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ARTICLE

A pilot study in menopausal women with prediabetes: refining the blood exerkine irisin assay in relationship with the calcium-phosphorus metabolism

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Abstract: Objective. In this pilot study, we aimed to analyze the blood irisin levels in relation to glucose and mineral metabolism assays in menopausal women with non-diabetic altered glucose regulation: impaired fasting glucose (IFG) or impaired glucose tolerance (IGT). Methods. This was a prospective, transversal, non-interventional, bi-centric (bi-country) study, between December 2024 and October 2025. Results. The patients (N=47) with IGT (N=21) or IFG (N=26) had a similar age (63±9.24 versus 63.46±8.16years, p=0.856), menopause duration (14.05±9.37 versus 16.76±8.07years, p=0.297), and body mass index with mean values within the obesity range (34.84±5.07 versus 31.34±6.46kg/sqm, p=0.765). Average 25-hydroxyvitamin D showed an insufficiency (28.62±8.09 versus 28.06± 6.99ng/mL). The IGT versus IFG group were found with statistically significantly higher 1-hour glycaemia in the oral glucose tolerance test (207.04±34.72 versus 179.40±33.91mg/dL, p=0.009), 2-hour insulin (101.44±67.26 versus 48.97±34.35 µUI/mL, p=001), fasting insulin (13.00±7.38 versus 8.36±3.96µUI/mL, p=0.008); HOMA-IR (3.53±2.32 versus 2.27±1.17; p=0.21) with mean levels sustaining insulin resistance. Conclusion. Circulating irisin was marginally elevated in the IGT versus the IFG group and positively correlated with body mass index in both. We found no correlation with the glucose profile, but with selective mineral metabolism assays. This pilot study requires an expansion of the sample size to pinpoint the potential practical utility of irisin as a biomarker.

Keywords: myokine, muscle, hormone, diabetes, glucose, insulin, calcium, 25-hydroxyvitamin D, cold, physical exercise

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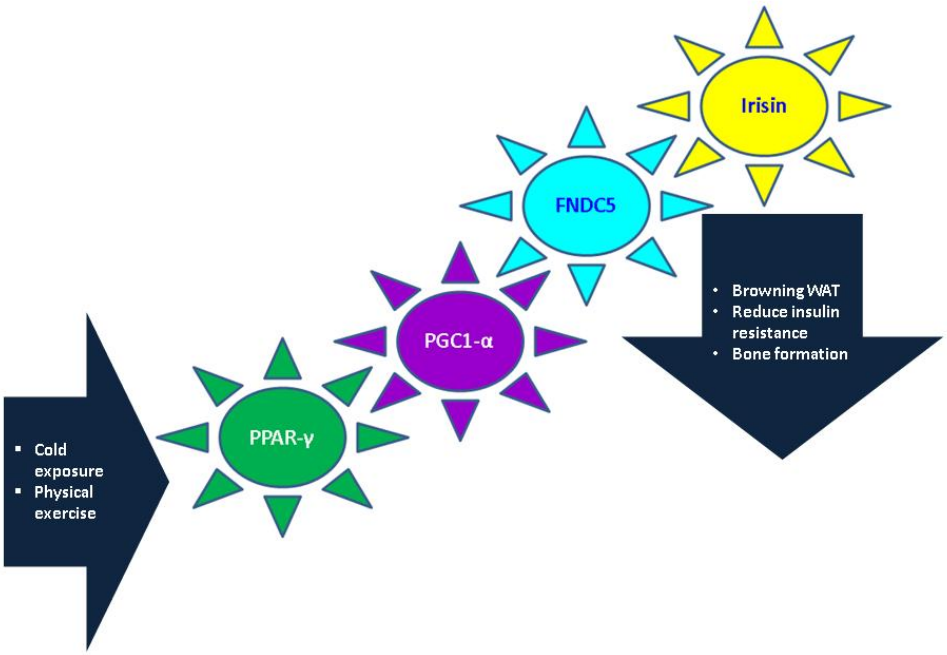
INTRODUCTION

One of the most intense areas of research in the modern era involves the skeletal muscle status, which became the novel endocrine gland of interest, noting that the complex spectrum of muscle-released exerkines (or myokines) dramatically changed with the detection of irisin in 2012 by Boström et al. [1-3]. Irisin was first identified as being secreted amid exposure to extreme conditions, particularly cold and physical exercise, but recently its blood level was analyzed in relation to various ailments in order to become a biomarker or prognostic marker in daily practice,

independently of the triggers such as extreme environments/conditioning programs [4-6].

Irisin is released from a larger precursor fibronectin type III domain-containing protein 5 (FNDC5) by activation due to transcriptional co-activator peroxisome proliferator-activated receptor-γ (PPAR-γ) co-activator-1 (PGC1-α), while adenosine monophosphate-activated (AMP) protein kinase, a main regulator of nutrient homeostasis, also activates irisin [7-9]. The current level of pathogenic knowledge primarily involves the role of irisin in transforming beige adipocytes into brown adipocytes, which further offers the substrate to produce heat from fatty acids and glucose in the human body [10-12]. (Figure 1)

Figure 1: The core findings in the irisin signal transduction pathways [1-12] (Abbreviations: FNDC5 = fibronectin type III domain-containing protein 5; PPAR-γ = peroxisome proliferator-activated receptor γ; PGC1-α = co-activator peroxisome proliferator-activated receptor γ co-activator-1; WAT = white adipose tissue



The most well-known stimulus of this physiological process is represented by the muscle activity and cold exposure [13,14]. This loop of regulating brown adipose tissue (BAT) thermogenesis is sustained by other peripheral mechanisms involving molecules such as leptin and fibroblast growth factor 21 (FGF21) in addition to the central thermogenesis [15-17]. On the contrary, the inhibitors of BAT thermogenesis are ghrelin, adiponectin, and plasminogen activator inhibitor-1 [18,19].

Overall, beige adipocytes, as a core element of thermogenesis upon browning of the white adipose tissue, are activated by myokines (e.g., irisin, interleulin-6), hepatokines (e.g., FGF21), cardiac proteins (e.g. brain natriuretic peptide), and neuronal pathways underlying sympathetic inputs (which are activated by cold exposure or adipokine leptin release, etc.) [20-21]. A combination of both cold and physical training induces body heat loss, with an improvement of the cardiovascular status and of the insulin resistance via irisin release from the muscle, as well as via activation of hepatic FGF21 (among others) [22-24]. However, this theoretical background is limited by an individual's tolerance to cold and the genetic and epigenetic-based muscle ability to release the hormone irisin [25,26].

Notably, irisin and interleukin-6 are involved in oxidative stress and cellular antioxidant defences, as well as the anti-aging process [27]. Moreover, irisin and FGF21 are related to beta-klotho interplay, which offers a connection to the mineral metabolism signal transduction pathways [28,29]. The exerkine was found to act as a bone-forming agent via the Wnt-catenin pathway interference, and further research involves its practical applications as a bone marker or interventional trials with irisin as an anti-osteoporotic agent [28-31]. An impairment of the muscle activity might cause muscle-related insulin resistance, which may be complicated by type 2 diabetes and other metabolic ailments; hence, irisin seems to be situated in the centre of these complex bone and glucose cross-roads [32].

To date, only a limited number of clinical trials in humans addressed irisin testing in everyday practice, as opposed to the numerous data in animal experiments, particularly murine studies (due to the biochemical similarity to the human irisin).

Objective

In this pilot study, we aimed to analyze the blood irisin levels in relation to glucose, lipids, and mineral metabolism levels in menopausal women diagnosed with altered glucose regulation (other than diabetes): either impaired fasting glucose (IFG) or impaired glucose tolerance (IGT).

MATERIALS AND METHODS

Study design

This study was a prospective, transversal, non-interventional, pilot, bi-centric (bi-country) study, conducted between December 2024 and October 2025 in two university hospitals.

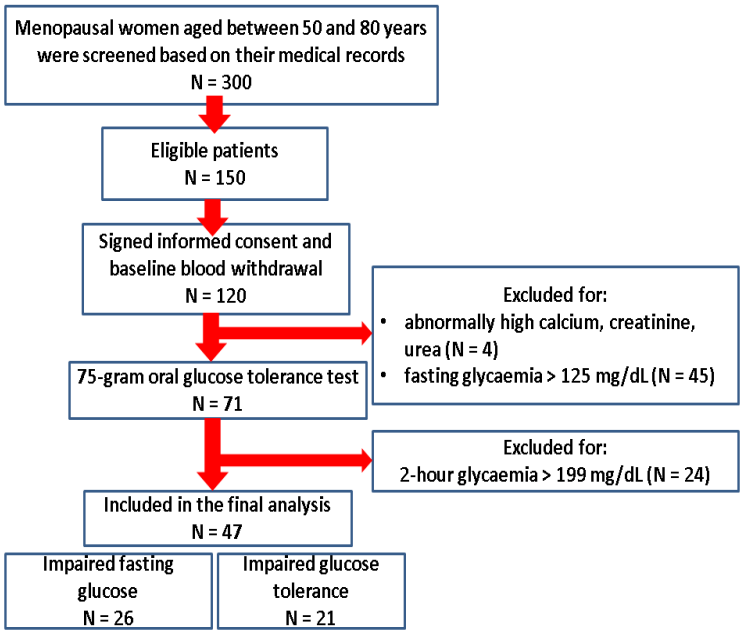
Study population

We enrolled active, apparently healthy menopausal adults who underwent a complete biochemical and hormonal panel according to the study protocol amid hospitalization at either of the two university centers involved in the research. We included females aged between 50 and 80 years, who were confirmed with a physiological or surgical menopausal status, having at least one year since (hormone replacement-free) secondary amenorrhea, and provided a written consent to participate in the study. We excluded individuals who were previously diagnosed with osteoporosis or osteopenia, bone metabolic conditions, cancers, chronic renal failure, endocrine diseases, such as parathyroid tumour, Cushing's disease, Cushing's syndrome, acromegaly, or prolactinoma, as well as previous or current diagnosis of type 1, type 2, or secondary diabetes mellitus. Also, the subjects who underwent at any point in life the following medications and/or procedures were ruled out: corticotherapy, insulin therapy, anti-obesity and anti-diabetic drugs, bariatric surgery, therapy to prevent or act against osteoporosis or reduce osteoporotic (fragility) fracture risk, and/or reduce hypercalcemia (e.g., oral or intravenous bisphosphonates, teriparatide, denosumab, calcitonin, romosozumab, abaloparatide).

Study protocol

The anamnesis and medical records provided during hospitalization included the spectrum of inclusion and exclusion criteria in terms of previous diseases and medications. Further on, eligible participants signed the informed consent to be enrolled in the study. Then, the specific blood tests were performed, as follows: fasting blood withdrawal was applied after an 8-to-12-hour overnight fasting, followed by a 75-gram oral glucose tolerance test (OGTT). OGTT was performed only in the subjects who presented a fasting glycaemia of less than 125 mg/dL. During OGTT, two additional blood withdrawals were performed after one hour and after two hours since oral glucose administration. The subjects who experienced a level of 2-hour glycaemia of 200 mg/dL or above were ruled out. The females who were confirmed with either IFG or IGT were included in the final analysis if fasting glycaemia was identified at a level of >100 mg/dL and <126 mg/dL, respectively, the patients had a value of 2-hour glucose in OGTT of >140 and <200 mg/dL [33]. (Figure 2)

Figure 2: Study protocol



The following parameters were collected: age, years since menopause, body mass index (in kg/sqm), glucose and lipid profile, mineral metabolism biochemical and hormonal assays, and bone turnover makers. (Figure 3A and 3B)

Figure 3A: Study parameters according to the protocol for the glucose-lipids metabolism; (Abbreviations: CLIA = Clinical Laboratory Improvement Amendments; OGTT = oral glucose tolerance test)

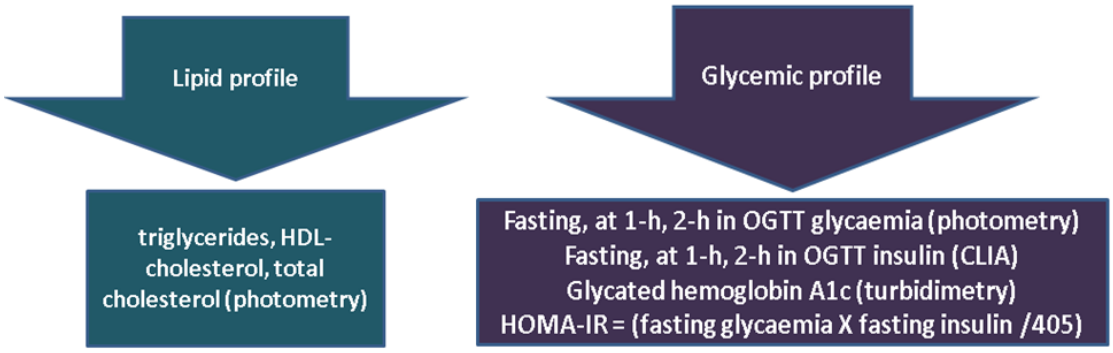
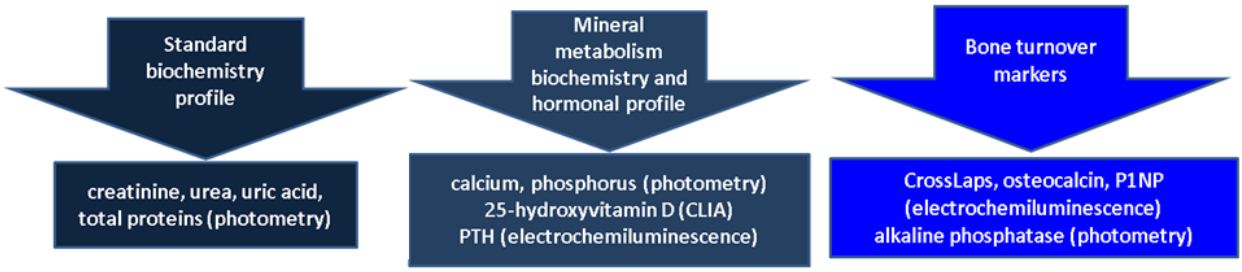
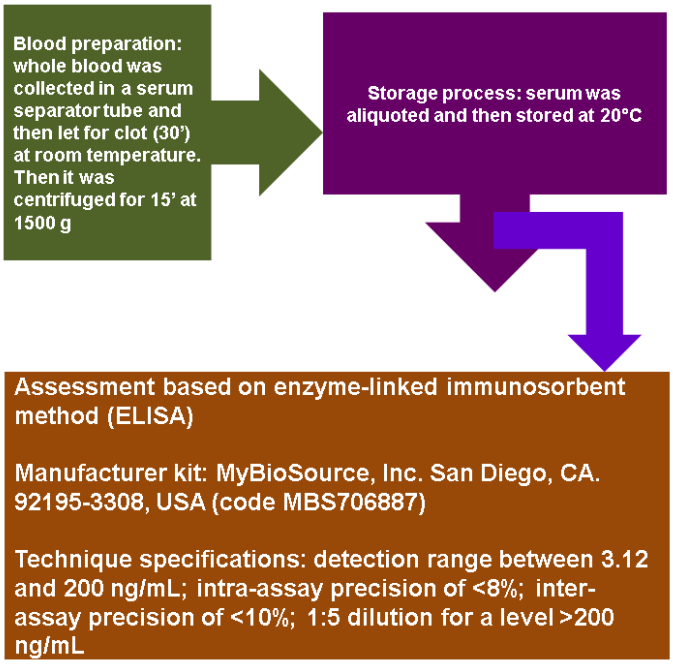


Figure 3B: Study parameters according to the protocol for standard biochemistry panel, as well as mineral metabolism exploration in terms of biochemical and hormonal assays, in addition to the panel of bone turnover markers; (Abbreviations: CLIA = Clinical Laboratory Improvement Amendments; PTH = parathyroid hormone)



Irisin was tested based on the MyBioSource kit via the enzyme-linked immunosorbent method (ELISA), and all blood samples were collected at the “C.I. Parhon” National Institute of Endocrinology, Bucharest, Romania, which provided the infrastructure for all irisin assays. (Figure 4)

Figure 4: Technique specifications for circulating irisin testing according to the study protocol



Statistical analysis

MedCalc® (Statistical Software version 23.3.7; MedCalc Software Ltd, Ostend, Belgium, 2025) was used for statistical analysis. Kolmogorov-Smirnov test was applied for normality/non-normality pattern, respectively, t-test for comparison of independent samples with normal distribution, as well as Mann-Whitney test for comparison of independent samples with non-normal distribution. The descriptive analysis (mean ± standard deviation) and Pearson correlation (for normal distribution), respectively, Spearman rank correlation (for non-normal distribution) were performed. The cut-off $p < 0.05$ established the statistical significance.

Ethical aspects

The study was ruled in accordance with the Declaration of Helsinki for medical research. Each patient signed the informed consent regarding participation in the study. Ethical Committees from each university center approved the study (number 32 from 09 September 2024 at "C.I. Parhon" National Institute of Endocrinology, Bucharest, Romania, and number 97 from 11 November 2024 at State "Nicolae Testemiţanu" University of Medicine and Pharmacy, Chisinau, Republic of Moldova).

RESULTS

The patients included in the final analysis were either found with IGT (N = 21) or IFG (N = 26). These two subgroups have a similar age (63 ± 9.24 versus 63.46 ± 8.16 years, p = 0.856) and menopause duration (14.05 ± 9.37 versus 16.76 ± 8.07 years, p = 0.297), as well as body mass index with mean values within the obesity range (34.84 ± 5.07 versus 31.34 ± 6.46 kg/sqm, p = 0.765). They were found with similar mineral metabolism assays and bone turnover markers, with an average 25-hydroxyvitamin D showing a mild insufficiency (28.62 ± 8.09 versus 28.06 ± 6.99 ng/mL, normal value above 30 ng/mL).

Glucose profile assessment was found statistically significantly increased 1-hour glycemia in the IGT group versus the IFG group (207.04 ± 34.72 versus 179.40 ± 33.91 mg/dL, p = 0.009). Fasting (baseline) insulin was statistically significantly higher in the IGT versus IFG group (13.00 ± 7.38 versus 8.36 ± 3.96 µUI/mL, p = 0.008; normal values between 1.9 and 23 µUI/mL), respectively, 2-hour insulin in OGTT (101.44 ± 67.26 versus 48.97 ± 34.35 µUI/mL, p = 001).

HOMA-IR was statistically significantly elevated in the IGT group when compared to the IFG group: 3.53 ± 2.32 versus 2.27 ± 1.17 (p = 0.21), with both mean levels sustaining insulin resistance. (Table 1)

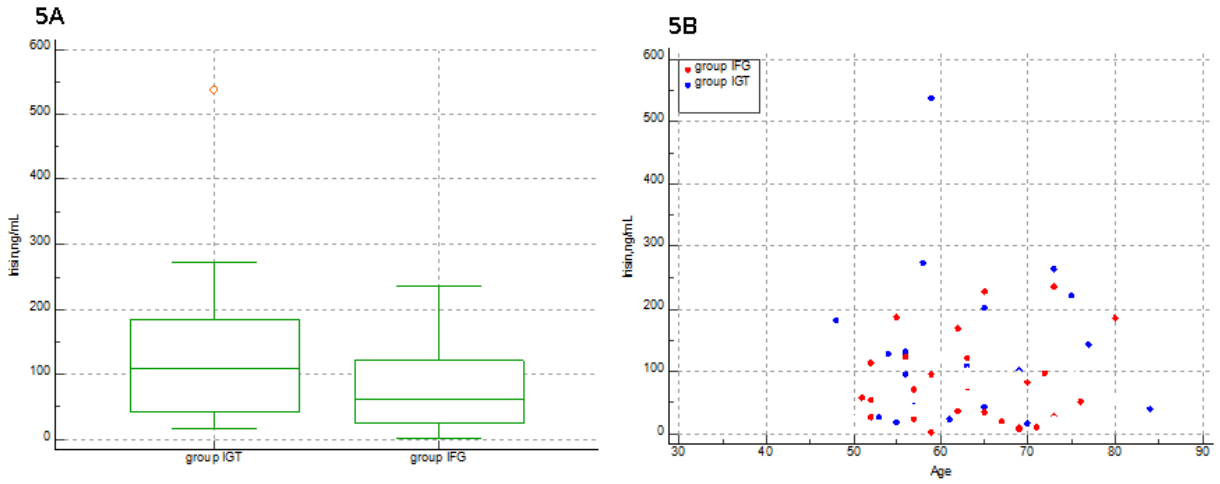
Table 1: The study parameters within the IGT group and the IGF group

Parameter	**Group IGT**	**Group IFG**	
Number of patients	21	26	
	mean ± SD or median (IQR)	mean ± SD or median (IQR)	p-value
Demographic features			
Age (years)	63 ± 9.24	63.46 ± 8.16	0.856
Years since menopause	14.05 ± 9.37	16.76 ± 8.07	0.297
Body mass index (kg/sqm)	34.84 ± 5.07	31.34 ± 6.46	0.765
Mineral metabolism and standard biochemical assays			
Total calcium (mg/dL)	9.57 ± 0.43	9.31 ± 1.34	0.400
Phosphorus (mg/dL)	3.42 ± 0.49	3.69 ± 0.62	0.114
Total proteins (g/dL)	7.50 ± 0.48	7.49 ± 0.47	0.904
Creatinine (mg/dL)	0.75 ± 0.17	0.77 ± 0.13	0.700
Urea (mg/dL)	40.16 ± 8.38	42.37 ± 12.10	0.481
Uric acid (mg/dL)	5.44 ± 1.20	7.20 ± 10.21	0.439
Mineral metabolism hormones			
parathyroid hormone (pg/mL)	42.77 ± 11.45	47.26 ± 20.24	0.369
25-hydroxyvitamin D (ng/mL)	28.62 ± 8.09	28.06 ± 6.99	0.781
Bone turnover makers			
total alkaline phosphatase (U/L)	77.04 ± 28.71	76.62 ± 22.71	0.955
osteocalcin (ng/mL)	20.60 ± 7.70	76.62 ± 22.71	0.331
P1NP (ng/mL)	55.57 ± 20.47	62.20 ± 29.40	0.394
CrossLaps (ng/mL)	0.38 ± 0.18	0.41 ± 0.17	0.544
Glucose metabolism assessment			
Fasting glycaemia (mg/dL)	108.35 ± 10.34	108.92 ± 6.25	0.816
Glycaemia - at 1-hour in OGTT	207.04 ± 34.72	179.40 ± 33.91	0.009
Glycaemia - at 2 hours in OGTT	162.61 ± 17.01	117.41 ± 18.81	<0.0001
Fasting insulin (µUI/mL)	13.00 ± 7.38	8.36 ± 3.96	0.008
Insulin - at 1-hour in OGTT	94.67 ± 53.60	76.45 ± 44.43	0.219
Insulin - at 2 hours in OGTT	101.44 ± 67.26	48.97 ± 34.35	0.001
Glycated hemoglobin A1c (%)	5.89 ± 0.43	5.73 ± 0.30	0.144
HOMA-IR	3.53 ± 2.32	2.27 ± 1.17	0.021
Lipid profile			
Total cholesterol (mg/dL)	202.46 ± 37.81	186.83 ± 55.06	0.256
Triglycerides (mg/dL)	125.31 ± 80.36	116.17 ± 52.66	0.655
HDL-cholesterol (mg/dL)	56.02 ± 14.79	57.87 ± 14.00	0.690
Circulating irisin (ng/mL)	133.78 ± 121.30	81.64 ± 69.57	0.07

Abreviations: HOMA-IR = Homeostasis Model Assessment of Insulin Resistance; OGTT = oral glucose tolerance test; Normal values: calcium = 8.8 – 10.2 mg/dL; phosphorus = 2.3 – 4.7 mg/dL; total proteins = 6.4 – 8.6 g/dL; creatinine = 0.5 – 1.2 mg/dL; urea = 22 – 43 mg/dL; uric acid = 2.6 – 6 mg/dL; parathormone = 17.3 – 74.1 pg/mL; 25-hydroxyvitamin D = >30 ng/mL; total alkaline phosphatase = 35 – 104 U/L; osteocalcin = 15 – 46 ng/mL; P1NP = 20.25 – 76.31ng/mL; CrossLaps = ng/mL; glycemia 80 – 115 mg/dL; fasting insulin = 1.9 – 23 µUI/mL; glycated hemoglobin A1c = 4.8 – 5.9%; HOMA-IR < 2; total cholesterol <200 mg/dL; tryglicerides <150 mg/dL; HDL-cholesterol = 35 – 65 mg/dL

Circulating irisin was borderline elevated in the IGT group in comparison with the IFG group: 133.78 ± 121.30 versus 81.64 ± 69.57 ng/mL (p = 0.07). (Figure 5)

Figure 5: Circulating irisin assays in the two study groups according to box plot charts (5A) and irisin distribution in relationship with the patients' age based on a scatter plot visual representation (5B)



Irisin statistically significantly and positively correlated with body mass index (r = 0.42, p = 0.05 in the IGT group, respectively, r =0.54, p = 0.004 in the IGF group). (Table 2)

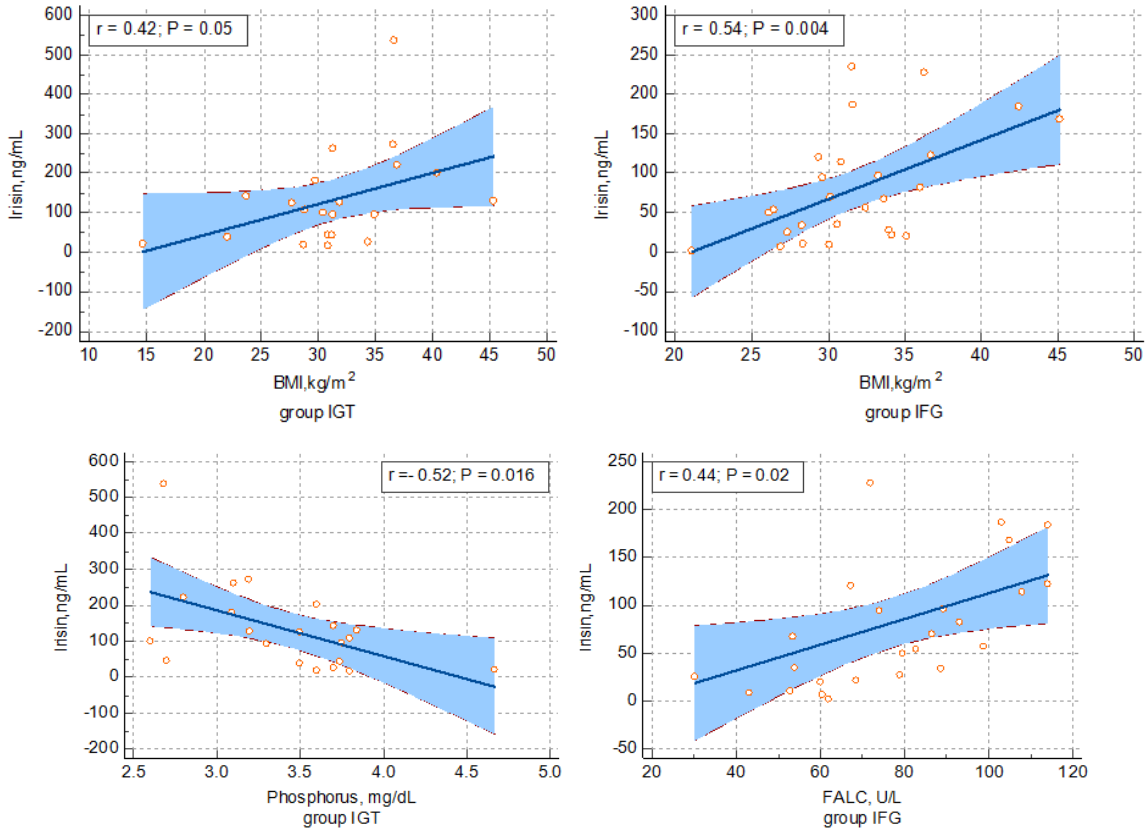
Table 2: The correlations between irisin and the study parameters in the IGT and IGF groups

Irisin correlation with:	**Group IGT**	**Group IFG**
Age (years)	r = -0.03 p = 0.89	r = 0.095 p = 0.64
Menopause duration (years since menopause)	r = -0.001 p = 0.99	r = -0.02 p = 0.88
Body mass index (kg/sqm)	r = 0.42 p = 0.05	r = 0.54 p = 0.004
Total serum calcium (mg/dL)	r = 0.04 p = 0.84	r = -0.36 p = 0.07
Phosphorus (mg/dL)	r = -0.52 p = 0.01	r = 0.168 p = 0.42
Uric acid (mg/dL)	r = 0.09 p = 0.69	r = 0.16 p = 0.44
Parathyroid hormone (pg/mL)	r = -0.28 p = 0.21	r = 0.30 p = 0.12
25-hydroxyvitamin D (ng/mL)	r = -0.19 p = 0.40	r = 0.04 p = 0.83
Total alkaline phosphatase (U/L)	r = -0.37 p = 0.09	r = 0.44 p = 0.02
Osteocalcin (ng/mL)	r = -0.34 p = 0.12	r = 0.01 p = 0.93
P1NP (ng/mL)	r = -0.28 p = 0.22	r = 0.01 p = 0.92
CrossLaps (ng/mL)	r = -0.13 p = 0.55	r = 0.02 p = 0.90
Fasting glycaemia (mg/dL)	r = -0.31 p = 0.16	r = 0.08 p = 0.68
Glycaemia - at 1-hour in OGTT	r = 0.05	r = 0.16

	p = 0.81	p = 0.42
Glycaemia - at 2 hours in OGTT	r = -0.20 p = 0.37	r = -0.43 p = 0.02
Fasting insulin (µUI/mL)	r = 0.43 p = 0.05	r = 0.04 p = 0.83
Insulin - at 1-hour in OGTT	r = 0.35 p = 0.12	r = 0.10 p = 0.60
Insulin - at 2 hours in OGTT	r = 0.24 p = 0.31	r = -0.365 p = 0.06
Glycated hemoglobin A1c (%)	r = 0.20 p = 0.37	r = 0.03 p = 0.85
HOMA-IR	r = 0.30 p = 0.18	r = 0.05 p = 0.77
Total cholesterol	r = -0.16 p = 0.48	r = 0.04 p = 0.81
Triglycerides	r = 0.13 p = 0.57	r = -0.03 p = 0.87
HDL-cholesterol	r = -0.03 p = 0.88	r = 0.26 p = 0.22

Irisin statistically significantly and positively correlated with total alkaline phosphatase (r = 0.44, p = 0.02) in the IGF group, and negatively with serum phosphorus (r = -0.52, p = 0.01) in the IGT group. (Figure 6)

Figure 6: Irisin correlation with body mass index, phosphorus, and alkaline phosphatase in study groups



DISCUSSION

In this pilot study (N = 47), we assessed menopausal pre-diabetic women with regard to the irisin assay in order to potentially find its utility in relation to glucose and mineral metabolism exploration. We identified a marginally different irisin level between IGT and IFG, which were age-matched groups. A more severe glucose status was pointed out in IGT versus IFG in terms of presenting higher one-hour glucose and two-hour insulin levels during OGTT, fasting insulin, as well as HOMA-IR.

Notably, the hypothesis that irisin might serve as a biomarker in prediabetes is still under research nowadays, and only heterogeneous results have been published so far. For instance, Ertuna et al. [34] enrolled 100 participants aged between 18 and 65 years (60 of them were confirmed with prediabetes and 40 were prediabetes/diabetes-free), followed for a three-month protocol of a healthy lifestyle. Irisin statistically significantly increased after three months ($p < 0.001$), and provided a diagnosis performance similar to HOMA index (sensitivity of 93.3%, specificity of 65%), while originally being lower in prediabetes than controls [34]. Moreover, Liu et al. [35] studied 41 males with prediabetes (mean age of 37.8 years) versus 45 men with normal results during OGTT (average age of 37.1 years), and identified a correlation between irisin and fasting glycaemia ($r = -0.725$, $p < 0.05$), and two-hour glycaemia during OGTT ($r = -0.36$, $p < 0.05$) only in the group confirmed with prediabetes [35]. In the REACTION study, He et al. [36] included 2350 newly diagnosed subjects with prediabetes and found that irisin was an independent predictor of developing diabetes, and a decrease in the basal level above the median irisin value was associated with a higher risk of diabetes (odds ratio of 5.077, 95% confidence interval between 2.112 and 12.206) [36]. Other observational studies also provided inconclusive results of irisin testing in prediabetes, mostly noting that a lower level of the protein is associated with a more severe picture of glucose anomalies [37].

Interestingly, we found a positive correlation between irisin and body mass index in both groups with prediabetes ($r = 0.42$, $p = 0.05$ in the IGT group, respectively, $r = 0.54$, $p = 0.004$ in the IGF group). Pardo et al. [38] showed that a more accurate correlation should be expected with body fat rather than body mass index [38]. Stengel et al. [39] identified a higher irisin in the obese versus the normal-weight population, with a positive correlation coefficient ($r = 0.5$, $p < 0.01$), which was similar to our results [39]. On the contrary, we previously mentioned the study conducted by Liu et al. [35] that confirmed a negative correlation between irisin and body mass index ($r = -0.325$, $p < 0.05$) only in the control (diabetes-negative) group, and not in the one with prediabetes [35].

We found no statistically significant results with respect to irisin and the lipid profile, while generally, a limited number of studies showed statistically significant results. For example, a small sample size study identified a correlation between irisin and triglycerides of -0.339 ($p < 0.05$) only in the control male group, not in the men cohort diagnosed with prediabetes, whereas a positive correlation with HDL-cholesterol was found ($r = 0.432$, $p < 0.05$) [35]. Another study in diabetic and obese participants identified that a lower irisin was associated with a higher level of triglycerides ($r = -0.343$, $p = 0.024$), and lower HDL-cholesterol ($r = 0.363$, $p = 0.17$) [40]. Additionally, Fu et al. [41] suggested that irisin is reduced in patients with carotid atherosclerosis [41].

With regard to the exploration of the mineral metabolism and the spectrum of traditional bone turnover markers, only alkaline phosphatase correlated with irisin in the IGF group ($r = 0.44$, $p = 0.02$), while lower phosphorus levels were associated with higher irisin in the IGT group $r = -0.52$, $p = 0.01$). Based on our findings, irisin should be differently positioned with the calcium-phosphorus assessment in the two categories of prediabetes. Many of the current studies focus on reporting the irisin, osteocalcin, and P1NP improvement upon physical training since they act as bone-forming contributors [42] or their profile in type 2 diabetic populations, which is expected to reduce their blood levels [43]. In the previously mentioned study, irisin positively correlated with P1NP ($r = 0.398$, $p < 0.05$), respectively, with osteocalcin ($r = 0.351$, $p < 0.05$) in males with prediabetes [35].

At limitations of this study, we mention the cross-sectional design in a relatively small sample size. This pilot study will be followed by a larger trial to pinpoint the impact of irisin testing in daily practice, not only in pre-diabetic, but also in type 2 diabetic subjects. Notably, we restricted enrollment to menopausal adults in order to avoid the bias of inter-individual variations in women of reproductive age and males with respect to the bone health assessment (specifically, the panel of bone turnover markers) and multiple hormonal influences such as estrogens, progesterone, and testosterone status [44].

Also, we did not quantify the level of daily (physical training), which might impact the baseline irisin value. Prior data suggested that improvement of the insulin resistance amid physical exercise might involve an increase in irisin, while the overall results are not homogeneous [45]. Moreover, at this point, many irisin assay variations are caused by a lack of standardization with respect to the method and kit of detection, and further studies are necessary.

CONCLUSION

Among the panel of glucose, lipids, and mineral metabolism parameters, we found in this pilot study that, despite a marginally significant higher irisin in IGT versus IFG, the hormone correlated with body mass index in both groups, and only selectively with mineral metabolism assays, and none of the glucose status biomarkers. To our best knowledge, only a limited number of well-designed prospective studies have been published so far in the field of prediabetes and the results are still inhomogeneous depending on the study population, variations of irisin kits, as well as known or unknown biases of detection such as co-morbidities, hormonal influences, muscle status variations in terms of mass, function, and level of training as well as body composition analysis.

Supplementary Materials

Not applicable

Author Contributions

Conceptualization, D.M., A.P., N.L.-O., L.S., M.C., S.V.S., O.-C.S., and V.C.; methodology, D.M., A.P., N.L.-O., L.S., M.C., S.V.S., O.-C.S., V.C., D.-M.P., and M.-L.C.; software, D.M., A.P., N.L.-O., L.S., M.C., S.V.S., O.-C.S., and V.C.; validation, D.-M.P., and M.-L.C.; formal analysis, D.M., A.P., N.L.-O., L.S., M.C., S.V.S., O.-C.S., and V.C.; investigation, D.M., A.P., N.L.-O., L.S., M.C., S.V.S., O.-C.S., V.C., D.-M.P., and M.-L.C.; resources, D.M., A.P., N.L.-O., L.S., M.C., S.V.S., O.-C.S., V.C.; data curation, D.-M.P., and M.-L.C.; writing—original draft preparation, D.M., and M.C; writing—review and editing, M.C.; visualization, D.-M.P., and M.-L.C.; supervision, D.M., A.P., N.L.-O., L.S., M.C., S.V.S., O.-C.S., V.C., D.-M.P., and M.-L.C.; project administration, M.C.; funding acquisition, M.C.; All authors have read and agreed to the published version of the manuscript. No generative AI was involved in the production of this article.

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Institutional Review Board Statement

The research was conducted in accordance with the Declaration of Helsinki. Each patient signed the informed consent in order to be enrolled in the study. The study was approved by the Ethical Boards of both centers (number 32 from 30 September 2024 at "C.I. Parhon" National Institute of Endocrinology, Bucharest, Romania, respectively, number 97 from 20 November 2024 at State "Nicolae Testemițanu" University of Medicine and Pharmacy, Chisinau, Republic of Moldova).

Informed Consent Statement

The patients signed the informed consent to participate in this study.

Data Availability Statement

All available data are in the article.

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Conflicts of Interest

The authors declare no conflict of interest.

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ARTICLE

A retrospective study in anti-osteoporotic drug naïve adults with a one-year history of low-trauma fall: associated bone metabolic panel amid the presence of osteoporotic fractures

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Abstract: Objective. In this retrospective study, we aimed to analyse bone profile in menopausal women who suffered a low-trauma fall and were found to have an osteoporotic fracture. Methods. This real-life setting included individuals with a fall within the last year before DXA scan [providing bone mineral density (BMD)/T-score], and excluded traumatic falls, prior anti-osteoporosis therapy, diagnosis of osteoporosis, cancers, and bone metabolic diseases. Results. 5-year age-group analysis was statistically significantly different (N=48, p=0.017): in the 70-74 year interval, 75% were in group F (fracture +ve), in the 50-54 year interval, 83.33% were in group nonF (fracture-free). Mineral metabolism assays and bone turnover markers pinpointed a similar profile, except for DXA: femoral neck T-score was lower in group F versus nonF (-1.75±0.72 versus -1.17±0.91, p=0.029). Receiver operating characteristic curve of femoral neck T-score for predicting a fracture showed an area under the curve of 0.691 (p=0.031). Conclusion. Menopausal women with a 1-year fall history with fractures were older, had a longer menopause duration, and had lower femoral neck BMD than fracture-free women. Type 2 diabetes and hypertension had similar prevalence, as did the vitamin D profile and bone turnover markers. Individuals with vertebral fractures (most common types) showed a lower lumbar BMD versus those with non-vertebral fractures.

Keywords: osteoporosis, DXA, fracture, bone turnover marker, fracture risk, osteocalcin, P1NP

INTRODUCTION

Osteoporosis and associated fragility (osteoporotic) fractures represent a major health, economic, and social burden in the modern era, which is projected to affect a higher number of people across the aging process, in association with a worldwide increasing population with a larger life span [1,2]. The main cost-drivers are the medications against osteoporosis for primary and secondary fracture prevention assimilated into the wider frame of fracture-associated costs, noting that more than half of the individuals who are candidates for specific medication (bisphosphonates, denosumab, teriparatide, romosozumab) actually remain untreated [3,4]. For

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instance, the average annual costs of treating osteoporotic fractures vary between 5000 and 6500 billion USD in European countries, Canada, and the USA [5]. In addition, indirect costs involve the osteoporosis-related disability, and related programs of physical rehabilitation, impaired quality of life, reduced productivity, as well as work engagement issues [5,6].

Essentially, the most important epidemiologic impact comes from primary (menopause- and age-related) osteoporosis, but other fracture risks might contribute to enhancing the loss of bone strength, such as chronic diseases, smoking, various neoplasia, and long-term drug use, etc. [7,8]. Recently, type 2 diabetes was found to increase the fracture risk, particularly in menopausal women, by affecting the bone microarchitecture rather than the bone mineral density (BMD), as reflected by the Dual-Energy X-Ray Absorptiometry (DXA) scan [9,10].

In this complex spectrum of osteoporotic fracture risks, it is mandatory to mention the risk of falls that offers the circumstances of a fracture in prior undiagnosed osteoporotic patients [11-13]. The risk of fall is more difficult to be quantified in daily multidisciplinary practice than most of the fractures contributors, and it involves numerous comorbidities, such as blood pressure or glycaemia variations during day time, sarcopenia, fraility, and any type of muscle mass and/or function disturbance, impaired vision, obesity and various gait anomalies, changes of the blood electrolytes levels, etc. [14-16].

Objective

In this study, we aimed to analyse the bone profile in menopausal women who suffered a recent low-trauma fall that was further complicated by an osteoporotic fracture.

MATERIAL AND METHODS

Study design

This was a retrospective, real-life study in menopausal women, between January 2024 and August 2025.

Study population

We included patients who suffered a low-trauma fall within the last year before having a DXA assessment. Inclusion criteria were: women of 50 years of older; confirmed menopausal status; patients' written consent to anonymously use the medical records according to the hospital rules for inpatients; available central DXA scan at three major sites (L1-L4 lumbar spine, femoral neck, and total hip); the subjects were registered with a non-traumatic fall during most recent 12 months (before DXA assessment).

Exclusion criteria were: traumatic falls; prior medication against osteoporosis at any point in life (alendronate, risendronate, zoledronate, denosumab, teriparatide, romosozumab), corticotherapy, insulin therapy, hormone replacement therapy (oestrogens); previous diagnosis of any malignancy, including primary or secondary bone cancers, as well as inherited/acquired bone metabolic disorders, type 1 or secondary diabetes mellitus, chronic kidney failure, active endocrine diseases (e.g. primary or tertiary hyperparathyroidism, hyperthyroidism, prolactinoma, acromegaly, Cushing's syndrome). Notably, the patients who were previously (before the fall) diagnosed with osteoporosis or osteoporotic fractures were ruled out, even though they did not follow any specific medication against osteoporosis or fracture prevention. Moreover, the subjects with multiple osteoporotic fractures were excluded. The individuals with unclear circumstances of the fall (e.g., domestic accident, street fall, etc.) were also eliminated.

Study protocol

Retrospective data collection included age, menopause duration (since last menstruation), the panel of mentioned comorbidities, biochemical and hormonal parameters, and body mass index (kg/sqm). Also, central DXA (GE Lunar prodigy machine)-based parameters, which provided bone mineral density and T-scores. Osteoporosis was diagnosed according to World Health Organization (WHO) standard criteria and current guidelines: lowest T-score at central DXA of -2.5 or less, respectively, osteopenia in women with the lowest T-score at DXA between -2.5 and -1 [17-19]. Non-interpretable DXA scan was considered if the subjects did not have the available BMD and T-score for lumbar spine, total hip (on the left side), and femoral neck (on the left side), or the scan was not feasible (e.g., severe coxarthrosis, prosthesis, neurosurgery for the spine with local implants, large kidney or gall bladder stones). In this particular instance, the patients were ruled out. (Figure 1)

All patients underwent X-Ray profile of the thoracic and lumbar spine. Prevalent fractures were analysed based on the medical records by two trained radiologists (dr. Costachescu and dr. Preda). The circumstances of the fall and the integration of the fracture as being osteoporotic (low-trauma or spontaneous) fragility fractures were categorized by the medical team according to the data provided during anamnesis at the moment of hospitalization, in addition to the patients' records. The analyzed fracture sites were: vertebral, hip, leg, forearm, arm, clavicle, etc. (Figure 2)

Individuals with a prevalent fragility fracture were included in group F, and those without a post-fall fracture represented group nonF.

Figure 1: Study parameters according to the mentioned methods

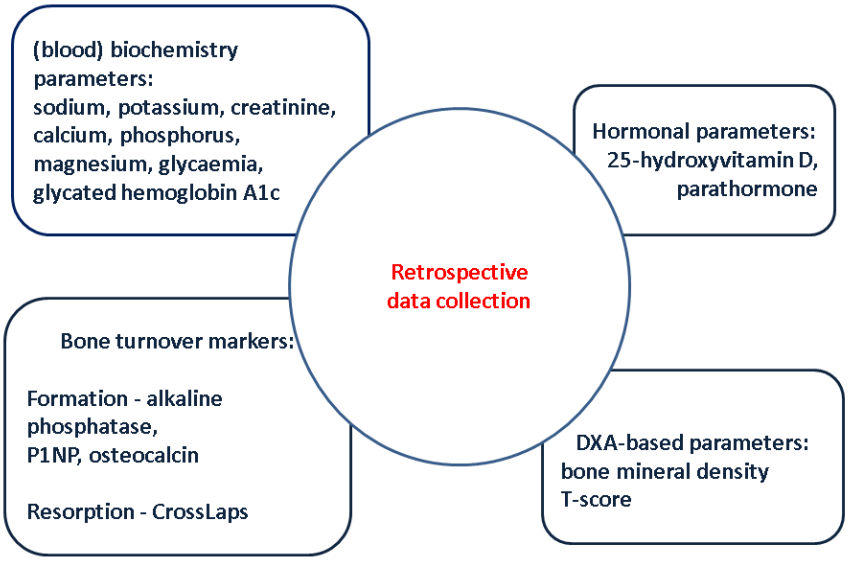
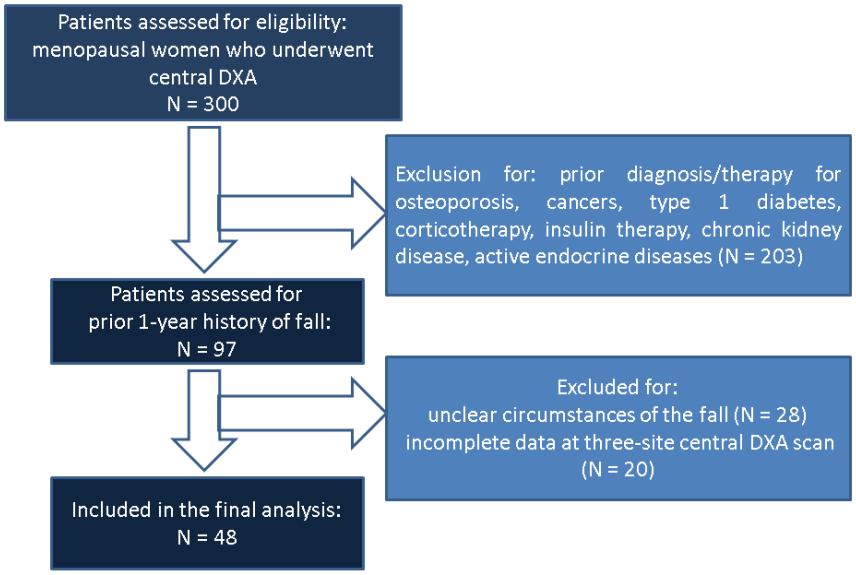


Figure 2: Study protocol



Statistical analysis

SPSS v.29.0.2.0 (IBM Corp., Armonk, NY, USA) and Excel v16.100.1 (Microsoft, Redmond, WA, USA) were used for the statistical analysis. Continuous variables were assessed with regard to their distribution for normality via the Kolmogorov-Smirnov test, and they were expressed as mean ± standard deviation (SD) in cases with a normal distribution, respectively, as median and quartiles (Q1, Q3) for the parameters with a non-normal distribution. The categorical variables (expressed as absolute numbers and percentages) were explored with respect to the between-group differences by applying the Chi-square test or Fisher's exact test. The independent sample t-test was used for the comparison of continuous variables between two independent groups, respectively, the Mann–Whitney U test, for the groups with other relationships. The receiver operating characteristic curve was used for different parameters in order to distinguish between the two analysed variables. A cut-off value of less than 0.05 was considered a statistically significant result.

Ethical aspects

Each patient had at least one hospitalization at "C.I. Parhon" National Institute of Endocrinology, Bucharest, Romania (whereas central DXA was performed). The Ethical Committee of this tertiary centre of endocrinology ("C.I. Parhon" National Institute of Endocrinology, Bucharest, Romania) approved the study (number 33 from 22 September 2025). The study was performed in accordance with the Declaration of Helsinki. Specific consent for participation in this study was waived due to the retrospective design.

RESULTS

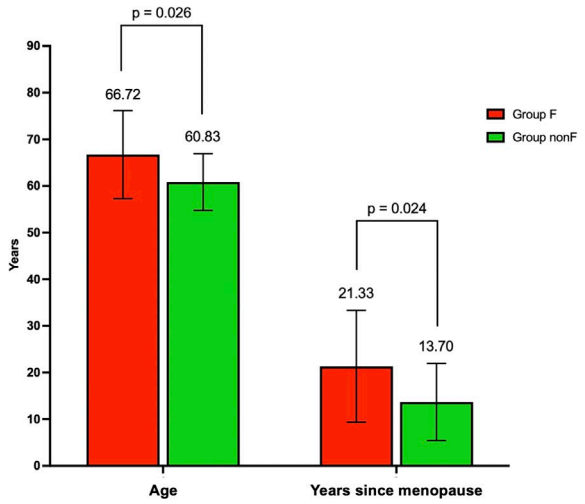
A total of 48 patients were included in the final analysis, having a mean age of 63.04 ± 7.95 years, respectively, and a menopause duration of 16.56 ± 10.38 years since menopause. 27.50% of the subjects had prevalent fragility fractures (group F, N = 18) and 62.50% were fracture-free (group nonF, N = 30). Body mass index was similar between the groups, as well as the cardio-metabolic panel, according to the study protocol. (Table 1)

Table 1: Demographic parameters of the entire sample, group F and group nonF

Parameter	Descriptive statistics (Units)	Entire sample (N = 48, 100%)	Group F (N = 18, 37.50%)	Group nonF (N = 30, 62.50%)	p-value
Age	Mean ± SD (years)	63.04 ± 7.95	66.72 ± 9.44	60.83 ± 6.07	**0.026**
Years since menopause	Mean ± SD	16.56 ± 10.38	21.33 ± 11.97	13.70 ± 8.26	**0.024**
Smoker status	N (%)	8 (16.67)	1 (5.56)	7 (23.33)	0.229
Dyslipidemia	N (%)	24 (50.00)	11 (61.11)	13 (43.33)	0.371
Type 2 diabetes mellitus	N (%)	2 (4.17)	2 (11.11)	0 (0.00)	0.136
High blood pressure	N (%)	30 (62.50)	11 (61.11)	19 (63.33)	0.878
Body mass index	Mean ± SD (kg/sqm)	25.52 ± 4.45	25.25 ± 4.93	25.68 ± 4.21	0.749

Age was statistically significantly higher in group F of 66.72 ± 9.44 years compared to group nonF of 60.83 ± 6.07 (p = 0.026), as well as years since menopause (21.33 ± 11.97 versus 13.70 ± 8.26, p = 0.024). (Figure 3)

Figure 3: Bar chart showing the mean and standard deviation of the patients' age and menopause duration in group F versus group



Analysed biochemical parameters were similar between the studied groups. (Table 2)

Table 2: Biochemical parameters of the entire sample, group F and group nonF

Parameter	Descriptive statistics (Units)	Entire sample (N = 48, 100%)	Group F (N = 18, 37.50%)	Group nonF (N = 30, 62.50%)	p-value	Normal range
Fasting glycaemia	Mean ± SD (mg/dL)	104.57 ± 24.55	105.48 ± 30.01	102.42 ± 20.16	0.756	74-106
Glycated hemoglobin	Mean ± SD (%)	5.48 ± 0.33	5.40 ± 0.34	5.54 ± 0.33	0.298	4.8-5.9
Serum creatinine	Mean ± SD (mg/dL)	0.78 ± 0.10	0.76 ± 0.08	0.79 ± 0.10	0.411	0.7-1.2
Serum sodium	Mean ± SD (mmol/L)	140.41 ± 2.10	140.75 ± 1.58	140.26 ± 2.31	0.592	136-145
Serum potassium	Mean ± SD (mmol/L)	4.46 ± 0.42	4.33 ± 0.39	4.52 ± 0.42	0.219	3.5-5.1

Total serum calcium had a similar value in group F versus group nonF (9.58 ± 0.37 mg/dL versus 9.57 ± 0.40 mg/dL (p = 0.910). Mean 25-hydroxyvitamin D was 22.82 ± 7.26 ng/mL in group F and 22.92 ± 10.66 ng/mL (p = 0.971) in group nonF. No between-group difference was found with respect to bone formation and resorption markers. (Table 3)

Table 3: Calcium-phosphorus metabolism and bone turnover markers in study population

Parameter	Descriptive statistics (Units)	Entire sample (N = 48, 100%)	Group F (N = 18, 37.50%)	Group nonF (N = 30, 62.50%)	p-value	Normal range
Total serum calcium	Mean ± SD (mg/dL)	9.57 ± 0.38	9.58 ± 0.37	9.57 ± 0.40	0.910	8.4-10.2
Ionized serum calcium	Mean ± SD (mg/dL)	4.17 ± 0.19	4.18 ± 0.20	4.16 ± 0.18	0.810	3.9-4.9
Serum phosphorus	Mean ± SD (mg/dL)	3.75 ± 0.53	3.73 ± 0.48	3.76 ± 0.57	0.872	2.5-4.5
Serum magnesium	Mean ± SD (mg/dL)	1.95 ± 0.16	1.99 ± 0.17	1.94 ± 0.16	0.440	1.6-2.6
25-hydroxyvitamin D	Mean ± SD (ng/mL)	22.88 ± 9.35	22.82 ± 7.26	22.92 ± 10.66	0.971	30-100
Parathormone	Mean ± SD (pg/mL)	49.10 ± 21.20	40.60 ± 17.07	54.76 ± 22.15	0.051	15-65
Osteocalcin	Median (Q1, Q3) (ng/mL)	28.67 (17.88, 36.25)	27.60 (15.98, 37.27)	29.23 (18.54, 33.86)	0.457	14-46
Alkaline phosphatase	Mean ± SD (U/L)	82.28 ± 28.09	84.73 ± 32.37	80.80 ± 25.79	0.674	35-129
P1NP	Median (Q1, Q3) (ng/mL)	60.58 (38.89, 84.75)	69.35 (32.84, 72.00)	59.33 (42.35, 84.75)	0.902	20.25-76.31
CrossLaps	Median (Q1, Q3) (ng/mL)	0.50 (0.36, 0.71)	0.53 (0.37, 0.79)	0.50 (0.35, 0.59)	0.476	0.330-0.782

Femoral neck T-score was statistically significantly lower in group F in comparison to group nonF (-1.75 ± 0.72 versus -1.17 ± 0.91, p = 0.029). (Table 4)

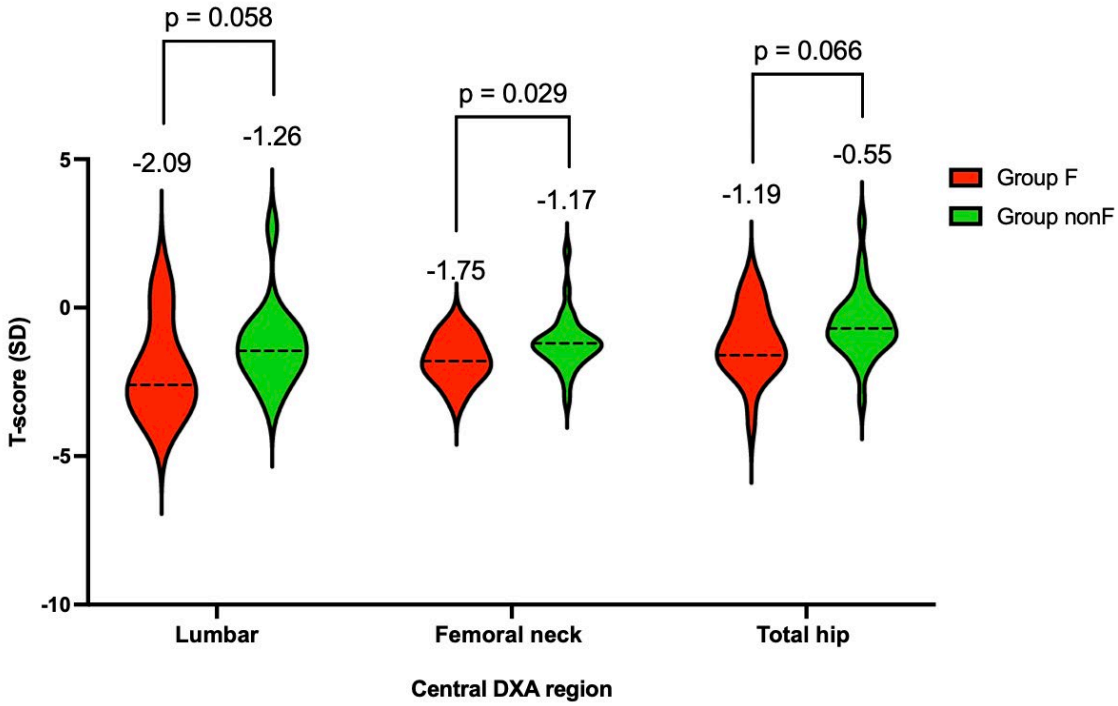
Table 4: Central DXA evaluation in the study population

Parameter	Descriptive statistics (Units)	Entire sample (N = 48, 100%)	Group F (N = 18, 37.50%)	Group nonF (N = 30, 62.50%)	p-value	Normal range
Total serum calcium	Mean ± SD (mg/dL)	9.57 ± 0.38	9.58 ± 0.37	9.57 ± 0.40	0.910	8.4-10.2
Ionized serum calcium	Mean ± SD (mg/dL)	4.17 ± 0.19	4.18 ± 0.20	4.16 ± 0.18	0.810	3.9-4.9

Serum phosphorus	Mean ± SD (mg/dL)	3.75 ± 0.53	3.73 ± 0.48	3.76 ± 0.57	0.872	2.5-4.5
Serum magnesium	Mean ± SD (mg/dL)	1.95 ± 0.16	1.99 ± 0.17	1.94 ± 0.16	0.440	1.6-2.6
25-hydroxyvitamin D	Mean ± SD (ng/mL)	22.88 ± 9.35	22.82 ± 7.26	22.92 ± 10.66	0.971	30-100
Parathormone	Mean ± SD (pg/mL)	49.10 ± 21.20	40.60 ± 17.07	54.76 ± 22.15	0.051	15-65
Osteocalcin	Median (Q1, Q3) (ng/mL)	28.67 (17.88, 36.25)	27.60 (15.98, 37.27)	29.23 (18.54, 33.86)	0.457	14-46
Alkaline phosphatase	Mean ± SD (U/L)	82.28 ± 28.09	84.73 ± 32.37	80.80 ± 25.79	0.674	35-129
P1NP	Median (Q1, Q3) (ng/mL)	60.58 (38.89, 84.75)	69.35 (32.84, 72.00)	59.33 (42.35, 84.75)	0.902	20.25-76.31
CrossLaps	Median (Q1, Q3) (ng/mL)	0.50 (0.36, 0.71)	0.53 (0.37, 0.79)	0.50 (0.35, 0.59)	0.476	0.330-0.782

Lumbar BMD and T-score were borderline statistically significantly lower in group F compared to group nonF (0.927 ± 0.184 g/sqcm versus 1.026 ± 0.164 g/sqcm, p = 0.059, respectively -2.09 ± 1.51 SD versus -1.26 ± 1.37 SD, p = 0.058). (Figure 4)

Figure 4: Violin plot showing mean values of lumbar T-score, femoral neck T-score and total hip T-score in group F and group nonF



The distribution of patients from group F and group nonF was statistically significantly different among the 5-year age groups (p = 0.017). (Table 5)

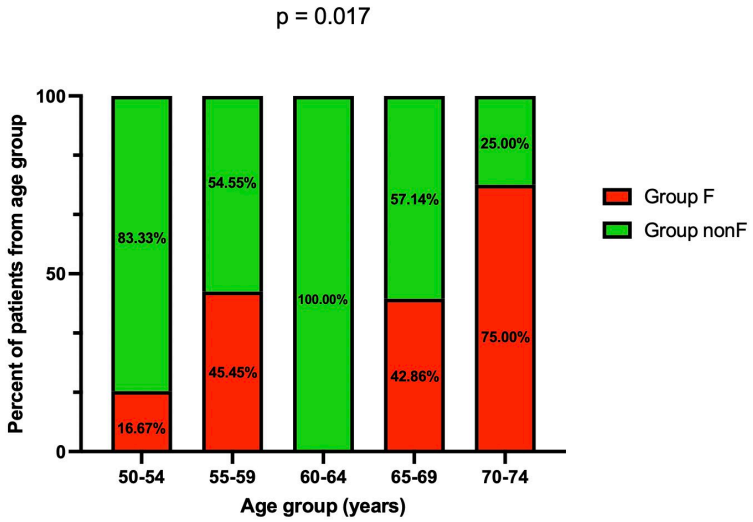
Table 5: Distribution of patients from group F and group nonF within each age group

Age group (years)	N (%)	Group F, N (%)	Group nonF, N (%)	p-value
50-54	6 (12.50)	1 (16.67)	5 (83.33)	**0.017**

55-59	11 (22.91)	5 (45.45)	6 (54.55)	
60-64	9 (18.75)	0 (0.00)	9 (100.00)	
65-69	14 (29.17)	6 (42.86)	8 (57.14)	
70-74	8 (16.67)	6 (75.00)	2 (25.00)	

The highest prevalence was found in the 60-64 years age-group with respect to the individuals from group nonF, while 75% of the subjects from 70-74 years age-group were identified in group F. (Figure 5)

Figure 5: Stacked bar chart showing the distribution of patients from group F and group nonF within the age group

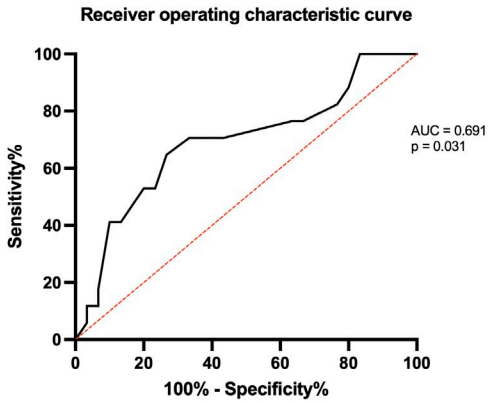


Receiver operating characteristic curve was plotted to determine an optimal femoral neck T-score value to predict the presence of a fragility fracture and it resulted in a value of -1.55 SD with a sensitivity of 64.71 (41.30-82.69) % and a specificity of 73.33 (55.55-85.82) %, with a Youden index of 0.380. (Table 6, Figure 6)

Table 6: Sensitivity and specificity of femoral neck T-score -1.55 SD cut-off value for predicting a fragility fracture

Cut-off value femoral neck T-score (SD)	AUC	Sensitivity (95% CI)	Specificity (95% CI)	Youden Index
-1.55	0.691	64.71 (41.30-82.69)	73.33 (55.55-85.82)	0.380

Figure 6: Receiver operating characteristic curve of femoral neck T-score for predicting a fragility fracture



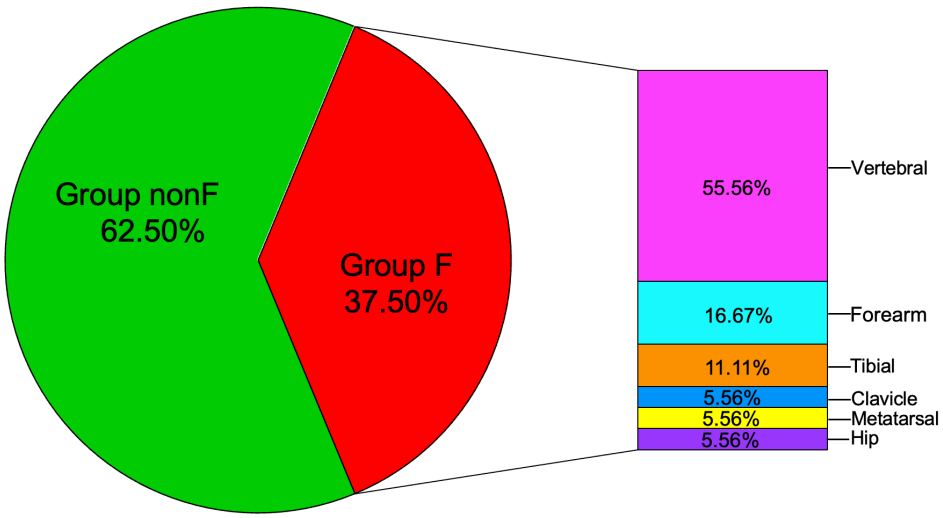
55.56% of patients from group F had vertebral fractures, 16.67% had forearm fractures, 11.11% had tibia fractures, 5.56% had a clavicle fracture, 5.56% had a metatarsal fracture, and 5.56% had a hip fracture. (Table 7)

Table 7: Frequency of fragility fracture location within group F

Fracture location	N (%)
Vertebral	10 (55.56)
Forearm	3 (16.67)
Tibia	2 (11.11)
Clavicle	1 (5.56)
Metatarsal	1 (5.56)
Hip	1 (5.56)

The analysis of WHO categories at central DXA showed that in group F, 55.56% (N = 10) of the patients had osteoporosis, and were equally distributed for each category, namely, osteopenia and normal DXA (22.22% per category, N = 4). In group nonF, the highest prevalence was for osteopenia (66.67%, N = 20), and was equally distributed in the other two categories (16.67%, N = 5). (Figure 7)

Figure 7: Bar of pie chart showing the frequency of fragility fracture location in group F



When analyzing the patients with vertebral versus non-vertebral fractures, the study variables, regardless demographic panel, comorbidities, blood assays and DXA-based parameters, were similar, except for a lower BMD and T-score at lumbar spine in subjects with vertebral versus non-vertebral fractures (0.849 ± 0.151 versus 1.023 ± 0.185 g/sqcm, p = 0.042, respectively, -2.72 ± 1.22 versus -1.30 ± 1.54, p = 0.044). (Table 8)

Table 8: Demographic parameters of the group with vertebral fractures and the group with non-vertebral fractures

Parameter	Descriptive statistics (Units)	Group with vertebral fractures (N = 10, 55.56)	Group with non-vertebral fractures (N = 8, 44.44%)	p-value
Age	Mean ± SD (years)	69.90 ± 10.65	62.75 ± 6.16	0.112
Years since menopause	Mean ± SD	25.60 ± 13.13	16.00 ± 8.25	0.091

Smoker status	N (%)	0 (0.00)	1 (12.50)	0.444
Dyslipidemia	N (%)	6 (60.00)	5 (62.50)	0.914
Type 2 diabetes mellitus	N (%)	2 (20.00)	0 (0.00)	0.477
High blood pressure	N (%)	7 (70.00)	4 (50.00)	0.630
Body mass index	Mean ± SD (kg/sqm)	25.65 ± 2.74	24.75 ± 6.98	0.712
Fasting glycaemia	Mean ± SD (mg/dL)	104.35 ± 28.51	106.13 ± 22.71	0.803
Glycated hemoglobin	Mean ± SD (%)	5.46 ± 0.34	5.30 ± 0.36	0.487
Serum creatinine	Mean ± SD (mg/dL)	0.73 ± 0.17	0.82 ± 0.20	0.125
Serum sodium	Mean ± SD (mmol/L)	141.00 ± 2.16	140.50 ± 1.00	0.689
Serum potassium	Mean ± SD (mmol/L)	4.35 ± 0.33	4.31 ± 0.45	0.903
Total serum calcium	Mean ± SD (mg/dL)	9.70 ± 0.39	9.42 ± 0.28	0.134
Ionized serum calcium	Mean ± SD (mg/dL)	4.27 ± 0.16	4.05 ± 0.19	0.053
Serum phosphorus	Mean ± SD (mg/dL)	3.77 ± 0.50	3.66 ± 0.49	0.716
Serum magnesium	Mean ± SD (mg/dL)	1.95 ± 0.22	2.03 ± 0.13	0.588
25-hydroxyvitamin D	Mean ± SD (ng/mL)	22.78 ± 8.44	22.87 ± 6.03	0.979
Parathormone	Mean ± SD (pg/mL)	41.43 ± 17.29	39.12 ± 18.56	0.820
Osteocalcin	Median (Q1, Q3) (ng/mL)	27.65 (15.11, 36.29)	25.71 (16.77, 40.25)	0.684
Alkaline phosphatase	Mean ± SD (U/L)	86.67 ± 29.44	81.83 ± 39.12	0.789
P1NP	Median (Q1, Q3) (ng/mL)	69.35 (29.59, 71.78)	101.68 (50.35, 153.00)	0.175
CrossLaps	Median (Q1, Q3) (ng/mL)	0.37 (0.30, 0.78)	0.58 (0.46, 0.77)	0.309
Lumbar BMD	Mean ± SD (g/sqcm)	0.849 ± 0.151	1.023 ± 0.185	**0.042**
Lumbar T-score	Mean ± SD (SD)	-2.72 ± 1.22	-1.30 ± 1.54	**0.044**
Lumbar Z-score	Mean ± SD (SD)	-1.05 ± 1.34	-0.10 ± 1.00	0.115
Femoral neck BMD	Mean ± SD (g/sqcm)	0.769 ± 0.082	0.839 ± 0.107	0.146
Femoral neck T-score	Mean ± SD (SD)	-1.97 ± 0.64	-1.44 ± 0.76	0.143
Femoral neck Z-score	Mean ± SD (SD)	-0.23 ± 0.57	-0.24 ± 0.39	0.960
Total hip BMD	Mean ± SD (g/sqcm)	0.823 ± 0.142	0.916 ± 0.130	0.194
Total hip T-score	Mean ± SD (SD)	-1.50 ± 1.12	-0.76 ± 1.03	0.184
Total hip Z-score	Mean ± SD (SD)	-0.01 ± 1.02	0.13 ± 0.57	0.750

DISCUSSION

In this study, we analysed a large spectrum of potential bone and metabolic contributors to falls and further on to osteoporosis and osteoporotic fractures. Our findings showed that the general cardiovascular and glucose profile, including glycaemia and glycated haemoglobin A1c, as well as sodium assays, were similar in group F versus nonF, while age and menopause duration were statistically significant higher (66.72 ± 9.44 versus 60.83 ± 6.07, p = 0.026, respectively, 21.33 ± 11.97 versus 13.70 ± 8.26 years, p = 0.024). Hypo-estrogenic status (since subjects with hormone replacement therapy were ruled out) represents a traditional menopause-related contributor to reduced bone strength, but not necessarily to an elevated risk of falls [20,21]. Age distribution according to five-year age-groups was statistically significantly different (p = 0.017); in the 70-74 years interval, 75% of the individuals were from group F, while in the 50-54 year interval, 83.33% of them were from group nonF. Of note, type 2 diabetes had a relatively low rate (varying between 4.17% in the entire cohort to a maximum of 11.11% in group F) at an average body mass index of 25 kg/sqm in each sub-group. On the contrary, arterial hypertension was registered in more than 60% of the people, a condition that may be complicated by falls in cases with diurnal variations of the blood pressure [22,23].

The analysis of the (blood) mineral metabolism and bone turnover markers pinpointed a similar landscape in patients with fractures when compared to those who were fracture-free. Vitamin D status, as a potential interplay with falls and fractures, highlighted an overall insufficiency (with average values in between 20 and 30 ng/mL). We did not quantify the daily vitamin D replacements since the available 25-hydroxyvitamin D represents the best pointer of the overall D-metabolism in individuals with normal kidney function and who are hyperparathyroidism-free [24-26].

This study also showed that, among the panel of analysed parameters, DXA-BMD remains the main discriminator: femoral neck T-score was lower in group F versus nonF (-1.75 ± 0.72 versus -1.17 ± 0.91, p = 0.029), while receiver operating characteristic curve of femoral neck T-score for predicting a fragility fracture showed an area under the curve of 0.691 (p = 0.031). Moreover, patients with prevalent vertebral fractures had a statistically significant lower lumbar BMD and T-score versus those with non-vertebral fractures (0.849 ± 0.151 versus 1.023 ± 0.185 g/sqcm, p = 0.042, respectively, -2.72 ± 1.22 versus -1.30 ± 1.54, p = 0.044). These DXA-derived results confirm the general data from literature, as well as the fact that the most prevalent fractures in a population with a mean age of 63.04 ± 7.95 years, vertebral fractures represent the most frequent site [27,28].

When it comes to active fracture prevention, the presence of an osteoporotic fracture clearly indicates the initiation of an anti-fracture drug, and, in this cohort, we identified patients who were anti-osteoporosis medication candidates, yet they were not treated so far. This treatment gap represents a major burden of the osteoporotic landscape nowadays, even in developed countries [29,30]. Notably, our country has one of the lowest rates of DXA devices per population, and this might also contribute to the delay of medication initiation [19,30]. Also, the reimbursement of medication to reduce the fracture risk is applied based on DXA results, specifically, the diagnosis of osteoporosis, and, in this instance, only 55% of the patients were identified with osteoporosis at DXA in group F. On the other hand, osteoporosis (uncomplicated with fractures) was also confirmed to have a lower prevalence (of 16.67%) in group nonF, and this finding requires drug initiation as the primary fracture prevention level in this instance. In addition to controlling the risk factors/associated ailments and improving the BMD, fall prevention also includes complex rehabilitation programs, as well as home care devices/services for surveillance [31-33].

A recent study published in 2025 showed that the prediction of a hip fracture as provided by the femoral neck bone mineral density in females, respectively, males is of 66.9%, respectively, of 66.5% (mean female population age of 72.5 years, respectively, of 72.3 years for males) [34]. Of note, hip represents a better predictor of hip fractures than lumbar spine bone mineral density, which, otherwise, stands for a better predictor of vertebral fractures [35]. Recently, predictive models based on deep learning machines were proposed for further study as replacements for single bone mineral density at DXA in order to achieve an adequate prediction of fragility fractures [36]. Moreover, Prince et al. [37] showed that other DXA parameters (than bone mineral density/T-score) might help de prediction of a femoral neck fracture: for instance, in 69 individuals who suffered a femoral neck fracture within the following 10 years, respectively, 59 participants who underwent a trochanter fracture (versus controls who were fracture-free) areal bone mineral density at baseline hip DXA was 7% lower at femoral neck for the first category of study population, respectively, 15% lower for the second category [37].

Limits of the study and further research

As a limitation, we mention the sample size and the retrospective design. Of note, we applied numerous exclusion criteria in order to obtain an adequate analysis of this real-life setting and to eliminate the bias that comes from integrating the detection of a fragility fracture upon a recent fall. Generally, the falls, as potential contributors to the fracture risk, are less likely to be clarified in daily practice, nor controlled, since numerous elements might be involved, such as, in this case, diabetes, high blood pressure, or even older age. Osteosarcopenia might be co-present, too, in these patients, and hence, the assessment of the muscle status in addition to a low BMD might help the understanding of this pathogenic loop in falls [32,33].

CONCLUSION

Menopausal women who suffered a fall during the last 12 months and who were further identified with an osteoporotic fracture were older and showed a longer menopause duration when compared to those who were fracture-free. The prevalence of type 2 diabetes and high blood pressure was similar, as well as vitamin D and bone turnover markers. A lower BMD was found at the femoral neck, while the individuals with vertebral fractures (which were the most common types) showed a lower lumbar BMD versus those with non-vertebral fractures. Further prospective, larger trials are necessary to pinpoint the burden of falls in real-life settings and to address the anti-osteoporotic therapy gaps.

Supplementary Materials

Not applicable.

Author Contributions

Conceptualization, M.-L.C., O.-C.S., M.K., M.C., E.-M.P., D.-M.P., and A.V.; methodology, M.-L.C., O.-C.S., M.K., M.C., E.-M.P., D.-M.P., and A.V.; software, M.-L.C., O.-C.S., M.K., M.C., E.-M.P., D.-M.P., and A.V.; validation, A.V.; formal analysis, M.-L.C., and A.V.; investigation, M.-L.C., O.-C.S., M.K. (radiologic assessment), M.C., E.-M.P. (radiologic assessment); resources, O.-C.S., and M.C.; data curation, M.-L.C., D.-M.P., and A.V.; writing—original draft preparation, O.-C.S., and M.C.; writing—review and editing, M.-

L.C., D.-M.P., and A.V.; visualization, M.-L.C., O.-C.S., D.-M.P., and A.V.; supervision, M.-L.C.; project administration, A.V.; funding acquisition, M.-L.C., O.-C.S., M.K., M.C., E.-M.P., D.-M.P., and A.V. All authors have read and agreed to the published version of the manuscript. No generative AI was involved in the production of this article.

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Institutional Review Board Statement

(ethical approval 33 from 09/29/2025)

Informed Consent Statement

Not applicable due to the retrospective design of the study.

Data Availability Statement

All available data are within the article.

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Conflicts of Interest

The authors declare no conflict of interest.

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REVIEW

Current Trends in Quantum Technologies for Advancing Bioimaging Techniques

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Abstract: Abstract Quantum computing (QC) has emerged as a transformative technology with the potential to surpass classical computational limits, especially in complex domains like medical imaging. This study investigates the integration of QC and quantum machine learning (QML) into medical imaging, with a focus on MRI, EEG, and CT. A structured literature review was conducted to identify recent developments, focusing on studies using accessible open-source data and English-language peer-reviewed publications. By leveraging quantum principles such as superposition and entanglement, hybrid quantum-classical models have demonstrated enhanced accuracy, speed, and efficiency in diagnostic tasks. In MRI, QML has improved early detection and classification of neurological diseases like Alzheimer's and brain tumours. In EEG analysis, quantum algorithms such as Quantum EEGNet and Quantum Support Vector Machines have shown superior performance in detecting disorders, including schizophrenia and autism spectrum disorder. Additionally, quantum algorithms for CT image reconstruction and classification have achieved faster processing with fewer artifacts and greater fidelity. Despite current hardware constraints, results highlight the promise of QC in addressing existing limitations in medical diagnostics. The findings support continued development of QML models and quantum-enhanced workflows, with potential to revolutionise clinical practice by offering more accurate, efficient, and personalised care in neurological imaging and analysis.

Keywords: Quantum Machine Learning; Convolutional Neural Network; Quantum Neural Network; Deep Learning; MRI; EEG; CT

INTRODUCTION

Quantum computing (QC) represents an emerging field that applies quantum-mechanical principles to complex computational problems, with the potential to surpass classical computing capabilities. [1]. The concept of QC was postulated in the 1980s in an article written by Nobel Laureate Richard Feynman, who suggested that the study of simulating quantum systems requires the construction of a quantum computer [2]. Moving forward, Peter Shor developed an algorithm presented at the 35th Annual Symposium on Foundations of Computer Science, which demonstrated that quantum computers could factor large numbers faster than any classical computer, showing potential in important domains such as cryptography [3]. Therefore, despite its promising capabilities, quantum computing raised significant concerns—particularly regarding its potential to compromise sensitive information in areas like cybersecurity [4] and data privacy [5]. In the present, QC found its purpose in diverse domains such as drug discovery and molecular simulation [6], machine learning and artificial intelligence [7], financial modelling in trading and investment [8], or climate prediction, astrophysics, and particle physics [9]. Following the success of Variational Quantum Eigensolver simulations used in the process of molecular development [10], scientists expanded the use of quantum technology capabilities to other medical domains, such as imaging techniques. Medical imaging is facing increasing pressure to deliver faster, more accurate diagnoses amid rising scan volumes and clinician shortages, driving a clinical need for automation and efficiency. At the moment, machine learning (ML) and deep learning (DL) have become essential tools, offering superior performance in image classification, segmentation, and disease detection, comparable to human expertise [11]. However, the continuous need for a better signal processing technique, which would be capable of offering higher resolution results in the domain of Magnetic Resonance Imaging (MRI) [12] or Electroencephalogram (EEG) [13], led to the development of hybrid quantum

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algorithms in medical imaging. Although quantum imaging is not directly dependent on QC, ongoing research in the field offers considerable promise for improving diagnostics through more precise and non-invasive medical assessments.

This review investigates the emerging role of QC and quantum applications in advancing medical imaging technologies, particularly in MRI and EEG signal processing, by examining how developments in quantum algorithms and hybrid computation have the potential to overcome classical limitations in diagnostic resolution, speed, and accuracy. It also investigates the broader context in which QC evolved—from theoretical foundations and cryptographic challenges to its current applications in molecular simulation and biomedical innovation.

MATERIALS AND METHODS

A meticulous medical literature search was conducted using the Web of Science scientific database between July 8th and July 12th 2025, targeting articles published within the last 5 years. The search strategy utilised various keyword combinations, including: “quantum computing”, “MRI”, “EEG”, “Quantum imaging”, “quantum mechanics”, “quantum phenomena”, and “machine learning”. The initial screening of all retrieved articles was conducted by reviewing their titles and abstracts to evaluate their relevance to the research topic. Studies were considered eligible for full-text analysis if they met specific inclusion criteria: written in English, full-text accessible, open access and categorised as narrative reviews or original articles related to current progress in the development of QC or quantum algorithms in medical imaging, the basics of quantum mechanics and quantum machine learning. Any articles falling outside these parameters were excluded from further evaluation. Each stage of the review was carried out independently by the researchers.

RESULTS AND DISCUSSION

Machine learning and the development of quantum hybrid systems

ML represents a subfield of AI that enables systems to learn from data and improve their performance on a specific task without being explicitly programmed for each decision. The techniques are classified into 3 categories: supervised learning, unsupervised learning, and reinforcement learning. ML algorithms are being increasingly adopted in medical imaging and pathology to enhance the speed and precision of disease diagnosis. Convolutional Neural Networks (CNNs), in particular, have become widely used for interpreting medical images from modalities such as X-rays, CT scans and MRIs [14]. These models are capable of autonomously identifying and categorising abnormalities such as tumours and lesions, supporting clinicians and radiologists in making faster and more accurate diagnostic decisions [15]. By learning from extensive collections of labelled medical images, ML systems can detect subtle patterns that may escape human observers, contributing to earlier diagnosis and potentially better patient outcomes.

The field of Quantum Machine Learning (QML) has emerged from the combination of AI and ML to improve algorithmic efficiency [16]. It builds upon the principles of quantum computing to enable inherent solution parallelism [17], facilitating optimal constraint solving in line with Moore’s law [18]. Quantum algorithms rely on Boolean algebra operators (such as OR, AND, NOT gates) alongside principles of quantum physics. Data representation in these systems is based on quantum bits (qubits), which are grounded in the theoretical properties of electron spin [19]. Notably, quantum computing allows for the encoding of information beyond classical binary states, including complex and negative values. Furthermore, taking advantage of quantum phenomena such as superposition and entanglement offers the potential for substantial speedups in performing ML tasks compared to traditional approaches. Quantum algorithms can accelerate essential operations such as matrix manipulation, which supports many techniques, including clustering and principal component analysis [20]. Adaptation of classical algorithms such as support vector machines [21] and neural networks [22] into quantum frameworks has shown promise in improving computational efficiency, especially for high-dimensional datasets. In fields like healthcare, where data is often vast and intricate, QML may dramatically enhance processes such as medical image analysis, genomics, and drug discovery [23],[24].

Quantum algorithms in MRI

MRI is a critical diagnostic tool for neurological disease, offering detailed visualisation of brain structure and function. In Alzheimer’s disease (AD), MRI helps detect early atrophy in key regions such as the hippocampus [25]. A model for the diagnosis and classification of different grades of severity (non-demented, mild demented, moderate demented, and very mild demented) of AD was conceived using a QML classifier and 5-qubit quantum hardware or simulator. The presented model surpassed the classical Support Vector Machine in both classification accuracy and training speed, especially when dealing with low-resolution MRI data, but further investigation is needed to assess its feasibility for integration into medical diagnostic systems [26]. The development of this technology would innovate the domain of diagnosis for AD, as the self-reported cognitive assessments commonly used are susceptible to response bias and misclassification [27],[28]. In order to detect mild early-stage cognitive impairment, Choi et al. used functional MRI technology and correlated it with a hybrid quantum-classical algorithm. In classical simulations, the hybrid model outperformed traditional CNNs in balanced accuracy and highlighted two brain regions (the right hippocampus and left parahippocampus) associated with cognitive decline [29]. For brain tumours, MRI provides precise localisation and tissue characterisation, essential for diagnosis and surgical planning [30]. Rajamohana et al. proposed a method that integrates classical and hybrid quantum-inspired graph neural

networks for tumour classification. The hybrid Quantum Graph Neural Networks achieved performance metrics on par with advanced classical neural networks, with training accuracy rising from 41% to 64% and validation accuracy improving from 31% to 52%. These results highlight the model's effectiveness in differentiating between normal and tumour images [31]. In tuberous sclerosis complex (TSC), MRI reveals cortical tubers and subependymal nodules, aiding early diagnosis and monitoring [32]. Lin et al. created a quantum neural network called QR-Net, which integrated CNNs with a quantum neural network layer for TSC classification. The model utilised two layers, the first one converting classic input into a quantum state, and a second one that used entanglement and qubit interactions to process the states. Compared to traditional 3D-ResNet models, QR-Net demonstrated superior performance in classifying TSC-related epilepsy, achieving higher accuracy and AUC scores across the study cohort [33]. MRI also plays a key role in Parkinson's disease by identifying subtle changes in the basal ganglia and brainstem [34]. MRI's high-resolution structural and functional insights are indispensable in understanding, diagnosing, and managing these complex diseases.

Quantum algorithms in EEG

EEG is a widely utilised, non-invasive method for recording the brain's electrical activity, playing a key role in both clinical diagnostics and neuroscience research. It has been essential in exploring brain function, identifying neurological disorders, and advancing brain-computer interface technologies. While traditional analysis approaches, such as ML and DL models like EEGNet, have shown strong performance across various EEG applications, they may struggle to fully capture the intricate, high-dimensional patterns inherent in EEG signals [35].

Quantum-EEGNet (QEEGNet) is an innovative hybrid neural network that combines quantum computing with the classical EEGNet framework to enhance EEG signal representation and analysis. By embedding quantum layers into the architecture, the model aims to capture more complex patterns in the EEG data, offering potential computational benefits. Evaluated on the BCI Competition IV 2a dataset, QEEGNet outperformed the traditional EEGNet across most subjects and showed greater robustness to noise, underlining the promise of quantum-enhanced models in advancing EEG-based research and applications [35]. Furthermore, another hybrid quantum-classical neural network model that integrates a variational quantum circuit into a deep neural network for EEG, electromyogram, and electrocorticogram analysis showed state-of-the-art competence with a small number of parameters for the variational quantum circuit [36].

Even though EEG is not currently involved in the diagnosis of Schizophrenia or Autism Spectrum Disorder (ASD) as a standalone diagnostic tool, it plays an increasingly important role in research and adjunctive clinical assessment. Aksoy et al. evaluated the performance of QML models, particularly Quantum Support Vector Machines (QSVM), for the early diagnosis of schizophrenia using EEG data. EEG signals from four selected channels were decomposed into five frequency sub-bands via Discrete Wavelet Transform (DWT), and statistical features were extracted from each sub-band to construct the feature set. Principal Component Analysis (PCA) was applied for dimensionality reduction, and the resulting features were encoded into quantum states using various feature maps for input into QSVM. The results demonstrated that, despite the limitations of current quantum hardware, QSVM achieved state-of-the-art classification accuracy, even outperforming classical algorithms, while utilising a reduced number of qubits. The study also highlights the potential benefits of QML in handling noisy EEG data and suggests further investigation into feature map configurations, quantum-classical hybrid models, and implementation on real quantum devices [37]. Another study investigated the use of EEG-based biomarkers for ASD classification, emphasising the selection of optimal electrode pairs and the application of advanced ML techniques. EEG signals from electrode combinations C3-C4, C3-Cz, and C4-Cz were analysed, with C4-Cz demonstrating superior performance when paired with QSVM. Feature extraction included peak frequency, Stockwell transform coefficients, and peak-to-peak amplitude, enhancing the discriminative power of the input data. The QSVM model, using amplitude embedding, achieved a classification accuracy of 98.9%, outperforming classical support vector machine baselines. These results highlight the potential of QML in EEG-based ASD diagnostics and point to future directions involving MEG integration, neural structured learning, and large-scale population studies [37].

Quantum algorithms in CT

The use of quantum technology in CT doesn't involve novel discoveries in the diagnosis of neurological diseases but presents different improvements in the domain of image acquisition [38], accuracy [39], processing [40], and storage [41]. Jun et al. present a quantum image reconstruction algorithm aimed at enhancing the fidelity of CT scans by mitigating artifacts inherent in classical reconstruction methods such as filtered back projection. The algorithm encodes the unknown CT image into a quantum state represented by qubits and minimises the discrepancy between the experimentally acquired sinogram and the Radon transform of the quantum state using a global energy optimisation framework. Implemented on gate-based quantum computers or quantum annealers, the method leverages quantum parallelism to efficiently explore the solution space. It is applicable to various CT configurations, including cone-beam geometries, and operates independently of the light source, provided the projection data is well-defined. This approach enables high-accuracy reconstruction with reduced artifacts, representing a significant advancement in quantum-enhanced medical imaging [38]. For the improvement of CT processing speed, Sengupta et al. proposed a model for classifying COVID-19 from CT images, distinguishing it from non-COVID pneumonia cases. Leveraging the efficiency of quantum simulation, the quantum neural network (QNN) achieved faster convergence and superior performance on large-scale, biased image classification tasks. Compared to classical DL models, the QNN showed an accuracy improvement of over 2.92% and an average recall of 97.7%. Training runtime on quantum-

optimised hardware was 52 minutes, significantly faster than the 1 hour and 30 minutes required on a K80 GPU. These results highlight the potential of QNNs in enhancing medical image classification, particularly in high-stakes diagnostic contexts like COVID-19 [40].

CONCLUSION

The integration of ML with quantum computing has opened new frontiers in the analysis and interpretation of complex medical data, especially in the field of neurological diagnostics. Traditional ML models, such as CNNs and SVMs, have already demonstrated significant success in tasks like medical imaging and EEG signal analysis. However, the emergence of QML offers the potential to further enhance computational efficiency, accuracy, and speed by leveraging quantum principles such as superposition, entanglement, and quantum parallelism.

Across multiple imaging modalities (MRI, EEG, and CT) hybrid quantum-classical models have consistently outperformed or matched their classical counterparts, often with fewer parameters and faster training times. From Alzheimer's and Parkinson's disease to epilepsy, brain tumours, and developmental disorders such as ASD, QML approaches have enabled more sensitive and specific classifications by uncovering patterns not easily discernible with conventional methods. Furthermore, advancements in quantum algorithms for CT image reconstruction and classification demonstrate the versatility of QML beyond diagnosis, extending into image enhancement and processing.

Despite current hardware limitations, these early successes underline the transformative potential of quantum-enhanced ML in medical diagnostics. Continued research into hybrid architectures, feature encoding strategies, and real-device implementation will be crucial for translating experimental results into clinical practice. As quantum hardware matures, the integration of QML into medical imaging and brain signal analysis promises to revolutionise diagnostic workflows, offering more accurate, timely, and individualised care.

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The authors declare no conflict of interest.

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Authors' contribution

Conceptualization, Carmen Adella Sîrbu; methodology, Carmen Adella Sîrbu; investigation, Vlad Buica; writing—original draft preparation, Vlad Buica; writing—review and editing, Carmen Adella Sîrbu;

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REVIEW

Diagnostic and Therapeutic Principles for Burns Involving Functional Areas: A Comprehensive Overview

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Abstract: Burns that affect functional areas present unique challenges in terms of diagnosis and therapy due to their particular mobility and aesthetic importance. In this article, we summarize the current principles in the diagnosis, treatment, and rehabilitation of burns that affect these regions. In this regard, the recent medical literature (clinical evidence) was analyzed to outline management strategies specific to each region. Early and correct evaluation of lesions, along with individualized treatment are essential for maintaining mobility and preventing sequelae. The results obtained are applicable in both civilian and military environments. Superficial burns respond well to conservative care with dressings, splints, and physiotherapy on the one hand, and on the other hand, deep burns benefit from excision and early grafting, the standard modern procedure that shortens hospitalization and greatly improves results. Long-term recovery depends on scar modulation, reconstructive surgery, and multidisciplinary rehabilitation addressing both functional and psychological aspects. Treatment of the burns of functional areas requires prompt, tailored, and multidisciplinary management to restore function and minimize disability. Standardized treatment algorithms and expanded research on long-term rehabilitation remain essential for improving burn care outcomes.

Keywords: burns, functional areas, face, hands, feet, perineum, joints, rehabilitation, reconstructive surgery, scar management

INTRODUCTION

Burns represent one of the most important types of trauma, being a public health problem and causing over 180,000 victims annually. The impact of this trauma is significant in civilian activity and much more important and frequent in military activity or in the case of conflagrations. The American Burn Association (ABA) and its European equivalent (EBA) have clearly mentioned as a severity factor the involvement of functional areas in burns, regardless of whether they have a large total affected area or not. Therefore, the need arose for an analysis of the specialized literature in the civilian field to evaluate and stratify the most recent recommendations on the treatment of these important and sensitive regions. Articles such as those mentioned above allow the development of a set of recommendations on the treatment of functional areas such as the hands, face, perineum, feet, and major joints, both in the civilian environment, where they were developed, and in the military field, by extrapolation, the pathology in question being similar.

MATERIALS AND METHODS

Purpose of the study

In this study, we sought to find the main therapeutic methods recently used for the surgical treatment of functional areas. Their treatment is of particular importance because these areas have complex functionality and often a major aesthetic impact, while a mediocre or a bad result would lead to a low quality of life for the surviving patients. The quality of the surgical treatment, despite not being the only aspect, is a decisive one in increasing the quality of life. The physiokinetotherapy and the psychological support are equally important, but do not constitute the subject of the

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current research. Databases used: We used the most recent articles on this subject that have appeared in the specialized literature during the last years in WoS and PubMed, comparing this data to the general principles that still apply and that have been described in previous years. Data selection: We have selected only the articles in English regarding the treatment in the field of Plastic Surgery, both in the surgical period and in the rehabilitation period. We excluded the "Case Report" articles and those not concerning the functional areas due to their low relevance. The result of this research was a significant number of articles from which the most relevant and recent references were kept, resulting in 69. Data extraction: From the aforementioned sources, the surgical procedures and techniques were extracted, with the most indications from various authors based on the consistently good results obtained in the field of burns treatment and research. Other possible therapeutic options were not ignored either, as alternative solutions may be very useful in the more complex cases that affect multiple functional areas, with severe implications that derive and which also require a staged treatment. We also highlighted a series of very new surgical treatment procedures that seem promising and may become standard in the future, and also added the experience of the research group that conducted this study. Data analysis: The data obtained were structured on anatomical regions, so that for each of them, the particularities and therapeutic solutions are clear and nuanced. We have stated the therapeutic possibilities with the most indications and also other possible options. This enumeration can become a therapeutic algorithm for this pathology, with both civilian and military applicability.

RESULTS

1. THE BURNED HAND

The hand represents an anatomical region of particular importance due to its function in daily activities. It also plays a major role in communication and the expression of emotions, as well as from an aesthetic standpoint, since it is difficult to conceal. Its function is absolutely indispensable, especially in today's digital age, where any impairment of the hands could compromise or hinder interaction with the surrounding environment, leading to social and intellectual isolation of the individual suffering from burns in these areas [1]. This area is often the most frequently affected in cases of major burns, with hand burns occurring in over 80% of cases [2,3]. Due to the severe implications and the frequency of injury, it is natural and essential that these burns are included among the main admission criteria of the American Burn Association (ABA). The loss of hand function has a significant impact, and patients require assessment in specialized centers, as such injuries often lead to major disabilities, rendering the patient unable to perform their occupation or carry out daily activities [1]. Despite the fact that they account for less than 3% body surface on each side, the major impact of hand burns arises from the morbidity they can cause. Therefore, the therapeutic approach applied in these cases is crucial, as prioritization of these areas is often neglected. Treatment must begin immediately after the injury occurs and may involve a surgical approach - such as performing decompression incisions - or an initial conservative approach, followed later by other treatment methods, including covering the resulting defects, immobilization with splints (either for posture or to prevent shearing of grafted areas), closely accompanied by physiokinetotherapy with the main objective of preservation and restoration of hand function [2,4]. In a study conducted by Sheridan et al., normal hand function was successfully regained in 97% of patients with superficial partial-thickness burns and in 81% of those with deep partial-thickness or full-thickness burns [5].

Epidemiology

A retrospective study conducted in a major burn center in Southwest China over a five-year period (2012–2017) supports the data mentioned above, providing a series of statistical findings focused mainly on hand burn injuries [6]. Out of a total cohort of 470 patients, the majority affected were male, 73.62%, while the most frequently represented age group was children under 10 years old, accounting for 29.57%. Hand burns occurred in 60.21% of cases outside the workplace, and their frequency increased significantly during the cold season, over a 3–4 month period from December to March, representing 55.11% of all burns recorded in a year. The etiologic agents were flames in 40.42% of cases, electrical current in 30.85%, and hot liquids in 20.21%. Another valuable piece of information highlighted once again is the impact that burn depth and the lack of immediate cooling of the burned area have on unfavorable outcomes, increasing the risk of amputation and prolonging the hospitalization period. In conclusion, this study emphasizes not only the importance of injuries to functional areas, in this case, the hands, but also the crucial role of prevention in influencing the course of burn injuries [6].

Clinical and Pathological Features

Hand burns often occur as a protective reflex, when patients instinctively use their hands to shield their heads from flames or hot liquids. As such, most burns are located on the dorsal side of the hands and less so on the palmar side. Another important aspect to take into consideration is the difference between the dorsal and volar skin. The dorsal skin of the hand is thinner and more mobile, allowing flexion movements at the joints but also carrying a higher risk of deeper burns. In contrast, the volar skin is more resistant to pressure and significantly more resistant to thermal energy due to its greater thickness and well-developed stratum corneum. It adheres tightly to the palmar aponeurosis and contains a high density of sensory nerve endings (Meissner, Merkel, Vater-Pacini corpuscles), which explains the major sensory deficits that may result from injury to this region [7,8]. Regardless of the affected side, another anatomical peculiarity lies in the short distance between the skin surface and the underlying structures - blood vessels, nerves, tendons, and joints, making them particularly vulnerable to thermal injury [2].

Therapeutic Principles

The treatment of hand burns must meet several essential conditions: [2]

- Prevention of wound deepening and of new wound formation
- Rapid coverage of the burn wounds
- Early functional rehabilitation with preservation of active and passive movements
- Prevention of infection
- Prevention of loss of functional structures

Treatment Principles: [2]

- Assessment of the burn's size and depth
- Application of dressings appropriate to the burn degree
- Determination of treatment indication - conservative versus surgical
- Surgical treatment - including escharotomy, necrectomy, and reconstructive procedures with grafts or flaps
- Early physiokinetotherapy
- Use of posture splints
- Secondary and tertiary surgical corrections - when indicated

The first stage in the treatment of major burn patients involves a thorough examination to assess the depth of the lesions. Based on this evaluation, the appropriateness of surgical treatment is determined, or a conservative approach is chosen. Although clinical evaluation is often relied upon, it has been shown to be accurate in only 60–75% of cases, even when performed by an experienced plastic surgeon specializing in burns [2]. There are also adjunct methods to objectively assess burn depth, offering a broad range of options - from biopsy and histological evaluation to perfusion measurement techniques such as angiography, thermography, video microscopy, and laser Doppler imaging [9–15]. Since burn injuries represent an area of major clinical interest, numerous studies have addressed this topic for over 50 years. Treatment can be both conservative and surgical, with long-standing debates regarding the choice between them and the timing of surgical intervention - early excision and grafting versus delayed grafting.

Surgical Treatment

The first studies described since 1982 the importance of early excision and grafting in third-degree burns with limited surface area, while emphasizing that in extensive burns, priority should be given to covering large areas and minimizing fluid and heat loss [16,17]. This concept is still applied and studied in our days. A study performed in 2019 compared the effects of early versus delayed excision and grafting. Conducted between January 2013 and December 2015, the study included 50 patients with bilateral grade IIB hand burns. Postoperative follow-up over a 3-month period assessed both objective and subjective outcomes using the Michigan Hand Questionnaire. Variables analyzed included hand function, pain scale, limitation of daily activities, overall patient satisfaction, and aesthetic outcomes. Although graft take rates were similar between groups, early excision and grafting proved superior in terms of patient satisfaction, significantly shorter hospital stays, and lower treatment costs [18].

Conservative Treatment

One of the most important issues in hand burn management is the evolution toward the sequelae stage, characterized by joint contractures that limit finger movement. The main causes leading to this stage include the initial trauma (its intensity and duration of exposure), wound infection, improper wound coverage, prolonged immobilization, and incorrect positioning of the hand during immobilization [2]. Often, as a result of edema secondary to trauma and fluid resuscitation, a series of intrinsic minus-type deformities may develop: flexion of the radiocarpal joint, hyperextension of the metacarpophalangeal joints, and flexion fixation of the interphalangeal joints, with the thumb fixed in an adducted position [19]. This underscores the importance of maintaining the hand in an optimal "intrinsic plus" position from the very first day: the wrist flexed at 20–30 degrees, metacarpophalangeal joints flexed at 80 degrees, full extension of the interphalangeal joints, and maximal abduction of the thumb. In alert and cooperative patients, immobilization is often necessary only during the night, provided that active and passive physiotherapy is performed twice daily. This approach can significantly reduce the incidence of permanent contractures and deformities [20].

Sequelae Stage

Treatment of the sequelae stage should ideally begin from the very first moment, through prevention. However, if this stage develops despite all preventive measures, several therapeutic methods can be used to limit the formation of hypertrophic scars. Among the predisposing factors for hypertrophic scar formation are: young age, secondary infection, skin tension, anatomical location (certain areas such as the axilla, neck, and shoulder have a higher risk), and burn depth, with higher incidence in deep partial-thickness and full-thickness burns [21,22]. The treatment of choice is often surgical, involving excision of scars or modification of tension vectors to lengthen the restrictive scars that may otherwise fix joints in unfavorable positions. Modern adjunctive methods include the use of lasers, which aim to improve scar texture, thickness, color, and local pruritus [23].

Reconstruction of the hands

The main hand sequelae were described by Achauer and include: claw deformity, palmar contractures, web space deformities, hypertrophic scarring, impairment resulting from amputations, and nail bed injury. Treatment of the sequelae phase is typically undertaken after full scar maturation, since premature intervention may lead to increased inflammation and additional scar formation. Therefore, surgical treatment generally begins six months after the injury [23]. Most hypertrophic scar contractures are treated using local flaps, with Z-plasty being the technique of choice - commonly performed at 60-degree angles, thereby lengthening the scar by approximately 75% [24]. These flaps are robust and viable, provided they are not excessively thinned and that the underlying subcutaneous tissue is preserved. To reduce local morbidity, surgeons prefer performing multiple smaller flaps rather than fewer large ones, which are generally indicated for other areas such as the axilla [23]. Z-plasty can also be used to treat web space deformities or in the palmar area where tendons or bone structures are exposed, and skin grafts cannot cover the post-excisional defects. More severe cases may require the use of distant flaps [25-28]. An interesting aspect related to patient perception concerns objective measurement tools for evaluating hand recovery. According to the American Society for Surgery of the Hand (ASSH), the Total Active Motion (TAM) is calculated for each finger by summing the degrees of active flexion and subtracting the sum of extension deficits across all joints. Although a normal value is around 260 degrees, patients often tolerate moderate decreases well and may still rate their hand function as “good” or “almost as good as before the injury.” Literature suggests that even a TAM value of 112 degrees is sufficient for performing most daily activities. Thus, patients may experience a notable reduction in this objective measure without perceiving a functional deficit in everyday tasks [29].

2. CEPHALIC EXTREMITY

The face represents an area of great interest, being the second most frequently affected functional region in burn injuries. The treatment of deep facial burns presents numerous challenges due to the anatomical complexity and functional diversity of this relatively small region. No standardized treatment protocol has been established, and as a result, sequelae often appear long after the trauma, frequently with severe consequences [30]. There has long been controversy regarding the choice between conservative and surgical treatment. In this regard, several studies have been conducted comparing final aesthetic outcomes and functional considerations [31]. One such study divided patients into four groups: those treated conservatively, healing within 21 days, those healing in more than 21 days, the third group underwent excision and grafting within 18 days, and the last group was operated upon more than 18 days after the injury. The best overall outcomes were achieved in the group of patients who healed conservatively within 21 days. Among the remaining groups, better results were obtained in grafted patients, who showed superior aesthetic outcomes. With regard to functional outcomes, no significant differences were observed between the groups. The most common complications were microstomia and ectropion, both reported in 17 of the 27 patients included in the study [31]. A 2012 study analyzed data collected over 12 years (1999–2010), focusing on full-thickness skin grafting for multiple facial burns involving several aesthetic units. The scalp was most commonly used as the donor site, providing an excellent color and texture match with the recipient areas. However, many authors have also noted long-term complications such as: significant loss of sensation, ectropion, gaps between the grafted regions and the hairline, visible graft edges, and hypertrophic scarring in the perioral area extending to the chin [32].

Periocular Burns

Numerous studies have demonstrated the importance of these anatomical regions as they are frequently involved in facial burns [30,33]. A study conducted by Still et al. (1995) described that 230 of the total of 1527 patients (representing 15.06%) required an ophthalmologic consultation either due to the burns or due to preexisting pathologies. 9.36% of the patients required medical care, with half of them having bilateral involvement, 11 patients requiring skin grafting or tarsorraphy, thus highlighting the incidence and the sequelae that require a close collaboration with the ophthalmologists [12, 34].

Nasal Burns

The nose is a very important anatomical region for both its functional and aesthetic aspects. Because of its location, it forms a very visible and complex aesthetic unit with multiple subunits. Aside from the aesthetic aspect, just as important is the functional role that it has, the reconstruction being an important issue. This three-dimensional structure is a complex one that requires substantial skills and experience for an adequate reconstruction. As such, satisfactory results are attained only after several staged procedures that achieve social reintegration and good functionality as well [35-37]. Despite the fact that its importance is indisputable when trying to establish a reconstructive algorithm, this region falls in sequence after the treatment of the periorificial regions [38,39].

Orofacial Burns

Partial-thickness burns in these regions may be treated by conservative means, but this option is severely limited in the case of full-thickness burns. A 2015 study by Clayton et al. compared a group of 12 patients with full-thickness burns of the oral commissure to a control group of 120 individuals of similar age. The final outcome was measured by assessing maximum mouth opening along both vertical and horizontal axes. While initial restriction of movement was severe, by the end of conservative treatment, which included rehabilitation therapy, there was significant improvement. However, even after recovery, mobility remained substantially reduced

compared to the control group, and the average treatment duration was 550 days, with half the patients requiring more than two years of rehabilitation [20].

Surgical Treatment

The management of facial burns has unique particularities related to the specialized anatomy of this region, with local vascularization significantly superior to that of other body areas and a higher density of skin appendages, especially in men. Due to these characteristics, excision and grafting are often delayed until days 12–15, even in deep partial-thickness or full-thickness burns. Many superficial and intermediate burns can heal spontaneously without sequelae within 15 days. The main exception is carbonized lesions, which justify early surgical intervention [23].

Treatment of Facial Burn Sequelae

Treating the effects of facial burn sequelae is often a lengthy and staged process commencing with a thorough assessment of the patient's available skin capital. The best outcomes are obtained when treating a major unit with a minor subunit in the same procedure, rather than two major units that are adjacent [40]. Reconstruction is most commonly performed using skin grafts, ideally from donor areas with similar color and texture to the face, including hair-bearing skin when appropriate. Preferred donor sites include the expanded supraclavicular region or the inner arm, as these areas provide grafts that closely resemble facial skin. In contrast, skin from the abdomen or thighs tends to be darker or more yellowish, producing noticeable contrast and therefore being avoided whenever possible [41]. Grafts should not be expanded, as this increases the risk of unaesthetic scarring that can attract attention. Full-thickness skin grafts are preferred, with small incisions made using a No. 11 scalpel blade to facilitate adaptation and drainage [23]. Flaps are primarily used in the sequelae stage, with very limited indications in the acute phase, such as cases involving exposure of bone, blood vessels, nerves, or the eyeball. Their use in emergencies is rare, since initial treatment should preserve as much tissue capital as possible for future reconstructive procedures [23,41].

Infections Associated with Facial Burns

Infections represent a major cause of morbidity and mortality among patients with severe burns [42]. Burns involving the face and neck frequently require intubation, often performed before hospital admission, or are associated with inhalation injuries - both of which are recognized risk factors for pneumonia. According to a study by Cotte et al., among 152 patients, pneumonia developed early in the disease course in 58 cases (38.2%), with the majority of the patients being intubated pre-hospital (65%). Among the patients who arrived at the ICU without prior intubation, 84% required subsequent intubation, with an average duration of mechanical ventilation of 18.1 ± 32.5 days. The main pathogens implicated in the development of early post-burn pneumonia were: Staphylococcus aureus - 56.9%, Streptococcus pneumoniae – 19%, Haemophilus influenzae – 19% and Escherichia coli – 6.9% [43] The study concluded that pre-hospital intubation is associated with a higher risk of early-onset pneumonia, which in turn leads to a significant increase in mortality Just as well it must be admitted the fact that delaying intubation in such cases may lead to respiratory compromise which may be life threatening.

Burn Stigmata

The advances in medicine have led to improvements in the survival rates of patients with severe burns. That means a majority of them are facing permanent sequelae. As such, in the three main stages of recovery, they shall require support [44]. The first phase, the resuscitation one, brings uncertainty of survival, anxiety, delirium, confusion, fear associated with pain and sleep disturbances. The second phase, which is that of rehabilitation, is influenced by the need for repeated surgical interventions and painful dressing changes. The third phase commences after discharge and means the challenges these patients face to reenter society in their new condition [45-47]. As such, psychological support becomes equally important for the patient as it is for their family, which may have a modified dynamic by the attempts to conceal the patient, leading to a delay in reintegration. Efforts in this direction lead to strengthening of the family's cohesion, facilitate the patient's reintegration in society, and reduce interpersonal conflict [47,48].

Emerging Areas of Interest

Modern medicine has led to an improvement in survival rates, but also to a bigger percentage of patients with sequelae requiring rehabilitation. Severe facial burns can lead to complications such as ectropion, oral commissure contracture, loss of facial sensitivity, poor oral or dental hygiene, excessive salivation, speech articulation difficulties, and problems with intubation. Some of these complications can be managed conservatively through rehabilitative therapies aimed at restoring the functionality of physiological sphincters, improving speech and oral control, and ultimately enhancing quality of life for facial burn survivors [49].

3. PERINEUM

The perineal region is an anatomically and functionally important area in the management of major burn patients. The ABA includes burns of this region among the major admission criteria, with the incidence of perineal burns ranging between 1.7% and 13% of all hospital admissions. These burns most frequently occur in patients with extensive total body surface area (TBSA) involvement, estimated between 21% and 56%, even though the external genitalia represent only about 1% of the total body surface. The perineum

is a region of particular concern because of its high association with urinary tract infections, an increased risk of nosocomial infections, and a higher recorded mortality rate among affected patients [50–52]. In terms of surface area, the external genitalia account for approximately 1% of the total body surface, while the entire perineal region comprises about 4–6%. Anatomically, this region is relatively protected from direct thermal injury, so isolated perineal burns are rarely described. When they do occur, they are most often associated with burns of the thighs and lower abdomen. Perineal burns occur more frequently in male patients and are associated with higher mortality, longer hospital stays, and an increased risk of secondary infection [53]. Treatment of perineal burns can be divided into two main approaches: conservative and surgical. Large-scale studies in the literature, such as the one conducted by Harpole et al., analyzed 71,895 patients, of whom only 1,245 had perineal burns (representing 10.4% of total cases). The authors recommended conservative treatment for the majority of cases, reserving surgical intervention - specifically excision and skin grafting - for patients with third-degree (full-thickness) burns [54].

4. BURNS OF THE FEET

Despite representing a small percentage of TBSA, burns to this region may lead to severe functional and clinical impact, more so in patients with high-risk conditions, such as those suffering from diabetes, which also heal more slowly. Early detection of the burns, optimal wound management, and good glycemic control may prevent deepening of superficial burns and avoid the need for excision, grafting, or amputation. A study by Jason Diab et al. described a higher incidence of these patients in the cold season, with a 1.7 times higher chance of burns in the winter (usually by hot liquid) compared to the non-diabetic patients, who mostly had burns in the warm season [55-57]. The goal is to reduce edema, pain, restore normal function and allow early mobilization. The treatment is often prolonged and usually requires a multidisciplinary approach with a diabetologist and endocrinologist consultations as well. The local management means cleansing and debridement, prevention of infection and bacterial colonization, pain control, and offloading pressure from the burned areas, which promotes healing by optimal moisture balance and support for epithelialization [58].

Conservative Treatment

The main objective is to prevent the formation of contractures, commissural closure, or macerations and ulcerations. This is obtained by balancing the exudate, reducing inflammation, and facilitating debridement while offering an adequate antimicrobial coverage and protecting the burned area [59]. One of the most important regions that needs protection is that of the ankle, which lacks means of defending itself due to a lack of an abundant adipose layer or muscular protection. The sequelae to this region may lead to gait abnormalities, posture modification, as well as pelvic and spinal deformities. Surgical treatment requires the excision of the scar tissue and tension vector modification by use of several Z-plasties, which can also be accompanied by the use of skin grafts to regain mobility and function [60,61].

5. MAJOR JOINTS

The huge impact that the burns to these regions bring makes prevention even more important. Once the burns have been described, the treatment is dependent on the degree. The more superficial ones are treated conservatively by epithelialization promotion using dressings developed for burns, dermal substitutes and splints. The deeper burns require early excision and grafting or the use of local flaps [62-64]. Due to the important functional consequences and major systemic response, these patients often require immobilization for a long period of time, which brings along a higher risk of contracture formation. Despite the fact that any joint may be affected by this, the joints that are most prone to deformities are the elbow, the shoulder, the hip, and the knee [65]. Even so, it is crucial to use posture splints in some cases. Studies have shown that splints significantly reduce the incidence of scar contractures, particularly when used for at least six months. Patients who refuse splinting or for whom splints were not prescribed are far more likely to require reconstructive surgical interventions later on [66]. In severe burns, extensive soft tissue defects with exposure of joints or bone may occur, requiring complex reconstructive techniques, such as local or distant flap coverage. These situations are most frequently reported following electrical burns [67]. Progression to the sequelae stage of joint involvement has been described in approximately one-third of patients with articular burns. Despite its prevalence, the specialized literature has not yet established a standardized protocol that accounts for the specificities of each anatomical region, and therefore, a comprehensive set of recommendations remains lacking [68]. It is, however, universally accepted that surgical treatment of scar contractures should only begin after the metabolic activity of the scar has ceased - typically more than one year after wound healing. As such, the risk to develop contractures is severely reduced [69].

CONCLUSION

Burns that affect functional areas represent a major therapeutic challenge, which has direct implications on the survival rate but especially on the long-term quality of life of survivors. The management of burns in these regions requires a multidisciplinary approach that integrates early assessment, appropriate prompt intervention and sustained rehabilitation. Despite significant advances in reconstructive surgical techniques and refined physiokinetotherapeutic protocols, the prevention of functional and aesthetic sequelae remains a difficult goal to achieve. The analyzed literature emphasizes the importance of early, individualized treatment plans adapted to the functional requirements specific to each anatomical region. Moreover, the psychosocial dimension of recovery after burns should not be underestimated, since rehabilitation after post-traumatic stress and disfigurement, as well as subsequent social

reintegration, presents a continuous, long-term activity that requires complex multidisciplinary care. Future research should focus on: standardization of treatment algorithms for functional areas; improving the quality of post-combustion scars; improving rehabilitation techniques; last but not least, the extension of long-term tracking data, in order to optimize the results through further research.

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ARTICLE

Exploring Sustainable Food Choices in Romania: Evidence from a Quantitative Cross-Sectional Study

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Abstract: This study explores behavioral and demographic predictors of sustainability engagement in sustainable food choices among Romanian adults, addressing a regional gap in Eastern European sustainability research, based on an online non-probability sample of 406 respondents collected through an online cross-sectional survey, with voluntary response and snowball sampling techniques, conducted between 15 October 2024 and 30 January 2025. From 527 initial responses, 121 questionnaires were excluded due to incomplete or inconsistent answers, resulting in a final analytic sample of 406 respondents. A modified version of the Food Choice Questionnaire (FCQ), termed the Consumers' Engagement in Food Choices Questionnaire (CEFCQ), was employed to capture sustainability-related behaviors and attitudes. Four hypotheses were tested using multiple linear regression. While snowball sampling is appropriate for exploratory research, we acknowledge its limitations in terms of representativeness at the national level. The respondents haven't received any incentives, and data privacy and confidentiality were guaranteed. A modified version of the Food Choice Questionnaire (FCQ) was employed to assess sustainability-related attitudes and behaviors, including food label awareness, shopping frequency, and food waste reduction practices. Drawing on existing literature, four hypotheses were formulated and tested using multiple linear regression analysis. Results indicate that label reading behavior and perceived importance of digitalization are positively associated with sustainability engagement, while shopping frequency is negatively associated. Educational attainment and digital marketplace engagement show no significant association after controlling for other variables. The model explains approximately 25% of the variance in sustainability engagement. Given the non-probabilistic sampling design and demographic skew toward female and highly educated respondents, findings are interpreted as exploratory. This study contributes region-specific evidence on sustainable food behavior in Romania and highlights the role of information-oriented behaviors over formal education in predicting sustainability engagement. Implications for consumer education, labeling policy, and targeted interventions aligned with the European Green Deal are discussed.

Keywords: sustainable food consumption; food choices; food waste reduction; environmental awareness; sustainability engagement; cross-sectional study

INTRODUCTION

Sustainable food consumption emerged as a central component of environmental and public health policy discussions, particularly in light of accelerating climate change, resource depletion, and shifting population dynamics. As global food systems experience mounting pressure, understanding how consumers make food choices and what motivates them to adopt sustainable practices has become essential for designing effective strategies that support long-term environmental and public health goals. These behaviors are directly linked to international policy

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frameworks such as the United Nations Sustainable Development Goals (SDGs), particularly SDG 12 (Responsible Consumption and Production) and SDG 3 (Good Health and Well-being).

Eastern Europe remains underrepresented in empirical research on sustainable food behavior. Romania presents a particularly relevant case study. As an Eastern European country undergoing rapid socio-economic transformation, Romania faces the dual challenge of aligning consumer behavior with sustainability objectives while addressing long-standing issues related to food waste, low environmental awareness, and inconsistent policy implementation. Despite agriculture representing around 3.9% of the national Gross Domestic Product (GDP) [1], sustainable food consumption remains an underexplored topic in the Romanian research landscape. Existing studies tend to focus on Western Europe, leaving a gap in the understanding of how Eastern European consumers perceive and engage with sustainability in everyday food decisions. This gap in the literature is significant, as regional differences in food consumption habits and sustainability practices can influence the success of European Union (EU) policies, such as the Green Deal. By focusing on Romania's specific context, this study addresses a critical regional gap and provides insights directly relevant to the implementation of sustainability strategies in Eastern Europe. Although the study may not introduce methodological innovations, it contributes to the field by adapting existing research frameworks to a regional context that has been largely overlooked. This makes the research not only relevant but also essential for shaping future policies in the region, with potential long-term impacts on local sustainability efforts.

Consumer engagement plays a crucial role in shaping food systems, influencing not only purchasing behavior but also broader issues such as food waste, packaging choices, and demand for sustainably sourced products. These behaviors directly impact progress toward international frameworks such as the United Nations Sustainable Development Goals (SDGs), especially SDG 12 (Responsible Consumption and Production) and SDG 3 (Good Health and Well-being) [2,3].

This study aims to address this research gap by investigating the extent to which behavioral and demographic factors influence sustainability awareness in food choices among Romanian consumers. Specifically, it examines the relationships between sustainability awareness and variables such as food label reading, shopping frequency, and education level. The study employs a modified version of the well-established Food Choice Questionnaire (FCQ), adapted to incorporate sustainability-related dimensions, including food waste reduction, environmental labeling, and digital information sourcing.

Given the identified research gap and the limited empirical evidence from Eastern Europe, this study tests four hypotheses concerning education, shopping behavior, digital engagement, and label use and addresses the following research question: To what extent do behavioral and demographic factors predict sustainability awareness in food choices among Romanian consumers?

Providing empirical evidence from an underrepresented national context, the study contributes to the literature on sustainable consumption and informs culturally relevant interventions for promoting responsible food choices.

LITERATURE REVIEW AND HYPOTHESES DEVELOPMENT

The Food Choice Questionnaire (FCQ) remains a foundational framework for understanding motivations underlying food selection, traditionally emphasizing health, convenience, sensory appeal, and price. Recent extensions integrate sustainability considerations, reflecting growing consumer awareness of environmental and ethical impacts. It has long been an important topic in consumer behavior literature, often grounded in the Food Choice Questionnaire (FCQ) developed by Steptoe [4], which identified key motivational factors influencing food decisions, such as health, convenience, sensory appeal, and price. These foundational dimensions remain relevant for understanding contemporary food choices, especially when extended to include sustainability concerns [5–7]. In the past decade, sustainability has emerged as a crucial dimension shaping consumer food preferences. Researchers [8, 9] have emphasized the integration of environmental and ethical concerns alongside traditional drivers of food choice. This evolution reflects a shift from pure nutritional and sensory factors to a more holistic understanding, encompassing ecological impact, animal welfare, and social justice [10–12].

To capture this complexity, researchers have either adapted the FCQ with sustainability-specific items or developed new instruments tailored to capture environmental awareness and responsible consumption (e.g., the CEFCQ in this study). Recent works [13, 14] highlight the balance between psychometric validity and survey efficiency, especially in large-scale or time-limited research contexts [15–17].

Comparative studies [18, 19] reveal substantial differences in sustainability engagement across countries, often linked to cultural values, economic development, and policy environments. For instance, Nordic consumers tend to prioritize environmental factors more than their counterparts in Southern Europe, underscoring the importance of localized approaches in research and marketing [20–22].

Eastern Europe represents a unique context where traditional values intersect with new sustainability discourses. Other studies [23, 24] suggest that while interest in sustainable consumption is growing, barriers such as limited knowledge, affordability, and weak policy enforcement remain significant. Qualitative research [25] has revealed inconsistencies between expressed attitudes and actual practices, underscoring the need for context-specific instruments and policies.

Romania, in particular, is underrepresented in sustainability research despite its rapid socio-economic transformations and persistent issues with food waste and low environmental awareness. Researchers [26] found that Romanian consumers show rising interest in organic and locally sourced foods, but knowledge gaps and price sensitivity often inhibit adoption. Other studies have identified differences in urban versus rural consumption patterns, revealing that sustainability in Romania is still at a formative stage [27].

Despite these contributions, there is still a lack of validated instruments tailored to the Romanian context. Existing studies often apply frameworks developed in Western Europe without accounting for local cultural and economic factors. This study addresses this gap by adapting the FCQ to include sustainability-specific dimensions such as food waste reduction, environmental labeling, and digital sourcing of information.

The FCQ was modified to reflect contemporary sustainability issues, including items on environmental labeling, food waste, and digital sourcing of information. Although not fully revalidated psychometrically, the adapted instrument was pilot-tested on a small Romanian sample (N = 11) to ensure clarity, cultural relevance, and internal consistency.

Based on the prior research, the following hypotheses are proposed [8,9,14]:

- H1: Educational attainment (ED_AT) is positively associated with sustainability engagement (S_E).
- H2: Digital marketplace engagement (DME) is positively associated with sustainability engagement (S_E).
- H3: Label reading behavior (LRB) and nutritional awareness (NA) are positively associated with sustainability engagement (S_E).
- H4: Shopping frequency (S_F) is positively associated with with sustainability engagement (S_E).

These hypotheses reflect prior findings in sustainable consumption research [6, 10] and are tested using multiple linear regression analysis. These hypotheses also reflect prevailing assumptions that education, information seeking, and frequent market exposure foster sustainable behavior.

In line with recent efforts to optimize the FCQ for large-scale or time-constrained applications [28–30], the questionnaire was pilot-tested with 11 respondents to evaluate clarity, internal consistency, and completion time. Minor refinements were made based on their feedback.

Shopping frequency has been associated with increased exposure to product information, which may in turn enhance sustainability awareness [8,11]. Similarly, food label reading has been identified as a behavioral cue for environmentally conscious purchasing [21]. Education level is often linked to sustainability knowledge, although recent studies suggest that its predictive power varies across contexts [14, 29].

MATERIALS AND METHODS

Study Design and Sample

A quantitative cross-sectional survey was administered online between October 2024 and January 2025. The survey link was disseminated through email and social media using voluntary response and snowball sampling techniques. A total of 527 responses were collected. To ensure data reliability and internal consistency, a quality screening procedure was applied. Responses were excluded if they contained missing data (e.g., unanswered items in key sections of the questionnaire) or if they exhibited logical inconsistencies across related questions, such as contradictory answers to conceptually linked items. Following this process, 105 questionnaires were removed, resulting in a final sample of 406 valid responses used for the analysis. The study adopted a quantitative, cross-sectional research design to examine Romanian consumers' engagement in sustainable food choices. Data were collected using a modified version of the widely validated Food Choice Questionnaire (FCQ) [4], adapted to incorporate sustainability-related dimensions and behavior-specific constructs.

The adapted instrument, referred to as the Consumers' Engagement in Food Choices Questionnaire (CEFCQ), includes 11 items grouped into four main sections: (1) demographic profile, (2) food purchasing behavior, (3) sustainability-related awareness, and (4) consumer attitudes toward food labeling, waste, and digitalization. Likert-type scales, multiple-choice, and count-based items were used.

Data were collected via an online survey conducted between October 2024 and January 2025, using non-probability sampling, voluntary response and snowball sampling techniques. While this approach does not ensure statistical representativeness at the national level, it provides valuable exploratory insights from a diverse sample (N = 406), sufficient in size for behavioral analysis.

The sample consisted predominantly of women (64.8%) and university-educated respondents (approximately 79%). Given the non-probability sampling design and demographic skew, findings are interpreted as exploratory and not statistically representative of the Romanian population.

Instrument and Measures

The CEFCQ was adapted from the FCQ to include sustainability-related items covering food waste reduction, labeling behavior, digital information use, and perceptions of organizational responsibility. The instrument comprised 11 items grouped into four domains: demographics, purchasing behavior, sustainability awareness, and labeling/digital attitudes.

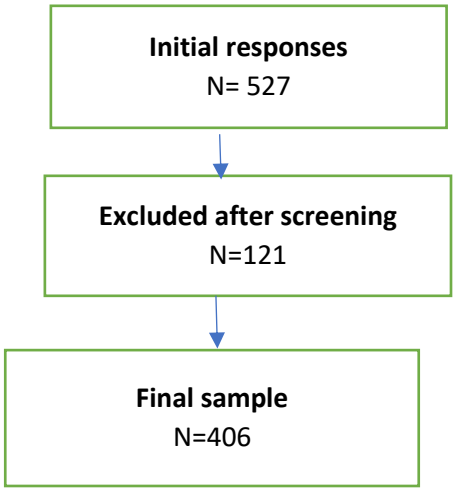
Given the formative nature of the sustainability engagement index, internal consistency reliability was not assessed. The index aggregates nine conceptually related sustainability domains and reflects the breadth of engagement rather than a latent construct. Prior to the main data collection, the adapted questionnaire was pre-tested on a small convenience sample of 11 Romanian adults in order to assess item clarity, linguistic appropriateness, and completion time. This pre-test was conducted as a cognitive and cultural check rather than a psychometric validation. Given the formative nature of the sustainability engagement index and the exploratory purpose of the study, internal consistency reliability and factor structure were not evaluated at this stage. Minor wording adjustments were made based on participant feedback before launching the main survey.

A non-probability sampling approach was used, combining voluntary response sampling with snowball dissemination via digital platforms. The survey was distributed online through email and social media (Facebook, WhatsApp, Instagram, LinkedIn), encouraging participants to forward the link within their networks. While the sample used in this study is not probabilistic and therefore does not permit statistical generalization to the broader national population, the final count of valid responses (n = 406) exceeds the minimum sample size of 384 typically recommended in Krejcie and Morgan's (1970) table for probabilistic sampling [31]. This minimum is generally applied to achieve a 95% confidence level with a ±5% margin of error. Although the current sample does not follow probabilistic methods, its size still offers a robust foundation for the analysis conducted in this study. However, due to the non-random nature of the sampling, no claims of statistical representativeness can be made. The findings should be interpreted as indicative patterns within the surveyed group rather than as population-level estimates, due to the fact that the current research is an exploratory one.

Variables

Data were collected between October 2024 and January 2025 using a self-administered online questionnaire hosted on Google Forms. The survey link was disseminated through email and social media using voluntary response and snowball sampling techniques. From 527 initial responses, 16 responses were excluded due to incomplete data and failure to meet predefined quality criteria. This resulted in 511 usable responses. A further quality screening (attention checks, straight-lining detection, and response-time thresholds) led to the exclusion of 105 additional cases. The final analytical sample, therefore, consists of 406 valid questionnaires, which is the sample used in all statistical analyses and reported in Figure 1 in accordance with STROBE guidelines.

Figure 1: Flow diagram of participant inclusion and exclusion according to STROBE guidelines



The first page provided information about the study's aim, confidentiality, voluntary participation, and the right to withdraw at any time. Ethical approval was obtained from the Metabolism Research Center under protocol number 0004/03.09.2024.

Statistical Analysis

Descriptive statistics (means, standard deviations, and correlations) are presented in Table 2. Multiple linear regression was used to test hypotheses. Model diagnostics included variance inflation factors (VIF), Durbin–Watson statistics, and Breusch–Godfrey tests for autocorrelation. Regression coefficients are reported with 95% confidence intervals.

All variables used in the study are described in Table 1, including their coding scheme and measurement level:

Table 1: Variable Operationalization

Variable	Definition	Type
Educational Attainment (ED_AT)	Self-reported education level (1 = Primary; 6 = Doctorate)	Ordinal
Shopping Frequency (S_F)	Frequency of food purchases (1 = Daily; 4 = Rarely)	Ordinal
Digital Marketplace Engagement (DME)	Preferred purchase channel (1 = Physical; 2 = Mixed; 3 = Online)	Categorical
Voluntary Recycling Behavior (VRB)	Type of shopping bag used (−1 = Disposable; 0 = Mixed; 1 = Reusable)	Ordinal
Label Reading Behavior (LRB)	Number of product label elements checked (log-transformed)	Count (log)
Nutritional Awareness (NA)	Number of nutritional label items consulted (0–9)	Count
Digitalization and Technology Perception (DIGI_TEH)	Perceived importance of technology in the food sector (Likert scale 1–5)	Ordinal
Warning Labels Acceptance (WL)	Support for warning labels on unhealthy food (0 = No; 1 = Yes)	Binary
Sustainability Engagement (S_E)	Number of sustainability dimensions engaged (0–9)	Count

The variable Educational Attainment is a demographic variable that captures the respondent's educational background: primary education = 1; secondary education = 2; post-secondary education = 3; bachelor's degree = 4; master's degree = 5; doctorate degree = 6. Our scale, while neglecting those without any formal education, is fair enough to capture the entire educational spectrum.

Shopping Frequency reflects the purchase habits, and it was defined as the number of shopping trips made by a respondent over a specific period of time. It was scaled as daily = 1; several times a week = 2; once a week = 3; rarely = 4.

Digital Marketplace Engagement is a variable that represents the way in which the respondent makes their food shopping. Responses were coded as a polytomous variable with 3 distinct categories (physical, mixed, online) and reflect the preferred purchase channel coded as 1 = physical; 2 = mixed; 3 = online.

Voluntary Recycling Behavior measures the respondent's choice of shopping bags, with values indicating the degree of engagement in environmentally friendly practices: a score of −1 represents the use of disposable bags, 0 indicates mixed usage of both disposable and reusable bags, and 1 reflects consistent use of reusable bags, signifying a stronger commitment to sustainability and recycling practices.

Label Reading Behavior is a count-type variable that measures the number of informational elements (e.g., ingredients, nutritional value, expiration date, price, manufacturer) selected by the respondent from a predefined list. To reduce distribution skewness and control for extreme variations, the variable was log-transformed (natural logarithm) before being included in the regression model.

Nutritional Awareness measures the total number of nutritional label elements (e.g., proteins, carbohydrates, fats, calories, fiber, salt, vitamins, etc.) read by the respondent from a product's label. Each respondent selected from a predefined list the types of information they usually consult, and the variable was coded from 0 (reads no information) to 9 (reads all listed categories). It is treated as a discrete quantitative variable (count-type), usable as a predictor in regression models.

Digitalization and Technology Integration measures the degree of importance attributed to the implementation of technology in the food industry, on a Likert scale from 1 to 5, where: 1 = Not important at all, 2 = Slightly important, 3 = Moderately important, 4 = Important, 5 = Very important. This is an ordinal variable used to assess respondents' perceptions of the role of technology and digitalization in the food sector. Some researchers highlighted its potential to reduce carbon emissions [33-35], while others caution that consumer behavior trends associated with affluence and consumption upgrading may exacerbate environmental degradation [36].

Warning Labels Acceptance is a binary dichotomous variable that measures the respondent's support for the introduction of warning labels on unhealthy food products, where a score of 0 indicates opposition to such labels, and a score of 1 reflects support for their implementation, signaling an openness to measures aimed at improving public health and informing consumer choices.

The dependent variable, Sustainability Engagement, is a count index (0–9) representing agreement with organizational involvement across sustainability domains. Independent variables include educational attainment, shopping frequency, digital marketplace engagement, label reading behavior (log-transformed), nutritional awareness, perceived importance of digitalization, voluntary recycling behavior, and acceptance of warning labels. Sustainability Engagement was operationalized as a composite index measuring respondents' beliefs about the extent to which organizations should be involved in various sustainability-related domains. The index consists of 9 items, each representing a distinct area of sustainable action: production, waste management, ecosystem protection, technological innovation, development of plant-based proteins, promotion of healthy eating, sustainable production, eco-friendly packaging systems, and sustainable distribution chains. For each domain where respondents agreed that organizations should be involved, one point was assigned (1 = yes, 0 = no). The total score ranges from 0 to 9, with higher scores indicating a broader and stronger perception of organizational responsibility in promoting sustainability. This index is a simple count measure, combining elements that reflect attitudes, behavioral intentions, and normative expectations. Although the items address different thematic areas, they are theoretically unified under the broader construct of perceived organizational engagement in sustainability efforts. The resulting index was treated as a quantitative variable in the analyses, labeled as Sustainability Engagement. Although the items reflect different sustainability domains, they are conceptually related and contribute formatively to the overarching construct. Since the purpose of the index is to capture a general orientation toward sustainability-related organizational engagement, internal consistency was not a requirement, and the index is considered formative rather than reflective.

A decrease in buying frequency allows customers to make savings, while an increase in buying frequency is related to a short food supply chain [32]. Digitalization and Technology integration (DIGI_TEH) is an ordinal variable used to assess respondents' perceptions of the role of technology and digitalization in the food sector. Some researchers highlighted its potential to reduce carbon emissions [33-35], while others caution that consumer behavior trends associated with affluence and consumption upgrading may exacerbate environmental degradation [36]. Warning Labels (W_L) is a dichotomous variable measuring respondents' acceptance of introducing warning labels on unhealthy foods, similar to those found on cigarette packs. Responses were coded as: 1 for positive feedback and 0 for negative feedback. Consumer tools such as health star ratings have been shown to support healthier food choices [37].

RESULTS

Descriptive statistics

Respondents reported moderate sustainability engagement (M = 4.87, SD = 2.31). Label reading behavior and perceived importance of digitalization showed the highest correlations with sustainability engagement (r = 0.42 and r = 0.31, respectively).

Descriptive statistics were computed for all variables. The core of the analysis consisted of a multiple linear regression model, using S_E (Sustainability Engagement) as the dependent variable. Independent variables were selected based on theoretical relevance and empirical findings in the literature.

To enhance model robustness, the analysis included standard diagnostic procedures:

- Multicollinearity check via Variance Inflation Factors (VIF)
- Autocorrelation test using the Durbin-Watson statistic
- Heteroskedasticity check using the Breusch-Godfrey test

The statistical analysis was conducted using the RealStats Excel Add-in, a validated analytical extension suitable for econometric modeling in spreadsheet environments. Where necessary, transformations (e.g., log for skewed count data) were applied to meet model assumptions.

Socio-Demographic Profile of Respondents

The data were collected from a Google Forms questionnaire of 406 respondents, distributed via WhatsApp and email. The respondents were male and female over 18 years old who voluntarily agreed to get involved in this study. They were assured that all the answers would be kept anonymous. The survey included a section of demographic information with closed-ended questions about their gender, their age, and their level of education (Table 2).

The possible bias (65% of respondents are female, university-educated, and digitally active) related to the sample limits the generalizability of the findings to the broader population. The gender imbalance may be explained by findings from the UK-based research company YouGov, which indicate that women are more likely than men to be the decision-makers regarding food consumption habits in the household [38]. Thus, the FCQ questionnaire will be invariably biased towards the female gender in the responses. While the sample may seem skewed towards university graduates, according to the 2021 national Romanian census, it should be noted that online surveys tend on average to attract more responses from professionals with better education [39]. This bias is widely documented in survey methodology literature and does not invalidate the internal consistency of the findings, although it does constrain external validity. Finally, we emphasize that the study’s objective is theoretical and exploratory rather than strictly

demographic representativeness. The results contribute to understanding the relationships between food choice motivations within a digitally engaged consumer segment, and future research should employ stratified or probability-based sampling methods to enhance population-level generalizability.

The next section comprises questions related to the consumers' engagement in sustainable food. All questions comprise an open-ended section where respondents can add their answers if they are not included within the multiple-choice questions.

The discrepancy between the total sample size (n = 406) and the demographic table (n = 406) reflects partial nonresponse to demographic questions. These respondents were retained for regression analyses where their responses to relevant variables were complete.

Table 2: Demographic Characteristics (n=406)

Age Group	Frequency	Gender	Frequency	Educational level	Frequency
18-24	24,31%			High School	14,43 %
25-34	15,42%	Male	35,18 %	Bachelor's Degree	50,00 %
35-44	17,39%			Master Degree	21,94 %
45-54	23,72%			Post-university Studies	6,52%
55-64	14,43%	Female	64,82 %	Doctorate	7,11%
>65	4,74 %				

This distribution of education levels in the sample aligns with findings from similar consumer behavior and sustainability studies, which also report relatively low representation of respondents with doctoral degrees [40-42].

Regression Analysis

The regression model (Table 3) was statistically significant ($F = 18.22$, $p < 0.001$) and explained 25.4% of the variance in sustainability engagement (Adjusted $R^2 = 0.254$). Label reading behavior ($\beta = 1.88$, 95% CI [1.49, 2.27], $p < 0.001$) and perceived importance of digitalization ($\beta = 0.34$, 95% CI [0.17, 0.51], $p < 0.001$) were positively associated with sustainability engagement. Shopping frequency showed a significant negative association ($\beta = -0.32$, 95% CI [−0.59, −0.05], $p = 0.024$). Educational attainment, digital marketplace engagement, nutritional awareness, voluntary recycling behavior, and warning label acceptance were not significant predictors.

Table 3: Regression Model

Multiple R	0.5182	D-lower	1.7971
R Square	0.2685	D-upper	1.8774
Adjusted R Square	0.2538	DW_TEST sig	Unclear
Standard Error	2.1364	BGT Lags	1.0000
Observations	406	BGT LM*	2.3546
Intercept	Yes	BGT df1	1.0000
F	18.2196	BGT df2	396

p-value	>0.0001	BGT p-value	0.1257
Sig	Yes	BGT LM	2.3998
D-stat	2.1511	BGT p-value	0.1214

As we can observe in the table below (Table 3), the multiple linear regression has an adjusted R-square of ~25%, meaning the variables explain about 25% of the sustainability indicator. The p-value of the F-test also indicates a statistically significant model. The Durbin-Watson test being inconclusive (D-stat = 2.1511) requires us to deploy a lagged Breusch-Godfrey Test with 1 lag for the purpose of the model.

Since the Breusch-Godfrey Test (BGT) yields an insignificant p-value at 1 lag, we can affirm that the model passes the residual autocorrelation test (Table 3).

Regarding the regression variables themselves, two tests were performed. The linear regression model works best with an

intercept when independent values can be zero [43]. The first involved checking for Simpson's paradox by checking the sign of the regression coefficients against those from the Pearson correlation matrix. The second test involved the multicollinearity coefficients for the independent variables (Figure 2).

No issues were detected with the variables, as both tests yielded negative results.

Figure 2: Regression Model

	Coefficient	***Simpson's paradox***	***t stat***	***p-value***	***Sig***	***Vif***
Intercept	0.1344		0.2137	0.8309	No	
ED_AT	0.0567	No	0.6278	0.5305	No	1.0250
S_F	-0.3178	No	-2.2631	0.0242	**Yes**	1.0199
D_M_E	0.5709	No	1.1087	0.2682	No	1.0241
V_R_B	0.2674	No	1.8292	0.0681	No	1.0648
L_R_B	1.8829	No	9.4893	>0.0001	**Yes**	1.1093
N_A	0.0227	No	0.3290	0.7423	No	1.0956
DIGI_TEH	0.3368	No	3.8923	0.0001	**Yes**	1.0169
W_L	0.5578	No	1.6112	0.1079	No	1.0096

Using a significance threshold of 5%, three out of the 9 predictors, including the intercept, were found to be statistically significant. All coefficients are positive, except shopping frequency (S_F), which has a negative and significant coefficient. A higher frequency of shopping is associated with a lower sustainability engagement, suggesting that people who are shopping frequently may not place as much importance on sustainable practices.

Contrary to our initial hypothesis, which posited that frequent shopping would reflect greater consumer involvement and, consequently, stronger sustainability engagement, the empirical results reveal a negative association. This outcome indicates that higher shopping frequency may instead reflect more impulsive or emotionally driven consumption behaviors, potentially limiting consumers' focus on sustainability-related attributes.

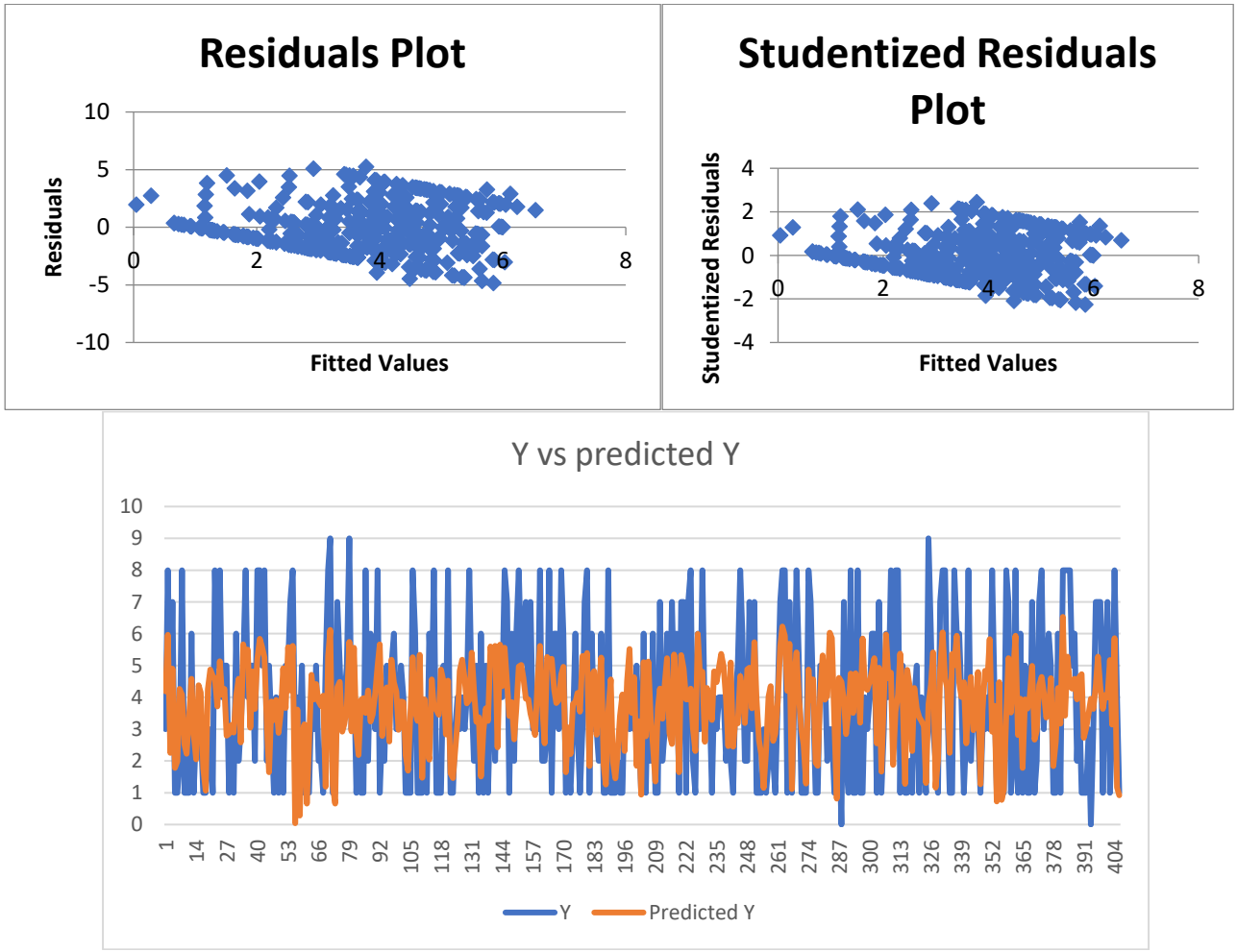
Surprisingly, Educational Attainment (ED_AT) has a positive, but not significant, relationship with sustainability engagement, rejecting hypothesis number 1.

Digital marketplace engagement (D_M_E) is not statistically significant, suggesting that how much a respondent engages with digital marketplaces does not have a significant impact on their sustainability engagement. In addition, Digitalization and Technology integration (DIGI_TECH) is highly significant and positively associated with sustainability engagement. Respondents who think technology plays an important role in the food sector tend to have a positive sustainability engagement.

Label reading behavior (L_R_B) has a positive and highly significant relationship with sustainability engagement. Respondents who read labels are more likely to have a stronger sustainability engagement. However, nutritional awareness (N_A) is not statistically significant.

Warning labels (W_L), even though positively correlated, are not statistically significant. This indicates that the acceptance of warning labels for unhealthy food products by the respondents is not a strong predictor of sustainability engagement in our sample.

Figure 3: Residuals Plots



According to the figure above, the predicted values of y fit nicely with the actual observations (Figure 3). The large residuals are to be expected for an exploratory study.

These findings underscore the complexity of sustainability-related consumer behaviors in emerging economies such as Romania. While traditional assumptions suggest that higher education leads to more sustainable behavior, our data show no statistically significant relationship between educational attainment and sustainability engagement, in line with recent studies from Eastern Europe that emphasize the gap between formal knowledge and actual behavioral practices [44]. In contrast, label reading behavior emerged as a robust predictor, suggesting that access to clear and trusted product information may play a more pivotal role than generalized education in shaping sustainable consumption. This reinforces the argument that informed choice, rather than academic background, may drive pro-sustainability behavior [45].

DISCUSSION

Food production relies heavily on key resources such as water, energy, and labor. Wasting food means wasting valuable inputs. Romania faces a significant challenge regarding food waste, driven by consumer behaviors, industry practices, or inefficiencies. However, legislative reforms (Law number 217 on reducing food waste since 17 November 2016 (revised in 2024); Regulation EU number 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC, and Commission Regulation (EC) No 608/2004) underline a sustained effort to address this issue. Moreover, while our model focused primarily on cognitive and behavioral predictors, emotional, cultural, and social factors, such as peer influence, values, or food traditions, should be integrated into future models to offer a more holistic view of sustainable food behavior.

This study provides exploratory evidence on behavioral predictors of sustainability engagement in food choices among Romanian consumers. Contrary to common assumptions, educational attainment was not a significant predictor once behavioral variables were considered. This finding aligns with recent evidence suggesting that formal education alone does not translate into sustainable consumption without accessible and actionable information.

Label-reading behavior emerged as the strongest predictor, underscoring the importance of transparent, credible product information. This supports prior research emphasizing informed choice as a key driver of pro-environmental behavior. The perceived importance of digitalization was also positively associated with sustainability engagement, suggesting openness to technological solutions in promoting sustainable food systems.

The negative association between shopping frequency and sustainability engagement contradicts the original hypothesis and indicates that frequent purchasing may reflect convenience-oriented behavior rather than sustainability considerations. This result suggests that interventions targeting purchasing routines may be as important as information campaigns.

Actual digital marketplace engagement is not significantly correlated with sustainability engagement, a finding that complements the study of Ananda et al. [46], who discovered that online shoppers waste more food compared to their counterparts who shop in situ.

Voluntary recycling behavior is not strongly correlated with sustainability engagement, implying the existence of complex sustainability engagement patterns that can be addressed by further research with other parameters.

Nutritional awareness was not found to be a significant predictor of sustainability engagement in our model. Such findings might be explained by the existence of buyers with specific dietary needs who have limited food choices available to them and for whom sustainability is of less concern than the average population.

While Ozbay and Duyar [47] found that education is a predictor of support for renewable energy production, an indicator of sustainability awareness, this paper finds an insignificant, albeit positive correlation between education and sustainability engagement. Such a finding might be due to effective media outreach campaigns and the interaction between age and educational attainment in the context of the Romanian sample.

While bag usage is prevalent in the Romanian context, it is important to distinguish such actions from a comprehensive implementation and sustainability engagement.

Contrary to our initial hypothesis, which posited that higher shopping frequency would be associated with greater sustainability engagement, based on the assumption that frequent shoppers may rely more on short supply chains, the results reveal a negative relationship. This unexpected finding suggests that sustainability-oriented consumption may be driven less by shopping frequency and more by deliberate purchasing strategies, such as localized shopping in smaller batches, which can reduce environmental impact through lower primary and secondary packaging use.

The findings of this study challenge common assumptions in the literature, particularly the notion that education level is a consistent predictor of sustainability awareness. Instead, behavioral variables such as shopping frequency and attention to food labeling appear to be more strongly associated with sustainable consumption patterns. By focusing on an underrepresented national context and using a tailored FCQ instrument, this study contributes new empirical evidence to the literature on sustainable food behavior.

The results have implications for researchers, policymakers, and stakeholders interested in promoting culturally relevant interventions aimed at encouraging sustainable food practices and reducing food waste in line with European Green Deal targets.

Cooperation between the government, businesses, and civil society is essential to reducing food waste and preserving resources. For instance, businesses are required to implement at least two measures before discarding food, such as reducing prices on near-expiry items, donating surplus food, or converting waste into animal feed or compost. A comprehensive plan is being developed to set targets, assign responsibilities and allocate resources for food waste reduction. The Ministry of Agriculture and Rural Development established a national platform for businesses to report food waste data, which was launched by June 30, 2025. The Ministry of

Agriculture and Rural Development, in cooperation with the Ministry of Education, is promoting campaigns such as „You can protect the planet as well! Together we start reducing food waste", targeting school students to encourage responsible consumption habits. Romania passed food waste legislation in February 2024, requiring supermarkets and restaurants to implement measures like discounting near-expiry food or donating surplus, aligning with EU goals to halve per capita food waste by 2030. Many educational campaigns were launched by the Ministry of Agriculture, InfoCons, and local councils to inform consumers and promote behavior change.

From a practical perspective, our research on consumer engagement in sustainability in food choices is pertinent for both businesses and policymakers who aim to promote sustainable behaviors. Based on our results, there are several takeaways that could be implemented in the real world, such as increasing consumer awareness and encouraging sustainable purchasing behaviors. For instance, food retailers could start developing educational programs and label literacy to assist customers in assessing the environmental impact of their choices.

Another practical implication refers to food companies and technology providers who should invest in digital platforms that deliver real-time sustainability data and offer personalized feedback on environmentally responsible options. Integration of digital tools can support behavioral shifts toward more sustainable purchasing patterns.

This study contributes to the literature on sustainable food consumption in several ways. First, it applies an adapted version of the FCQ that incorporates sustainability-specific items validated for the Romanian context. Second, it examines a set of behavioral and digital variables, such as label reading, shopping frequency, and digital marketplace engagement, that have been understudied in Eastern Europe. Third, it provides empirical evidence from Romania, an underrepresented country in sustainability research, thereby addressing a clear regional research gap. These contributions support the development of more localized and behaviorally-informed sustainability interventions.

While this study provides valuable insights into Romanian consumers' sustainability engagement and food-related behaviors, it is not without limitations. The use of a non-probability convenience sample limits the generalizability of the findings. Future research should consider employing stratified or randomized sampling methods to better capture demographic and regional diversity. Another limitation is the cross-sectional design, which restricts our ability to infer causality. Longitudinal studies would be beneficial to track shifts in behavior over time, especially in response to public policy, educational interventions, or market innovations.

Ultimately, our findings support the development of culturally tailored, evidence-based strategies to reduce food waste and advance the goals of the European Green Deal and the broader transition to sustainable food systems.

Future studies could explore the relationship between shopping behaviors, digital engagement, and sustainability engagement through longitudinal data. Additionally, examining the impact of socio-economic factors such as income, urban vs rural location, could help refine the understanding of how different consumer segments engage with sustainable food choices.

CONCLUSIONS

This study highlights the primacy of information-oriented behaviors, particularly label reading and digital awareness, in predicting sustainability engagement in food choices among Romanian consumers. Formal education and online purchasing alone appear insufficient to foster sustainable behavior. The findings support policies emphasizing labeling clarity, consumer education, and digital tools aligned with the European Green Deal. Limitations include non-probabilistic sampling and cross-sectional design. Future research should employ representative samples and longitudinal designs to assess causal relationships and temporal dynamics in sustainability engagement. Incorporating stratified sampling strategies across urban and rural regions and across income groups would improve external validity and allow for population-level inference. Moreover, future studies should integrate additional psychosocial and contextual variables, such as values, social norms, household constraints, and price sensitivity, to capture the multidimensional nature of sustainable food behavior. Mixed-method approaches combining survey data with qualitative interviews or experimental designs could further elucidate the mechanisms underlying the attitude–behavior gap and inform the design of more effective, context-sensitive policy interventions.

Conflicts of interest and sources of funding

The authors declare no conflict of interest. This research received no external funding.

Authors' contribution

Conceptualization, L.M.D. and C.C.; methodology L.M.D., C.A.Z., R.L, AI and C.C; validation, L.M.D. and C.C; formal analysis, L.M.D. and C.C; investigation, L.M.D. and C.C; resources, L.M.D. and C.C; data curation, L.M.D. and C.C; writing—original draft preparation, L.M.D. and C.C; writing—review and editing, L.M.D. and C.C; project administration, L.M.D. and C.C. All authors have read and agreed to the published version of the manuscript. No generative AI was used during the production of this article.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of RESEARCH METABOLIC CENTER (No. 0004/03.09.2024).

Patient consent for publication

Not applicable.

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REVIEW

Modern definition and treatment of HFpEF – what is valid in 2025 and what to expect in 2026

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Abstract: Heart failure with preserved ejection fraction (HFpEF) is a complex pathology that has undergone a paradigm shift in the last few years. As technology develops and diagnosis algorithms become more refined, early diagnosis leads to prompt medical management. This management has also changed in recent years. Moreover, HFpEF phenotyping attempts further nuance the management of patients with this disease. Whereas guidelines are not so firm on the medical classes of drugs that should be employed in HFpEF compared to HFrEF, new trials are on the way to change this. This literature review focuses on the evolution of diagnosis criteria for HFpEF, the clinical scores proposed by current guidelines, and also on the medical management of this pathology, focusing on medical management and how it has changed recently, by highlighting landmark trials that have been published on the topic or that are going to be published on the topic.

Keywords: HFpEF; heart failure; diastolic dysfunction; ISGLT2; GDMT; landmark trials; heart failure phenotyping

INTRODUCTION

Heart failure with preserved ejection fraction (HFpEF) is a challenging syndrome in contemporary cardiology, due to the heterogeneous nature of the disease. Compared to heart failure with reduced ejection fraction (HFrEF), it is more difficult to diagnose. It requires integration of clinical, echocardiographic and biochemical data in order to suggest the diagnosis. Due to its heterogeneity, treatment options have not been as thoroughly studied as in HFrEF. However, new trials are starting to refine the treatment protocols of HFpEF, and it remains to be seen how the conclusions of these trials will be integrated into the new guidelines for management of HFpEF developed by international cardiology societies and international heart failure societies [1].

DIAGNOSIS OF HFpEF

The definition of HFpEF has been refined with the accumulation of increasing evidence. It is currently defined as a clinical syndrome manifesting with heart failure symptoms (exertional dyspnea, fatigue, reduced exercise capacity, peripheral edema of no other cause) associated with hemodynamic changes (spontaneous or provokable increased left ventricular filling pressures, alongside elevated natriuretic peptides) in the presence of a normal left ventricular ejection fraction (≥50%) [2,3].

There are multiple risk factors for developing HFpEF. Out of these, the most frequent are [4]:

- Advanced age
- Female sex
- Obesity
- Atrial fibrillation
- Chronic kidney disease

The clinical examination usually shows signs and symptoms of heart failure (dyspnea, peripheral edema, reduced exercise capacity of no other cause). The ECG shows no

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pathognomonic findings, although it can reveal the pathophysiology of the disease in some situations, showing left ventricular hypertrophy criteria (such as a positive Sokolow-Lyon index or Cornell Index), atrial fibrillation or low QRS voltage in limb leads suggestive of cardiac amyloidosis. Moreover, diffuse low QRS voltage may be seen in obese patients. These changes can already hint towards a phenotype of HFpEF [5,6].

Whereas laboratory assessment can reveal multiple risk factors, such as kidney disease or diabetes mellitus, the most important biomarkers in HFpEF are natriuretic peptides (BNP/B-type natriuretic peptide and NT-proBNP/N-terminal-proBNP). However, compared to HFrEF, the importance of natriuretic peptides is more nuanced, as there are no clear cutoff criteria. The ESC guidelines recommend low cutoff values for screening – NT-proBNP of 125 pg/mL or BNP of 35 pg/mL; these cutoffs have modest specificity in chronic heart failure, but have a very high negative predictive value in the acute setting. Moreover, these cutoffs have less negative predictive value in the chronic setting, especially with the increasing age of the patient. Thus, some authors suggest different cut-off values for young (<50 years, an NT-proBNP cut-off of 50 pg/mL), middle-aged (50-75 years, an NT-proBNP cut-off of 75 pg/mL), and elderly patients (>75 years, an NT-proBNP cut-off of 250 pg/mL). Subsequently, other factors increasing natriuretic peptide levels independent of heart failure should be taken into consideration. Some of these are presented in Table 1 [7–10].

Table 1: Causes of increased natriuretic peptides

Cardiac causes of increased natriuretic peptides	**Noncardiac causes of increased natriuretic peptides**
Acute coronary syndromes	Age
Atrial fibrillation	Female gender
Valvular heart disease	Renal impairment
Cardiomyopathies	Pulmonary embolism
Myocarditis	Obstructive sleep apnea
Cardioversion	Systemic bacterial infections
	Chemotherapy; toxins; critical illness; burns

Echocardiographic assessment is the cornerstone of HFpEF diagnosis, as it offers a non-invasive means of identifying structural and functional cardiac abnormalities, while also confirming the preserved left ventricular ejection fraction. The definition of HFpEF includes objective evidence of cardiac structural and/or functional abnormalities consistent with the presence of either LV diastolic dysfunction and/or raised LV filling pressure. Left ventricular volumes and diameters should be recorded to accurately assess systolic function and ventricular geometry. Furthermore, both concentric and eccentric left ventricular myocardial hypertrophy may also be identified (by measuring left ventricular mass index and relative wall thickness), and in that case, screening for cardiac amyloidosis might be taken into consideration. Other structural parameters assessed are left atrial volumes and left atrial volume index, measured at end-systole. Diastolic function parameters are very important for highlighting increased left ventricular filling pressures. The most frequent functional parameters assessed are: early mitral inflow velocity (E), E/A ratio in sinus rhythm, E/average e' ratio, and tricuspid regurgitation velocity. However, newer algorithms for assessing LV filling pressures are under development, taking into account other echocardiographic parameters, such as pulmonary S/D ratio or left atrial strain [4,11–14].

There are 2 scores mentioned in the 2021 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure, although a recommendation for their usage is not offered. These scores are HFA-PEFF and H2-FPEF.

HFA-PEFF is recommended by the Heart Failure Association and includes a 4-step algorithm

1. P – Pretest assessment – assessing signs and symptoms of HF, risk factors, ECG, standard echocardiography (to confirm normal ejection fraction and to exclude other causes of heart failure)
2. E – Echocardiographic (comprehensive) and natriuretic peptide assessment (if not measured in step 1)
3. F1 – Functional testing in case of uncertainty – exercise stress echocardiography to assess stress diastology and/or invasive hemodynamic measurements to assess pulmonary capillary wedge pressure (PCWP)
4. F2 – Final etiology

Step 2 includes 3 measurement domains, with Major (2 points) and Minor (1 point) criteria. These domains are functional, morphological and natriuretic peptide, as presented in Table 2.

A score of at least 5 in step 2 suggests the diagnosis of HFpEF, whereas a score between 2 and 4 recommends a diastolic stress test or invasive hemodynamic measurements. It is important to note that the score in each domain is not cumulative; the maximum score in each domain is taken into account.

Step 3 requires functional testing to assess elevated LV filling pressure during stress or invasive hemodynamic tests at rest or at stress. An average E/e' ≥ 15 (adding 2 points) or tricuspid regurgitation velocity > 3.4 m/s (adding 3 points) during non-invasive exercise stress echocardiography is considered positive. The score obtained in step 3 after exercise stress echocardiography is added to the score from step 2. A total score of at least 5 points confirms HFpEF.

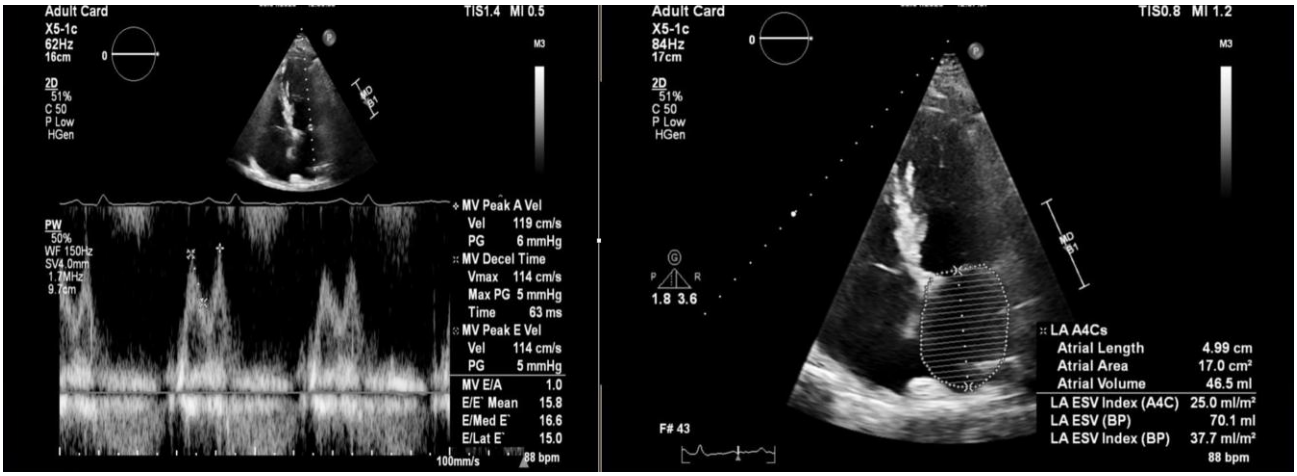
A pulmonary capillary wedge pressure ≥ 15 mmHg at rest or ≥ 25 mmHg during exercise by invasive hemodynamic measurement is considered positive, and the diagnosis of HFpEF is confirmed.

Table 2: HFA-PEFF step 2[OV1.1]

1. Functional measurement domain		
e' – peak early diastolic velocity of mitral annular motion (cm/s)	Major (2 points)	Septal e'< 7 or lateral e'<10 for <75 years Septal e'<5 or lateral e' < 7 for ≥ 75 years
	Minor (1 point)	N/a
E/E' – peak early diastolic velocity of mitral inflow divided by the mean value of e' recorded at the septal and lateral mitral annulus	Major (2 points)	E/e' ≥ 15
	Minor (1 point)	E/e' ratio between 9 and 14
Tricuspid regurgitation velocity (m/s) or Pulmonary arterial systolic pressure (mmHg)	Minor (1 point)	Peak velocity > 2.8 PAPs > 35 mmHg
LV GLS (% as positive value)	Minor (1 point)	GLS < 16
2. Morphological measurement domain		
LAVI – left atrial volume index (ml/m^2)	Major (2 points)	Sinus rhythm: > 34/ atrial fibrillation: > 40
	Minor (1 point)	Sinus rhythm: 29-34/ atrial fibrillation > 34-40
LVH – left ventricular hypertrophy LV max index – LVMI (g/m^2) LV relative wall thickness (RWT)	Major (2 points)	Male: LVMI ≥ 149 and RWT > 0.42; Female: LVMI ≥ 122 and RWT > 0.42
	Minor (1 point) – at least 1 positive criterion	Male: LVMI ≥ 115/RWT >0.42/LV wall thickness ≥ 12 mm Female: LVMI ≥ 95/RWT >0.42/LV wall thickness ≥ 12 mm
3. Natriuretric peptide domain		
Serum concentration of BNP OR Serum concentration of NT-proBNP (pg/mL)	Major (2 points)	Sinus rhythm: BNP>80/ atrial fibrillation: >240 OR Sinus rhythm: NT-proBNP>220/ atrial fibrillation: >660
	Minor (1 point)	Sinus rhythm: BNP 35-80/ atrial fibrillation: 105- 240 OR Sinus rhythm: NT-proBNP>125-2250/ atrial fibrillation: >375-660

An example of elevated LV filling pressures estimated by transthoracic echocardiography from the personal collection of the authors is presented in Figure 1.

Figure 1: TTE suggestive of elevated filling pressures



The other score mentioned in the 2021 ESC guidelines for heart failure is the H2FPEF score, which is more clinically accessible. The focus of this score is the clinical probability of HFpEF based on associated diseases and characteristics of the patient, with less focus on echocardiographic parameters. It must also be noted that the H2FPEF score does not include plasmatic natriuretic peptide dosing. The score incorporates and rates the following items:

- Heavy – BMI>30 kg/m2 – 2 points
- Hypertensive – treatment with at least 2 antihypertensive medications – 1 point
- Atrial Fibrillation (permanent/paroxysmal) – 3 points
- Pulmonary hypertension – PAPs>35 mmHg – 1 point
- Elder – age > 60 years – 1 point
- Filling pressures – E/e' ratio > 9 – 1 point

Regarding the concordance of these scores, there are multiple studies comparing them. In a meta-analysis published by Estes-Schmatzl et al. in 2025, the 2 diagnostic algorithms produced discordant results in the same patient population in 28-41% of cases. The H2FPEF score had a higher sensitivity, identifying patients with obesity or atrial fibrillation HFpEF phenotypes, whereas the HFA-PEFF algorithms had a higher specificity, as they required more complex and comprehensive imaging assessment. Moreover, in another article published in 2022 by Amanai et al, the only H2FPEF score could be used to discriminate between patients with dyspnea and those with poor exercise capacity. These practical insights suggest complementary roles, rather than competitive ones, for these 2 scores in clinical practice, as H2FPEF is more suitable for primary care and general cardiology, whereas HFA-PEFF could be used in specialized heart failure clinics [15–20].

Furthermore, as machine learning and AI become more accessible in medicine, the implementation of AI algorithms for HFpEF might become widespread. There are multiple proposed models being developed. One model is the EchoGo Heart Failure model, which uses neural network analysis of an apical 4-chamber transthoracic echocardiogram video to predict HFpEF with a sensitivity of 82% and specificity of 81%. Another model, called AIM-HFpEF, integrates data from electronic health records and analyses them using natural language processing, with a sensitivity of 89% and a specificity of 62% for the diagnosis of HFpEF [21,22].

RECENT LANDMARK TRIALS IN HFPEF

After 2021, there was a paradigm shift in the treatment for HFpEF, with the validation of SGLT2 inhibitors in patients with chronic heart failure with preserved ejection fraction. In 2021, EMPEROR-PRESERVED showed a reduction in cardiovascular death or heart failure hospitalization in patients treated with empagliflozin, while also improving health-related quality of life. The primary outcomes were reached by reducing heart failure hospitalization, as the reduction of cardiovascular deaths did not reach statistical significance alone, only in association with HF hospitalization [23–25]

After the validation of empagliflozin in 2021, the results of the DELIVER trial showed benefits in reducing cardiovascular death or worsening heart failure, while maintaining efficacy on the whole spectrum of ejection fraction by using another SGLT2 inhibitor, dapagliflozin [26–29].

These two trials led to ESC publishing a focused update of the heart failure guidelines in 2023, recommending SGLT2 inhibitors with a class I recommendation, with an A level of evidence [1].

Furthermore, 2024 marked the publication of 2 trials assessing GLP-1 agonists in HFpEF patients with obesity. The STEP-HFpEF trial followed semaglutide in patients with obesity-related HFpEF. The primary endpoints reached were a reduction in body weight and an increase in perceived health-related quality of life, as assessed by KCCQ-CSS. In a subgroup analysis, the body weight reduction in women was higher. However, it must be noted that only 3.6% of patients received SGLT2 inhibitors for HFpEF, and it is difficult to assess whether the benefits were driven by weight loss or improvement in HF symptoms. This study highlights the benefit of semaglutide in improving HF-related symptoms and exercise function, regardless of baseline health status or sex, but requires a larger study in order to follow cardiovascular events, as STEP-HFpEF was underpowered for clinical events [30–32].

Another GLP-1 agonist trial was the SUMMIT trial, published in 2024, which compared the safety and efficacy of tirzepatide among patients with HFpEF and obesity. Tirzepatide is a GLP-1 and GIP agonist, and it was shown in this trial that it can reduce cardiovascular death and a worsening HF event compared to placebo, while also increasing patient-oriented quality of life and functional capacity in obese patients with HFpEF. This molecule has also been shown to reverse left ventricular remodeling by reducing left ventricular mass and paracardiac adipose tissue on cardiac magnetic resonance studies. Moreover, the reduction of high-sensitivity C-reactive protein in the tirzepatide group is theorized to be a separate mechanism of action of this molecule [33–35].

However, there is no recommendation from ESC to prescribe GLP-1 receptor agonists in patients with heart failure yet, but a recommendation for the prescription of GLP1-RA with proven cardiovascular benefits (at that moment – liraglutide, semaglutide, dulaglutide, epfeglenatide) for patients with type 2 diabetes mellitus and atherosclerotic cardiovascular disease, with a class I, level of evidence A according to the ESC clinical consensus statement on obesity and cardiovascular disease [36].

Another landmark trial published in 2024 is FINEARTS-HF, which investigated the efficacy and safety of finerenone, a nonsteroidal mineralocorticoid receptor antagonist, in reducing the risk of cardiovascular death and total worsening heart failure events in patients with HFmrEF, HFpEF and HFimpEF. The primary endpoint of the study was reached and there was no modification of the benefit across the range of LVEF analyzed. Moreover, the patients reported an increase in perceived quality of life and there were no differences in benefits between sexes [37–40].

There are multiple studies in development assessing various strategies and molecules in HFpEF. One of them is AMBER-HFpEF, which assesses the safety, tolerability, pharmacokinetics, and pharmacodynamics of ulacamten, which is a selective, oral cardiac myosin inhibitor. However, this is only a Phase 2 study, enrolling only 60 patients with symptomatic HFpEF [41].

Chronic, systemic low-grade inflammation is recognized as a key element in HFpEF pathophysiology. Another study, HERMES, is a phase 3 trial assessing ziltivekimab, which is an anti-inflammatory molecule targeting IL-6 signaling, with a composite primary outcome of cardiovascular death, heart failure hospitalization, or urgent heart failure visit. There are also multiple studies regarding the anti-inflammatory effects of colchicine on the quality of life, vascular, and cardiac function in patients with HFpEF, such as COLHEART-PRESERVED. [42–46].

Senolytic agents, which eliminate senescent cells, are being studied preclinically in laboratory models of HFpEF in reversing cardiac and vascular remodeling in aging models. However, more data is required before initiating large-scale trials in the foreseeable future [47].

These pharmacology trials are in close relationship with recent attempts to further classify HFpEF into distinct phenotypes that may benefit from different treatments and interventions. Furthermore, pooled data from these studies, whether they reached the end point (such as EMPEROR-PRESERVED for empagliflozin, DELIVER for dapagliflozin, or FINEARTS-HF for finerenone) or did not (such as PARAGON-HF for sacubitril/valsartan in HFpEF and TOPCAT for spironolactone), have been used in phenotyping attempts of patients with HFpEF.

PHENOTYPES OF HFPEF

There have been multiple attempts to phenotype HFpEF, just like HFrEF was phenotyped in the past. This helps better understand the pathophysiology of the disease and the appropriate medical management, but also the outcomes of these patients.

One attempt was performed in a subanalysis of the TOPCAT trial, where three distinct phenotypes were identified: one with normal LV geometry, filling pressures, atrial stiffness and natriuretic peptides, but with other clinical features or pathologies that can explain the symptoms, one with atrial fibrillation, usually in elderly women, and one with diabetes and obesity [48].

Another study, performed on a cohort of 2147 patients at Peking University Third Hospital and using a neural network AI model identified three phenogroups: one with multiple metabolic comorbidities, left ventricular hypertrophy and diastolic, but also systolic dysfunction, one with female patients with atrial fibrillation and structural abnormalities in the atria and the right ventricle, with diastolic dysfunction, whereas the 3rd group included younger men with unhealthy lifestyle, hyperlipidemia and liver dysfunction. The first phenogroups presented lower systolic function, as measured by LVEF and Sm, as measured by Tissue Doppler. The second phenogroup exhibited the most significant diastolic dysfunction, with higher E/e' ratios, LAVI, and PCWP. They were also more likely to have significant valvular regurgitation or atrial fibrillation [49].

Larson et al, in 2024, developed a different category of phenogroups by performing invasive hemodynamic cardiopulmonary testing. There were 4 groups, but with overlapping characteristics: cardiometabolic, left atrial myopathy, stiff vascular and pulmonary vascular disease, each having distinct hemodynamic characteristics and determinants of exercise intolerance. Furthermore, there were patients who fulfilled the criteria for more than one phenogroup, and these patients had worse outcomes and functional capacity [50,51] .

The cardiometabolic subgroup's pathophysiology is centered around type 2 diabetes mellitus and obesity, as these diseases lead to chronic low-grade inflammation that alters LV remodeling, accelerating concentric hypertrophy and diastolic dysfunction. Moreover, these patients usually exhibit increased visceral adiposity, causing adipokine imbalances that further increase and maintain the pro-inflammatory status of these patients. Moreover, obese patients need a greater volume of circulating blood, increasing stroke volume and leading to increased myocardial wall stress, further accelerating left ventricular remodeling and hypertrophy [52,53]. These metabolic changes highlight the benefits of weight loss by bariatric surgery, when indicated, or in milder cases of obesity, the usage of glucagon-like peptide-1 receptor agonists that alter adipokine secretion and reduce hunger by slowing gastric emptying and modulating hunger centers in the central nervous system.

Atrial fibrillation is frequently associated with HFpEF and has been described as a phenotype of HFpEF in various studies. There are several elements of the pathophysiology of atrial fibrillation that are common with HFpEF and may lead or accelerate the development of this disease, in a vicious cycle. Some of these mechanisms are the pro-inflammatory state, which is common between the two diseases, but also cardiac microvascular endothelial cell lesions, with impaired cardiac microvascular dysfunction, cardiac remodeling, and diastolic dysfunction, with alteration in the calcium metabolism of cardiomyocytes. The pro-inflammatory microenvironment in the atria in patients with early atrial lesions, with increased TNF-α, TGF-β, IL1B, and IL6, is also important in the pathophysiology of HFpEF. The inflammatory cells in the atrial myocardium expressed TGF-β, which triggers differentiation of fibroblasts to myofibroblasts, which produced more collagen and decreased the local concentration of matrix metalloproteinases, which reduced total cellularity by increasing the collagen/cell ratio in the atria. Furthermore, this local inflammation can further increase the risk of atrial fibrillation by leading to atrial fibrosis. Furthermore, in atrial fibrillation, LV filling is altered not only by losing the atrial systole, but also by the increased ventricular rate, which is often associated with atrial fibrillation. This can, in time, transform an atrial cardiomyopathy into atrial fibrillation-induced ventricular cardiomyopathy, which is associated with impaired systolic function of the left ventricle. There are also multiple comorbidities that are associated with both HFpEF and atrial fibrillation, accelerating the vicious circle by inducing a significant pro-inflammatory state in the body. These comorbidities are arterial hypertension (or other causes of arterial stiffness), diabetes mellitus, obesity, or renal disease. Obesity is important, especially in association with atherosclerosis, which is itself an inflammatory disease with a vicious cycle in which diabetes, arterial stiffness, and dyslipidemia are frequently associated. These diseases cause vascular inflammation, causing monocyte infiltration and macrophage activation, causing microvascular dysfunction and increased afterload, which triggers LV remodeling by concentric or eccentric hypertrophy. An important disease that triggers chronic inflammation is chronic kidney disease, which has multiple risk factors, including atrial fibrillation and HFpEF (age, obesity, type 2 diabetes mellitus). This chronic inflammation leads to increased reactive oxygen species, decreased availability of nitric oxide, and microvascular dysfunction, mechanisms that are common among chronic low-grade pro-inflammatory diseases. Furthermore, overactivation of the renin-angiotensin-aldosterone system also contributes to LV remodeling. Moreover, increased monocyte percentages have been associated with asymptomatic diastolic dysfunction and correlated with diastolic dysfunction on cardiac transthoracic ultrasound [54–62].

These pathophysiological changes in the atrial fibrillation phenogroup with HFpEF highlight the importance of rate control and rhythm control. However, data regarding catheter ablation for patients with atrial fibrillation and HFpEF is contradicting. While a study from the Swedish Heart Failure Registry identified lower risk of all-cause mortality or heart failure hospitalization regardless of the ejection fraction, a meta-analysis performed by Oraii et al and published in 2024 in JAMA Cardiology, pooling 2465 participants from 12 randomized control trials found no differences in cardiovascular death, all-cause death or heart failure events in the HFpEF subgroup, which included 913 participants [63,64].

Another hemodynamic phenogroup described was the one with arterial stiffness. One significant cause of arterial stiffness is arterial hypertension. There are multiple links between these and HFpEF, as arterial hypertension increases afterload and left ventricular hypertrophy and fibrosis due to microvascular dysfunction. These left ventricular changes lead to left ventricular remodeling. Endothelial senescence has been identified as an important element in the pathophysiology of several metabolic and cardiovascular diseases. Endothelial cells are exposed to multiple stimuli that can lead to senescence. These stimuli can be hemodynamic, such as shear stress or disturbed blood flow (in bifurcations or in stenosed arteries), or chemical (inflammatory cytokines, reactive oxygen species, metabolites). At a molecular level, senescence is caused by multiple, successive rounds of cell division that shorten telomeres, as the majority of human adult somatic cells have very low levels of telomerase, which can replenish the telomeric reserve [65].

One of the principal effects of endothelial senescence is inflammation, leading to low-grade diffuse inflammation that has been associated with multiple diseases, such as atherosclerosis or HFpEF. Moreover, there is a vicious cycle where these pathologies, all proinflammatory, can accelerate one another. Hypertension and aging are closely related, as endothelial dysfunction associated with senescence leads to an increased concentration of vasoconstricting endothelin-1 and angiotensin-2 compared to vasoprotective and

vasodilatory molecules such as NO. Furthermore, increased pulse pressure leads to a significant exposure of endothelial cells to mechanical stress that can accelerate endothelial cell damage, leading to a positive feedback loop that accentuates hypertension. Multiple studies have identified hypertension as a significant cause of left ventricular remodeling by concentric or eccentric hypertrophy, while also increasing left ventricular myocardial stiffness, leading to increased filling pressures and diastolic dysfunction that is present in many cases of HFpEF. There are multiple studies underway with molecules that target endothelial senescent cells, but none are yet focused on HFpEF. However, anti-inflammatory molecules may also have a positive effect on cellular senescence. As previously mentioned, there are multiple studies ongoing assessing anti-inflammatory molecules and HFpEF, such as ziltivekimab and colchicine [65–71].

CONCLUSION

Heart failure with preserved ejection fraction diagnosis and treatment have been constantly refined by important trials assessing diagnostic scores and medical treatment of this disease, such as EMPEROR-PRESERVED, DELIVER, FINEARTS-HF, or SUMMIT. With multiple molecules currently being studied, the future of HFpEF treatment guidelines might change, leading to multiple treatment options for these patients.

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Authors' contribution

Conceptualization, A-E. M., S-I. D.; resources, A.P., S.C.; writing—original draft preparation, B-V.V., I.T.I; writing—review and editing, S.C, A.P.; tables and figures, I.T.I., A.P.; supervision, A-E.M., S-I.D. All authors have read and agreed to the published version of the manuscript. No generative AI was used for the production of this article.

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ARTICLE

Reliability and Validity of the Persian Version of the Nurse–Patient Therapeutic Communication Questionnaire

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Abstract: This study aimed to assess the validity and reliability of the Therapeutic Communication Questionnaire for nurses. This cross-sectional analytical research was conducted in Kashan in 2023. After the translation process, the questionnaire was distributed among the target community. The questionnaire was designed in electronic form (Porsline) and consisted of three sections: demographic information, the Therapeutic Communication Questionnaire for nurses with patients, and a questionnaire on barriers to effective ("nurse–patient") communication. After completing the questionnaire, the data were entered into SPSS (version 16). Out of 347 participants, 123 (35.47%) were male, with a mean age of 27.07 ± 3.97 years, respectively. The Cronbach's alpha coefficient for the questionnaire was 0.89, and the test–retest reliability (intraclass correlation coefficient, ICC (3,1) was 0.80. The Bartlett's test value was 10368.72, which was statistically significant (P<0.001). The Kaiser-Meyer-Olkin Measure (KMO) index in the results analysis was 0.86. Exploratory factor analysis using principal axis factoring with Promax rotation, combined with scree plot and parallel analysis, supported a three-factor solution explaining 42.46% of the variance. (Only factors 1 and 2 had acceptable reliability.) The questionnaire shows good validity and reliability for Persian speakers. However, cultural differences must be considered, as attitudes influence the nature of communication.

Keywords: nursing; therapeutic communication; questionnaire validity; questionnaire reliability; Persian translation

INTRODUCTION

Communication is an essential skill in clinical environments, especially in medical professions [1]. Effective interaction and communication between healthcare professionals, particularly nurses, and patients can play a significant role in the process of patient care and treatment [2]. Additionally, adequate and appropriate communication with patients can enhance the quality of health care services and increase patient satisfaction with medical staff and provided services [3, 4].

In some circumstances, professionalism and acquiring necessary communication skills with patients may be influenced by factors such as the nursing profession, environment, and the patient [5]. Studies indicate that proper and effective communication with patients correlates with increased patient satisfaction and well-being [6, 7]. Conversely, inadequate communication may lead to medication errors and unintended harm [2]. Given the importance of interaction and communication between patients and nurses, there is a need for special attention to communication competence in the nursing profession [4].

Recent studies have shown that although healthcare professionals receive training in communication with patients, the application of these skills in clinical settings when dealing with patients is minimally reported [8, 9].

Perhaps one reason for the weakness in "nurse–patient" communication is the lack of awareness of this interaction and ignorance of the extent of the relationship, partly due to the shortage of measurement tools. Currently, there are some questionnaires for assessing the communication styles or attitudes of "nurse–patient" communication, some of which have been evaluated in Persian [10, 11].

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However, until now, there has been no questionnaire specifically designed to measure the therapeutic relationship between nurses and patients. Therefore, the aim of this study is to determine the validity and reliability of the Therapeutic Communication Questionnaire for nurses working with patients in the hospitals of Kashan city in Iran.

MATERIALS AND METHODS

This cross-sectional study assesses the validity and reliability of the Therapeutic Communication Questionnaire for nurses interacting with patients in hospitals located in Kashan city in 2023. After obtaining approval and necessary coordination with the administrative and managerial departments of the hospitals, the research was initiated. The research sample included nurses and paramedics (Bachelor of Science in Operating Room Technology and Bachelor of Science in Anesthesia) in the hospitals of Kashan city. Using the convenience method, samples were selected from available nurses and paramedics. Individuals unwilling to participate were excluded from the study. The questionnaire was in English. The questionnaire was translated into Persian in several stages, including (a) using bilingual translators with a psychological background who were familiar with both cultures; (b) translation from English to Persian by two translators and back-translation from Persian to English by two separate translators with a management background; and (c) peer review of the original English, Persian, and translated versions to assess clarity, discrepancies, and semantic errors. The questionnaire was sent to 350 people, of whom 347 responded completely. The questionnaire was designed in electronic form (Porsline) and consisted of three sections: demographic information, the Therapeutic Communication Questionnaire for nurses with patients, and a questionnaire on barriers to effective “nurse–patient” communication. It was distributed to the target group through common messaging apps. Access to each nurse was facilitated through communication with the department heads, who shared the questionnaire link along with written instructions with their respective teams. Each participant's link was unique, and the time to complete the questionnaires was also removed. Duplicate responses were also removed. This study was approved by Kashan University of Medical Sciences (IR.KAUMS.NUHEPM.REC.1401.028). The Persian version consists of 49 items. The factors were called dimensions that included the following elements: 1) professional (1–20); 2) contextual and situational (21–34); and 3) patient (35–49).

Correspondence with the Original Author

The Therapeutic Communication Questionnaire for “nurse–patient” was obtained after searching databases, reviewing the questionnaire, and ensuring there was no existing Persian version. Contact was made with the responsible author of the research via email, explaining our request. The questionnaire was then provided to us.

Research Tool

Therapeutic Communication Questionnaire for “nurse–patient”: This tool was developed by Verónica V. Marquez-Hernándeza [3]. The final version of the questionnaire consists of 49 items, each rated on a scale from 1 to 4. The questionnaire is divided into three sections, with factors 1, 2, and 3 comprising 20, 14, and 15 items, respectively. The questionnaire assesses professional (items 1-20), contextual and situational (items 21-34), and patient-related (items 35-49) aspects. A Likert scale of 1 - 4 was used, where 1 indicates not important at all, and 4 indicates very important. Some questions were reverse-scored based on the original author's opinion. These questions include 21, 24, 25, 36, 39, 42, and 49. The higher scores indicate better therapeutic communication.

Translation Process to Persian

The original English version was translated into Persian, reviewed by two Persian translators experienced in English text translation, and further examined by a group of Persian language experts proficient in English. The translated Persian version was then translated back into English by the two bilingual experts. The final Persian translation was obtained after comparing the original and translated expressions and incorporating suggestions.

Content Validity

To assess content validity, the questionnaire was reviewed by three experts. If the content validity index (CVI) was less than 0.7, the item was rejected. If it ranged between 0.7 and 0.79, it needed modification, and if it was above 0.79, it was considered acceptable [12].

Questionnaire on Barriers to Effective Nurse-Patient Communication (Questionnaire Used for Concurrent Validity)

The data collection tool is a two-part questionnaire that includes the personal information of patients and questions related to the nurses' perspectives on communication barriers. The questionnaire consists of 30 multiple-choice questions, which are evaluated in four areas: individual and social factors (8 items), job characteristics (9 items), clinical conditions of the patient (4 items), and environmental factors (9 items). At the end, an open-ended question is asked. The options range from a maximum score of 5 (completely agree) to a minimum score of 1 (completely disagree). The questionnaire's face and content validity were determined using the opinions of experts and the content validity index (CVI) (0.81). The Cronbach's alpha coefficient for this questionnaire was found to be 0.96, indicating high reliability [13].

Reliability

External reliability was determined by having 30 nurses complete the questionnaire, and test-retest reliability was measured over a 10-day interval. A correlation of 0.7 or higher indicated that the tool had reliable properties (14). Internal reliability (Cronbach's alpha) was calculated using a scale from 0 to 1, and a coefficient above 0.7 was considered[15].

Convergent validity

Convergent validity was assessed using the Therapeutic Communication Questionnaire for nurses with patients and the Pearson correlation coefficient.

Construct validity

Exploratory factor analysis was employed to examine the structural validity, and the construction validity of questionnaire items was examined using exploratory factor analysis. The Principal Axis Factoring extraction method with Promax (oblique) rotation(κ=4) was applied. Sampling adequacy and correlation among items were evaluated using the Kaiser-Meyer-Olkin measure and Bartlett's test of sphericity.

In summary, this research employed a thorough approach to ensure the translation, validation, and reliability testing of the Therapeutic Communication Questionnaire for nurses with patients in the hospitals of Kashan city.

RESULTS

The findings indicate that out of the total 347 participants, 123 individuals (35.47%) were male and 224 individuals (64.53%) were female, who completed the questionnaire. The participants' average age was 27.07 ± 3.97 years, and the lowest and highest ages in the study were 24 and 47 years old, respectively. Average and standard deviation of nurses' work experience: 3.55±3.10 years. The minimum and maximum work experience were 2 and 19 years, respectively.

The obtained results from the average scores of each domain indicate that in the questionnaire on barriers of effective nurse-patient communication, the highest score, relative to the number of items, pertains to the clinical factors domain (12.5 out of 20 points), whereas the lowest score is related to job-related characteristics (21.45 out of 45 points). In the nurse-patient therapeutic communication questionnaire, the highest score is associated with professional (66.19 out of 80 points), and the lowest score is related to the contextual and/or situational domain (41.70 out of 45 points) (Table 1).

Table 1: Mean and standard deviation of the Questionnaires

Barriers to Effective Nurse-Patient Communication	Mean score	Standard Deviation
Individual and social factors	23.72	5.28
Job characteristics	21.45	6.53
Clinical conditions of the patient	12.50	3.08
Environmental factors	21.51	6.73
Total score of the Questionnaire	79.19	17.12
Nurse–PatientTherapeuticCommunication Questionnaire	Mean score	Standard Deviation
Professional (1–20)	66.19	9.15
Contextual and situational (21–34)	41.70	5.16
Patient (35–49)	42.36	4.38
Total score of the Questionnaire	150.53	16.93

At the item level, floor effects were minimal across all items (<20%). Ceiling effects were observed for several items, with up to 50% of respondents selecting the highest response option. However, at the scale level, no floor or ceiling effects were detected, as the observed total score means ranged from 1.5 to 3.7, with no respondents achieving the minimum or maximum possible scores. The factor loadings of the final three-factor solution were obtained using principal axis factoring with Promax rotation. Only factor loadings with absolute value≥ 0.30 are retained (Table2).

Table 2: Descriptive statistics, final factor structure, item retention status, and correlation of the final Persian version

Item No.	Mean	SD	Final Factor	Loading||	SD	Ceiling %	Floor %	Corrected Item-Total Correlation
1	3.27	0.710	Factor 1	0.470	Retained	40.7	1.4	0.433
3	3.27	0.707	-	0.212	Removed	40.4	1.7	0.565
4	3.52	0.715	Factor 1	0.449	Retained	61.6	2.6	0.528
5	3.41	0.783	Factor 1	0.581	Retained	55.6	4	0.507
6	3.42	0.694	Factor 1	0.564	Retained	52.1	1.4	0.571
7	3.36	0.676	Factor 1	0.651	Retained	45.8	1.4	0.553
8	3.09	0.750	Factor 1	0.611	Retained	37.5	1.4	0.582
9	2.70	1.061	Factor 1	0.337	Retained	28.9	16.3	0.321
10	3.44	0.687	Factor 2	0.334	Retained	53.6	1.4	0.483
11	3.23	0.798	Factor 1	0.693	Retained	41.8	4	0.532
12	3.53	0.681	Factor 1	0.416	Retained	61.9	1.7	0.510
13	3.29	0.763	Factor 1	0.857	Retained	45	2.3	0.567
14	3.40	0.711	Factor 1	0.642	Retained	51	1.7	0.526
15	3.43	.0625	Factor 1	0.690	Retained	49.3	0.9	0.662
16	3.29	0.802	Factor 2	0.360	Retained	47	3.2	0.629
17	3.28	0.764	Factor 2	0.357	Retained	44.4	2.3	0.549
18	3.46	0.713	Factor 1	0.774	Retained	55.6	2.6	0.688
19	3.33	0.693	Factor 1	0.848	Retained	43.8	1.4	0.601
20	3.21	0.726	Factor 1	0.558	Retained	37.5	1.4	0.666
21	2.61	1.010	Factor 2	0.418	Retained	22.6	16	0.312
22	2.93	0.880	Factor 3	0.662	Retained	30.1	4.9	0.487
23	3.14	0.708	Factor 2	0.557	Retained	30.7	2	0.683
25	2.61	0.935	Factor 1	0.422	Retained	18.9	12.6	0.213
26	3.21	0.626	-	0.179	Removed	31.2	0.9	0.583
27	3.33	0.742	Factor 3	0.530	Retained	46.4	2.3	0.538
28	2.98	0.868	Factor 1	0.420	Retained	30.4	6	0.484
29	3.24	0.743	Factor 1	0.428	Retained	40.4	1.7	0.565
30	3.23	0.777	Factor 2	0.723	Retained	41.5	2.3	0.551
32	3.27	0.718	Factor 1	0.394	Retained	41.3	1.4	0.625
33	3.09	1.019	Factor 1	0.444	Retained	45.8	10.3	0.503
34	3.29	0.696	-	0.210	Removed	41.3	1.4	0.675
36	2.55	0.937	Factor 2	0.639	Retained	17.8	13.5	0.359
37	3.01	0.843	Factor 2	0.577	Retained	27.5	8	0.595
38	3.20	0.820	Factor 2	0.566	Retained	41.5	4	0.488
39	3.05	0.906	-	0.216	Removed	35.5	8	0.298
40	3.25	0.851	Factor 3	0.628	Retained	45.8	5.4	0.486
41	3.14	0.867	Factor 3	0.423	Retained	39.5	5.7	0.513
42	2.51	0.992	Factor 1	0.421	Retained	18.1	18.3	0.173
43	3.02	0.839	Factor 3	0.769	Retained	30.4	5.7	0.478
44	2.92	0.967	Factor 3	0.451	Retained	31.8	10.9	0.216
45	3.00	0.887	Factor 2	0.702	Retained	34.1	4.9	0.505
46	3.09	0.768	Factor 3	0.543	Retained	30.9	3.2	0.582
47	2.88	0.878	Factor 2	0.555	Retained	25.5	7.4	0.574
48	3.22	0.735	Factor 3	0.589	Retained	38	1.4	0.594
49	3.17	0.826	Factor 2	0.600	Retained	38.3	5.5	0.555

The questionnaire, after undergoing the translation process, was assessed by nurses for its clarity, and the obtained results indicated the face validity of the questionnaire.

The content validity of the questionnaire was calculated to be 0.94. Cronbach's alpha coefficients for the three extracted factors were 0.927, 0.856, and 0.027, respectively. After examination, the third factor showed poor internal consistency, suggesting that the corresponding items may represent a heterogeneous construct and require further refinement. The overall reliability of the full

questionnaire was α=0.89 and ICC (3,1) =0.80, 95% CI = 0.89, 0.92, indicating acceptable stability. McDonald's omega coefficients were also computed, yielding similar results to Cronbach's alpha (ω_total=0.88). The MDC95 values of test-retest reliability were 2.11.

The convergent validity between the Therapeutic Communication Questionnaire and the Barriers to Effective Communication Questionnaire was examined using Pearson correlation. A small but statistically significant negative correlation (r = –0.23, 95% CI (0.135-0.314), P<0.001) was observed between therapeutic communication and perceived barriers, consistent with the expectation that higher communication competence is associated with fewer barriers. (Table 3).

Table 3: Correlation between the nurse-patient therapeutic communication questionnaire and the obstacles to effective nurse-patient communication questionnaire.

Correlation	Questionnaire of Therapeutic Communication Between the Nurse and Patient	
Questionnaire on Barriers to Effective Nurse-Patient Communication	r	(-0.225)
	Sig	(P<0.001)

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

Based on the exploratory factor analysis (Principal Axis Factoring with Promax rotation), three factors were retained according to the scree plot and parallel analysis, which together explained 42.46% of the total variance (Table 4). Factors with eigenvalues greater than one were initially extracted (13 components > 1), but the scree plot clearly supported a three-factor solution consistent with the theoretical structure of the original questionnaire (Figure 1). (Only factors 1 and 2 had acceptable reliability). The statement that the overall questionnaire has "good reliability" is misleading unless it is qualified to exclude Factor 3 (Figure1).

Table 4: Exploratory Factor Analysis: Total Variance Explained by Three Factors

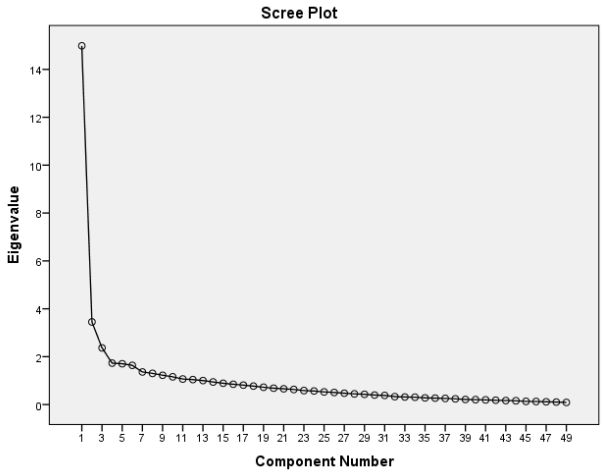
Component	Initial Eigenvalues			Extraction sums of squared loadings		
	Total	% of variance	Cumulative %	Total	% of variance	Cumulative %
1	(14.99)	(30.59)	(30.59)	(14.60)	(29.79)	(29.79)
2	(3.45)	(7.04)	(37.63)	(3.02)	(6.16)	(35.95)
3	(2.37)	(4.83)	(42.46)	(1.98)	(4.05)	(40)

The Kaiser-Meyer-Olkin (KMO) value was 0.86, and Bartlett's test of sphericity was significant (χ2=10368.72; df=1176; P<0.001), confirming sampling adequacy and correlation among items (Table 5).

Table 5: KMO values and Bartlett's Test

Indices	Measurement
Sample adequacy measurement	(0.86)
Bartlett's test	(10368.72)
degrees of freedom	(1176)
Significance level	(P<0.001)

Figure 1: Scree plot chart



DISCUSSION

The main purpose of this study is to provide a suitable tool for assessing the therapeutic relationship between nurses and patients in Persian-speaking nurses. The results indicate that this questionnaire is satisfactory in terms of validity, reliability, and acceptability, making it a suitable tool for use in scientific research by other researchers. Considering that no study related to the questionnaire of the therapeutic relationship between nurses and patients has been conducted in Iran so far, to our knowledge, this study is the first of its kind.

To assess the internal reliability of the questionnaire, Cronbach's alpha test was used. The Cronbach alpha coefficient for the entire questionnaire was 0.89, indicating a high level of internal consistency. This is consistent with other studies, such as the one by Manuel González-Cabrera and colleagues, where the Cronbach's alpha was reported as 0.82 in the design and validation of a questionnaire on delivering bad news in nursing, published in 2020 [16]. Additionally, Mojtaba Fattahi Ardakani et al. reported a Cronbach's alpha of 0.86 in their study on the psychometric properties of the health action process approach in the effective “nurse–patient” relationship in 2019 [11].

The research findings in this area are inconsistent across different cultures, with part of them related to methodology and research. In this test, there was no significant problem, and the sample group had no major issue in understanding the questions. Almost all questions were comprehensible to them, indicating the face validity of the test. To examine the reliability of the questionnaire, the ICC test was used. The overall ICC result for the test was 0.80, indicating satisfactory reliability of the questionnaire, similar to the study conducted by Najafi M and colleagues [17].

The content validity of the questionnaire was obtained by distributing the questionnaire among three experts, of which 94% of the total questionnaire questions were deemed closely or highly relevant. Some questions and sentences were modified, but no questions were removed. The amount of content validity is also similar to the study conducted by Najafi M and colleagues [17].

To identify the factors of this questionnaire and assess its structural validity, factor analysis was used. The findings indicated that 13 items account for 42.46% of the total variance of the questionnaire. This signifies the desirable internal validity of the questionnaire. In similar studies in the field of designing and validating questionnaires on delivering bad news in nursing, an experimental study published in 2020 by Manuel González-Cabrera revealed that out of 28 items, 11 items had eigenvalues greater than 1, explaining 71.60% of the questionnaire's variance [16].

In the current research, exploratory factor analysis was employed to assess the structural validity of the questionnaire. Three factors were identified for the questionnaire items, indicating that the questionnaire is three-dimensional. The elements referred to as dimensions encompassed the following factors: 1) professional (1–20); 2) contextual and/or situational (21–34); and 3) patient (35–49). The identified factors corresponded conceptually to professionalism, contextual/occupational, and patient-related dimensions, explaining 42.46% of the total variance (only factors 1 and 2 had acceptable reliability). The third factor does not meet the minimal reliability criteria ($\alpha \approx 0.03$) and therefore should not be used as a separate subscale; the instrument should be interpreted primarily in terms of the total score and the two reliable domains. The statement that the overall questionnaire has “good reliability” is misleading unless it is qualified to exclude Factor 3.

The internal consistency of the first two dimensions was strong ($\alpha = 0.93$ and 0.86), whereas the third dimension showed low reliability ($\alpha = 0.03$), possibly due to few or heterogeneous items but acceptable McDonald’s ω. The negative correlation with the communication barrier questionnaire ($r = -0.23$) confirmed that both tools measure related but opposite constructs, providing evidence for concurrent validity. The current research confirms the suitability of the used items.

In this study, the Bartlett's test statistic was obtained as $\chi^2 = (10368.72)$; df= (1176), as reported in other similar studies. In Manuel González-Cabrera's study of designing and validating a questionnaire on delivering bad news in nursing, published in 2020, Bartlett's test was significantly meaningful as well [16]. Also, in María del Carmen Giménez-Espert's study on developing and validating a psychometric tool for assessing nurses' attitudes toward patient communication, published in 2018, Bartlett's test showed a significant value as $\chi^2 = 10352.40$; df=820 [16]. In Genoveva Granados-Gámez's study, published in 2009, on developing and validating a questionnaire for analyzing nurse–patient relationship, significant values were also obtained [3].

One limitation of this study was the focus solely on nursing staff and the exclusion of nursing students. It is recommended that future research examine the perspectives of nursing students as future nurses. The study sample is not representative of all Iranian nurses (e.g., by age, region, and experience), and external validity should be interpreted with caution.

CONCLUSION

The questionnaire for assessing the therapeutic relationship between “nurse–patient” demonstrated high levels of content validity, reliability, and structural validity in the Persian-speaking population. The study underscores the need for reliable tools that are culturally appropriate, and the presented questionnaire can serve as a valuable instrument for research in the field of “nurse–patient” relationships in the Iranian context.

Conflicts of interest and sources of funding

The authors declare no conflict of interest

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No artificial intelligence tools were used to write the article.

Authors' contribution

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

Ethics approval and consent to participate

This study has received the ethics code IR.KAUMS.NUHEPM.REC.1401.028 from the Research Ethics Committee of Kashan University of Medical Sciences.

Informed consent was obtained from all participants.

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REVIEW

The crucial role of nucleoprotein in designing a protective vaccine and precision diagnosis for Crimean-Congo hemorrhagic fever virus

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Abstract: Crimean-Congo hemorrhagic fever virus (CCHFV) is a highly pathogenic agent that causes severe hemorrhagic disease with high mortality. Outbreak control requires rapid and accurate diagnosis together with effective vaccination strategies. The nucleoprotein (NP) is a central component of viral replication and of innate and adaptive immune responses, making it a promising target for diagnostic and vaccine development. NP is highly conserved across CCHFV isolates, which supports broad molecular and serological detection. Several studies have demonstrated that NP-based assays provide sensitive and specific results, a critical requirement for outbreak management. In vaccine research, NP has been shown to induce strong cellular immune responses that contribute to long-term protection. Multiple platforms, including recombinant proteins, DNA constructs, and viral vectors, have successfully incorporated NP with promising preclinical immunogenicity. Despite this progress, challenges remain in ensuring safety and achieving consistent immune responses across populations. Advances in nanotechnology, structural vaccinology, and bioinformatics may further improve the effectiveness of NP-based products. In conclusion, NP represents a key element for both reliable diagnostics and the induction of protective immunity. Future research should focus on optimizing NP-based platforms for translation into clinical applications and improving global preparedness against CCHFV.

Keywords: Crimean Congo, biological threat, nucleoprotein, vaccine development

INTRODUCTION

Crimean-Congo hemorrhagic fever virus (CCHFV) is a tick-borne virus that can cause severe disease in humans and livestock [1]. CCHFV possesses a tri-segmented negative-sense RNA genome and is classified within the Orthonairovirus genus of the Nairoviridae family. Clinical symptoms include fever, diarrhea, fatigue, and drowsiness, with severe cases presenting with kidney lesions, liver failure, and lung damage, and a mortality rate of approximately 30% [1,2]. CCHFV poses a widespread threat to global public health due to its potential prevalence, high mortality, nosocomial infections, and difficulties in treatment and prevention [3]. Currently, there is no licensed vaccine against Crimean-Congo hemorrhagic fever. In 2019, the World Health Organization estimated that 3 billion people worldwide are at risk of CCHFV infection [4]. CCHFV causes acute illness in humans, primarily transmitted through tick bites. In animals, it is asymptomatic but poses a major threat to humans in close contact, especially in farms, clinics, and abattoirs. Previous studies have highlighted the immunogenic potential of the CCHFV nucleoprotein (NP) [3,5,6]. NP is abundantly expressed during infection and plays a critical role in the viral lifecycle by packaging the viral genome and antigenome [7]. The significant immune responses elicited by NP, including neutralizing antibodies and CD4+/CD8+ T cells, indicate its suitability as a vaccine antigen [8, 9]. The current study aims to enhance vaccine design against CCHF by incorporating NP alongside glycoproteins [10]. Utilizing bioinformatics approaches, the identification of conserved, immunogenic epitopes within NP could optimize vaccine formulations and diagnostic assays [11]. This integrated strategy may improve protective efficacy and advance prevention/control efforts for this important pathogen. In summary, NP represents a promising antigen for combating CCHFV through vaccination and diagnosis. The

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findings from ongoing research provide insights to guide the rational development of effective countermeasures against this global health threat.

Geographic Distribution

CCHF is one of the most important emerging tick-borne viral diseases, with sporadic human cases regularly reported across various regions globally. While CCHFV circulates naturally among several animal hosts, humans represent a dead end for the virus. The virus is maintained through a silent transmission cycle involving ticks, vertebrates, and subsequent tick reinfection. People in close contact with infected animals or their products face a heightened risk of acquiring the disease. Delayed diagnosis of CCHF can also lead to outbreaks through secondary nosocomial infections [2,6]. CCHFV has an extensive geographic presence as an emerging/re-emerging pathogen. Reported regions include Africa, the Balkans, the Middle East, and Asia. Within the Middle East, cases have been documented in Iran, Iraq, Saudi Arabia, Oman, and the United Arab Emirates. Eastern European nations with local transmission include Albania, Bulgaria, Greece, Kosovo, Turkey, Georgia, and Russia. Its potential for aerosol spreads, high fatality rates, and lack of approved countermeasures qualify CCHFV as a priority pathogen of biosecurity concern [2,8,12].

1. BACKGROUND, GENOMIC AND PROTEOMIC ANALYSIS OF CCHFV

1.1. History

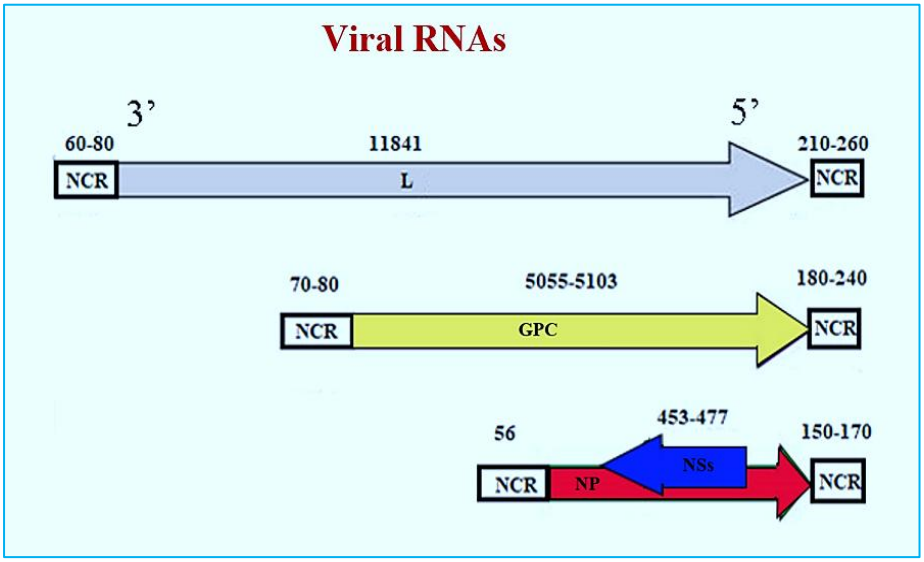
CCHFV was first recognized in 1944 during an outbreak among military personnel in Crimea, Russia, and later isolated in the Congo region in 1956 [13,14]. Subsequent studies confirmed its identity as a highly pathogenic tick-borne virus, leading to the current designation as CCHFV [6,13,15]. Since then, CCHFV has been established as a major public health threat, particularly in Africa, Asia, and Eastern Europe, where its geographic range and incidence are expanding [3]. Mortality is usually 30–50% and can reach 80% in some outbreaks [4]. Still, no approved vaccine or effective antiviral exists [15], and the risk of hospital transmission shows the urgent need for better tools.

1.2. Genome and Proteins

CCHFV possesses a tripartite, linear, single-stranded RNA genome of negative polarity that is encapsidated by viral NP to form distinctive spherical ribonucleoprotein complexes (RNPs) (shown in Figure 1) [3,5]. The small (S) segment is approximately 1,700 nucleotides in length and encodes NP in the viral sense, as well as a non-structural protein (NSs) in the opposite, complementary sense [7]. NP binds to viral RNA and is critical for genome packaging, while NSs act as interferon antagonists to suppress host immune responses [8]. The medium (M) segment measures approximately 5,400 nucleotides [10]. It directs the production of a 160 kDa GPC (glycoprotein precursor) that is cleaved by cellular proteases into the mature glycoproteins glycoprotein N (Gn) and glycoprotein C (Gc), which are found on the virion envelope. The M segment additionally encodes a non-structural protein (NSm) involved in glycoprotein maturation [15]. The largest (L) segment is approximately 12,100 nucleotides in length [1]. It produces an approximately 200 kDa RNA-dependent RNA polymerase with cap-snatching endonuclease and methyltransferase activities essential for transcription and replication of the viral genome [16-18]. Also, the M segment of the virus contains a large GPC, which is processed into seven proteins, including the structural glycoproteins Gn and Gc. These surface glycoproteins are potential vaccine candidates as they have been shown to be targets of neutralizing antibodies. [11,16,19]. GPC also yields a 38 kDa glycoprotein (GP38) and a heavily glycosylated mucin-like domain through additional processing steps [20,21].

CCHFV is an enveloped virus containing a tri-segmented, negative-sense RNA genome [3]. The virion possesses surface glycoproteins Gn and Gc, which facilitate receptor binding and host cell entry. Some evidence suggests an additional protein, GP38, may associate with the viral envelope, though its localization and function remain unclear [7]. The three genomic segments - small (S), medium (M), and large (L) - encode distinct viral proteins. The S segment expresses the NP in one reading frame and an NSs in the opposite frame [8]. Segment M is more complex, directing the production of GPC that host proteases process into glycoproteins Gn and Gc, a mucin-like domain (MLD), GP38, and the NSm involved in glycoprotein maturation [10]. Notably, the L segment of CCHFV is unusually large for bunyaviruses and produces an RNA-dependent RNA polymerase (RdRp) containing an ovarian tumor (OTU) protease domain [1]. The RdRp facilitates transcription and replication of the viral genome [11]. Following receptor-mediated endocytosis, CCHFV undergoes pH-dependent membrane fusion and releases its ribonucleoprotein complexes into the host cytosol [19]. Viral mRNAs initiate translation while the RdRp synthesizes antigens and NP and L proteins package new genomes [3]. Mature virions bud from the Golgi apparatus before exiting via exocytosis [22]. CCHFV proteins also subvert host cell apoptosis and immunity, representing important targets for therapeutic intervention [19].

Figure 1: . Schematic representation of the Crimean–Congo hemorrhagic fever virus (CCHFV) genome.



The CCHFV genome has three segments of negative-sense RNA: small (~1.6 kb), medium (~5.4 kb), and large (~12.1 kb). They code for NP (nucleoprotein), GPC (glycoprotein precursor), and L (large) proteins, respectively, and are flanked by non-coding regions (NCRs). Figure created by the authors for illustrative purposes.

2. INVESTIGATION ON NP AS A KEY GENE IN CCHFV TRI-SEGMENTED GENOME

2.1. NP

NP of CCHFV binds genomic and antigenomic RNAs and interacts with the viral RNA-dependent RNA polymerase (L protein) to form RNPs [4], which are enveloped by a lipid bilayer containing surface glycoproteins [6]. NP can self-assemble into nucleocapsid-like structures and plays essential roles in viral packaging, transcription, and replication, as well as in immune evasion through modulation of apoptosis and suppression of interferon induction [4,6]. Due to its high abundance during infection and strong sequence conservation, NP is highly immunogenic and represents a key viral structural protein for studying CCHFV biology [4,7].

2.2. Conservation of the CCHFV tri-segmented genome

CCHFV shows extensive genetic diversity, categorized into seven clades based on the genetic relatedness of the S segment. These clades are geographically clustered, with three in Africa, two in Asia, and two in Europe. The nucleotide variation between strains can be as high as 20% for the S segment, 31% for the M segment, and 23% for the L segment [8,9]. Genetic analysis of partial M sequences reveals a close relationship between Iranian isolates at less than 1.4% nucleotide diversity [8]. However, the M segment and GPC demonstrate up to 19.3% nucleotide and 14.8% amino acid differences between virus genotypes. Distinct geographic genotypes correlate with observations of S segment comparisons, though greater diversity exists in the M segment and proteins. This likely reflects different tick vectors and vertebrate hosts across virus life cycles [8]. Previous work mapped the main antigenic region of NP between amino acids 201–306, which reacted strongly with convalescent human sera. Comparisons among CCHFV isolates in this region reveal a high level of conservation, with homology ranging from 92–100% [11].

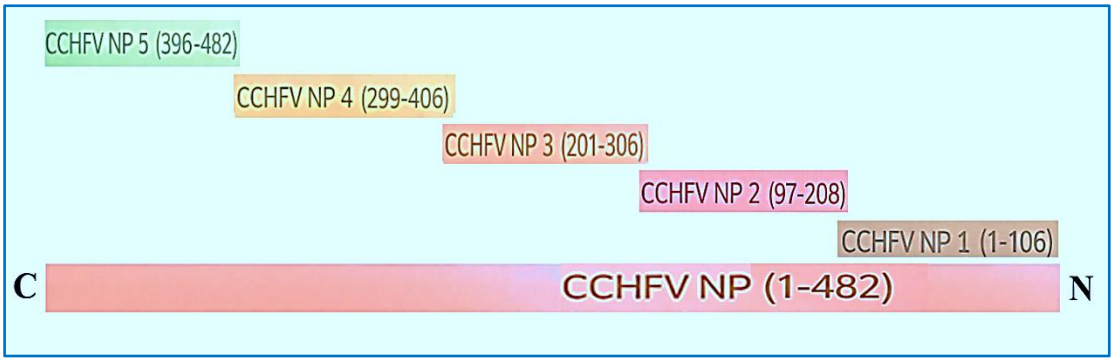
2.2.1. NP-Based Serological Diagnostics

NP of CCHFV is a highly conserved and immunogenic antigen, making it ideal for serological diagnostic assays. One study developed an optimized ELISA for detecting IgG antibodies against CCHFV NP. Most conventional serological methods require manipulation of live virus under BSL-4 conditions, but recombinant NP (rNP) expressed in a baculovirus system enables safe detection of NP-specific antibodies [11]. In this study, rNP derived from the Chinese CCHFV strain 8402 (91.9–92.5% amino acid homology with strain AP929) demonstrated high sensitivity and specificity when compared with the indirect immunofluorescence assay (IFA). The His-tagged rNP allows production without BSL-4 facilities, enhancing accessibility and safety [11]. Sequence analysis identified the NP 201–306 amino acid region as highly conserved and strongly antigenic across global CCHFV strains, supporting its utility in detecting antibodies from diverse lineages. Overall, the validated rNP-based IgG ELISA provides a sensitive, specific, and practical tool for CCHFV serodiagnosis and epidemiological surveillance, addressing key limitations of previous diagnostic methods [10,11].

3. NP CONSERVATION AND SEROLOGICAL APPROACHES FOR CCHFV DETECTION

NP of CCHFV is highly conserved and immunogenic, making it an ideal target for serological diagnostic assays. Conserved hyper-antigenic regions within NP allow precise identification of linear and conformational epitopes, facilitating the development of epitope-based diagnostics. NP-based assays include ELISA, IFA, PRNT, HI, and Western blot, while PCR remains essential for viral detection and strain differentiation [9]. Accurate diagnosis is challenging due to non-specific symptoms, assay limitations, and biosafety requirements. In Kenya, NP-based ELISA demonstrated high seropositivity in livestock and lower rates in rodents and febrile patients, with isolates from the Africa 3 lineage indicating multi-species circulation and the need for ongoing surveillance [5,9,23]. Recombinant NP (rNP) spanning amino acids 201–306, derived from Chinese strains, has been shown to detect antibodies against diverse global CCHFV strains, highlighting its cross-reactivity and broad applicability in serodiagnostic platforms, as illustrated in Figure 2 [10,11].

Figure 2: Schematic overview of truncated nucleoprotein (NP) fragments used for epitope mapping.



The amino acid positions and designations of the truncated NP fragments, which were expressed as fusion proteins with GST on the N-terminal side, are indicated. Figure adapted from Wei et al., 2010 and Saijo et al., 2002 [10,11].

4. DRUG OPTIONS FOR TREATING CCHF

Severe CCHF is a hemorrhagic fever characterized by uncontrolled inflammation and cytokine storms, resulting in significant immunopathology. Ribavirin remains the only approved treatment, underscoring the urgent need for effective vaccines and novel therapies [13]. Limited clinical attempts have explored anti-inflammatory interventions to mitigate the hyperinflammatory response. In one comparative study, high-dose methylprednisolone combined with ribavirin improved outcomes compared with ribavirin alone, with corticosteroids appearing particularly beneficial in severely ill patients, although cohort sizes were small. Experimental data from type I interferon–blocked mice indicate that genetic deletion of the TNF receptor or antibody-mediated blockade of TNF signaling can protect against lethal disease, suggesting that clinically approved anti-TNF agents and other cytokine-targeted drugs warrant further evaluation, as shown in Table 1. Antibody-based therapies have also shown promise: plasma or antibodies from CCHF survivors, as well as mouse and human monoclonal antibodies, can benefit severely ill patients. However, some antibodies effective in neonatal models failed in lethally infected adults, and protection does not necessarily require neutralizing activity. Notably, the protective effect of a GP38-targeting monoclonal antibody was dependent on complement activity [1].

Table 1: The Drugs approved for CCHF treatment.

Therapeutic agent	Pharmacological class	Molecular target	Evidence from preclinical studies	Clinical-level evidence	Key remarks
Ribavirin	Nucleoside analogue antiviral	RNA-dependent RNA polymerase (RdRP)	Variable and controversial efficacy reported in rodent models	Clinical outcomes remain inconsistent across patient populations	Overall, limited benefit; early initiation may be critical; potential use only within combination therapies
Favipiravir	Nucleoside analogue antiviral	RNA-dependent RNA polymerase (RdRP)	Demonstrated antiviral activity in rodent and non-human primate models	Clinical benefit has not been conclusively established	Delayed administration is effective in animal studies; robust clinical trials are required

2′-Deoxy-2′-fluorocytidine	Nucleoside analogue antiviral	RNA-dependent RNA polymerase (RdRP)	Preclinical assessment has not yet been performed	No clinical evidence is currently available	Additional preclinical investigations are necessary
Molnupiravir	Nucleoside analogue antiviral	RNA-dependent RNA polymerase (RdRP)	No significant antiviral activity observed in rodent models	Clinical efficacy data are lacking	Further clinical development appears unlikely
Convalescent plasma or survivor-derived antibodies	Neutralizing and non-neutralizing antibody-based therapy	Viral structural proteins	Preclinical efficacy has not been systematically evaluated	Limited and inconclusive clinical findings were reported	Further preclinical and clinical studies are needed to clarify the therapeutic potential
Monoclonal antibodies	Neutralizing and non-neutralizing antibody-based therapy	Viral structural proteins	Limited supportive evidence from rodent studies	No validated clinical data available	Comprehensive preclinical and clinical validation is required
Corticosteroids	Anti-inflammatory agents	Host immune response modulation	Preclinical investigations have not been reported	Clinical benefit remains uncertain	Current evidence does not support routine use; controlled studies are warranted

5. CCHF VACCINATION

CCHF is a severe, often fatal disease endemic in parts of Africa, Asia, the Middle East, and Southern Europe. Its increasing incidence has led the World Health Organization (WHO) to designate it as a priority for the development of countermeasures [19,24]. While viral envelope glycoproteins are logical vaccine targets due to their role in host cell entry, their antigenic variability facilitates immune evasion. In contrast, the NP is highly conserved and abundantly expressed, making it an attractive vaccine antigen [19]. Multiple vaccine platforms, including subunit, viral vector, DNA, and mRNA vaccines, have been evaluated in preclinical studies, showing variable protective efficacy [1,16,17]. RNA-based vaccines expressing NP alone have achieved complete protection against clinical disease, while combining NP with the glycoprotein precursor further enhanced protection against both disease and viral replication, with even a single 100 ng RNA dose proving protection [25]. Similarly, a DNA vaccine encoding NP and glycoproteins from the CCHFV Hoti strain, administered intramuscularly with electroporation, was well tolerated in cynomolgus macaques and induced robust antibody and T-cell responses. These immune responses correlated with the absence of viremia, reduced tissue viral loads, and improved hematological parameters post challenge [25,26].

5.1 Inactivated Vaccine Platform

The only experimental inactivated CCHFV vaccine to date is the Bulgarian vaccine, developed in the 1970s by propagating a virulent strain in suckling mouse brains, followed by chloroform inactivation and heat treatment at 58 °C to denature viral proteins while preserving conformational epitopes [18,23]. This vaccine has been administered on a limited basis in Bulgaria but remains unapproved by major regulatory authorities. Wider development has been constrained by sporadic case occurrence outside endemic areas and the lack of small animal models that fully mimic human disease [22,27,28]. In a key study, healthy individuals immunized with a four-dose schedule displayed markedly higher frequencies of interferon-gamma (IFN-γ) secreting $CD4^+$ and $CD8^+$ T cells, as well as increased IgG titers, compared with those receiving a single dose [23]. Neutralizing antibody titers in the four-dose group were two- to four-fold higher than in the single-dose group, suggesting that booster doses are important for optimal protection. While the first dose induced detectable antibodies, subsequent doses significantly enhanced both humoral and cellular immunity. These findings indicate that the inactivated vaccine platform can elicit protective immune responses when optimally administered, and further refinement of production methods and immunization regimens could improve safety, availability, and immunogenicity for the Bulgarian vaccine or novel formulations [23,29].

5.2. Adenoviral Vector Vaccine Platform

Adenoviruses are well-characterized viral vectors with high carrying capacity and broad cell tropism, enabling efficient antigen delivery for vaccine development [23]. For CCHFV, adenoviral vectors expressing the N gene induced strong IgG and T-cell responses in mice, providing partial protection against lethal challenge [30]. Despite challenges such as pre-existing immunity and limited transgene expression, continued optimization could enhance their potential for CCHFV vaccine design [23]. In this platform, NP served as the

primary antigen, demonstrating its capacity to induce both humoral and cellular immune responses when delivered via adenoviral vectors.

5.3 DNA Vaccine Platform

DNA vaccines are attractive for CCHFV due to their ability to induce protective immunity via in vivo antigen expression, straightforward design, and lack of interference from pre-existing vector immunity [23]. Although no recombinant DNA vaccines have been approved for bunyaviruses, two leading CCHFV candidates have shown strong immunogenicity in animal models [31]. One encodes ubiquitin-fused Gn, Gc, and NP, while another uses transcriptionally competent virus-like particles (tc-VLPs). In mice, DNA vaccination incorporating NP elicited a balanced Th1/Th2 response and conferred protection against lethal challenge despite modest neutralizing antibody titers. Boosting with tc-VLPs shifted immunity toward a Th2 profile post-challenge. These results highlight NP's role in driving cellular immunity, particularly a Th1 response, which may be critical for durable protection. Continued optimization of antigen selection, delivery systems, and immunization regimens could further improve the efficacy of DNA vaccines for CCHFV. This evidence supports including NP as a core antigen in DNA-based CCHFV vaccine strategies to achieve balanced and long-lasting immunity.

5.4. Subunit Vaccine Platform

The CCHFV envelope polyprotein (EPP) is cleaved into five components: mucin-like variable region, Gp38, Gn, NSm, and Gc, all of which can serve as targets for subunit vaccine design. In one in silico study, protein sequences of EPP and RdRP from the Nigeria/IbAr10200/1970 strain were analyzed for T- and B-cell epitope prediction, MHC binding affinity, and multi-epitope vaccine construct design [32,33]. Selected epitopes were linked with appropriate linkers, fused to a β-defensin adjuvant, and optimized for antigenicity, allergenicity, solubility, and physicochemical stability. Table 2 lists eight predicted cytotoxic T-lymphocyte (CTL) epitopes, and Table 3 summarizes four helper T-lymphocyte (HTL) epitopes with their cytokine-inducing profiles (IL-4, IL-10). Molecular docking and dynamics simulations confirmed strong and stable binding with human immune receptors (TLR2, TLR3, TLR4), and codon optimization suggested high expression potential in Escherichia coli K12. The final 427–amino acid construct incorporated CTL and HTL epitopes along with ten B-cell epitopes, designed to elicit balanced immune responses, including IL-4–mediated B-cell activation and IL-10–driven regulation of inflammation. Although this construct targeted glycoprotein-derived epitopes rather than NP, incorporating conserved NP epitopes into future subunit designs could broaden immune coverage and durability, since NP has demonstrated strong T-cell immunogenicity in other vaccine platforms [23,32,33].

Table 2: Selected CTL epitopes for multi-epitope-based vaccine construction and their antigenicity, immunogenicity, toxicity, allergenicity, conservancy, cross-reactivity with the human proteome, and interacting MHC Class-I allele prediction.

Source protein	CTL epitope sequence	Residue range	HLA supertype	Composite prediction score	Predicted antigenicity score	Predicted immunogenicity score	Toxicity prediction	Allergenicity assessment	Epitope conservation (%)	Associated MHC class I alleles	Cross-reactivity evaluation
EPP	KKIEISGLK	1438–1446	B27	0.9605	0.9491	0.13994	Predicted to be non-toxic	Not applicable	100.00	HLA-A*30:01 HLA-A*03:01 HLA-A*11:01	No cross-reactivity detected
EPP	HREVEINVL	411–419	B39	2.5097	0.7096	0.31548	Predicted to be non-toxic	Not applicable	100.00	HLA-B*08:01 HLA-B*40:01	No cross-reactivity detected
EPP	TEAIVCVEL	1231–1239	B44	1.4217	1.1768	0.22941	Predicted to be non-toxic	Not applicable	100.00	HLA-B*40:01 HLA-B*44:03 HLA-B*44:02	No cross-reactivity detected
RDRP	YLYIVITLY	1411–1419	A1	1.7622	0.9588	0.32448	Predicted to be non-toxic	Not applicable	100.00	HLA-B*15:01 HLA-A*30:02 HLA-A*03:01 HLA-A*26:01 HLA-A*01:01 HLA-B*35:01 HLA-A*32:01 HLA-B*57:01 HLA-A*68:01 HLA-B*58:01 HLA-A*11:01 HLA-B*53:01 HLA-A*33:01 HLA-A*02:01 HLA-A*23:01 HLA-B*44:02 HLA-A*24:02 HLA-B*44:03	No cross-reactivity detected
RDRP	VTDIVVGAI	902–910	A1	1.4576	1.0628	0.27164	Predicted to be non-toxic	Not applicable	100.00	HLA-A*01:01 HLA-B*51:01 HLA-A*68:02 HLA-A*02:06 HLA-A*32:01	No cross-reactivity detected
RDRP	LMSFLNWRV	3263–3271	A2	1.3555	1.1247	0.26439	Predicted to be non-toxic	Not applicable	100.00	HLA-A*02:01 HLA-A*02:03 HLA-A*02:06 HLA-A*68:02 HLA-A*32:01	No cross-reactivity detected
RDRP	YHSIAELTM	42–50	B39	2.0951	0.6095	0.22589	Predicted to be non-toxic	Not applicable	100.00	HLA-B*35:01 HLA-B*08:01 HLA-B*51:01 HLA-A*24:02 HLA-B*53:01	No cross-reactivity detected

										HLA-A*23:01 HLA-B*40:01	
RDRP	IQNRITGLY	2775–2783	B62	1.4651	0.6585	0.23824	Predicted to be non-toxic	Not applicable	100.00	HLA-B*15:01 HLA-A*30:02 HLA-A*01:01 HLA-A*32:01 HLA-A*03:01 HLA-B*57:01 HLA-A*26:01 HLA-B*58:01 HLA-A*30:01 HLA-A*11:01 HLA-B*35:01 HLA-B*44:03 HLA-B*44:02 HLA-B*53:01	No cross-reactivity detected

Table 3: Predicted HTL epitopes and their antigenicity, toxicity, cytokine-inducing properties, conservancy, cross-reactivity, and interacting MHC class-II alleles.

Protein	Epitopes	Position	Antigenicity	Toxicity	IC50 (nM)	IL4 pred	IL10 pred	IFN-epitope	Conservancy (%)	Interacting MHC class II alleles	Cross-reactivity
RDRP	EVLINRNSLKARSE	1240–1254	1.1352	Non-toxic	13.3	Inducer	Inducer	Positive	100.00	HLA-DRB1*08:02 HLA-DQA1*02:01/DQB1*03:01 HLA-DRB5*01:01 HLA-DRB1*13:02 HLA-DRB1*15:01	Zero
RDRP	VHEKRNOPPSVENVO	700–714	1.2275	Non-toxic	5	Inducer	Inducer	Positive	80.00	HLA-DQB1*02:02 HLA-DRB1*02:02 HLA-DRB3*02:02 HLA-DRB1*09:01	Zero
EPP	KAFSAMPKTSLCFYI	1508–1522	0.5604	Non-toxic	10	Inducer	Inducer	Positive	100.00	HLA-DRB1*09:01 HLA-DRB1*11:01	Zero
EPP	ILFFMFGWRILFCFK	1610–1624	1.1537	Non-toxic	6.3	Inducer	Inducer	Positive	86.67	HLA-DQA1*01:01/DQB1*05:01 HLA-DRB3*01:01 HLA-DPA1*01:03/DPB1*02:01 HLA-DPA1*03:01/DPB1*04:02	Zero

5.5. Virus-Like Particle Vaccine Platform

NP can self-assemble into VLPs when overexpressed in insect cells, although the assembly mechanisms remain incompletely understood [4]. VLPs mimic the native virion structure while lacking infectious material, combining high immunogenicity with an excellent safety profile [2,23]. For some viruses, nucleocapsids or glycoproteins autonomously form VLPs during replication, and VLP-based vaccines have demonstrated protective efficacy for other pathogens. The baculovirus expression system supports high-level protein production, facilitating CCHFV VLP formation. The conserved 235–305 amino acid region of NP contains strong antigenic domains, making it a suitable immunogen for VLP design [23]. Preclinical candidates include DNA constructs and modified vaccinia Ankara (MVA) vectors encoding the full GPC or individual NP subunits, which conferred full or partial protection in murine models [19]. MVA, a replication-deficient vaccinia strain, supports high-level transgene expression and has shown promise for CCHFV vaccines, though immune responses require optimization [23–25]. Inactivated virus preparations and combined DNA/VLP regimens achieved partial protection, while subunit or MVA-N vaccines failed to protect [19]. Glycoprotein-focused strategies induced antibodies without protection, underscoring the need to incorporate conserved antigens such as NP [19,27]. Table 4 summarizes all vaccine platforms evaluated for CCHFV to date, and Table 5 provides a comparative overview of these platforms along with the specific role of NP in CCHFV vaccine development. Overall, VLP approaches merit continued development through optimized antigen selection, production processes, and identification of correlates of protection [22,23,29].

5.6 NP in Vaccine Design

NP of CCHFV is highly conserved and abundantly expressed, making it a prime candidate for inclusion in vaccine platforms. It has been evaluated in multiple formats, including recombinant NP subunits, DNA vaccines encoding NP, viral-vectored vaccines (e.g., adenovirus, MVA) expressing NP, and VLPs generated by NP overexpression in baculovirus–insect-cell systems [4,6,7,19,23,25,26]. Preclinical studies demonstrate that NP can induce strong $CD4^+$ and $CD8^+$ T cell responses and contribute to protection. However, NP alone may not completely block viral replication; combining NP with glycoprotein antigens frequently improves protection against both disease symptoms and viremia [19,23,25,26].

In VLP-based approaches, the conserved NP region (aa 235–305) functions as an immunodominant core that supports particle assembly and enhances antigenicity [4,23]. Thanks to its strong immunogenicity and cross-strain conservation, NP is considered a key component in multi-antigen vaccine designs aimed at eliciting balanced humoral and cellular immune responses. As summarized in Table 5, NP's specific role across different vaccine platforms underscores its significance in CCHFV vaccine development.

Table 4: CCHF vaccination platforms and their functions.

Type of vaccine	Antigen	Function
DNA vaccine	M segment	Equally Th1 and Th2 response
mRNA vaccine	S segment	Induce both humoral and cellular immune responses
Exosome-based vaccine	Whole virus	T-cell activities
Inactive vaccine	Whole virus	T-cell activities
Plant-expressed vaccine	GN-GC	Antibody response
Subunit vaccine	GN-GC	Neutralizing antibody response
VSV-based vaccine	GN-GC	Induce both humoral and cellular immune responses
MVA vaccine	M, S segment	Induce both humoral and cellular immune responses
VLP vaccine	NP	Induce both humoral and cellular immune responses
Adenoviral vector vaccine	NP	(a) Upregulated IgG response (b) Protection via CD4+ and CD8+ T cells and non-neutralizing antibody responses

GN-GC, glycoprotein N-glycoprotein C; MVA, modified vaccinia virus Ankara; NP, nucleoprotein; VLP, virus-like particle; VSV, vesicular stomatitis virus. Information summarized and adapted from the published literature cited in the text.

Table 5: Comparative Summary of Vaccine Platforms and the Role of NP in CCHFV Development

Vaccine Platform	Description	Role of NP	Advantages	Limitations/Challenges
DNA Vaccine	Plasmid-based delivery of viral antigen genes for in vivo expression.	The NP gene is expressed to induce cellular immunity; it is often combined with glycoproteins for broad immunity.	Induces balanced Th1/Th2 response; easy to produce; no pre-existing vector immunity interference.	Moderate neutralizing antibody response alone; optimization needed for durability and potency.
Subunit Vaccine	Purified recombinant viral proteins (e.g., glycoproteins Gn/Gc) are used as vaccine antigen.	NP may be included as a conserved antigen to enhance T cell responses, though most subunits focus on glycoproteins.	Safe, well-characterized, potentially strong antibody responses.	Subunit of Gn/Gc alone is sometimes insufficient; NP addition is needed to improve protection.
Virus-Like Particle (VLP) Vaccine	Non-infectious particles mimicking virion structure, assembled from expressed viral proteins including NP.	NP self-assembles into VLP cores, enhancing immunogenicity by mimicking natural virus morphology and promoting strong cellular immunity.	High immunogenicity without live virus; mimics authentic virion structure.	Complex manufacturing; need to confirm proper assembly and stability.
Adenoviral Vector Vaccine	Recombinant adenovirus delivering NP and/or glycoprotein antigens in vivo to stimulate an immune response.	NP expressed to elicit CD4+, CD8+ T cell and antibody responses; partial protection was reported.	Robust induction of cellular immunity; well-studied vector.	Pre-existing anti-vector immunity may reduce efficacy; partial protection from NP alone.
Inactivated Vaccine	Chemically or physically inactivated whole virus vaccines.	NP is present in the whole virion; it contributes to cellular immunity with other viral components.	Comprehensive antigen presentation.	Safety concerns, complex production, and lack of widespread approval.

mRNA Vaccine	Synthetic mRNA encoding NP and other antigens delivered for in vivo expression.	NP included to enhance cellular immunity alongside glycoproteins (not detailed extensively here but relevant given platform trend).	Rapid design, scalable manufacture, potent immune responses.	Stability and delivery challenges; emerging technology for CCHFV.

NP, nucleoprotein; VLP, virus-like particle. Information summarized and adapted from the published literature cited in the text.

6. IMMUNITY

6.1. NP-Mediated Immune Mechanisms

The cellular immune response to CCHFV is driven by the activation of both natural killer (NK) cells and cytotoxic T lymphocytes (Tc cells), with NK cells often emphasized due to their prominent role in early infection. Antibodies contribute by recognizing antigens on infected cell surfaces, which may occur through two pathways: (i) intracellular processing of viral proteins, including NP, and presentation via MHC class I; or (ii) antigen presentation during viral budding. NP elicits both humoral and cellular immune responses through multiple mechanisms, including NK cell activation via antibody-dependent cellular cytotoxicity (ADCC), Tc cell stimulation, and balanced Th1/Th2 polarization. When used as a vaccine antigen, NP can also be presented via MHC class II on antigen-presenting cells (APCs), promoting cytokine production (e.g., IL-2, IL-4, IL-6, IL-10, IL-12, TNF-α, IFN-γ, IL-17), memory Tc formation, and cross-presentation for enhanced cytotoxic responses. High NP expression and accessibility on the cell surface reinforce its value as a target for immune monitoring and vaccine development [7,34].

6.2. Antibody-Mediated Effector Mechanisms (ADCC, ADCP, CDC)

Antibody-mediated mechanisms bridge innate and adaptive immunity in CCHFV infection. ADCC involves NK cell–mediated killing of infected cells via Fc gamma receptor (FcγR) engagement with antigen-specific antibodies, functioning similarly to Tc cells. Antibody-dependent cellular phagocytosis (ADCP) is mediated by macrophages and dendritic cells through Fc receptor–dependent uptake of antibody-coated targets, enhancing antigen presentation and activating both cellular and humoral responses. Complement-dependent cytotoxicity (CDC) relies on the complement cascade, initially triggered by natural antibodies, to eliminate infected cells prior to full activation of adaptive immunity [7,34].

6.3. T Cell Epitope Mapping in CCHFV Survivors

A study of eleven male survivors of CCHFV infection in South Africa examined peripheral blood mononuclear cells (PBMCs) collected 10 months to 13 years post-infection. Cells were stimulated with 36 peptide pools covering NP, glycoprotein N (GN), and GC. Enzyme-linked immunospot (ELISpot) assays detected IFN-γ responses to 16 peptides, mapping to 10 distinct epitopic regions, most within NP.

Two peptides, N262–280 and N298–316, elicited strong responses in multiple survivors, predominantly mediated by $CD8^+$ T cells, as confirmed by CD8 depletion assays. No single immunodominant epitope was identified, and response breadth/magnitude did not clearly correlate with time since infection; however, participants tested more than a decade post-infection showed weaker responses. These epitopic regions are illustrated in Figure 3 and summarized in Table 6 [27,20].

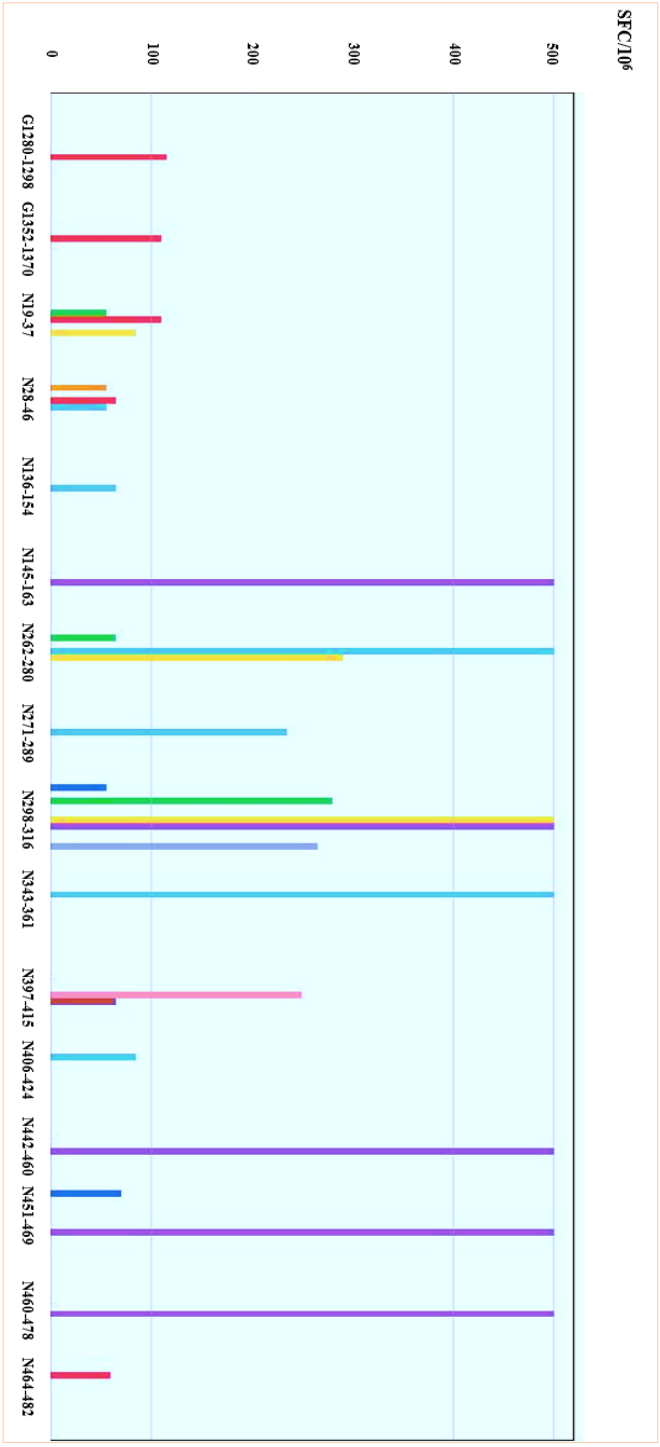
In summary, ten distinct T cell epitopes of CCHFV were identified in survivors, predominantly in the NP, with two in GC. Peptides N262-280 and N298-316 elicited the strongest $CD8^+$ T cell responses, as confirmed by depletion assays, which are shown in Figure 4 [27].

Blocking IFN-γ signaling in infected mice significantly increased mortality, underscoring its critical role in viral control [34]. The predominance of NP epitopes, similar to findings in Hantaan virus, may relate to NP abundance during replication and supports its potential as a multi-epitope vaccine target. In contrast, limited GN/GC responses and the failure of previous subunit vaccines suggest glycoproteins alone may be insufficient for T cell–mediated protection. The study identified long-lived T cell responses to CCHFV, focusing on epitope mapping rather than precise quantification. Although 19mer peptides may underestimate responses compared to optimal 9mers, this did not affect the objectives. $CD8^+$ T cells were confirmed as the main responders in depletion assays, though broader phenotypic confirmation is needed. Most epitopes were located in NP, with only two in GC and none in GN. Memory T cell responses persisted up to 13 years post-infection, similar to findings in Puumala virus, suggesting potential for durable vaccine-induced protection. The exclusively male cohort reflected past South African epidemiology, with high-risk groups including farmers, veterinarians, and individuals exposed to ticks or infected animal materials. The NP epitopes described here are the first identified after natural CCHFV infection and provide valuable targets for vaccine design and immunogenicity assessment [27].

Table 6: Epitopic regions

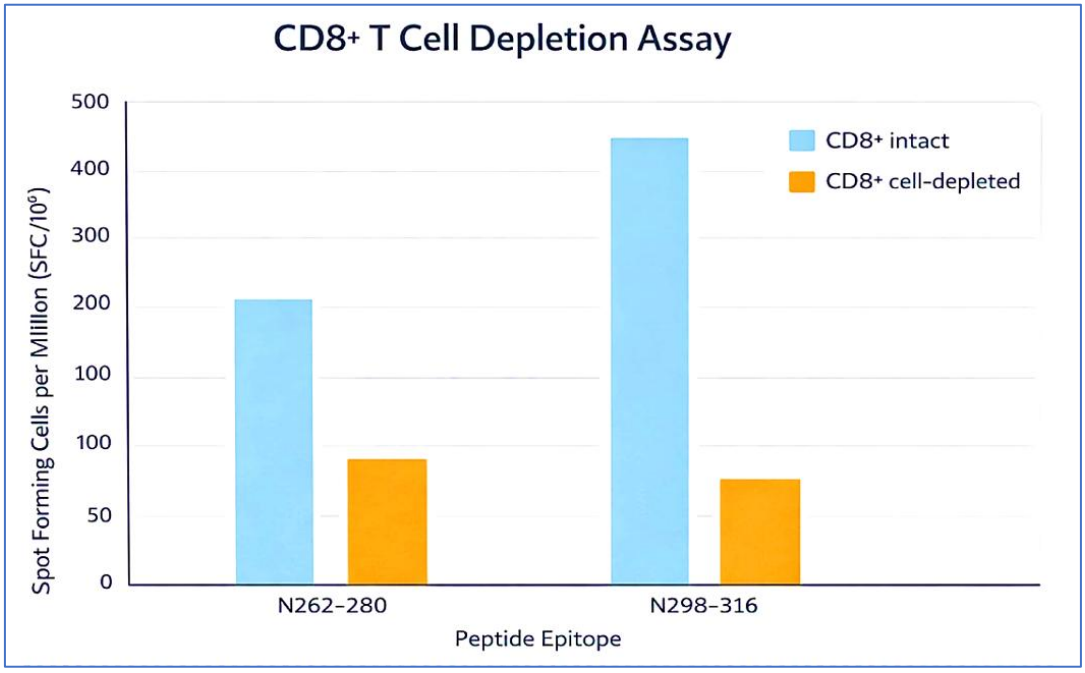
Peptide	Number of positive responses	Amino acid sequence	Range of magnitude of responses (SFC/10^6)
N19–37	3	EFKKGNGLVDTFTNSYSFC	55–110
N28–46	3	DTFTNSYSFCESVPNLDRF	55–65
N136–154	1	DIGFRVNANTAALSNKVLA	65
N145–163	1	TAALSNKVLAEYKVPGEIV	>500
N262–280	3	DKHKDEVDARASADSMITNL	65–>500
N271–289	1	ASADSMITNLLHKIAKAQE	235
N298–316	5	RAQGAQIDTAFSSYYWLYK	55–>500
N343–361	1	KMKKALLSTPMKWGKKLYE	>500
N397–415	2	VANPDDAAQGSGHTKSILN	65–250
N408–424	1	GSGHTKSILNLRTNTETNN	85
N442–460	1	NIQDMDIVASEHLLHQSLV	>500
N451–469	2	SEHLLHQSLVGKQSPFQNA	70–>500
N460–478	1	VGKQSPFQNAYNVKGNATS	>500
N464–482	1	SPFQNAYNVKGNATSANII	60
G1280–1298	1	TLHPRIIEEGFFDLHVQKV	115
G1352–1370	1	DGCDLDYYCNMGDWPSCTY	110

Figure 3: Detection of CCHFV-specific T cell responses by IFN-γ ELISpot assay.



The magnitude of responses in spot-forming cells per million (SFC/10^6) is shown for each peptide to which study participants responded positively. Figure reproduced/adapted from Goedhals et al., 2017 [27].

Figure 4: Confirmation of $CD8^+$ T cell–mediated responses by depletion assay.



IFN-ɣ ELISpot responses ($SFC/10^6$) are shown before and after CD8+ T cell depletion for peptides N262-280 and N298-316. Figure adapted from Goedhals et al., 2017 [27].

LIMITATIONS AND DIRECTIONS FOR FUTURE STUDIES

Despite the significant potential of NP-based CCHFV diagnostics and vaccine development, many technical challenges remain to be addressed before these methods can be translated into practical clinical tools.

First, CCHFV is genetically heterogeneous, driven by an error-prone RNA polymerase and frequent recombination, which pose a major challenge and may undermine the antigenic conservation of NP epitopes across strains. This heterogeneity makes it more difficult to develop broadly protective NP-based vaccines and diagnostic tests and requires constant monitoring of circulating strains and subsequent adaptation of target epitopes [8,18].

Second, additional obstacles arise from the complexity of manufacturing emerging NP-based platforms, especially VLPs. Although VLPs are highly immunogenic, they are subject to complex production and purification processes and can be unstable during process development, aspects that can affect both vaccine consistency and regulatory approval [4,20].

Third, only a limited number of NP-targeted vaccine candidates currently have extensive in vivo efficacy data or clinical trials. Despite encouraging preclinical results, these vaccines still need to be tested in lethal, clinically relevant animal models and then in humans to verify safety, immunogenicity, and long-term protection [16,19,22].

Fourth, safety remains a priority, especially when using live vector platforms that express NP, which may raise concerns regarding neurovirulence or unwanted host responses. Moreover, regulatory pathways for innovative NP-based vaccine technologies have not yet been streamlined [17,22,26].

Future research should focus on multi-epitope and multi-antigen vaccine platforms that integrate NP with other viral proteins to boost efficacy. Recent developments in immunoinformatics, bioengineering, and nanotechnology promise to optimize NP antigen presentation and vaccine delivery [24,25,32,33]. At the same time, advances in highly sensitive, rapid NP-based diagnostics can facilitate early detection and outbreak control [6,11,12]. Addressing these limitations through multidisciplinary efforts is essential to realize the full potential of NP in controlling CCHFV and reducing the global burden of CCHF.

CONCLUSION

CCHF is a severe tick-borne disease with a high case fatality rate, ranging from 10–50% for tick-borne transmission and up to 80% for nosocomial transmission, affecting over 30 countries across Europe, Asia, and Africa [35]. Recent diagnostic advances, including a

novel SYBR Green-based one-step real-time RT-PCR assay, have successfully detected CCHFV in clinical samples with high sensitivity, capable of detecting fewer than 20 copies of viral RNA per reaction [36].NP of CCHFV is highly conserved, abundantly expressed, and strongly immunogenic, making it a promising candidate for subunit vaccines. Partial efficacy up to 78% has been reported using a prime-boost vaccination strategy targeting NP [19]. NP can self-assemble into VLPs and stimulate both humoral and cellular immune responses, highlighting its potential as a core vaccine component. Moreover, rNP has proven useful in serosurveillance and diagnostic applications by detecting IgG and IgM antibodies, serving as a safe alternative to whole-virus antigens [3,37]. However, focusing exclusively on NP may be insufficient to induce sterilizing immunity or fully block transmission. Multivalent vaccine strategies incorporating additional conserved antigens, such as the L protein, could provide broader protection. Critical challenges remain in defining which NP epitopes elicit the most protective versus non-neutralizing immune responses, optimizing antigen folding and purity for large-scale production, and establishing correlates of protection in humans and animal models. Ethical and technical limitations hinder direct human challenge studies, further complicating efficacy assessment [5,19].

In conclusion, while NP demonstrates significant promise as a vaccine target and diagnostic tool, further research is needed to refine vaccine design, evaluate combinatorial strategies, and fully understand its protective potential against CCHFV. Continued preclinical and clinical studies are essential to advance safe and effective vaccines and to mitigate the public health impact of this high-consequence pathogen [37].

Conflicts of interest and sources of funding

The authors declare that they have no competing interests.

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Authors' contribution

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

Ethics approval and consent to participate

All assessments were conducted in accordance with ethical principles and under the supervision of the University's Ethics Committee (Ethic NO. IR.BMSU.BLC.1403.001).

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REVIEW

The Evolving Landscape of Multiple Sclerosis Therapy

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Abstract: The therapeutic landscape for multiple sclerosis has evolved markedly in recent years, with an expanding arsenal of disease-modifying therapies offering clinicians more tools to manage the disease. This progress presents both opportunities and complexities, as treatment decisions increasingly require individualized strategies balancing efficacy, safety, and long-term outcomes. While current therapies effectively reduce inflammation and delay disease progression, they fall short in halting neurodegeneration, highlighting a critical unmet need. Treatment paradigms range from escalation strategies prioritizing safety to early high-efficacy approaches aimed at aggressive disease control. Data increasingly support early initiation of high-efficacy DMTs in patients with poor prognostic indicators, but concerns over long-term safety and tolerability remain. Shared decision-making, informed by patient preferences and evolving evidence, is central to modern MS care. The future is promising, with new therapies in advanced stages of research, which seek to exceed the limits of current therapy, to act in a targeted manner, limiting both inflammation and neurodegeneration, for better control of disease activity, with an improved safety profile.

Keywords: Multiple sclerosis; Disease-modifying therapies; Treatment strategies; Neurodegeneration; High-efficacy therapy; Escalation approach; Personalized medicine; Shared decision-making; Long-term safety; Neuroprotection.

INTRODUCTION

Multiple sclerosis (MS), an autoimmune disease of the central nervous system, predominantly affects young people, especially women, and is the leading cause of non-traumatic disability among them (1). Although the etiology is unknown, studies show that it occurs as a consequence of a complex interaction between genetic and environmental factors (2). The clinical manifestations and the evolution of the disease are extremely variable and unpredictable.

Despite this heterogeneity, treatment options were once limited, making therapeutic decisions relatively straightforward. However, in recent years, the treatment of MS patients has evolved significantly due to the availability of new disease-modifying therapies (DMTs). While this progress marks a significant advancement, it also presents new challenges for clinicians, who must now navigate complex treatment decisions tailored to individual patients. Compounding these challenges is the uncertainty surrounding the long-term safety of newer DMTs.

Current treatments do not stop the underlying neurodegeneration, even though they successfully reduce inflammation and provide clinical and radiological benefits. The goal of ongoing research is to close this gap. New treatments with a higher safety profile are needed because the existing ones have long-term immunologic hazards and side effects.

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MULTIPLE SCLEROSIS PATHOGENESIS

Under physiological conditions, the central nervous system (CNS) is considered an immune-privileged environment, as the passage of cells and molecules across the blood–brain barrier (BBB) is tightly regulated. However, in MS, this protective mechanism becomes compromised. Autoreactive B and T lymphocytes are able to cross the BBB and infiltrate the CNS, where they induce lesions, particularly in the white matter (3). These lesions are characterized by inflammation, demyelination, and axonal loss (4). Notably, MS has significant immunopathological variability: certain forms are dominated by inflammation and relapses, whilst others are driven predominantly by neurodegeneration, as shown in progressive forms of the illness (5), (6). Although the full pathophysiologic mechanisms remain unclear, crucial stages in lesion formation have been discovered, and current DMTs are meant to target various locations.

The autoimmune process in MS is initiated in the peripheral immune system. Antigen-presenting cells, primarily dendritic cells, present self-antigens (myelin-derived autoantigens) to lymphocytes, which may then become autoreactive (7), (8). Under normal conditions, physiological defense mechanisms such as immune tolerance act to prevent this. Regulatory T cells, regulatory B cells, and natural killer (NK) cells play key roles in identifying and eliminating autoreactive lymphocytes. However, when the function of these regulatory cells is impaired, autoreactive cells can escape immune surveillance and contribute to disease progression (9), (10), (11). As a result, DMTs have been developed to target the antigen presentation process or to enhance the function of regulatory T cells

The role of T lymphocytes in the pathogenesis of MS is well established and represents a major target of DMTs (12), (13). B lymphocytes also contribute to the disease process, generating antibodies as evidenced by the increased intrathecal synthesis of immunoglobulins, seen as oligoclonal bands in the cerebrospinal fluid (CSF). Additionally, inflammatory infiltrates containing B cells have been identified in the meninges of MS patients. These infiltrates are associated with more severe clinical manifestations, greater cortical involvement, and enhanced neurodegeneration (6).

Sphingosine 1-phosphate (S1P) receptors on the surface of T lymphocytes play an important role in regulating their migration. During activation and clonal expansion, the expression of S1P receptors increases, enabling T cells to exit the lymph nodes and migrate to sites of inflammation, where they carry out their immune functions (14). This mechanism underlies the therapeutic action of S1P receptor modulators in MS. By inhibiting these receptors, such drugs sequester lymphocytes within lymph nodes, thereby limiting the inflammatory response (15).

The precise mechanisms by which activated immune cells cross the BBB in MS are not completely understood yet. Potential contributing factors include dysfunction of tight junction proteins and alterations in BBB efflux pump activity (16), (17), (18). MS lesions typically form around small veins and venules, which are sites particularly susceptible to immune cell infiltration.

Glial cells also contribute to maintaining CNS homeostasis (19). Under pathological conditions, activated microglia and macrophages release proinflammatory cytokines, produce reactive oxygen and nitrogen species, and promote excitotoxicity by impairing glutamate reuptake, all of which contribute to neuronal injury (3). Additionally, excitatory neurons become more vulnerable due to ion channel dysregulation, mitochondrial dysfunction, and reduced inhibitory signaling (20). Emerging research also highlights a possible role for meningeal lymphatic vessels in transporting immune cells and macromolecules from the cerebrospinal fluid to peripheral immune sites, though this pathway remains poorly understood and is not yet a target of current disease-modifying therapies (21).

AVAILABLE DISEASE-MODIFYING THERAPIES IN MULTIPLE SCLEROSIS

MS was the first neurological disorder to benefit from the advancement of effective disease-modifying medicines. The DMT approved for the treatment of MS was interferon beta-1b in 1993, followed three years later by glatiramer acetate and interferon beta-1a. These treatments represented a significant milestone, reducing annual relapse rates by 20–30% and delaying the progression to MS in individuals with clinically isolated syndrome (22). Over time, the number of available therapies has grown dramatically. In general, improved treatment efficacy is associated with higher hazards, particularly with immunosuppressive medicines, which may increase the incidence of infections or malignancies. By contrast, interferon beta and glatiramer acetate are considered immunomodulators and are associated with a more favorable safety profile. Choosing a treatment for MS is challenging and must be individualized for each patient. The decision should always consider the overall risk–benefit profile, comorbidities, and, importantly, the patient's preferences, as part of a shared decision-making process.

Beta interferons, the first known therapy for multiple sclerosis, are administered via subcutaneous or intramuscular injections. Although their exact mechanism of action is not fully understood, they appear to modulate the immune system by interacting with specific receptors on the surface of leukocytes. They decrease antigen presentation to T cells and promote the activity of regulatory T cells. Additionally, they reduce the passage of inflammatory cells across the BBB, increase the production of anti-inflammatory cytokines, and inhibit the release of pro-inflammatory cytokines (23). Interferons are considered safe during pregnancy and lactation.

Glatiramer acetate is a synthetic polymer administered subcutaneously. It is an immunomodulator with a peptide structure that resembles myelin basic protein. As a result, it competes with self-antigens for binding to MHC class II molecules and redirects the autoimmune response away from myelin. Although its exact mechanism remains unclear, it is known to shift the immune environment

toward an anti-inflammatory state and promote regulatory T cell activity (24). Glatiramer acetate requires no routine lab monitoring, has no known drug interactions, and is not associated with opportunistic infections or malignancies. Like interferon beta, it is considered safe for use during pregnancy and breastfeeding.

Teriflunomide inhibits the proliferation of activated T and B lymphocytes. It interferes with the de novo synthesis of pyrimidines in rapidly dividing cells by inhibiting dihydroorotate dehydrogenase (DHODH), a mitochondrial enzyme. Additionally, it exerts effects independent of DHODH inhibition by suppressing protein tyrosine kinase activity and modulating cytokine production (25). It is administered orally. Due to its long half-life (18–19 days) and enterohepatic recirculation, elimination from the body can take up to eight months. It is contraindicated in pregnancy because of its teratogenic potential.

Fumarates, primarily dimethyl fumarate, are also administered orally and function by activating the Nrf2 pathway, which plays a key role in the cellular response to oxidative stress. This activation provides neuroprotective effects by reducing oxidative damage to neurons and astrocytes (26). Fumarates are not recommended during pregnancy or lactation.

S1P receptor modulators, including fingolimod (non-selective), siponimod, ozanimod (selective for S1P1 and S1P5), and ponesimod (selective for S1P1), regulate immune cell trafficking by down-modulating S1P receptors on lymphocytes. This results in the functional sequestration of lymphocytes within lymph nodes, reducing their migration into the bloodstream and CNS. These agents also cross the BBB, where they may influence neurogenesis, glial function, and potentially promote remyelination (27). Despite their efficacy, S1P modulators carry risks such as cardiovascular side effects and disease rebound upon discontinuation. They are contraindicated during pregnancy.

Cladribine, an oral therapy, is particular in its mode of action, immune reconstitution therapy. This approach involves two distinct phases. In the first phase, cladribine induces a targeted reduction of lymphocytes over several weeks. This results in selective immunosuppression, primarily affecting T cell–mediated cellular immunity and B cell–mediated humoral responses. This immunosuppressive effect is achieved through cladribine's cytotoxic mechanism: once phosphorylated within lymphocytes, it disrupts essential intracellular processes by interfering with DNA synthesis and repair, inhibiting ribonucleotide reductase, and ultimately inducing apoptosis (28). The second phase consists of a gradual repopulation and remodeling of the immune system over the following months. During this time, peripheral lymphocyte populations recover, resulting in a qualitatively and quantitatively altered immune profile (29), (30). This distinctive mechanism enables cladribine to provide sustained disease control and prolonged remission with a limited number of treatment cycles. However, despite well-established treatment protocols, long-term data remain limited, particularly for patients who do not respond adequately or may require additional treatment courses. Due to its cytotoxic effects, cladribine is contraindicated during pregnancy.

Natalizumab is a monoclonal antibody directed against α4β1 integrins (VLA-4), which are expressed on the surface of B and T lymphocytes. By binding to these integrins, it blocks their interaction with adhesion molecules such as VCAM-1 on the vascular endothelium, thereby preventing lymphocyte migration across the BBB (24). It is administered via intravenous infusion and, more recently, subcutaneously, and is considered a highly effective therapy. However, its high efficacy must be weighed against the significant risk of opportunistic infections, most notably progressive multifocal leukoencephalopathy (PML). Additionally, natalizumab carries a substantial risk of disease rebound upon treatment discontinuation, an important consideration when planning a therapeutic switch.

Ocrelizumab and ofatumumab are monoclonal antibodies targeting CD20, a surface antigen expressed on pre-B cells, mature B cells, and memory B cells. They induce B cell depletion at these stages through mechanisms such as antibody-dependent cellular cytolysis and complement activation (31).

Alemtuzumab is a monoclonal antibody targeting CD52 on the surface of lymphocytes and monocytes. It induces profound immunosuppression through complement-mediated cytolysis (32). Depletion occurs rapidly within days of treatment, followed by partial immune recovery over the next 8–12 months. $CD4^+$ T cells are the slowest to repopulate (33), (34). This delayed immune reconstitution contributes to the long-term reduction in inflammation associated with multiple sclerosis. However, due to the depth of immunosuppression it causes, the associated risks are significant and cannot be overlooked. For this reason, alemtuzumab is typically reserved for patients who have failed at least two prior DMTs.

Mitoxantrone is a cytotoxic agent that intercalates into DNA and inhibits topoisomerase II, thereby disrupting DNA repair and inducing cell death (35). Due to its dose-dependent risk of cardiotoxicity and secondary leukemia, its use has become rare and highly restricted.

Autologous hematopoietic stem cell transplantation (AHSCT) has been used as a therapeutic approach in carefully selected patients with autoimmune diseases since 1995 (36). It involves the elimination of autoreactive T cells, followed by immune system repopulation and the promotion of a more tolerant immune phenotype (37), (38), functioning as a form of immune reconstitution therapy. By restoring the balance between proinflammatory and regulatory immune cells, AHSCT suppresses CNS inflammation and may offer additional neuroprotective effects (39), (40). Stem cells used in this therapy are also believed to contribute to CNS repair. Their ability to migrate to brain lesions and differentiate into neuronal and myelin-producing cells supports remyelination and tissue regeneration (41) (42). Initially tested in patients with chronic progressive MS, outcomes were less favorable in that group. However,

retrospective studies have shown significantly better results in younger patients with aggressive, relapsing disease and shorter disease duration (43). As such, the ideal candidates for AHSCT are young, ambulatory individuals with highly active RRMS (39), (44). Studies suggest that up to 70–80% of these patients may achieve long-term remission, lasting four to five years or more (44).

THERAPEUTIC APPROACHES IN MULTIPLE SCLEROSIS

Therapeutic approaches primarily follow one of two strategies: escalation therapy or early high-efficacy treatment.

The escalation strategy involves starting with a treatment that offers moderate efficacy but a favorable safety profile (45). Disease activity is closely monitored through clinical assessment and MRI, with treatment intensified only if relapses or progression occur, thus prioritizing safety and minimizing exposure to high-risk medications.

In contrast, the strategy of initiating highly effective therapy early in patients with moderate to high disease activity allows for rapid control of inflammation from the outset, significantly improving long-term outcomes (46), (47). However, this approach also exposes patients to more intensive immunosuppression, increasing the risk of adverse effects, which must be carefully monitored. Meta-analyses and retrospective studies suggest that initiating high-efficacy DMTs at a younger age is linked to better treatment responses, slower disability progression, and delayed transition to secondary progressive MS (48), (49), (50). Data from national registries, such as the Swedish registry, support the observation that patients treated early with highly effective therapies reach significant disability milestones later in life (2), (51). To identify patients most likely to benefit from this approach, several poor prognostic factors have been established. These include demographic variables (age over 40, male sex, non-White ethnicity, and comorbidities), clinical indicators (frequent relapses, severe attacks requiring steroids or hospitalization, significant impact on daily function, involvement of multiple functional systems, severe motor, cerebellar, or brainstem symptoms, and incomplete relapse recovery), and radiological features (new gadolinium-enhancing T1 or T2 lesions, high T2 lesion burden, spinal cord lesions, and brain atrophy) (52).

Therapeutic Switching in MS

In MS, switching DMTs is primarily driven by lack of efficacy or issues with tolerability. Historically, treatment response was assessed using tools such as the Rio and Modified Rio scores, and more recently using the No Evidence of Disease Activity (NEDA) criteria. NEDA-3 means the absence of clinical relapses, disability progression, and MRI activity (new or larger T2 lesions and T1 gadolinium-enhancing lesions), while NEDA-4 includes the absence of rapid brain atrophy (53). A patient receiving DMT treatment who experiences a high relapse rate, moderate to severe relapses with functional impairment or involvement of the motor system, cerebellum, brainstem, or sphincters, incomplete recovery, or MRI activity should be evaluated for a possible treatment change. These indicators suggest suboptimal treatment response and warrant re-evaluation of the therapeutic approach to prevent long-term disability accumulation (52).

Before evaluating the effectiveness of a DMT, it is essential to allow sufficient time for the therapy to take full effect. The time to clinical response varies: natalizumab typically acts within one month; interferon-beta, teriflunomide, dimethyl fumarate, fingolimod, and ocrelizumab within three months; glatiramer acetate requires about six months; and cladribine and alemtuzumab reach full efficacy only after completing a two-year treatment course (52).

When considering a treatment switch, clinicians must account for lingering immunologic effects from the previous therapy, especially in the case of long-acting agents. The pharmacodynamics and mechanism of action of each DMT can influence both the effectiveness and safety of subsequent treatments. Short-acting DMTs (e.g., interferon, glatiramer acetate, dimethyl fumarate) clear within days to weeks, whereas medium-duration agents (e.g., fingolimod and natalizumab) may require weeks to months. Long-acting therapies, such as mitoxantrone, alemtuzumab, ocrelizumab, and teriflunomide, can exert effects lasting months to years. Careful balance is needed between washout duration and the risk of disease reactivation (55).

Discontinuation of DMT

The decision to stop DMT in MS is complex. The concept of immunosenescence refers to an age-related decline in immune system function that may both reduce MS inflammatory activity and increase susceptibility to adverse effects from immunotherapy. The DISCOMS study explored this issue by enrolling 259 patients aged over 55 with at least five years of stable disease. Participants were randomized to either continue or discontinue their DMT and monitored for two years. While the study did not yield definitive conclusions, it suggested that discontinuing therapy may be a reasonable consideration for older patients with prolonged disease stability. However, it did note a slightly increased risk of new MRI activity following discontinuation (54).

Shared Decision-Making in MS Therapy

Choosing the appropriate therapy in multiple sclerosis involves a shared decision-making process, where the physician carefully considers the patient's values, preferences, and lifestyle when selecting from evidence-based treatment options. This joint strategy has proven to enhance adherence and patient satisfaction. However, shared decision-making is not a one-time event but an ongoing dialogue, as MS is a chronic and evolving disease. Patients should be informed from the time of diagnosis that treatment may need to change over time for various reasons—such as suboptimal response, progression of the disease (e.g., from relapsing-remitting to

secondary progressive), safety concerns, or adverse effects. In cases of suboptimal response, clinicians should be vigilant for signs such as increased relapse rates, incomplete recovery, or new MRI lesions, all of which may warrant prompt and effective adjustments to the treatment plan.

THERAPIES UNDER DEVELOPMENT AND FUTURE PERSPECTIVES

Current treatments for MS primarily target inflammation, the dominant pathophysiological mechanism in the early stages of the disease. With the advent of highly effective DMTs, many patients with relapsing MS remain relapse-free and show no new demyelinating lesions on MRI. However, as the disease progresses, neurodegeneration becomes an increasingly central component of its pathology (55).

Unfortunately, existing therapies are largely ineffective in addressing these degenerative processes, and progression independent of relapse activity (PIRA) often continues. Even more concerning is that this subtle progression may not be fully captured by current clinical assessments, imaging techniques, or biomarkers, allowing disability to accumulate silently. Therefore, there is a pressing need for new therapies that target all aspects of MS pathology at every stage of the disease, not only inflammation, but also axonal damage and neuronal loss (56).

In terms of targeting the immune response, current anti-CD20 therapies have limitations, as they do not affect all stages of B-cell development. As a result, there is growing interest in therapies that also target the plasmablast and plasma cell stages, which are believed to play a significant role in the chronic phase of MS. One example is anti-CD19 therapy, which is discussed below (59).

Future treatment approaches also aim to reduce the significant adverse effects associated with current DMTs, such as immunosuppression-related infections, hepatotoxicity, and potential oncogenic risks. Developing safer, more tolerable therapies could significantly improve the long-term quality of life for individuals living with MS (57).

Novel monoclonal antibodies are currently under investigation in early clinical trials. Elezanumab targets repulsive guidance molecule A (RGMa), a protein that inhibits neural regeneration following CNS injury or inflammation (58). Temelimab is a monoclonal antibody targeting the human endogenous retrovirus W envelope protein (HERV-W-ENV), which may be involved in the pathogenesis of multiple sclerosis and other neurodegenerative diseases. Inebilizumab, a monoclonal antibody against CD19, acts on a broader spectrum of B cells, including plasmablasts and plasma cells that escape CD20-targeted treatments (58).

Cell-based therapies are also under development. Beyond AHSCT, mesenchymal stem cell therapy, using the regenerative and immunomodulatory properties of multipotent cells found in various tissues, is being explored in phase 2 trials. Oligodendrocyte progenitor cell therapy, aimed at enhancing remyelination, is also under investigation, although current results remain inconclusive (58).

One of the most revolutionary developments is the use of chimeric antigen receptor T (CAR-T) cell technology, which is derived from oncology (59). These genetically modified T cells are engineered to recognize and destroy specific cell targets, offering a potentially long-lasting therapeutic effect due to their ability to proliferate and persist in vivo (60). In MS, CAR-T cells targeting B cell markers such as CD19 show particular promise and are currently undergoing phase 2 clinical trials (61).

A significant limitation of current MS therapies is their limited ability to address compartmentalized inflammation within the CNS, which plays a critical role in long-term disability (62), (63). While existing treatments are effective in modulating peripheral immune responses and preventing relapses in relapsing forms of MS, they show reduced efficacy in progressive forms of the disease, where chronic inflammation within the CNS persists (62).

Bruton's tyrosine kinase (BTK) is a new therapeutic target. It is expressed in B lymphocytes, where it regulates their development and function, but is also present in other immune cells, including T cells, macrophages, dendritic cells, mast cells, and microglia, where it contributes to activation and phagocytosis, processes implicated in myelin damage (64), (65). Its role in ongoing neuroinflammation is supported by post-mortem analyses of brain tissue from patients with progressive MS, which have shown increased BTK expression in active white matter lesions (66).

Bruton's tyrosine kinase inhibitors (BTKi) are small molecules capable of crossing the BBB. This enables them to act both peripherally on the immune system and centrally within the CNS, targeting inflammation at both levels (67). First-generation BTKi, originally developed for oncology, lack selectivity and are associated with significant adverse effects, such as increased risks of bleeding, infection, and atrial fibrillation, which limit their suitability for long-term use in chronic autoimmune diseases (68). In contrast, second-generation BTKi offer greater selectivity and improved safety profiles (69). They are currently being investigated in phase 3 clinical trials across all forms of MS, including relapsing-remitting, secondary progressive, and primary progressive MS. If proven effective, these agents could represent a major advancement in MS therapy, particularly by expanding treatment options for progressive forms of the disease, where current choices remain limited (70).

CONCLUSIONS

Currently, with multiple therapeutic options available, the treatment of MS is personalized. Growing evidence supports the early initiation of highly effective therapies in appropriately selected individuals. Despite significant advances in controlling inflammation, current treatments do not halt neurodegeneration, underscoring the need for next-generation therapies that directly target disease progression. Treatment decisions are increasingly complex, requiring shared decision-making that aligns medical evidence with patient preferences, risk tolerance, and lifestyle. Future therapeutic goals include improving long-term safety, reducing immunologic risks, and developing tools to detect and treat progressive disease more effectively.

Conflicts of interest and sources of funding

The authors declare no conflict of interest.

Authors' contribution

Conceptualization, A.P., C.-A.S., and O.V.; methodology, A.P.; software, A.P.; validation, A.P., C.-A.S., V.L., and O.V.; formal analysis, A.P.; investigation, A.P., V.L., A.B., and O.V.; resources, C.-A.S. and F.C.P.; data curation, A.P., A.B., and O.V.; writing—original draft preparation, A.P. and A.B.; writing—review and editing, A.P., C.-A.S., V.L., A.B., F.C.P., and O.V.; visualization, A.P. and F.C.P.; supervision, C.-A.S.; project administration, C.-A.S. All authors have read and agreed to the published version of the manuscript. No generative AI was used in the production of this article.

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ARTICLE

The Romanian pulmonologists - nicotine addiction and the mirroring of their relationship with the patient

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Abstract: Background: Repeated surveys are essential for tracking trends in smoking prevalence among physicians, especially pulmonologists, who play a pivotal role in cessation counseling. Methods: In 2024, we conducted a descriptive cross-sectional surveillance study during the Romanian National Congress of Pneumology. A total of 332 registered pulmonologists were invited, and 226 (68.1%) provided a complete 30-item online questionnaire. The survey assessed smoking behavior and smoking-cessation counseling practices among Romanian pulmonologists for smokers and users of alternative nicotine products. Results: In a 2024 survey, current smoking rates were 4.9% (11/226) for conventional cigarettes, 2.2% (5/226) for heated tobacco products (HTPs), and 0.9% (2/226) for electronic cigarettes (e-cigarettes). Counseling practices were strongest for the initial steps of the 5A model: 93.4% (211/226) consistently asked about smoking status, and 88.1% (199/226) advised cessation. However, fewer assessed readiness to quit (65%, 147/226), monitored long-term abstinence (66.4%, 150/226), or assisted through referral and treatment. Only 36.3% (82/226) referred patients to the national STOP SMOKING program, and just 15.9% (36/226) declared to recommend Quitline programs. Conclusions: Smoking prevalence among Romanian pulmonologists has declined substantially over the past two decades. Despite high adherence to asking and advising patients, significant gaps remain in assistance and follow-up. Strengthened training and system-level support are needed to optimize cessation counseling.

Keywords: tobacco addiction; STOP SMOKING program; alternative nicotine products; electronic cigarettes; heated tobacco products.

INTRODUCTION

A "smoke-free Europe" has long been a priority and a vision guiding the European Commission's policies on public health, the environment, employment, and research. Significant progress has been made over the past decade, largely due to legislative actions and persistent health promotion efforts [1].

Romania became a member of the European Union in January 2007, and by 2016, notable advances had been made in protecting the population from secondhand smoke exposure. Romania ratified the WHO Framework Convention on Tobacco Control on 27 January 2006 and joined the group of countries offering comprehensive smoking cessation support in 2008 [2].

Beyond the multi-level strategies of tobacco control, the role of healthcare professionals—particularly physicians—in smoking prevention and cessation is well recognized. In Romania, data on smoking prevalence among health professionals are inconsistent. However, pulmonologists have been relatively well-studied, though much of the data comes from poster presentations at international conferences. Smoking prevalence among lung specialists has decreased over time, as evidenced by various rounds of surveillance.

In 2002, the prevalence of smoking among Romanian physicians was reported at 43.2%, with 16.6% identified as ex-smokers and 40.2% as non-smokers [3]. At the National Institute of Pneumology, 31.3% of medical personnel were smokers, and 5.2% were ex-smokers [4]. A 2005 study across 18 pulmonology clinics in Romania found a smoking prevalence of 30.8% among staff [5]. That same year, a survey conducted in Romanian maternity wards revealed that 35.5% of the personnel were

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smokers [6]. By 2009, smoking prevalence among pulmonologists had dropped to 11.3% [7]. In contrast, a 2014 study among cardiologists reported a higher prevalence of 26.5% [8]. Since then, no systematic evaluation of pulmonologists' cessation counseling practices has been published.

This creates an important knowledge gap: while it is known that physician smoking prevalence has declined, it remains unclear to what extent Romanian pulmonologists implement comprehensive smoking cessation strategies—including the 5A model (Ask, Advise, Assess, Assist, Arrange)—and how they address newer nicotine delivery products.

Given the absence of longitudinal or interventional components, the present study is strictly descriptive in nature and does not examine causal pathways, determinants of behavior, or psychosocial mechanisms. Its purpose is limited to documenting current smoking prevalence and counseling practices among pulmonologists.

MATERIALS AND METHODS

Pulmonologists, as healthcare professionals, play a key role in both advising patients on smoking cessation and serving as role models. However, some continue to use tobacco products themselves. To explore this issue, we conducted a survey using a structured questionnaire.

The study was launched during the Romanian National Congress of Pneumology in November 2024, and all pulmonologists (n = 332), registered as congress participants, were invited to take part in the survey. Recruitment was conducted through a congress newsletter and an electronic invitation containing a survey link, as well as in-person announcements at the event. Participants accessed the questionnaire through the emailed link. Eligibility criteria included being a licensed medical doctor who diagnoses, treats, and manages diseases of the lungs and respiratory system, practicing in Romania at the time of the survey. Exclusion criteria were incomplete or duplicate submissions. A "response" was defined as full completion of all 30 survey items. Data collection took place over a one-month period using a self-administered, internet-based questionnaire consisting of 30 items (Table 1). The questionnaire assessed personal tobacco use, attitudes toward smoking cessation, and clinical practices related to counseling patients who use tobacco or alternative nicotine products. The questionnaire was adapted from earlier surveys conducted among physicians on smoking behavior and cessation counseling practices. To reflect recent developments, items were updated to include new nicotine and tobacco products such as e-cigarettes and HTPs. The content was reviewed by senior pulmonologists for clarity and relevance; however, the instrument was not formally validated or pilot-tested prior to field use, which we acknowledge as a limitation.

All analyses were descriptive. Proportions were calculated directly from the number of respondents (n/N) and expressed as percentages. No specialized statistical software was used, and therefore, inferential analyses such as confidence intervals or regression models were not performed. As the study does not aim to draw causal inferences or examine psychosocial determinants, we acknowledge this as a limitation of the study, which restricts the generalizability of the findings and prevents deeper exploration of predictors of counseling practices.

Table 1: Summary of the questionnaire structure

SECTION	Items	Content	Response type
PROFESSIONAL AND TOBACCO USE	1-5	Training level, tobacco, and e-cigarette consumption habits	Categorical
CLINICAL SMOKING PRACTICES	6-17	Frequency of assessing and advising patients about smoking	Likert scale (Always-Never)
COUNSELING ATTITUDES	18-26	Beliefs about responsibility and efficacy of counseling	Likert scale (Strongly disagree-Strongly agree)
PERCEIVED BARRIERS	27-30	Barriers related to discomfort, patient reaction, and time	Likert scale (Strongly disagree-Strongly agree)

RESULTS

Of the 332 invited pulmonologists from various university centers across Romania, 68% (n = 226) provided complete responses. The sample consisted of 70% (n = 158) female and 30% (n = 68) male respondents. Regarding the respondents' level of training, the majority were senior specialists 60.6% (n = 137), but the sample also included specialist physicians (n = 46) and resident physicians (n = 43) (Figure 1).

Current smokers were defined as individuals who had smoked for at least six months during their lifetime and were still smoking at the time of the survey.

Non-current smokers included:

- Former (ex-) smokers: had smoked for at least six months but had not smoked in the past six months;
- Recent quitters: had smoked for at least six months but had quit within the past six months;
- Never smokers: had never smoked or fewer than 100 cigarettes in their lifetime.

Among respondents, 8% (n = 18) reported current use of tobacco or tobacco industry–derived novel products, with a predominance of conventional tobacco users (Figure 2a). Although resident physicians represent the new generation, one might expect them to gravitate toward heated tobacco products or electronic cigarettes. However, as shown in Figure 2b, they also predominantly use conventional cigarettes.

Figure 1: According to the participants' level of training: 60.6% (n = 137) were senior specialists, 20.4% (n = 46) were specialist physicians, and 19% (n = 43) resident physicians.

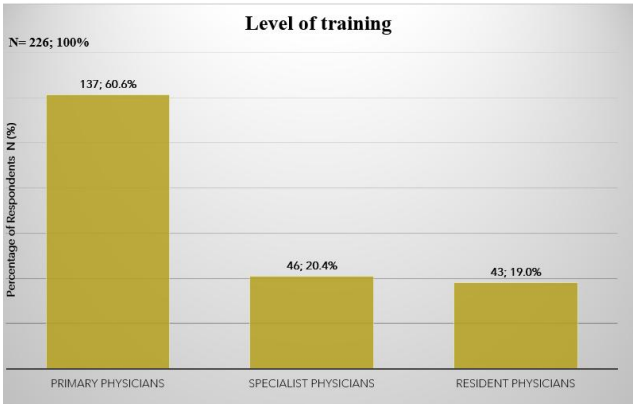
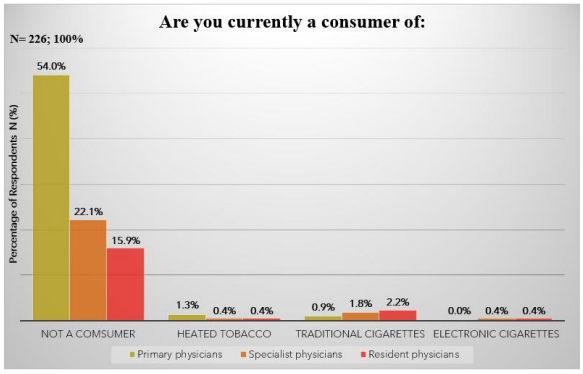
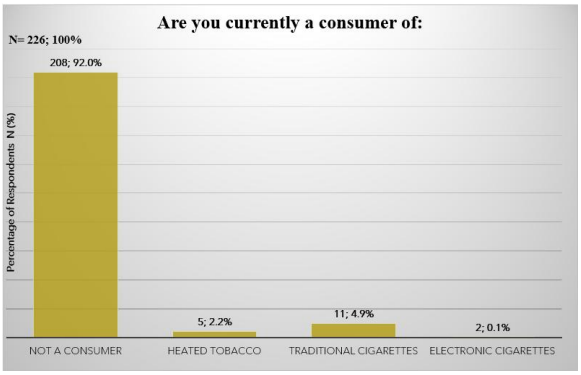


Figure 2: Distribution by level of training and by type of tobacco product used: a) 92% (n = 208) not a consumer; HTPs 2.2% (n = 5); conventional cigarettes 4.9% (n = 11); e-cigarettes 0.9% (n = 2); b) by level of training

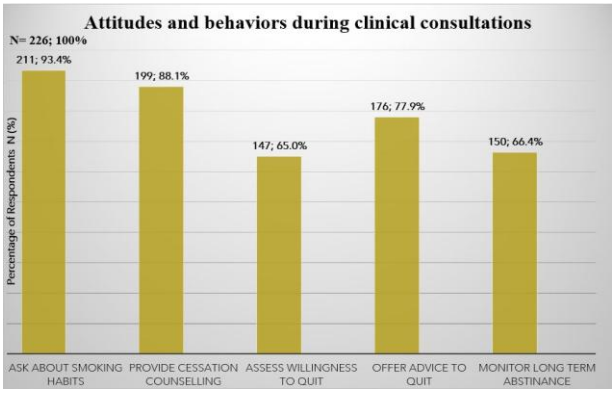


a)



b)

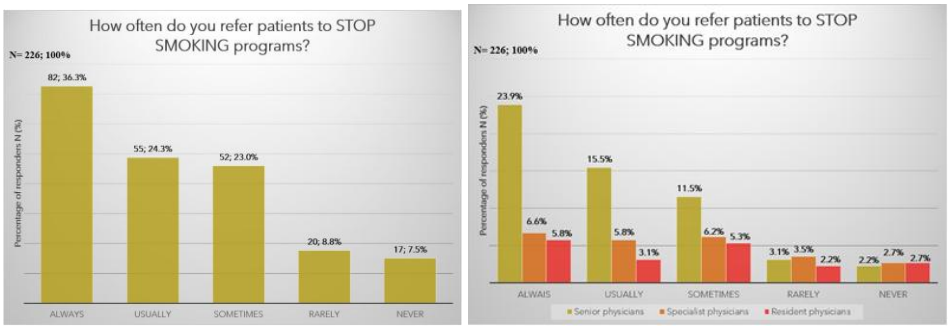
Figure 3: Distribution of responses regarding physicians' frequency of asking and advising patients about smoking (n = 226). Only the "always" category was retained to represent consistent counseling practice; "usually" responses were reported separately and were not combined with "always".



Our primary focus was on physicians' interactions with their patients regarding nicotine dependence. Of the 30 questionnaire items, 28 explored this aspect, specifically addressing attitudes and behaviors during clinical consultations. A total of 93.4% (n = 211) of respondents reported that they consistently ask patients about their smoking habits, and 88.1% (n = 199) stated that they always provide cessation counseling during consultations. However, only 65% (n = 147) routinely assess patients' readiness to quit, and 77.9% (n = 176) offer specific advice to support cessation. Notably, the percentage of physicians who monitor patients' long-term abstinence dropped to 66.4% (n = 150) (Figure 3).

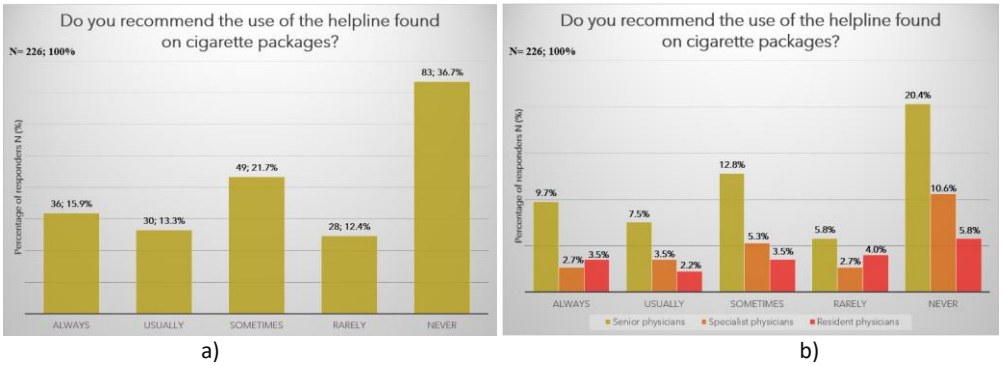
Importantly, the majority of respondents reported being unfamiliar with the STOP SMOKING program hotline (Figure 4). Only 15.9% (n = 36) of physicians stated that they always recommend the use of the telephone helpline to their patients (Figure 5), and just 36.3% (n = 82) refer patients to a formal STOP SMOKING program (Figure 4).

Figure 4: Physicians' responses to referring the patient to STOP SMOKING program: a) total and b) by level of training



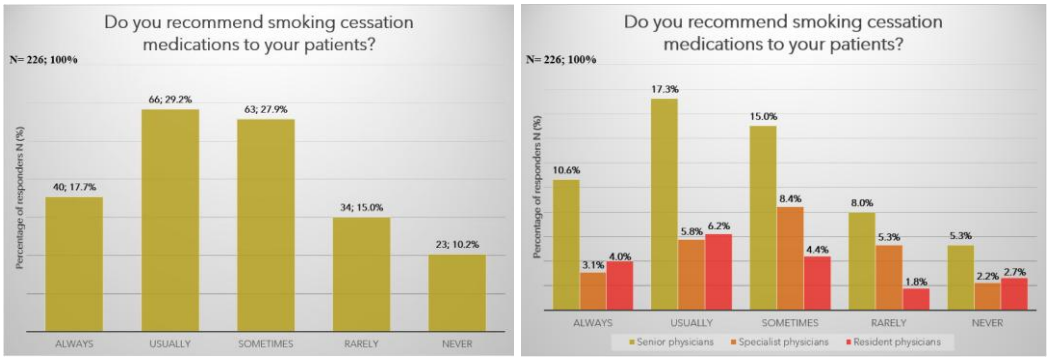
a) b)

Figure 5: Physicians' responses to referring the patient to Quitline on cigarette packs: a) total and b) by level of training



a) b)

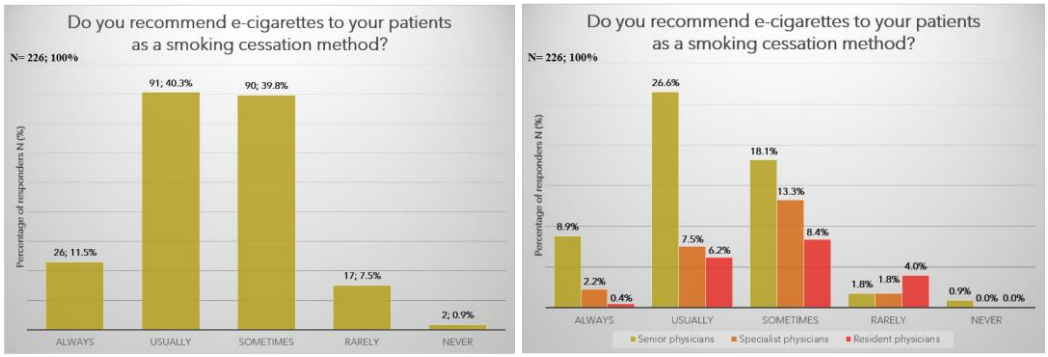
Figure 6: Physicians' responses to recommending medication to support tobacco cessation: a) total and b) by level of training



a) b)

Another point of interest relates to the recommendation of e-cigarettes as a cessation aid. Currently, 11.5% (n = 26) of physicians support this option (Figure 7).

Figure 7: Physicians' responses to recommend e-cigarettes to support cessation: a) total and b) by level of training



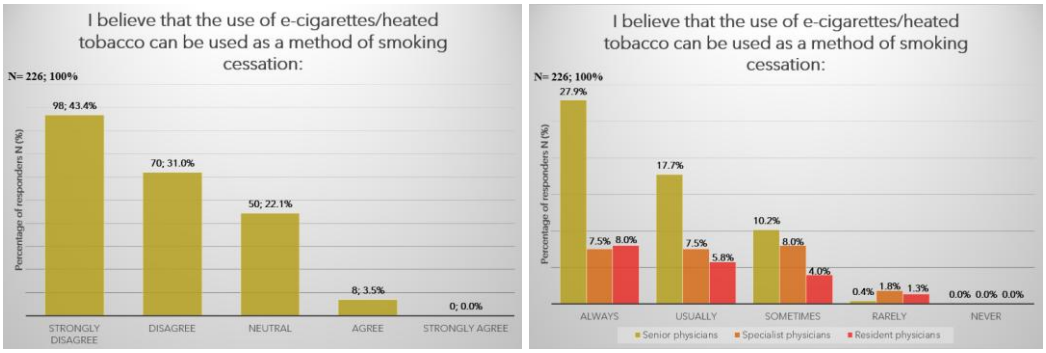
a) b)

However, only a small fraction—3.5% (n = 8) —express confidence (agree) in the effectiveness of e-cigarettes or HTPs as viable methods for smoking cessation (Figure 8).

More positive results emerged when assessing physicians' attitudes toward maintaining a smoke-free home environment—a key factor in preventing both passive smoking and relapse. A total of 58.9% (n = 133) of respondents stated it is always necessary, while 28.8% (n = 65) responded usually.

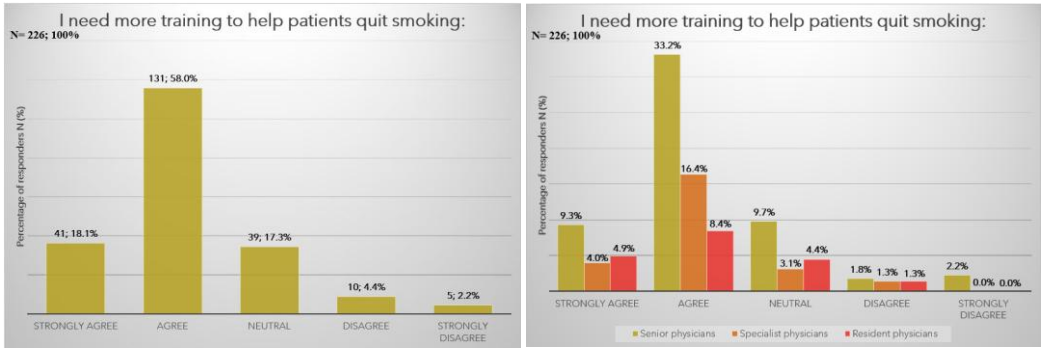
An important finding relates to physicians' interest and knowledge in cessation methods: 27% (n = 60 lack of knowledge; n = 1 not interested) of participants reported either a lack of knowledge or disinterest in available quitting strategies. Nevertheless, 93.8% (n = 127 strongly agree; n = 85 agree) acknowledged it is their responsibility to advise patients on quitting, and 96.5% (n = 110 strongly agree; n = 105 agree) believed they play an important role in smoking cessation counseling.

Figure 8: Physicians' views on e-cigarettes and HTPs as a smoking cessation tool: a) total and b) by level of training



a) b)

Figure 9: Physicians' responses regarding the need for additional training to support cessation: a) total and b) by level of training



a) b)

When probed further, 95.1% (n = 133 strongly agree; n = 82 agree) agreed that counseling remains important even if only a portion of patients follow through with cessation. Regarding preparedness, 76.1% (n = 133 strongly agree; n = 82 agree) of respondents expressed a need for training in smoking cessation counseling (Figure 9), and 78.3% (n = 48 strongly agree; n = 129 agree) believed they should adopt a more active role in supporting patients attempting to overcome nicotine dependence.

The final section of the questionnaire focused on physicians' perceptions of patient reactions during discussions about smoking. According to respondents, 39.4% (n = 5 strongly agree; n = 8 agree; n = 76 neutral) of patients do not appreciate receiving smoking cessation advice, while 35.8% (n = 5 strongly agree; n = 10 agree; n = 66 neutral) believe that addressing smoking habits does not strengthen the physician–patient relationship. Additionally, 9.3% (n = 3 strongly agree; n = 7 agree; n = 11 neutral) of physicians reported feeling uncomfortable asking patients about their smoking status.

Further insights revealed some unexpected findings: 25.2% (n = 4 strongly agree; n = 21 agree; n = 32 neutral) of respondents felt that counseling patients on smoking cessation is not an efficient use of consultation time, and 50% (n = 14 strongly agree; n = 46 agree; n = 53 neutral) reported that they allocate too little time to this aspect of care.

It is nevertheless surprising that resident physicians—a group we would expect to be aligned with contemporary trends toward a smoke-free world—do not appear fully aware of the role they hold in this transition. As shown in the previously analyzed figures, their involvement in guiding patients toward smoking cessation does not stand out in any meaningful way. One would expect them to refer patients to cessation programs, to recommend the Quitline, to discuss pharmacotherapy, or at least to direct them toward non-combustible nicotine products, even though these are not cessation methods per se. Equally striking is the fact that medical personnel, including pulmonologists, do not fully recognize the gaps in their own training when it comes to counseling, guiding, and following patients throughout their cessation journey.

To provide a clearer overview of counseling practices, we consolidated the main survey outcomes into Table 2. This table summarizes the proportion of pulmonologists who reported consistently applying key smoking cessation practices, using n/N notation. The results show very high adherence for the initial steps of asking about tobacco use (93.4%) and advising patients to quit (88.1%). However, subsequent steps of the 5A model—such as assessing readiness to quit (65%), arranging follow-up (66.4%), and assisting through referral to structured cessation programs (36.3%) or pharmacotherapy (17.7%)—were much less frequently implemented. Notably, only 15.9% of respondents reported recommending Quitline, and 11.5% recommended e-cigarettes as cessation aids.

Table 2: Key smoking cessation counseling practices among Romanian pulmonologists (N = 226).

Indicator	n/N	%
Always ask patients about smoking status	211/226	93.4
Always advise patients to quit	199/226	88.1
Assess readiness to quit	147/226	65.0
Offer specific cessation advice	176/226	77.9
Monitor long-term abstinence	150/226	66.4
Refer to the STOP SMOKING program	82/226	36.3
Recommend Quitline	36/226	15.9
Recommend pharmacological treatment	40/226	17.7
Recommend nicotine replacement therapy	49/226	21.7
Recommend e-cigarettes as a cessation aid	26/226	11.5

DISCUSSION

This descriptive surveillance study provides updated national data on smoking behavior and cessation counseling among Romanian pulmonologists. The findings confirm a substantial decline in smoking prevalence over the past two decades—from 30.8% in 2005 [5] to 7.9% in 2024 (Table 3)—a trend that aligns with reports from other European pulmonology groups, where rates typically range between 3.5% and 10% [10,11]. This is notable given Romania's broader epidemiological context, with an estimated 4.4 million tobacco users in 2022, ranking the country 37th globally and 10th in the WHO European Region [13,14].

Table 3: Trend in smoking prevalence among Romanian pulmonologists

Year	Source	Prevalence of Smoking (%)
2005	Munteanu & Mihălțan [5]	30.8
2009	Munteanu et al. [7]	11.3
2024	Present study	7.9

International comparisons further emphasize the variability of smoking among healthcare professionals. A meta-analysis of 246 studies involving 497,081 physicians reported an overall smoking prevalence of 21%, with the highest rates among medical students (25%) and family practitioners (24%) [9]. In Italy, smoking prevalence among healthcare workers increased from 15.9% in 2020 to 24.7%, including significant use of e-cigarettes (21%) [12,15]. Similarly high rates have been observed in Turkey (50.4% among hospital staff) [16] and China (26% among physicians) [17]. However, cross-country comparisons must be interpreted cautiously due to differing study years and methodologies.

Despite encouraging declines, any tobacco use among lung specialists remains relevant, given evidence that physicians' smoking behavior can influence their engagement in cessation counseling [19–21]. The broader Romanian context reinforces this concern: GATS 2018 reported that 65.9% of individuals attempting to quit did so without assistance, while 15.1% used e-cigarettes and 10.4% used HTPs as cessation aids. These patterns suggest gaps in professional guidance, including limited awareness or utilization of the STOP SMOKING program and uncertainty regarding alternative nicotine products [18]. Comparable international data show strong adherence to asking patients about smoking—for example, 98% of SEPAR members in Spain routinely inquire [10]—and evidence indicates that systematic assessment improves readiness for cessation counseling [17,22].

While smoking prevalence among Romanian pulmonologists is low, the study identifies clear gaps in cessation-related practices. Although the majority ask (93.4%) and advise (88.1%) patients, subsequent steps—including assessing readiness (65%), recommending pharmacotherapy (17.7%), and arranging follow-up (66.4%)—are less consistently applied. Similar patterns have been documented in other settings where time limitations, insufficient training, and system-level constraints impede delivery of comprehensive cessation care [12,18,23,27].

The finding that 27% of respondents lack knowledge or interest in cessation methods highlights the need for structured professional development. Evidence from Romania and other countries shows that targeted training significantly improves clinicians' confidence and implementation of cessation strategies [12,28]. The recent introduction of a national certification program in smoking cessation counseling (Ministry of Health Ordinance No. 1.677/2025) offers an important opportunity to address these gaps, provided that adequate support and dissemination are ensured.

System-level challenges were also evident. Only 36.3% of pulmonologists reported referring patients to the STOP SMOKING program, and 15.9% recommended the Quitline. Limited engagement with these services likely reflects their fluctuating availability, reduced visibility, and insufficient integration within routine care. Evidence from other countries shows that coordinated systems combining physician advice, pharmacotherapy, and Quitline or digital support enhance cessation outcomes [24–26]. Strengthening the stability, accessibility, and communication of national cessation resources is therefore crucial.

Overall, the results portray a professional group aligned with tobacco-control principles but constrained by educational and organizational barriers. Addressing these limitations—through enhanced training, reinforced national cessation infrastructure, and improved referral pathways—could substantially strengthen the role of Romanian pulmonologists in national tobacco-control efforts.

LIMITATIONS

This study has several limitations that should be considered when interpreting the findings. All analyses were descriptive, and no inferential statistics were performed. As such, comparisons between subgroups or across countries are illustrative only and cannot be interpreted as statistically significant differences. The international studies cited (e.g., from Spain, Turkey, and Switzerland) were conducted in different years, with varying methodologies and sample characteristics, which limits the validity of direct cross-country comparisons. Additionally, the questionnaire was adapted from prior physician surveys and underwent expert review to ensure face and content validity; however, no pilot testing was conducted. The absence of pilot testing may have limited our ability to identify potential issues with item interpretation or survey flow. As a descriptive surveillance study, the design does not support causal

inference, modeling of psychosocial constructs, or identification of predictors; all findings should be interpreted solely as observed frequencies and self-reported practices. Finally, as participation was voluntary and limited to pulmonologists attending a national congress, selection bias may have influenced the results, and the findings may not be fully generalizable to all Romanian pulmonologists.

CONCLUSIONS

Training in smoking cessation and the preparation of healthcare professionals for patient counseling should be integrated into the core curricula of all health professions. This remains a significant challenge in Romania, particularly in light of recent budget cuts by the Ministry of Health to the STOP SMOKING program and the declining number of physicians adequately trained to support patients in smoking cessation. The ability of Romanian physicians to fulfill their public health mission in tobacco control now largely depends on three critical factors: the quality of their training, the inclusion of tobacco control and cessation counseling in university curricula, and the availability of structured, approved cessation support programs for their patients.

At the same time, addressing disparities in perceived wellness among Romanian populations at high risk for tobacco use, including physicians themselves, is essential. Special attention should be given to understanding the factors contributing to persistent or increasing smoking rates among pulmonologists and how this group perceives and manages their own wellness and professional role in tobacco control efforts. While Romanian pulmonologists demonstrate substantial involvement in smoking cessation, opportunities remain for enhancing training, strengthening system-level support, and better integrating national cessation programs into daily practice. Addressing both organizational and educational barriers will be critical to improving smoking cessation outcomes in Romania and aligning practices with international standards.

Romanian pulmonologists report markedly reduced smoking prevalence compared with two decades ago and demonstrate a strong commitment to asking and advising patients about tobacco use. However, significant gaps remain in assistance and follow-up, particularly regarding pharmacological support and referrals to structured cessation services. Addressing these gaps through enhanced training, better integration of national programs, and system-level reforms could strengthen the role of pulmonologists in tobacco control and contribute to improved patient outcomes.

Conflicts of interest and sources of funding

The authors declare no conflict of interest.

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No generative AI was used during the writing of this article.

Authors' contribution

Conceptualization, FD.M. and AA.C.; methodology, FD.M.; validation, FD.M., AA.C. and AL.I.; formal analysis, FD.M., AA.C. and AL.I.; investigation, FD.M., AA.C. and AL.I.; resources, FD.M., AA.C. and AL.I.; data curation, AA.C.; writing—original draft preparation, AA.C. and AL.I..; writing—review and editing, AA.C.; visualization, FD.M., AA.C. and AL.I.; supervision, FD.M.; project administration, FD.M. All authors have read and agreed to the published version of the manuscript. All authors have equally quoted.

Ethics approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki. The research protocol was reviewed and approved by the Ethics and Scientific Research Committee of the "Marius Nasta" National Institute of Pneumology, Bucharest, Romania (Approval No. 14946/29.07.2025).

Patient consent for publication

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

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

Review Articles

RJMM welcomes reviews of important topics across the scientific basis of medicine and advances in clinical practice. Most published reviews are in response to the editorial invitation, including thematically related "miniseries" of reviews. Authors considering submitting a review for RJMM are advised to canvas their possible review with the Editor-in-Chief or a colleague editor; this avoids early rejection if the subject matter is not deemed a high priority for the Journal at the time of submission. Reviews are suggested to limit to 3500–5000 words, with an abstract of up to 200 words and up to 100 references and figures or tables.

Meta-Analyses or Systematic Reviews

RJMM particularly welcomes the submission of Meta-Analyses and Systematic Reviews, which underpin evidence-based medicine. Guidelines for the preparation of meta-analyses and Systematic Reviews are similar to other reviews, and articles are subject to the usual peer review process. Meta-analyses and Systematic Reviews are suggested to be limited to 3500–5000 words, with an abstract of up to 200 words and up to 100 references and figures or tables.

Original Articles (including clinical trials)

RJMM welcomes original articles concerned with clinical practice and research in the fields of medicine. Papers can cover the medical, surgical, radiological, pathological, biochemical, physiological, ethical, and/or historical subject areas. Clinical trials are afforded expedited publication if deemed suitable. RJMM also deals with the basic sciences and experimental work, particularly that with clear relevance to disease mechanisms and new therapies. Original articles are suggested to be limited to 3000 words, with an abstract of up to 200 words and up to 100 references and figures or tables.

Education and Imaging

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

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

MANUSCRIPT PREPARATION

Style

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

Spelling

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

Units

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

Abbreviations

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

Trade names

Trade names should not be used. Drugs should be referred to by their generic names, rather than brand names.

Parts of the Manuscript

The manuscript should be submitted in separate files: the title page and the main text file according to the Word model.

Title page

The title page should contain (i) a short informative title that contains the major keywords. The title should not contain abbreviations; (ii) the full names of the authors (if possible, not more than 5 authors per title); (iii) the author's institutional affiliations at which the work was carried out; (iv) the full postal and email address, plus telephone number, of the author to whom correspondence about the manuscript should be sent; (v) disclosure statement; and (vi) acknowledgments. The present address of any author, if different from that where the work was carried out, should be supplied in a footnote.

Disclosure statement

The source of financial grants and other funding should be acknowledged, including a frank declaration of the authors' industrial links and affiliations. In the case of clinical trials or an article describing the use of a commercial device, therapeutic substance, or food must state whether there are any potential conflicts of interest for each of the authors: failure to make such a statement may jeopardize the article being sent out for peer review.

Acknowledgments

The contribution of colleagues or institutions should also be acknowledged. Thanks to anonymous reviewers are not allowed.

Main text

As papers are double-blind peer-reviewed, the main text file should not include any information that might identify the authors. The main text of the manuscript should be presented according to the word model. Figures and supporting information should be submitted as separate files. Footnotes to the text are not allowed, and any such material should be incorporated into the text as parenthetical matter.

Abstract and keywords

Original articles must have a structured abstract that states in 200 words or fewer the purpose, basic procedures, main findings, and principal conclusions of the study. If appropriate, divide the abstract with the headings: Background and Aim, Methods, Results, and Conclusions. The abstracts of reviews need not be structured. The abstract should not contain abbreviations or references. Three to ten keywords should be supplied below the abstract and should be taken from those recommended by the US National Library of Medicine's Medical Subject Headings (MeSH) browser – http://www.nlm.nih.gov/mesh/meshhhome.html.

Text

Authors should use the word form when editing the submitted material.

References

The Vancouver system of referencing should be used. In the text, references should be cited using Arabic numerals in square parentheses in the order in which they appear. If cited only in tables or figure legends, number them according to the first identification of the table or figure in the text. In the

reference list, the references should be numbered and listed in order of appearance in the text. Cite the names of all authors when there are six or fewer; when seven or more, list the first three followed by et al. Names of journals should be abbreviated in the style used in MEDLINE. References to unpublished data and personal communications should appear in the text only.

Use Index Medicus as the style guide for references and other journal abbreviations.

Always insert the DOI of the article, if available.

Tables

Tables should be self-contained and complement, but not duplicate, the information contained in the text. Number tables consecutively in the text in

Arabic numerals. Type tables on a separate page with the legend above. Legends should be concise but comprehensive – the table and legend must be understandable without reference to the text. Column headings should be brief, with units of measurement in parentheses; all abbreviations must be defined in footnotes. Footnote symbols: †, ‡, •, ‣ should be used (in that order) and *, **, *** should be reserved for P-values. Statistical measures such as SD or SEM should be identified in the headings.

Figure legends

Type figure legends on a separate page. Legends should be concise but comprehensive – the figure and its legend must be understandable without reference to the text. Include definitions of any symbols used and define/explain all abbreviations and units of measurement. Indicate the stains used in histopathology. Identify statistical measures of variation, such as standard deviation and standard error of the mean.

Figures

All illustrations (line drawings and photographs) are classified as figures. Figures should be numbered using Arabic numerals and cited in consecutive order in the text. Each figure should be supplied as a separate file, with the figure number incorporated in the file name.

Preparation of Electronic Figures for Publication:

Although low-quality images are adequate for review purposes, publication requires high-quality images to prevent the final product from being blurred or fuzzy.

SUBMISSION REQUIREMENTS

Manuscripts should be submitted online at office@rjmm.ro. A cover letter containing an authorship statement should be included. The cover letter should include a statement covering each of the following areas:

– Confirmation that all authors have contributed to and agreed on the content of the manuscript, and the respective roles of each author. It is not accepted that the idea of equal contribution.

– Confirmation that the manuscript has not been published previously, in any language, in whole or in part, and is not currently under consideration elsewhere.

– A statement outlining how ethical clearance has been obtained for the research, particularly concerning studies involving human subjects and animal experimentation.

The institutional ethics committees approving this research must comply with acceptable international standards (such as the Declaration of Helsinki), and this must be stated.

– For research involving pharmacological agents, devices, or medical technology, a clear Conflict of Interest statement about any funding from or pecuniary interests in companies that could be perceived as a potential conflict of interest in the outcome of the research.

– For clinical trials, these have been registered in a publicly accessible database (see more under 'ETHICAL CONSIDERATIONS (Further Information)' later in these guidelines).

If the above items are not included in the cover letter, manuscripts cannot be sent for review. Please also note that the cover letter does not require a detailed or lengthy description of the content or structure of the manuscript itself.

Two Word files need to be included upon submission: A title page file and the main text file that includes all parts of the text in the sequence indicated in the section 'Parts of the manuscript', including tables and figure legends but excluding figures, which should be supplied separately.

The main text file should be prepared using the Word model that is to be found on the web page (https://revistamedicinamilitara.ro/author-submission/).

Each figure should be supplied as a separate file, with the figure number incorporated in the file name. For submission, high-resolution figures (at least 300 d.p.i.) saved as .eps, .jpg, or .tif files are required.

PUBLICATION PROCESS AFTER ACCEPTANCE

Accepted Articles

Accepted Articles are published online after final acceptance, and appear in PDF format only.

Proofs

Once the paper has been typeset, the corresponding author will receive an email alert containing the proof of acceptance. It is therefore essential that a working e-mail address is provided for the corresponding author.

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