow tract; **LGE,** late gadolinium enhancement; **HFpEF,** heart failure with preserved ejection fraction. *Adapted from Azevedo 2021; Bennett, 2023; Felix, 2025; Lopes, 2024; Teresi, 2025.[19,24–27]*

but no clinically significant resting or provocable LVOT gradient can be demonstrated[1,3].

Transthoracic echocardiography remains the primary imaging modality for the diagnosis of HCM. Current American Heart Association/American College of Cardiology (AHA/ACC) and European Society of Cardiology (ESC) guidelines define HCM as unexplained left ventricular wall thickness ≥15 mm in one or more myocardial segments, with or without right ventricular hypertrophy, that cannot be accounted for by abnormal loading conditions such as hypertension or aortic stenosis [2,26,28–30].

Cardiac magnetic resonance (CMR) imaging is recommended as part of the baseline assessment because of its superior spatial resolution and reproducibility, which enable accurate identification of apical or anterolateral hypertrophy, apical aneurysm, and myocardial fibrosis. CMR also plays an important role in distinguishing sarcomeric HCM from phenocopy conditions [27,28,31].

Genetic testing has become a central component of HCM evaluation, particularly in borderline cases and for family screening. Current guidelines recommend comprehensive or targeted next-generation sequencing panels in individuals with a definite or probable clinical diagnosis of HCM [28]. Identification of a pathogenic variant supports screening of first-degree relatives. Family members who test negative for the familial variant may be discharged from serial cardiac surveillance, whereas genotype-positive individuals require longitudinal follow-up to monitor for phenotypic conversion [28,32]. Despite the identification of more than 1,500 pathogenic variants in different genes, an estimated 40–60% of clinically diagnosed patients still lack a genetic diagnosis [32,33].

A multimodal diagnostic approach also facilitates recognition of disease stage. A commonly used clinical staging model divides HCM into four broad phases [10,19,27]: Stage I, genotype-positive/phenotype-negative disease, also referred to as the pre-phenotypic or non-hypertrophic phase; Stage II, genotype-positive/phenotype-positive disease, corresponding to the classic HCM phenotype and representing the most common clinical presentation; Stage III, adverse remodelling, characterised by myocardial fibrosis, worsening diastolic dysfunction, progressive atrial and ventricular dilatation, and a decline in ejection fraction to approximately

50–65%, with increased risk of heart failure and arrhythmias; and Stage IV, end-stage HCM, which occurs in a minority of patients, approximately 5–10%.

The epidemiology of HCM varies across countries and populations, reflecting disease heterogeneity, differences in screening practices, and under-recognition of subclinical disease. As a result, the true prevalence is likely underestimated. According to the ESC, prevalence ranges from approximately 1 in 500 to 1 in 200 individuals when current imaging and genetic criteria are applied [28]. Large registries such as the Sarcomeric Human Cardiomyopathy Registry (SHaRe) have substantially improved understanding of disease burden and natural history by providing data from specialised centres worldwide [34].

In Portugal, the Portuguese Registry of Hypertrophic Cardiomyopathy (PRo-HCM) remains the main national source of epidemiological data. In 2018, this registry included 1,042 patients diagnosed across several centres. The mean age at diagnosis was 53 years, approximately one- third of patients had a family history of disease, and LVOT obstruction was present in about 35% of cases [35].

Over recent years, HCM treatment has evolved from symptom control alone towards a more mechanistically oriented approach. Current guidelines emphasise treatment individualisation, guided by symptom burden, haemodynamic profile, complications, and 5-year sudden cardiac death risk estimation, while also increasingly incorporating strategies that may modify disease progression [2,28,34].

Conventional pharmacological therapy remains the cornerstone of symptomatic management. β-blockers are considered first-line treatment in symptomatic obstructive HCM because they reduce LVOT gradients and improve exercise tolerance. When β-blockers are ineffective or poorly tolerated, non-dihydropyridine calcium channel blockers such as verapamil may be used. Disopyramide may be added in selected patients with persistent obstruction because of its potent negative inotropic effect. Associated arrhythmias, especially atrial fibrillation, often require dedicated rhythm-control strategies, including amiodarone in selected cases [13,28,36].

For patients who remain severely symptomatic despite optimal medical therapy, septal reduction therapies are an important option. When performed at experienced centres, surgical septal myectomy achieves excellent symptomatic and haemodynamic outcomes, with low procedural mortality and morbidity. Alcohol septal ablation offers a less invasive alternative for selected patients with suitable coronary anatomy, particularly older individuals or those at higher surgical risk, although procedural success is somewhat lower [37].

More recently, cardiac myosin inhibitors have introduced a major shift in the therapeutic landscape of HCM. Mavacamten and aficamten directly target the hypercontractile state by reducing the number of functionally available myosin heads, attenuating excessive cross-bridge formation, and stabilising the myosin "OFF state". By acting at the level of sarcomeric mechanics, these agents reduce LVOT gradients, improve diastolic function, and alleviate symptoms, representing the first pharmacological therapies to directly target a core disease mechanism rather than its downstream consequences [38].

Nevertheless, current therapies still primarily act on the established phenotype. By contrast, gene-based therapies will act directly on the molecular substrate of disease. Although these strategies remain largely in preclinical or early clinical stages, they seek to correct or silence the primary molecular defect rather than simply manage its consequences [1,18,29,39].

2. TARGETED THERAPEUTIC STRATEGIES

Targeted therapeutic strategies for HCM can be broadly divided into three mechanistic levels: protein-level modulation, downstream pathway modulation, and gene- or RNA-targeted therapies. Together, these approaches move treatment beyond conventional symptomatic control towards therapies that directly address the molecular basis of disease.

The most clinically advanced mechanism-targeted therapies currently available for HCM are the cardiac myosin inhibitors mavacamten and aficamten, which act at the sarcomere level by attenuating hypercontractility and restoring a more physiologic balance of myosin head availability. Both agents reduce the number of actin–myosin cross-bridges by stabilising the energy-efficient super-relaxed (SRX) "OFF state" of myosin (Fig. 2) [36,38]. Mavacamten binds to the catalytic domain of the myosin head, inhibits ATPase activity, and promotes formation of the interacting-heads motif (IHM), thereby reducing myosin head availability and ATP consumption [40]. Aficamten acts through a similar principle, reducing contractility by slowing phosphate release, decreasing ATP turnover, and altering myosin head conformation, ultimately resulting in fewer myosin heads entering the active contractile cycle [38]. By counteracting sarcomeric hypercontractility, these agents directly target one of the central pathophysiological mechanisms of HCM.

A second therapeutic layer targets downstream signalling pathways activated by sarcomeric dysfunction, including pro-hypertrophic pathways such as MAPK, TGF-β, and Ca^{2+}/calmodulin-dependent calcineurin signalling, as well as the metabolic stress responses that contribute to the HCM phenotype [41]. Although no approved therapies currently act directly on these pathways in HCM, experimental approaches such as RNA interference (RNAi), antisense oligonucleotides (ASOs), and small-molecule pathway modulators aim to attenuate or prevent maladaptive hypertrophic remodelling [42].

The most upstream strategies are gene- and RNA-targeted therapies, which seek to correct, suppress, or bypass the causal molecular defect itself. These include gene replacement, gene editing, allelic silencing, splicing modulation, RNA editing, and non-coding RNA based therapies. Collectively, these approaches represent a spectrum of molecular interventions ranging from permanent genomic correction to reversible transcript-level modulation, each with distinct mechanistic advantages and translational challenges [23,43,44]. As illustrated in (Fig. 3), these strategies aim to intervene early in the pathogenic cascade by acting at the DNA, RNA, or protein level.

A) GENE REPLACEMENT

Gene replacement therapy is particularly relevant for *MYBPC3* related HCM, in which pathogenic variants commonly result in haploinsufficiency. In this setting, delivery of a functional wild-type copy of the gene can restore cMyBP-C expression and directly address the underlying molecular defect [45,46]. In this approach, full-length wild-type *MYBPC3* complementary DNA (cDNA) is packaged into a viral vector and delivered to cardiomyocytes, where it is transcribed and translated into functional protein that compensates for reduced endogenous expression [42].

Adeno-associated viral vectors (AAVs) are especially attractive for this purpose because of their strong cardiotropism and potential for sustained transgene expression. In recombinant AAV vectors, viral genes are replaced by the therapeutic transgene. Following delivery, the AAV genome generally persists episomally in the nucleus rather than integrating into the host genome, allowing long-term expression while reducing the risk of insertional mutagenesis [47,48].

Preclinical studies have shown that AAV-mediated *Mybpc3* delivery can rescue sarcomeric function and prevent development of the HCM phenotype in mouse models carrying frameshift *Mybpc3* mutations [42]. Mearini and colleagues demonstrated in a knock-in mice

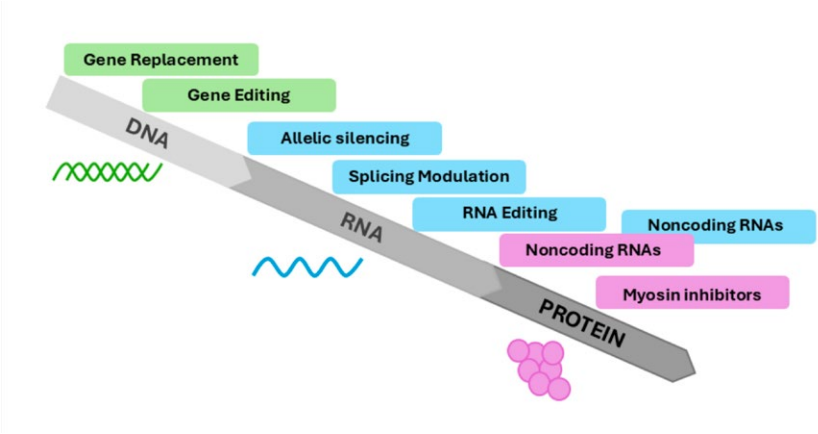

FIGURE 3. Targeted therapies for HCM target the molecular mechanisms underlying the disease at gene level (green boxes), RNA level (blue boxes) or protein level (pink boxes).

that a single systemic injection of AAV9-*Mybpc3*, driven by the cardiac troponin T promoter, increased *Mybpc3* mRNA and wild-type cMyBP-C protein levels, with effects persisting for more than 34 weeks [49]. In human iPSC-derived cardiomyocytes, the same strategy produced a twofold increase in *MYBPC3* mRNA and cMyBP-C protein expression, which was sufficient to suppress the hypertrophic phenotype at the cellular level [50].

This strategy has now entered clinical translation. In the ongoing MyPEAK-1 phase 1b/2 trial, patients with symptomatic HCM carrying pathogenic or likely pathogenic *MYBPC3* variants received a single intravenous infusion of TN-201, an AAV9 vector encoding *MYBPC3* cDNA. Early results indicate that TN-201 is generally well tolerated, with evidence of myocardial transgene expression, increased cMyBP-C protein levels, and favourable biomarker trends. However, patient numbers remain small and follow-up are still limited [51].

B) GENE EDITING, BASE EDITING, AND PRIME EDITING

Gene editing aims to correct the pathogenic variant directly within the genome. The best- studied platform is CRISPR/Cas9, together with more recent high-precision derivatives such as base editing and prime editing [12].

The CRISPR/Cas system was originally identified in bacteria as an adaptive immune mechanism against viruses. In genome editing, CRISPR/Cas9 introduces a double-strand DNA break at a genomic locus specified by a guide RNA (gRNA), enabling highly specific targeting [52]. Following DNA cleavage, endogenous repair pathways are activated, primarily non-homologous end joining (NHEJ) and homology-directed repair (HDR). NHEJ is active throughout most of the cell cycle and generally introduces small insertions or deletions (indels), often resulting in gene disruption. This feature can be exploited therapeutically to silence dominant-negative alleles [53,54]. By contrast, HDR enables precise sequence correction when an exogenous therapeutic donor DNA is provided but is less efficient in post-mitotic cardiomyocytes [44].

A landmark proof-of-concept study by Ma et al. reported correction of a pathogenic MYBPC3 variant in human heterozygous embryos, suggesting that early embryonic cells may preferentially exploit HDR, with reduced mosaicism. However, the mechanisms underlying these observations remain debated, and both the reproducibility of the findings and the associated ethical issues have been questioned [43].

CRISPR/Cas9 has also been applied in vitro to model and correct HCM-associated variants. Pavlova and colleagues created iPSC-CMs carrying the likely pathogenic *MYBPC3* p.N515del variant and successfully corrected this in-frame deletion using CRISPR/Cas9. Comparison of the mutant and corrected isogenic lines showed that the variant was associated with increased cell size, whereas correction restored a normal phenotype without detectable off-target effects or karyotypic abnormalities [55]. In vivo, CRISPR/Cas9-mediated HDR targeting of the murine *Mybpc3* p.W1098X mutation produced low correction efficiency, but still yielded

modest functional benefit, suggesting that even partial editing may be biologically meaningful [56].

To overcome the limitations associated with double-strand DNA breaks and HDR dependence, newer platforms have been developed. Base editing uses a catalytically impaired Cas9 or Cas9 nickase fused to a deaminase enzyme, enabling targeted base transitions without inducing double-strand DNA breaks [57]. Chai et al. showed that adenine base editing could correct the pathogenic *MYH7* p.R403Q variant in iPSC-CMs and in a humanised mouse model, rescuing disease-associated phenotypes with minimal off-target activity [57].

Prime editing further expands the scope of precise editing by enabling base substitutions, as well as small insertions and deletions, without double-strand breaks or donor DNA templates. This system uses a Cas9 nickase fused to a reverse transcriptase and guided by a prime editing guide RNA (pegRNA), which both targets the site and encodes the desired sequence with the genetic modification. Because prime editing does not rely on HDR, it may be particularly advantageous in cardiomyocytes [58,59]. Although not yet directly applied to HCM, successful prime editing-mediated correction of pathogenic variants in dilated cardiomyopathy iPSC-CMs has already been reported, supporting its future potential in inherited cardiomyopathies [58].

C) ALLELIC SILENCING

Allelic silencing is particularly attractive for HCM caused by dominant-negative mutations, because it aims to selectively suppress the mutant allele while preserving the wild-type allele. By reducing production of the pathogenic protein and maintaining wild-type one, this strategy seeks to restore sarcomeric function. Two main molecular approaches have been explored: RNA interference (RNAi) and antisense oligonucleotides (ASOs) [60].

RNAi is a natural mechanism of post-transcriptional gene silencing in which small RNA molecules guide the RNA-induced silencing complex (RISC) to specific transcripts, resulting in mRNA degradation or translational repression. Small interfering RNAs (siRNAs) generally act through near-perfect complementarity, leading to highly specific cleavage of target mRNA, whereas endogenous microRNAs (miRNAs) typically bind with partial complementarity and regulate broader gene networks [60–62].

Using this principle, Migliore et al. designed allele-specific siRNAs targeting two pathogenic *TNNT2* missense mutations and demonstrated selective knockdown of mutant transcripts in reporter-based assays. Although these experiments were performed in HEK293 cells rather than cardiomyocytes, they provided proof of principle that single-nucleotide discrimination is feasible, although highly dependent on precise sequence optimisation [63].

ASOs provide an alternative allele-selective strategy. These short, chemically modified single-stranded oligonucleotides bind complementary RNA sequences and recruit RNase H, which cleaves the RNA strand of the RNA–DNA duplex and thereby degrades the target transcript. Their chemical modifications enhance stability, nuclease resistance, and specificity [64]. Anderson et al. demonstrated the feasibility of SNP-guided ASO-mediated silencing in *MYH7*, using common linked single-nucleotide polymorphisms as allele-specific markers to selectively suppress mutant transcripts in both human iPSC-derived cardiomyocytes and a humanised mouse model [65].

Direct comparison of siRNA- and ASO-based silencing in *MYH7*-R403Q iPSC-CMs showed that siRNAs achieved greater reduction of mutant transcript levels and more pronounced improvement in hypertrophic and contractile phenotypes, whereas ASOs showed higher allele specificity with more modest phenotypic rescue. These findings emphasise the balance between silencing potency and selectivity [64].

Importantly, both siRNA- and ASO-based therapies have already reached clinical application in transthyretin amyloid cardiomyopathy, where they reduce hepatic transthyretin production. Patisiran and vutrisiran act through RNAi-mediated degradation of *TTR* mRNA, whereas eplontersen is an ASO currently under evaluation in the phase 3 CARDIO-TTRansform trial[66]. Although cardiac amyloidosis differs mechanistically from HCM, these studies support the broader clinical feasibility of transcript-directed cardiovascular therapies.

D) SPLICING MODULATION

Splicing modulation targets pre-mRNA processing rather than the DNA sequence itself. Typically achieved using ASOs, this approach redirects the endogenous splicing machinery to promote exon inclusion or exon skipping, restore reading frames, reduce aberrant transcript formation, or prevent nonsense-mediated decay [67–70].

A well-established clinical precedent is nusinersen for spinal muscular atrophy, which promotes exon 7 inclusion in the *SMN2* transcript and restores production of functional SMN protein [67,71]. Applying the same principle to HCM, Gedicke-Hornung et al. used a knock-in *Mybpc3* mouse model carrying a splice-disrupting exon 6 mutation and demonstrated that ASO-mediated exon skipping reduced aberrant transcripts and increased production of a partially functional isoform. These findings provided proof of concept that splicing modulation may represent a causal therapeutic strategy for selected MYBPC3 mutations [72].

E) RNA EDITING

RNA editing has emerged as a promising strategy that targets pathogenic transcripts without altering the underlying DNA sequence. Because it acts at the RNA level, it offers reversible and potentially safer modulation than permanent genome editing. CRISPR-Cas13 systems, together with guide RNAs, enable highly specific recognition and degradation or modification of mutant transcripts, making this approach especially attractive for dominant- negative HCM variants such as those in *MYH7* [73,74].

Yang et al. developed a high-precision Cas13 variant capable of distinguishing transcripts differing by only a single nucleotide. In a murine model carrying the *Myh6* p.R872H variant, this system selectively reduced the mutant transcript while preserving wild-type expression, thereby overcoming a major limitation of earlier Cas13 systems, namely insufficient allele specificity [62].

F) NON-CODING RNA BASED THERAPIES

Non-coding RNAs, particularly miRNAs, regulate multiple gene networks involved in sarcomeric function, calcium handling, hypertrophic signalling, and fibrosis, and therefore contribute to cardiac remodelling in HCM [75].

Among these, miR-133 has emerged as an important regulator of cardiac hypertrophy and fibrosis in cardiomyocytes and cardiac fibroblasts [75]. Experimental studies have shown that miR-133 expression suppresses fibrotic and hypertrophic remodelling by modulating the TGF-β/Smad and PI3K/Akt pathways, as well as histone deacetylases and β-adrenergic signalling [76]. In particular, miR-133 reduces *TGF-β1* expression and downregulates connective tissue growth factor gene (*CTGF*), thereby limiting fibroblast activation and extracellular matrix deposition. However, this regulation is bidirectional, since TGF-β signalling can itself suppress miR-133 expression, creating a complex feedback loop [76].

Although non-coding RNA based therapies do not directly correct the causal mutation, they target convergent downstream mechanisms and may therefore complement gene replacement, gene editing, or allelic silencing approaches. As such, they may become part of combination strategies aimed both at correcting the primary defect and attenuating the maladaptive remodelling that defines clinical HCM.

3. PRECLINICAL MODELS AND DELIVERY PLATFORMS FOR TARGETED THERAPIES

The development of targeted therapies for HCM depends on experimental models that reproduce key genetic and phenotypic features of the disease, as well as on delivery systems capable of safely transporting therapeutic material to cardiomyocytes. In preclinical research, the two main model systems are animal models and induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs). These complementary platforms are essential for studying disease mechanisms, testing therapeutic efficacy, and evaluating safety [77].

Animal models, particularly genetically engineered mice and pigs, have been instrumental in investigating how sarcomeric mutations give rise to the HCM phenotype. Knock-in models carrying pathogenic variants in *MYH7* or *MYBPC3* genes allow the study of genotype–phenotype relationships and provide an in vivo platform for testing gene replacement, gene editing, and RNA-targeted therapies [77]. For example, porcine models carrying the *MYH7* R723G variant reproduce key features of HCM, including myocyte disarray and early myocardial abnormalities [22]. Similarly, *Mybpc3* knockout models have shown that loss of cMyBP-C leads to hypertrophy, arrhythmias, and a severe HCM phenotype, supporting the concept of *MYBPC3* haploinsufficiency as a major disease mechanism [78,79]. Despite their value, animal models remain limited by interspecies differences in cardiac physiology, whereas large-animal models, although closer to humans, are more costly and technically demanding [29,77].

iPSC-CMs provide a complementary human cellular platform for modelling HCM and testing targeted therapies in vitro. These cells are generated by reprogramming somatic cells from patients with sarcomeric

mutations into pluripotent stem cells and differentiating them into cardiomyocytes [21]. iPSC-CMs carrying pathogenic *MYH7* or *MYBPC3* variants reproduce major cellular hallmarks of HCM, including hypertrophy, myofibrillar disarray, abnormal calcium handling, and altered contractility [80]. They are particularly useful for mutation-specific studies because CRISPR/Cas9 can be used to generate or correct variants, producing isogenic cell lines that differ only at the disease-causing locus [81,82]. Their major limitation is their relative immaturity, as they more closely resemble fetal than adult cardiomyocytes, although maturation strategies such as three-dimensional culture, micropatterning, and mechanical stimulation have improved their physiological relevance [83–85].

Efficient delivery of therapeutic genetic material to cardiomyocytes is another critical requirement for gene-based therapies in HCM. Currently, the main delivery systems explored are viral vectors and non-viral vectors. Among viral platforms, adeno-associated viruses (AAVs) are the most widely used in cardiac gene therapy because of their strong cardiotropism and ability to support sustained transgene expression, usually as episomal DNA, thereby reducing the risk of insertional mutagenesis [47,86]. AAV9 is particularly effective for myocardial delivery after systemic administration and has been widely used for *MYBPC3* gene replacement, CRISPR/Cas9 delivery, and RNA based approaches [47,87]. A major limitation of AAV vectors is their restricted packaging capacity, although this can sometimes be overcome by using cDNA constructs [42,88].

Other viral vectors have a more limited role. Adenoviral vectors can efficiently transduce cardiomyocytes and remain episomal, but their clinical applicability is restricted by strong immunogenicity and pre-existing neutralising antibodies[89,90]. Lentiviral vectors provide stable genomic integration and are useful in vitro, particularly in iPSC-CM studies, but their use in vivo is limited by insertional mutagenesis risk, lower efficiency in adult cardiomyocytes, and the need for invasive delivery [91–93].

Among non-viral systems, lipid nanoparticles (LNPs) are the most advanced platform. They can encapsulate mRNA, ASOs, siRNAs, and CRISPR/Cas9 ribonucleoprotein complexes, do not integrate into the genome, and are easier to manufacture than viral vectors [94–96]. Their main limitation is preferential hepatic accumulation after systemic administration, which reduces delivery to the myocardium. For this reason, cardiac-targeted LNPs are being developed using ligands that enhance myocardial uptake [97,98]. Compared with AAVs, LNPs generally produce faster but more transient expression, a feature that may be advantageous for applications such as genome editing, where prolonged nuclease exposure could increase off- target effects [98,99].

Overall, animal models and iPSC-CMs provide the essential preclinical framework for evaluating targeted therapies in HCM, whereas vector design remains a central determinant of translational success. Progress in both model systems and delivery technologies will be critical for moving gene and RNA based therapies from experimental proof of concept to clinical application.

4. ADVANTAGES AND LIMITATIONS OF TARGETED GENE THERAPIES

Targeted gene therapies offer important conceptual advantages over conventional pharmacological or invasive treatments because they act directly on the molecular mechanisms that drive disease onset and progression. Unlike standard therapies, which mainly relieve symptoms or reduce left ventricular outflow tract obstruction, gene and RNA based strategies aim to correct the primary cause of sarcomeric dysfunction and may therefore prevent downstream hypertrophy, fibrosis, and diastolic impairment [2]. In principle, these approaches will also offer the possibility of durable or even curative benefit after a single intervention, unlike conventional therapies that require lifelong administration and lose effect when discontinued [36,86].

A major strength of these therapies is their potential for personalised, mutation-specific intervention. Allele-specific silencing can selectively suppress pathogenic MYH7 alleles while preserving wild-type expression, and base editing may allow correction of single-nucleotide variants in an individualised manner [65,81]. In addition, because these strategies can be designed to act preferentially in cardiomyocytes, they may reduce systemic exposure and cumulative toxicity compared with chronic pharmacological therapy [2,100]. They may also be integrated with other treatment modalities, including symptom-directed therapies, as part of combined approaches [73].

Perhaps the most transformative feature of targeted therapies is their potential to alter the natural history of HCM, particularly if used at early stages such as genotype-positive, phenotype-negative disease. Sub-

tle abnormalities in energy use, relaxation, and diastolic function may be present before overt hypertrophy develops, raising the possibility that early intervention could delay or prevent structural remodelling [18,41]. Realising this potential will require sensitive biomarkers and multimodal strategies capable of detecting pre-phenotypic myocardial changes and monitoring therapeutic response; in this context, cardiac magnetic resonance may help identify subclinical abnormalities before hypertrophy is present [75,101].

Despite this promise, major limitations remain. Efficient and selective delivery to cardiomyocytes is still a central challenge. Although AAV9 shows marked cardiac tropism, it does not ensure exclusive myocardial specificity, and additional strategies such as cardiomyocyte- specific promoters and capsid engineering are often needed to reduce off-target expression, particularly in the liver and skeletal muscle [45,87,102,103]. Moreover, the dense extracellular matrix of the heart and the limitations of systemic or invasive delivery approaches continue to restrict homogeneous myocardial distribution, while non-viral systems such as lipid nanoparticles still show limited uptake by adult cardiomyocytes [97,104].

Another major barrier is the marked genetic and phenotypic heterogeneity of HCM. Different variants, even within the same gene, may act through distinct mechanisms such as haploinsufficiency or dominant-negative effects, complicating the development of universal therapies. Variable expressivity, incomplete penetrance, modifier genes, and genotype-negative cases further limit patient stratification and therapeutic timing [17,18,31]. In addition, the adult heart is largely post-mitotic, which constrains repair strategies that rely on cell division- dependent mechanisms [105].

Safety also remains a major concern. Pre-existing immunity to viral vectors may reduce therapeutic efficacy, and the high doses required for cardiac transduction can trigger inflammatory responses and limit redosing [90,106]. Gene-editing platforms also carry risks of off-target effects, unintended genomic alterations, and long-term genotoxicity, even with newer approaches such as base and prime editing [102]. Prolonged or excessive expression of therapeutic proteins or nucleases may further disrupt myocardial stability or increase arrhythmic risk, which has driven the development of self-limiting vectors, inducible systems, and tissue-specific promoters [107,108].

Finally, important ethical, regulatory, and practical challenges remain, especially regarding treatment of genotype-positive, phenotype-negative individuals, many of whom may never develop overt disease. Uncertainty in penetrance complicates risk–benefit assessment and patient selection. At the same time, large-scale manufacturing, mutation-specific development, regulatory approval, and cost remain substantial barriers to broad implementation, raising concerns about future healthcare inequities [30,39].

Overall, targeted gene therapies hold unique potential to shift HCM management from symptomatic treatment towards true disease modification. However, their successful clinical translation will depend on overcoming major challenges in delivery, specificity, safety, patient selection, and scalability.

5. CONCLUSION

Hypertrophic cardiomyopathy is a paradigmatic inherited myocardial disease in which pathogenic sarcomeric variants drive a prolonged pathogenic cascade that precedes structural remodelling by many years. Although current therapies have significantly improved symptom burden and clinical outcomes, they largely target the phenotypic consequences of disease rather than its primary molecular basis. Cardiac myosin inhibitors represent an important mechanistic advance, but their clinical role remains mainly confined to established disease.

Gene and RNA based therapies offer the opportunity of disease modification by targeting the causal substrate of HCM. Preclinical studies support the potential of gene replacement, genome editing, allelic silencing, and RNA-directed approaches to restore sarcomeric function and attenuate or prevent pathological remodelling, particularly when applied early in the disease course. Major translational challenges remain, including delivery, specificity, safety, scalability, and patient selection. Nevertheless, these strategies mark a critical shift from symptomatic treatment towards mechanism-based prevention and may ultimately redefine the management of inherited cardiomyopathies.

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